ELSEVIER
SAUNDERS

OBSTETRICS AND GYNECOLOGY CLINICS OF NORTH AMERICA

theclinics.com

ELSEVIER
SAUNDERS

Obstet Gynecol Clin N Am
31 (2004) xv–xvi

OBSTETRICS AND
GYNECOLOGY
CLINICS
OF NORTH AMERICA

Preface

Endocrinology of Pregnancy

Aydin Arici, MD Joshua A. Copel, MD
Guest Editors

Recent expansion of biomedical knowledge on the interactions between the fetus, placenta, and the mother have transformed our view of pregnancy in general. Recent basic and clinical investigations have improved significantly our understanding on how hormones affect the pregnancy, and on how pregnancy affects the fetal and maternal hormones. Because pregnancy may be seen as the ultimate hormonally mediated event, the topic of endocrinology of pregnancy is particularly relevant.

The expansion of knowledge that has occurred during the last two decades has pushed medicine toward subspecialization. On the other hand, a general obstetrician-gynecologist is continuously facing challenges to resolve endocrinologic problems during pregnancy. It is well known that physiologic changes of pregnancy may mask clinical findings and laboratory results of endocrinologic problems.

The endocrinology of pregnancy has become one of the areas that straddles multiple specialties; the authorship of this issue reflects this. The aim of this issue is to present a concise review of latest knowledge on the endocrinology of pregnancy to the reader. One needs to gather experts in perinatology, reproductive endocrinology, medical endocrinology, and neonatology to address topics that are quite broad in scope. A diverse group of internationally recognized experts have come together to discuss the cutting edge knowledge in their

0889-8545/04/$ – see front matter
doi:10.1016/j.ogc.2004.09.004

respective specialties. We are grateful to all of the authors, all of whom took the time to contribute to this issue despite their other responsibilities.

Finally, we greatly appreciate the support of Carin Davis and the staff at Elsevier for their outstanding editorial competence. We hope that this issue will serve women with their babies as well as the physicians who care for them.

Aydin Arici, MD
Division of Reproductive Endocrinology and Infertility
Department of Obstetrics, Gynecology, and Reproductive Sciences
Yale University School of Medicine
333 Cedar Street
P.O. Box 208063
New Haven, CT 06520-8063, USA
E-mail address: aydin.arici@yale.edu

Joshua A. Copel, MD
Division of Maternal Fetal Medicine
Department of Obstetrics, Gynecology, & Reproductive Sciences and Pediatrics
Yale University School of Medicine
333 Cedar Street
P.O. Box 208063
New Haven, CT 06520-8063, USA
E-mail address: joshua.copel@yale.edu

ELSEVIER
SAUNDERS

Obstet Gynecol Clin N Am
31 (2004) 727–744

OBSTETRICS AND
GYNECOLOGY
CLINICS
OF NORTH AMERICA

Luteal phase defect: myth or reality

Orhan Bukulmez, MD[a], Aydin Arici, MD[b,*]

[a]*Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, The University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Boulevard, Dallas, TX 75390-9032, USA*

[b]*Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology and Reproductive Sciences, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510, USA*

Luteal phase defect (LPD) was described by Jones in 1949 [1]; it is characterized by failure to develop fully mature secretory endometrium. This entity is defined as a defect of the corpus luteum to secrete progesterone in high enough amounts or for too short a duration. This results in an inadequate or out-of-phase transformation of the endometrium which precludes embryo implantation. Therefore, LPD is believed to be a cause of infertility and spontaneous miscarriage. Abnormalities of the luteal phase have been found in 3% to 10% of the female population that has primary or secondary infertility and occurs in up to 35% of those who have recurrent abortion [2].

As a clinical entity, however, LPD is poorly characterized. LPD may be identified in many women who have proven fertility. There is no definite consensus in the diagnosis of the condition. Some investigators emphasize the importance of endometrial histology in diagnosis and claim that the actual serum progesterone levels have no value as long as the endometrium is in-phase. Other investigators however, believe that only progesterone levels that are greater than a certain threshold can assure the optimal preparation of endometrium for implantation. LPD also has been believed to be one of the stages of ovulatory disturbance that starts with anovulation and continues as oligo-ovulation, LPD, and normal ovulation [3]. This article reviews the controversies that surround LPD.

* Corresponding author.
E-mail address: aydin.arici@yale.edu (A. Arici).

doi:10.1016/j.ogc.2004.08.007

Issues in etiopathogenesis

The proposed mechanisms of LPD include decreased levels of follicle-stimulating hormone (FSH) in follicular phase, abnormal luteinizing hormone (LH) pulsatility, decreased levels of LH and FSH during the ovulatory surge, decreased response of endometrium to progesterone, and elevated prolactin levels [4]. Furthermore, LPD has been linked to several factors (eg, inadequate endometrial progesterone receptors and endometritis) and drugs (eg, clomiphene citrate, gonadotropin releasing hormone (GnRH) agonists and antagonists).

Some investigators reported increased LH pulse frequency and abnormal follicular phase LH:FSH ratio [5], whereas others claimed inadequate LH surge [6] as possible etiologic factors for LPD. These findings were not confirmed in other studies [7,8]. Reported follicular phase FSH deficiency with decreased preovulatory estradiol levels as a cause for LPD [6] also was not demonstrated by other investigators [8,9].

Approximately one half of all LPDs have been attributed to the improper function of the GnRH pulse generator in the hypothalamus [10]. Following ovulation, the increased serum progesterone levels oversuppress the GnRH pulse generator which results in too few LH pulses, and therefore, improper luteal function. Hyperprolactinemia has also been implicated in LPD by interfering with GnRH secretion. Latent hyperprolactinemia by interfering with GnRH also has been associated with LPD [10].

In a primate model, 12-day physical and psychologic stress challenge induced LPD which was marked by the decrease in area under the curve for luteal phase serum progesterone levels. The reduction in overall luteal phase progesterone secretion was not associated with a shorter luteal phase which indicated that premature luteolysis did not occur. This reduction however was attributed to the observed decrease in luteal LH levels, which was ultimately related to the stress-induced dysfunction of the hypothalamic-pituitary-adrenal axis [11]. Mild hyperprolactinemia and exaggerated prolactin release in response to stress also has been associated with LPD or short luteal phase [10,12].

Experimental interference with the profile of gonadotropic stimulation during the follicular phase of the cycle by either using a GnRH agonist [13] or administering a crude follicular fluid preparation [14] reduced the progesterone secretion during the luteal phase. Other investigators demonstrated a decrease in immunoreactive FSH levels during the follicular phase in patients with LPD diagnosed by endometrial histology [15]. After the normal folliculogenesis, progesterone secretion can be decreased by interference with gonadotropic support by GnRH antagonist administration during the midluteal phase [16,17].

Abnormal LH pulse frequency has been linked to LPD [18]. LPD also has been associated with decreased inhibin levels in the follicular phase and a subnormal midcycle LH surge [4].

In the corpus luteum, the most abundant cell types are endothelial cells and the pericytes. Resident cells that stem from white blood cell line and fibroblasts also are present [19]. Only a minority of cells are the steroidogenic cells which are of

two types [20,21]. The large luteal cells originate from the follicular granulosa cells. These cells are not responsive to LH but produce several autocrine and paracrine peptides and eicosanoids. They also produce progesterone and estradiol, in turn, guaranteeing the basal production of these two hormones. The second cell type is the small luteal cells that are derived from the follicular theca cells. These cells acquire LH receptivity and respond to LH pulses with increased estradiol and progesterone secretion. In some patients, LPD is believed to be related to the failure of small luteal cells to respond to LH [10]. An ovarian cause for LPD—in the form of accelerated luteolysis—was suggested as one of the mechanisms [9]. The reasons for early luteal regression were linked to white blood cells and cytokines that are involved actively in the corpus luteum [22,23].

It is clear that any disturbance of ovulatory function may produce LPD in the research setting. The question remains whether each or some of these factors in a given individual is persistent enough to cause "chronic" LPD that leads to infertility or recurrent miscarriage.

Diagnosis

The optimal means of diagnosing LPD is controversial. It is defined historically as a lag of more than 2 days in the histologic development of endometrium compared with the day of the cycle. This lag should occur in more than one cycle. Several indicators and laboratory findings have been proposed for the diagnosis of LPD. These include shortened luteal phase in basal body temperature (BBT) charts, decreased luteal phase serum progesterone levels, and discrepancies in endometrial histologic findings.

Basal body temperature chart

BBT measurements were claimed to be useful in the diagnosis of short luteal phase; however, controversy exists regarding the appropriate criteria to use [9]. Progesterone increases the set-point of the hypothalamic thermoregulatory center. A serum progesterone level that is greater than 2.5 ng/mL may increase the BBT up to 1°F; this forms the basis of the BBT chart. Traditionally, a biphasic BBT chart with sustained increased temperature for 12 to 15 days is considered to be normal. Determining the length of the luteal phase was proposed to be the simplest approach for the evaluation of luteal function, although its predictive values have been questioned [24].

It was reported that 5.2% of women who have normal ovulatory cycles have luteal phases that are shorter than 9 days [7]. Such luteal phases were observed commonly in women who were younger than 24 and older than 45 years of age. When the temperature elevation is maintained for less than 11 days, the quality of ovulation and the resulting corpus luteum has been considered to be inadequate [7]. In 95 patients who had unexplained infertility, however, there were no differences in the length of the luteal phase when compared with 92 control

women who had normal ovulatory cycles [24]. The occurrence of luteal phase duration of up to 11 days were 9% and 8% in women who had unexplained infertility and in controls, respectively [24].

In 30 regularly menstruating women, different BBT patterns and luteal phase lengths were found in 36% and 67% of the observed consecutive cycles, respectively [25]. In addition, estrogen and progesterone levels and endometrial dating showed substantial variability in the consecutive cycles of each patient. This indicates that the conditions of the luteal phase are not the same in every cycle.

In studies, neither the rate of increase in the postovulatory temperature nor the magnitude of temperature elevation correlated with endometrial histology. The overall correlation of BBT charts with endometrial histology was as low as 25% [26]. BBT charts are not reliable enough to be considered as the diagnostic tool for LPD.

Endometrial histology

The original description of LPD in 1949 incorporated BBT charts, urinary pregnanediol levels, and endometrial biopsy as diagnostic tests [1]. The classic approach to diagnose LPD uses the histologic dating method of Noyes et al [27,28] in endometrial biopsy specimens. This original criterion was described in relation to BBT charts. Reproducibility to within 2 days of BBT charts was obtained in more than 80% of the 8000 biopsy specimens that were studied. The diagnosis is made histologically when endometrial maturation lags 2 or more days behind the expected day of ovulation and the subsequent onset of menses [29,30]. With this technique, the prevalence of LPD in an infertile population has ranged from 3.5% to 38.9% [30–32].

The optimal time for performing an endometrial biopsy has not been determined. In an earlier study, nearly one half of the abnormal endometrial biopsies that were performed during the midluteal phase had reverted to normal when repeated in the late luteal phase [33]. Some investigators recommended late luteal biopsy 11 to 12 days after positive urinary LH testing, although the endometrial histology may be increasingly variable as menstruation approaches [3]. When retrospective and prospective dating methods for the diagnosis of LPD were compared, the retrospective method (determination of LH peak by daily assay) identified 42% of biopsy specimens as out-of-phase, whereas the prospective method (calculation based on the onset of next menstrual period) identified only 10% as out-of-phase [34]. The results of repeat endometrial biopsies vary during each cycle in the same patient by 15 to 30% [35]. Therefore, two out-of-phase endometrial biopsies from two cycles have been recommended for the diagnosis of LPD.

There also has been a disagreement over whether to use a 2-day lag or a greater than 2-day lag to diagnose LPD. Five regularly menstruating women of proven fertility underwent a total of 39 endometrial biopsies [36]. Using a 2-day or greater lag in endometrial maturity to define LPD, the incidence of single and sequential out-of-phase endometrial biopsies was 51.4% and 26.7%, respectively.

Using a 3-day or greater lag to define a LPD, the incidence of single and sequential out-of-phase endometrial biopsies was 31.4% and 6.6%, respectively. Furthermore, these incidences in normal, fertile women were close to the rates observed in infertile populations [36].

There is significant inter- and intraobserver variability in the results of histologic dating. The duplicate endometrial biopsies from 25 women were dated by five evaluators on two separate occasions [37]. Inconsistencies between the evaluators accounted for 65% of the observed variability, whereas 27% was due to inconsistencies in duplicate readings by the same evaluator [37]. The significant inter- and intraobserver variability in the results of histologic dating, the issue of cycle-to-cycle variation of biopsy results, the debates in the proper timing of the biopsy, the disagreements over the diagnostic criteria of days of lag in the specimen, and the similar biopsy findings in fertile and infertile women compromise the dependability of endometrial histology in the diagnosis of LPD.

Progesterone levels

The serum progesterone levels are subject to large fluctuations as a result of pulsatile hormone release [38]. On the basis of a single progesterone determination during the midluteal phase, a false LPD may be diagnosed approximately 15% of the time [10]. Some investigators suggest that because the decreased progesterone levels are seen regularly before the occurrence of an LH pulse, it is more appropriate to draw two or three blood samples within a 3-hour period to decrease the probability of a falsely diagnosed LPD down to 2% to 0.5% [10].

In 457 patients who had regular menstrual cycles and normal ovulation as confirmed by transvaginal ultrasound, the distribution of midluteal phase serum progesterone levels were bimodal with two peaks at approximately 7 ng/mL and 11 ng/mL. The arbitrary cut-off for a normal progesterone level was set at greater than 8ng/mL. Life table analysis of the data showed that the patients who had decreased midluteal progesterone levels had decreased spontaneous fecundity [10].

Studies that compared daily luteal serum progesterone levels in women who had unexplained infertility with those who had normal ovulatory or conception cycles reported different cut-off values to define abnormal progesterone levels [39,40]. Some investigators defined abnormal progesterone levels as less than 5 ng/mL for 5 or more days in the luteal phase, whereas other investigators concluded that an abnormal level during the luteal phase was less than 10 ng/mL.

The corpus luteum is unresponsive to LH pulses during the early luteal phase. The response to LH develops between Day 4 and Day 6 after ovulation [41]. It has been suggested that if a single determination of progesterone level can be done on one of the days when the corpus luteum becomes responsive to LH, a correct diagnosis of LPD may be more likely [10]. When a midluteal progesterone level of less than 10 ng/mL was considered to be abnormal, the probability of falsely diagnosing LPD was as low as 4% [10]. The same group

concluded that LPD may occur in infertile patients at irregular and unknown intervals and may be chronic in only approximately 6% of these women [8].

The use of a single or serial progesterone levels as a diagnostic test has been criticized because of the pulsatile nature of progesterone secretion and the transient decrease in progesterone levels following daily events like food ingestion [42]. Progesterone levels vary up to 10-fold during the 2- to 3-hour pulse interval in the luteal phase [43]. In this respect, multiple daily progesterone measurements with the calculation of integrated progesterone levels during the luteal phase may be more accurate but are not applicable clinically.

The sensitivity and specificity of common clinical tests that are used for the diagnosis of LPD were assessed in 58 strictly defined normal women and 34 women who were evaluated for various reasons, including infertility and recurrent abortion [5]. BBT charts, maximum preovulatory follicle sizes, dated endometrial biopsies, and serum progesterone levels (single and multiple) were used in an attempt to predict which patients had decreased integrated progesterone levels during the luteal phase. Luteal integrated progesterone levels—an estimate of total progesterone output over the luteal phase—were determined by summing daily serum progesterone levels starting with the day after the LH surge and ending with the day before the next menstrual period. First, the normal range of integrated progesterone values was determined in a pool of 58 normal volunteers. The investigators calculated an arbitrary cut-off that was inspired from an earlier article that stated the prevalence rate of LPD as 10% [9]. Because 10% of the women in this pool had integrated progesterone values less than 80 ng · days/mL, the cut-off was set as such; however, various cut-off values that were reported in the literature were calculated in a variety of ways in different female populations and were higher than this threshold [12,44,45]. The patient population that was studied, however, had a prevalence rate of LPD of 21% with the cut-off value of less than 80 ng · days/mL [5].

In the study detailed above, unacceptably low sensitivity and/or specificity values were calculated for BBT chart, luteal phase length, and preovulatory follicle diameter for the diagnosis of LPD. Timed endometrial biopsy had marginal sensitivity (29%–57%) and specificity (44%–56%)—whether dated by next menstrual period or midcycle events, which included the day of LH surge or ovulation as determined by ultrasound. The best test for the prediction of decreased integrated progesterone was a single serum progesterone level from the midluteal phase (5 to 9 days after ovulation) that was less than 10 ng/mL (31.8 nmol/L) (sensitivity 86%, specificity 83%) or a sum of three random serum progesterone measurements that was less than 30 ng/mL (95.4 nmol/L) (sensitivity 100%, specificity 80%). The out-of-phase timed endometrial biopsy combined with a single midluteal progesterone level that was less than 10 ng/mL had a sensitivity of 71% and specificity of 93% [5]. In this study, the best dating criterion for endometrial biopsies was next menstrual period rather than the midcycle events. The endometrial biopsy was recommended as a second-line test, especially when LPD needs to be evaluated in a cycle that is treated with ovulation induction or supplemental progesterone [5]. Along with the concerns

that were described earlier, this study was criticized for using daily measurement of plasma progesterone as the reference test against all other tests that should be assessed [46]. The issues raised were that the receptivity of endometrium to progesterone could vary independent of serum progesterone levels and that histologic delay could be present with physiologic progesterone [47] or despite supraphysiologic progesterone levels [48]. Furthermore, the integrated serum progesterone is not a good indicator of endometrial histology [49].

Measuring urinary pregnanediol glucuronide, a metabolite of progesterone, in the first urine voided daily during the luteal phase was recommended to diagnose LPD. This approach may eliminate variability that is due to pulsatile secretion and may be more indicative of the total progesterone production by the corpus luteum [50–52]. Although this approach is an attractive tool in the research setting, its clinical applicability is difficult. In addition, the proportion of progesterone that is converted and excreted as pregnanediol glucuronide varies with age, stage of menstrual cycle, and other factors [3].

Ultrasound

It was recommended to monitor ovarian follicle size with pelvic sonography during the cycle to detect LPD. The follicle diameter was monitored throughout the follicular phase until the day of ovulation; this was indicated by an acute decrease in follicle diameter, abrupt increase in free intraperitoneal fluid, or appearance of intrafollicular echoes. A maximum mean preovulatory follicle diameter of less than 17 mm was considered to indicate LPD [53,54]. In a more recent study, however, a maximum preovulatory follicle size of 17 mm or less was unacceptably insensitive in the diagnosis of LPD [5]. There is no minimum follicle size that separates all normal women from those who have LPD. Studies regarding the assessment of the luteal phase by using transvaginal color and pulsed Doppler ultrasound did not show any significant benefit [55,56].

Clinical conditions that are associated with luteal phase defect

Recurrent abortion

Recurrent abortion is defined as the loss of three or more consecutive pregnancies before the twentieth week of gestation. This condition may be associated with LPD that is marked by retarded endometrial development in the peri-implantation period.

The diagnosis of LPD has been based on the histologic study of a timed luteal phase biopsy according to the method of Noyes et al [27]. In studies that examined timed endometrial biopsy specimens in women who had recurrent abortion, the incidence of LPD ranged from 17.4% [57] to 28% [58]. The evaluation of late luteal phase endometrial biopsies that were performed on regularly

menstruating, fertile women who had no history of pregnancy loss demonstrated a 26.7% incidence of at least a 2-day lag in sequential cycles [36].

In a prospective case series of 197 women who had a history of two consecutive first-trimester spontaneous abortions, preconceptional, single midluteal (5 to 9 days after ovulation) phase serum progesterone (cut-off level for progesterone was less than 10 ng/mL for LPD diagnosis) and estrogen levels did not predict future pregnancy loss [59].

In a recent study that aimed to investigate whether endometrial expression of specific cellular and molecular markers differ in women who have in-phase and out-of-phase endometrium that is consistent with LPD, endometrial biopsies were obtained from 36 women who had unexplained, recurrent first-trimester abortion. Endometrial biopsies were obtained accurately between 6th and 11th days following LH surge (LH + 6 to + 11). There were no differences in endometrial expression of CD45, CD4, and CD3 cells; estrogen receptor; progesterone receptor; leukemia inhibitory factor; and interleukin-6 between in-phase and retarded endometrium [60]. Although an earlier study showed increased epithelial cell expression of progesterone receptor in women who had recurrent abortion and LPD [61], this study did not find any difference in progesterone receptor and estrogen receptor expression between in-phase and LPD endometrium [60]. The differences between the two studies have been related to the variability of the timing of endometrial biopsies and the use of a newer progesterone receptor antibody. Most importantly, the study showed no difference in luteal progesterone levels in women who had in-phase or retarded endometrium [60]. In contrast, LPD was associated with decreased mid-cycle plasma estrogen levels which may indicate poor oocyte quality and a poorly functioning corpus luteum, although it secreted normal amounts of progesterone.

The observations on the artificial cycles suggested that optimum estrogen priming is essential during the follicular phase to achieve appropriate endometrial development during the luteal phase [62]. Because most cases of LPD were not associated with decreased progesterone, but rather, with an abnormal response of endometrium to progesterone, treatment has been targeted at improving the endometrial responsiveness by enhancing the priming of endometrium in the follicular phase. In a small retrospective study, controlled ovarian stimulation with human menopausal gonadotropin improved the endometrial maturation and increased pregnancy rate in patients who had recurrent miscarriage [63]. Although various treatments have been described for LPD, including ovulation induction with clomiphene citrate or gonadotropins, human chorionic gonadotropin injection at the time of expected ovulation, and progesterone supplementation during the luteal phase and the first trimester of the pregnancy, the data are inadequate to support any conclusion [64,65]. A meta-analysis of randomized trials of pregnancies that were treated with progestational agents failed to find any evidence for their positive effect on the maintenance of pregnancy [66]. In view of the uncertainties in establishing the diagnosis of LPD, the empiric treatment of unexplained recurrent abortion with clomiphene citrate was suggested, again without any valid scientific evidence [67].

Despite the many controversies that surround the association of recurrent abortion and LPD, the work-up recommendation for recurrent pregnancy loss still includes luteal phase endometrial biopsy 10 days after the LH surge for endometrial dating [68]. This recommendation and practice should be readdressed.

Infertility

The frequency of LPD in women who have infertility—when strictly defined—is no greater than that found by chance in normal cycles [69]. In a series of 1492 biopsies in 1055 women, 26 biopsies were in conception cycles [70]. With an in-phase biopsy, 15 of 20 pregnancies went to term; however, 4 of 6 pregnancies in women who had an out-of-phase biopsy also went to term. Furthermore, the term pregnancy rates were identical in women who had treated or untreated LPD that was diagnosed with endometrial dating [70].

In 126 cases of unexplained infertility, serial study of plasma hormones and midluteal endometrial biopsies revealed retarded endometrium in 34.1% of the patients. Approximately 78% of the patients who had retarded endometrium showed normal progesterone levels [71].

It was suggested that there may be degrees of LPD. With a lag of 5 days or more, treatment with clomiphene citrate yielded a conception rate of 79%; however, in women who had less severe defects, the same treatment was associated with a conception rate of 8.9% [72].

If a patient has persistent LPD that is accompanied by hyperprolactinemia, bromocriptine is recommended as a treatment option [68]. Although vaginal progesterone and oral dehydrogesterone have been used successfully to induce endometrial maturation in patients who were diagnosed with LPD [73,74], the association between the treatment for out-of phase endometrium and pregnancy in infertile patients is lacking [70,75].

The assessment of endometrial function is a highly controversial area in infertility. Inducing ovulation may improve the hormonal profile of the patient; this may not be associated with a receptive endometrium for implantation [76]. Conversely, postmenopausal and hypogonadal women who are given hormone replacement therapy and donor oocytes can achieve higher implantation rates than women who have normal cycles, even if the respective donors for both groups have comparable pregnancy rates [77].

The pathogenesis of LPD has been linked to inadequate corpus luteum function or inadequate endometrial response. The former has been explained further as due to impaired follicle development, insufficient LH surge, impaired luteotropic system, increased luteolysis, or primary dysfunction of the corpus luteum [78]. The pathogenesis-oriented treatments include estrogen or progesterone replacement, ovulation induction, luteal phase support with human chorionic gonadotropin, progesterone, GnRH pulse, and bromocriptine. In terms of achievement of successful pregnancies, little efficacy was associated with progesterone replacement; however, acceptable pregnancy rates were accom-

plished with ovulation induction. This scenario suggests that the primary cause of LPD in infertility is poor oocyte quality that is due to impaired follicle development. Although clinicians have considered LPD to be one of the most important causes of infertility for several decades, no convincing evidence exists for this relationship.

Luteal suppression in assisted reproduction

GnRH agonists increase pregnancy rates for in vitro fertilization (IVF) cycles by preventing premature surges of endogenous LH through pituitary suppression during controlled ovarian stimulation [79]. In this way, time is allowed for a larger number of oocytes to reach maturity before retrieval. GnRH agonists also work by increasing the length of time for gonadotropin-independent follicular growth resulting in synchronous development of a large cohort of follicles with the ability to respond to exogenous gonadotropins. In spite of these favorable effects, GnRH agonists may create an iatrogenic LPD [80]. The use of GnRH agonists causes the suppression of pituitary LH secretion for as long as 10 days after the last dosage. Without an LH signal, the corpus luteum may be dysfunctional. Without proper progesterone and estrogen stimulation, endometrial receptivity may be compromised [81]. Therefore, luteal supplementation with various agents has been used to prevent this abnormality.

In a recent meta-analysis, luteal supplementation with human chorionic gonadotropin and intramuscular (IM) progesterone significantly improved fertility outcomes as compared to no treatment in women undergoing IVF [82]. Oral progesterone supplementation during the luteal phase had less benefit than vaginal progesterone or IM human chorionic gonadotropin. The oral progesterone, however, also had decreased efficacy and a greater number of side effects than the IM progesterone.

It was hypothesized that IM human chorionic gonadotropin might be superior to progesterone alone as luteal support. Because human chorionic gonadotropin rescues the corpus luteum, it allows the continuation of estrogen and progesterone secretion and may maintain the secretion of other unknown products from the corpus luteum [83]. In a recent meta-analysis, no differences were found between IM human chorionic gonadotropin administration during the luteal phase when compared with IM or vaginal progesterone [82]. Some studies reported significant increases in hyperstimulation rates when human chorionic gonadotropin was used for luteal support [84,85]. Hence, there is no evidence that i.m. human chorionic gonadotropin as luteal support is superior to progesterone alone. The meta-analysis also showed that IM progesterone contributed to higher cumulative pregnancy and delivery rates than vaginal progesterone [82]. The optimal length of treatment for luteal support is still controversial; it may be limited to the luteal phase or through 10 to 12 weeks' gestation.

The recent availability of GnRH antagonists for the prevention of a premature LH increase in IVF was believed to be advantageous because gonadotropin levels recover within 24 hours after stopping the GnRH antagonist [86]. It was

speculated that luteal phase supplementation may not be required in cycles in which GnRH antagonist cotreatment is applied [87]. In a recent prospective study, the nonsupplemented luteal phase characteristics in patients who were cotreated with GnRH antagonists were analyzed in women who were randomized to recombinant human chorionic gonadotropin, recombinant LH, or an endogenous LH surge that was induced by a GnRH agonist bolus for the induction of final oocyte maturation. The luteal phase was inadequate in all groups that had decreased pregnancy rates. The investigators strongly recommended luteal support with GnRH antagonist cotreatment [88].

Recent concepts in endometrial evaluation

For a long time the premenstrual dating of endometrium was considered to be the gold standard for the evaluation of LPD. Recently, the relationship between the histologic changes and the endometrial receptivity has been questioned [89].

The evaluation of endometrial dating by Noyes criteria [27,28], was derived from observations in a predominantly infertile population; scant validating evidence exists despite its widespread use over 5 decades. The flaws of timed endometrial biopsy include its dependence on a subjective histologic interpretation; variation in the handling of glandular stromal disparity among different investigators; and a moderate reproducibility of readings, even when the same specimen is read several times by a single pathologist [5,34]. In addition, timed endometrial biopsy has been validated as the definitive test for LPD by comparing its results with unproven criteria, such as BBT charts and single progesterone measurements with various methods [27,90]. Therefore, histologic dating seemed to be a crude index of endometrial receptivity. Recent studies have been directed to find more objective measures of endometrial receptivity.

The midluteal assessment of endometrium with relevant markers was evaluated to define better endometrial receptivity. The measurement of glycodelin A (previously called placental protein, PP14) in endometrial flushings was recommended in the identification of an endometrial defect [91]. In this regard, $\alpha v\beta 3$ integrin expression and pinopod formation have been the proposed markers for uterine receptivity [92,93].

It is accepted that the endometrium is receptive to blastocyst implantation during a short period during the luteal phase that is known as the implantation window. Based on the IVF and embryo transfer data, this period lasts for approximately 4 days (between Days 5.5 and 9.5 following ovulation) [94]. Traditionally, this putative window of implantation has been defined by histologic features [27,75]. Because there have been many discrepancies in this definition, studies have focused on molecular markers that are believed to be important in endometrial receptivity. In a recent study, an increased level of $\alpha v\beta 3$ integrin expression and pinopods were found on postovulatory Days 6 to 7, irrespective of whether endometria were in-phase or out-of-phase [95].

The diminished endometrial receptivity that results in failed or defective implantation has been proposed as a mechanism of infertility that is not related to anovulation or tubal or male factors. LPD has been considered to be one of the many causes of an unreceptive endometrium. The studies of the biochemical markers of endometrial receptivity demonstrated that even when the morphologic development of endometrium proceeds normally, its functional maturation may be impaired. This discrepancy between endometrial histology and its functional maturation was observed in patients who had mild endometriosis [96] and unexplained infertility [97]. Progesterone receptor is down-regulated differentially in endometrial epithelium and stroma and loss of epithelial progesterone receptor coincides with the time of embryo implantation [98,99]. Several other studies have been published regarding the patterns of endometrial estrogen and progesterone receptor expression in LPD. The results of these studies varied widely [74,100–102]; small sample size, different patient populations, and differences in the timing of endometrial biopsies and the methodologies that were used may explain the conflicting results. The development and use of monoclonal antibodies that were more specific to steroid receptors seemed to make the findings of recent studies more valid.

In a more recent study, histologic delay that was consistent with LPD was associated with a failure of progesterone receptor down-regulation and a lack of $\alpha v\beta 3$ integrin expression [61]; however, in patients who had minimal or mild endometriosis, the down-regulation of progesterone receptor was not associated with the timely expression of $\alpha v\beta 3$ integrin. Hence, many alternate routes may affect endometrial receptivity at the molecular level; this complicates further the evaluation and diagnosis of LPD.

Among the patterns of integrin expression that were studied in human endometrium, $\alpha v\beta 3$ integrin appears precisely as the implantation window begins (~cycle Day 20) [103]. This marker may not be expressed in patients who have LPD as diagnosed by histologic dating as well as in some infertile women who have normal endometrial dating [96,97].

The potential significance of the newly proposed markers of endometrial receptivity was challenged recently. A study was conducted to investigate the intra-subject variability and inter-cycle reproducibility of histologic dating and endometrial receptivity markers, which included $\alpha v\beta 3$ integrin expression determined by immunohistochemistry and pinopod formation that was assessed under scanning electron microscopy [104]. Fifteen patients who had primary infertility underwent three endometrial biopsies in consecutive spontaneous cycles on postovulation Day 7 as determined by serial transvaginal ultrasound. $\alpha v\beta 3$ Integrin expression and pinopod formation in the endometrium of infertile patients were poorly reproducible and were highly variable from one cycle to another. Furthermore, the reproducibility for the new markers of endometrial receptivity was similar to that for traditional histologic dating [104]; hence, their potential usefulness as targets for infertility treatments was debated.

In another study, the correlation of midluteal endometrial histologic dating and $\alpha v\beta 3$ integrin expression with subsequent fecundity was examined [105].

One hundred consecutive infertile patients underwent two endometrial biopsies, 4 days apart (mid- and late luteal); these were timed from the day of ovulation as determined by transvaginal ultrasound. All patients were followed for 18 to 24 months. Twenty five midluteal biopsies were out-of-phase. Endometrial glandular $\alpha v\beta 3$ integrin expression was observed in 50% of midluteal specimens; expression was more frequent among in-phase biopsies. All late luteal biopsies expressed integrin. Thirty-eight women had spontaneous pregnancy. There was a lack of correlation between the presence or absence of $\alpha v\beta 3$ integrin and the outcome for infertile women, irrespective of whether endometrial biopsies were in-phase or out-of-phase [105]. The value of endometrial evaluation, histologically and immunohistochemically, for $\alpha v\beta 3$ integrin in patients who had infertility was questioned.

Summary

Although the diagnosis of LPD has been described convincingly in the research setting, it remains a controversial clinical entity. In clinical practice, the diagnosis of LPD has been attempted by several methods—BBT charts, progesterone levels indirectly, and endometrial biopsy as a direct and invasive method. All of these methods are retrospective; the interpretation of endometrial biopsies—even with the recently proposed molecular markers—has not been satisfactory. Therefore, no reliable method exists to diagnose LPD. When LPD is found, most physicians are inclined to incriminate it as the cause of infertility or recurrent abortion, although there is no convincing scientific evidence to support these associations. Does the LPD appear consecutively or sporadically? This question further complicates discussions on the diagnosis and treatment of LPD.

No specific treatment is intended to manage LPD. The treatment of LPD with progestin replacement has not been correlated with conception. The treatment decisions mostly are empiric. Treatment modalities that are recommended for unexplained infertility (eg, ovulation induction, assisted reproduction) have been successful in achieving pregnancy in women who have LPD. These issues undermine the efforts to diagnose the condition.

LPD is a reality in assisted reproduction cycles with GnRH agonist/antagonist suppression. Otherwise, there is no convincing evidence to define LPD as a distinct clinical entity that leads to reproductive problems. It is not justified to include costly and cumbersome tests to diagnose LPD in patients who have infertility or recurrent abortion.

References

[1] Jones GES. Some newer aspects of the management of infertility. JAMA 1949;141:1123–9.
[2] Insler V. Corpus luteum defects. Curr Opin Obstet Gynecol 1992;4(2):203–11.

[3] Ginsburg KA. Luteal phase defect. Etiology, diagnosis, and management. Endocrinol Metab Clin North Am 1992;21(1):85–104.
[4] Soules MR, McLachlan RI, Ek M, Dahl KD, Cohen NL, Bremner WJ. Luteal phase deficiency: characterization of reproductive hormones over the menstrual cycle. J Clin Endocrinol Metab 1989;69(4):804–12.
[5] Jordan J, Craig K, Clifton DK, Soules MR. Luteal phase defect: the sensitivity and specificity of diagnostic methods in common clinical use. Fertil Steril 1994;62(1):54–62.
[6] Ayabe T, Tsutsumi O, Momoeda M, Yano T, Mitsuhashi N, Taketani Y. Impaired follicular growth and abnormal luteinizing hormone surge in luteal phase defect. Fertil Steril 1994; 61(4):652–6.
[7] Lenton EA, Landgren BM, Sexton L. Normal variation in the length of the luteal phase of the menstrual cycle: identification of the short luteal phase. Br J Obstet Gynaecol 1984;91(7): 685–9.
[8] Hinney B, Henze C, Kuhn W, Wuttke W. The corpus luteum insufficiency: a multifactorial disease. J Clin Endocrinol Metab 1996;81(2):565–70.
[9] McNeely MJ, Soules MR. The diagnosis of luteal phase deficiency: a critical review. Fertil Steril 1988;50(1):1–15.
[10] Wuttke W, Pitzel L, Seidlova-Wuttke D, Hinney B. LH pulses and the corpus luteum: the luteal phase deficiency (LPD). Vitam Horm 2001;63:131–58.
[11] Xiao E, Xia-Zhang L, Ferin M. Inadequate luteal function is the initial clinical cyclic defect in a 12-day stress model that includes a psychogenic component in the Rhesus monkey. J Clin Endocrinol Metab 2002;87(5):2232–7.
[12] del Pozo E, Wyss H, Tollis G, Alcaniz J, Campana A, Naftolin F. Prolactin and deficient luteal function. Obstet Gynecol 1979;53(3):282–6.
[13] Sheehan KL, Casper RF, Yen SS. Luteal phase defects induced by an agonist of luteinizing hormone-releasing factor: a model for fertility control. Science 1982;215(4529):170–2.
[14] Stouffer RL, Hodgen GD, Ottobre AC, Christian CD. Follicular fluid treatment during the follicular versus luteal phase of the menstrual cycle: effects on corpus luteum function. J Clin Endocrinol Metab 1984;58(6):1027–33.
[15] Cook CL, Rao CV, Yussman MA. Plasma gonadotropin and sex steroid hormone levels during early, midfollicular, and midluteal phases of women with luteal phase defects. Fertil Steril 1983;40(1):45–8.
[16] Mais V, Kazer RR, Cetel NS, Rivier J, Vale W, Yen SS. The dependency of folliculogenesis and corpus luteum function on pulsatile gonadotropin secretion in cycling women using a gonadotropin-releasing hormone antagonist as a probe. J Clin Endocrinol Metab 1986;62(6): 1250–5.
[17] McLachlan RI, Cohen NL, Vale WW, Rivier JE, Burger HG, Bremner WJ, et al. The importance of luteinizing hormone in the control of inhibin and progesterone secretion by the human corpus luteum. J Clin Endocrinol Metab 1989;68(6):1078–85.
[18] Soules MR, Clifton DK, Cohen NL, Bremner WJ, Steiner RA. Luteal phase deficiency: abnormal gonadotropin and progesterone secretion patterns. J Clin Endocrinol Metab 1989; 69(4):813–20.
[19] Adashi EY. The potential relevance of cytokines to ovarian physiology: the emerging role of resident ovarian cells of the white blood cell series. Endocr Rev 1990;11(3):454–64.
[20] Ohara A, Mori T, Taii S, Ban C, Narimoto K. Functional differentiation in steroidogenesis of two types of luteal cells isolated from mature human corpora lutea of menstrual cycle. J Clin Endocrinol Metab 1987;65(6):1192–200.
[21] Hansel W, Dowd JP. Hammond memorial lecture. New concepts of the control of corpus luteum function. J Reprod Fertil 1986;78(2):755–68.
[22] Wuttke W, Theiling K, Hinney B, Pitzel L. Regulation of steroid production and its function within the corpus luteum. Steroids 1998;63(5–6):299–305.
[23] Wuttke W, Spiess S, Knoke I, Pitzel L, Leonhardt S, Jarry H. Synergistic effects of prostaglandin F2alpha and tumor necrosis factor to induce luteolysis in the pig. Biol Reprod 1998;58(5):1310–5.

[24] Smith SK, Lenton EA, Landgren BM, Cooke ID. The short luteal phase and infertility. Br J Obstet Gynaecol 1984;91(11):1120–2.
[25] Ishimaru T, Mahmud N, Kawano M, Yamabe T. Sporadic appearance of luteal-phase defect in prospective assessment. Horm Res 1992;37(Suppl 1):32–6.
[26] Downs KA, Gibson M. Basal body temperature graph and the luteal phase defect. Fertil Steril 1983;40(4):466–8.
[27] Noyes RW, Hertig MD, Rock MD. Dating the endometrial biopsy. Fertil Steril 1950;1:3–25.
[28] Noyes RW, Hamano JO. Accuracy of endometrial dating, correlation of endometrial dating with basal body temperature and menses. Fertil Steril 1953;4(6):504–17.
[29] Jones GS, Madrigal-Castro V. Hormonal findings in association with abnormal corpus luteum function in the human: the luteal phase defect. Fertil Steril 1970;21(1):1–13.
[30] Wentz AC. Endometrial biopsy in the evaluation of infertility. Fertil Steril 1980;33(2):121–4.
[31] Jones GS. The luteal phase defect. Fertil Steril 1976;27(4):351–6.
[32] Cumming DC, Honore LH, Scott JZ, Williams KP. The late luteal phase in infertile women: comparison of simultaneous endometrial biopsy and progesterone levels. Fertil Steril 1985; 43(5):715–9.
[33] Kusuda M, Nakamura G, Matsukuma K, Kurano A. Corpus luteum insufficiency as a cause of nidatory failure. Acta Obstet Gynecol Scand 1983;62(3):199–205.
[34] Li TC, Rogers AW, Lenton EA, Dockery P, Cooke I. A comparison between two methods of chronological dating of human endometrial biopsies during the luteal phase, and their correlation with histologic dating. Fertil Steril 1987;48(6):928–32.
[35] Balasch J, Vanrell JA, Creus M, Marquez M, Gonzalez-Merlo J. The endometrial biopsy for diagnosis of luteal phase deficiency. Fertil Steril 1985;44(5):699–701.
[36] Davis OK, Berkeley AS, Naus GJ, Cholst IN, Freedman KS. The incidence of luteal phase defect in normal, fertile women, determined by serial endometrial biopsies. Fertil Steril 1989; 51(4):582–6.
[37] Gibson M, Badger GJ, Byrn F, Lee KR, Korson R, Trainer TD. Error in histologic dating of secretory endometrium: variance component analysis. Fertil Steril 1991;56(2):242–7.
[38] Knobil E. The neuroendocrine control of the menstrual cycle. Recent Prog Horm Res 1980; 36:53–88.
[39] Landgren BM, Unden AL, Diczfalusy E. Hormonal profile of the cycle in 68 normally menstruating women. Acta Endocrinol (Copenh) 1980;94(1):89–98.
[40] Olive DL. The prevalence and epidemiology of luteal-phase deficiency in normal and infertile women. Clin Obstet Gynecol 1991;34(1):157–66.
[41] Hinney B, Henze C, Wuttke W. Regulation of luteal function by luteinizing hormone and prolactin at different times of the luteal phase. Eur J Endocrinol 1995;133(6):701–17.
[42] Nakajima ST, Gibson M. The effect of a meal on circulating steady-state progesterone levels. J Clin Endocrinol Metab 1989;69(4):917–9.
[43] Filicori M, Butler JP, Crowley Jr WF. Neuroendocrine regulation of the corpus luteum in the human. Evidence for pulsatile progesterone secretion. J Clin Invest 1984;73(6):1638–47.
[44] Wu CH, Minassian SS. The integrated luteal progesterone: an assessment of luteal function. Fertil Steril 1987;48(6):937–40.
[45] Schweiger U, Laessle RG, Tuschl RJ, Broocks A, Krusche T, Pirke KM. Decreased follicular phase gonadotropin secretion is associated with impaired estradiol and progesterone secretion during the follicular and luteal phases in normally menstruating women. J Clin Endocrinol Metab 1989;68(5):888–92.
[46] Castelbaum AJ, Lessey BA. Corpus luteum defect—"alloyed gold standard. Fertil Steril 1995; 63(2):427–8.
[47] Massai MR, de Ziegler D, Lesobre V, Bergeron C, Frydman R, Bouchard P. Clomiphene citrate affects cervical mucus and endometrial morphology independently of the changes in plasma hormonal levels induced by multiple follicular recruitment. Fertil Steril 1993;59(6):1179–86.
[48] Toner JP, Hassiakos DK, Muasher SJ, Hsiu JG, Jones Jr HW. Endometrial receptivities after leuprolide suppression and gonadotropin stimulation: histology, steroid receptor concentrations, and implantation rates. Ann N Y Acad Sci 1991;622:220–9.

[49] Batista MC, Cartledge TP, Merino MJ, Axiotis C, Platia MP, Merriam GR, et al. Midluteal phase endometrial biopsy does not accurately predict luteal function. Fertil Steril 1993;59(2): 294–300.
[50] Chatterton Jr RT, Haan JN, Jenco JM, Cheesman KL. Radioimmunoassay of pregnanediol concentrations in early morning urine specimens for assessment of luteal function in women. Fertil Steril 1982;37(3):361–6.
[51] Blacker CM, Ginsburg KA, Leach RE, Randolph J, Moghissi KS. Unexplained infertility: evaluation of the luteal phase; results of the National Center for Infertility Research at Michigan. Fertil Steril 1997;67(3):437–42.
[52] Santoro N, Goldsmith LT, Heller D, Illsley N, McGovern P, Molina C, et al. Luteal progesterone relates to histological endometrial maturation in fertile women. J Clin Endocrinol Metab 2000;85(11):4207–11.
[53] Check JH, Goldberg BB, Kurtz A, Adelson HG, Rankin A. Pelvic sonography to help determine the appropriate therapy for luteal phase defects. Int J Fertil 1984;29(3):156–8.
[54] Geisthovel F, Skubsch U, Zabel G, Schillinger H, Breckwoldt M. Ultrasonographic and hormonal studies in physiologic and insufficient menstrual cycles. Fertil Steril 1983;39(3): 277–83.
[55] Merce LT, Garces D, Barco MJ, de la Fuente F. Intraovarian Doppler velocimetry in ovulatory, dysovulatory and anovulatory cycles. Ultrasound Obstet Gynecol 1992;2(3):197–202.
[56] Tinkanen H. The role of vascularisation of the corpus luteum in the short luteal phase studied by Doppler ultrasound. Acta Obstet Gynecol Scand 1994;73(4):321–3.
[57] Tulppala M, Bjorses UM, Stenman UH, Wahlstrom T, Ylikorkala O. Luteal phase defect in habitual abortion: progesterone in saliva. Fertil Steril 1991;56(1):41–4.
[58] Li TC. Recurrent miscarriage: principles of management. Hum Reprod 1998;13(2):478–82.
[59] Ogasawara M, Kajiura S, Katano K, Aoyama T, Aoki K. Are serum progesterone levels predictive of recurrent miscarriage in future pregnancies? Fertil Steril 1997;68(5):806–9.
[60] Tuckerman E, Laird SM, Stewart R, Wells M, Li TC. Markers of endometrial function in women with unexplained recurrent pregnancy loss: a comparison between morphologically normal and retarded endometrium. Hum Reprod 2004;19(1):196–205.
[61] Lessey BA, Yeh I, Castelbaum AJ, Fritz MA, Ilesanmi AO, Korzeniowski P, et al. Endometrial progesterone receptors and markers of uterine receptivity in the window of implantation. Fertil Steril 1996;65(3):477–83.
[62] Li TC, Cooke ID, Warren MA, Goolamallee M, Graham RA, Aplin JD. Endometrial responses in artificial cycles: a prospective study comparing four different oestrogen dosages. Br J Obstet Gynaecol 1992;99(9):751–6.
[63] Li TC, Ding SH, Anstie B, Tuckerman E, Wood K, Laird S. Use of human menopausal gonadotropins in the treatment of endometrial defects associated with recurrent miscarriage: preliminary report. Fertil Steril 2001;75(2):434–7.
[64] Karamardian LM, Grimes DA. Luteal phase deficiency: effect of treatment on pregnancy rates. Am J Obstet Gynecol 1992;167(5):1391–8.
[65] Kutteh WH. Recurrrent pregnancy loss. In: Carr BR, Blackwell RE, editors. Textbook of reproductive medicine. 2nd edition. Stamford (CT): Appleton & Lange; 1998. p. 679–92.
[66] Goldstein P, Berrier J, Rosen S, Sacks HS, Chalmers TC. A meta-analysis of randomized control trials of progestational agents in pregnancy. Br J Obstet Gynaecol 1989;96(3):265–74.
[67] Speroff L, Glass RH, Kase NG. Recurrent pregnancy loss. In: Speroff L, Glass RH, Kase NG, editors. Clinical gynecologic endocrinology and infertility. Baltimore: Lippincott Williams & Wilkins; 1999. p. 1043–55.
[68] Roberts CP, Murphy AA. Endocrinopathies associated with recurrent pregnancy loss. Semin Reprod Med 2000;18(4):357–62.
[69] Wentz AC, Kossoy LR, Parker RA. The impact of luteal phase inadequacy in an infertile population. Am J Obstet Gynecol 1990;162(4):937–43 [discussion 943–5].
[70] Balasch J, Fabregues F, Creus M, Vanrell JA. The usefulness of endometrial biopsy for luteal phase evaluation in infertility. Hum Reprod 1992;7(7):973–7.

[71] Kusuhara K. Clinical importance of endometrial histology and progesterone level assessment in luteal-phase defect. Horm Res 1992;37(Suppl 1):53–8.
[72] Downs KA, Gibson M. Clomiphene citrate therapy for luteal phase defect. Fertil Steril 1983; 39(1):34–8.
[73] Balasch J, Vanrell JA, Marquez M, Burzaco I, Gonzalez-Merlo J. Dehydrogesterone versus vaginal progesterone in the treatment of the endometrial luteal phase deficiency. Fertil Steril 1982;37(6):751–4.
[74] Jacobs MH, Balasch J, Gonzalez-Merlo JM, Vanrell JA, Wheeler C, Strauss III JF, et al. Endometrial cytosolic and nuclear progesterone receptors in the luteal phase defect. J Clin Endocrinol Metab 1987;64(3):472–5.
[75] Balasch J, Vanrell JA. Corpus luteum insufficiency and fertility: a matter of controversy. Hum Reprod 1987;2(7):557–67.
[76] Bonhoff A, Naether O, Johannisson E, Bohnet HG. Morphometric characteristics of endometrial biopsies after different types of ovarian stimulation for infertility treatment. Fertil Steril 1993;59(3):560–6.
[77] Edwards RG, Morcos S, Macnamee M, Balmaceda JP, Walters DE, Asch R. High fecundity of amenorrhoeic women in embryo-transfer programmes. Lancet 1991;338(8762):292–4.
[78] Mori H. New aspects of the physiology and pathology of the luteal phase: an overview. Horm Res 1992;37(Suppl 1):3–4.
[79] Hughes EG, Fedorkow DM, Daya S, Sagle MA, Van de Koppel P, Collins JA. The routine use of gonadotropin-releasing hormone agonists prior to in vitro fertilization and gamete intrafallopian transfer: a meta-analysis of randomized controlled trials. Fertil Steril 1992;58(5): 888–96.
[80] Macklon NS, Fauser BC. Impact of ovarian hyperstimulation on the luteal phase. J Reprod Fertil Suppl 2000;55:101–8.
[81] Tavaniotou A, Albano C, Smitz J, Devroey P. Impact of ovarian stimulation on corpus luteum function and embryonic implantation. J Reprod Immunol 2002;55(1–2):123–30.
[82] Pritts EA, Atwood AK. Luteal phase support in infertility treatment: a meta-analysis of the randomized trials. Hum Reprod 2002;17(9):2287–99.
[83] Hutchinson-Williams KA, DeCherney AH, Lavy G, Diamond MP, Naftolin F, Lunenfeld B. Luteal rescue in in vitro fertilization-embryo transfer. Fertil Steril 1990;53(3):495–501.
[84] Araujo Jr E, Bernardini L, Frederick JL, Asch RH, Balmaceda JP. Prospective randomized comparison of human chorionic gonadotropin versus intramuscular progesterone for luteal-phase support in assisted reproduction. J Assist Reprod Genet 1994;11(2):74–8.
[85] Mochtar MH, Hogerzeil HV, Mol BW. Progesterone alone versus progesterone combined with HCG as luteal support in GnRHa/HMG induced IVF cycles: a randomized clinical trial. Hum Reprod 1996;11(8):1602–5.
[86] Oberye JJ, Mannaerts BM, Huisman JA, Timmer CJ. Pharmacokinetic and pharmacodynamic characteristics of ganirelix (Antagon/Orgalutran). Part II. Dose-proportionality and gonadotropin suppression after multiple doses of ganirelix in healthy female volunteers. Fertil Steril 1999;72(6):1006–12.
[87] Elter K, Nelson LR. Use of third generation gonadotropin-releasing hormone antagonists in in vitro fertilization-embryo transfer: a review. Obstet Gynecol Surv 2001;56(9):576–88.
[88] Beckers NG, Macklon NS, Eijkemans MJ, Ludwig M, Felberbaum RE, Diedrich K, et al. Nonsupplemented luteal phase characteristics after the administration of recombinant human chorionic gonadotropin, recombinant luteinizing hormone, or gonadotropin-releasing hormone (GnRH) agonist to induce final oocyte maturation in in vitro fertilization patients after ovarian stimulation with recombinant follicle-stimulating hormone and GnRH antagonist cotreatment. J Clin Endocrinol Metab 2003;88(9):4186–92.
[89] Castelbaum AJ, Wheeler J, Coutifaris CB, Mastroianni Jr L, Lessey BA. Timing of the endometrial biopsy may be critical for the accurate diagnosis of luteal phase deficiency. Fertil Steril 1994;61(3):443–7.
[90] Annos T, Thompson IE, Taymor ML. Luteal phase deficiency and infertility: difficulties encountered in diagnosis and treatment. Obstet Gynecol 1980;55(6):705–10.

[91] Dalton CF, Laird SM, Estdale SE, Saravelos HG, Li TC. Endometrial protein PP14 and CA-125 in recurrent miscarriage patients; correlation with pregnancy outcome. Hum Reprod 1998; 13(11):3197–202.
[92] Lessey BA, Ilesanmi AO, Lessey MA, Riben M, Harris JE, Chwalisz K. Luminal and glandular endometrial epithelium express integrins differentially throughout the menstrual cycle: implications for implantation, contraception, and infertility. Am J Reprod Immunol 1996;35(3): 195–204.
[93] Nikas G. Pinopodes as markers of endometrial receptivity in clinical practice. Hum Reprod 1999;14(Suppl 2):99–106.
[94] Aplin JD. The cell biological basis of human implantation. Baillieres Best Pract Res Clin Obstet Gynaecol 2000;14(5):757–64.
[95] Creus M, Ordi J, Fabregues F, Casamitjana R, Carmona F, Cardesa A, et al. The effect of different hormone therapies on integrin expression and pinopode formation in the human endometrium: a controlled study. Hum Reprod 2003;18(4):683–93.
[96] Lessey BA, Castelbaum AJ, Sawin SW, Buck CA, Schinnar R, Bilker W, et al. Aberrant integrin expression in the endometrium of women with endometriosis. J Clin Endocrinol Metab 1994;79(2):643–9.
[97] Lessey BA, Castelbaum AJ, Sawin SW, Sun J. Integrins as markers of uterine receptivity in women with primary unexplained infertility. Fertil Steril 1995;63(3):535–42.
[98] Lessey BA, Killam AP, Metzger DA, Haney AF, Greene GL, McCarty Jr KS. Immunohistochemical analysis of human uterine estrogen and progesterone receptors throughout the menstrual cycle. J Clin Endocrinol Metab 1988;67(2):334–40.
[99] Garcia E, Bouchard P, De Brux J, Berdah J, Frydman R, Schaison G, et al. Use of immunocytochemistry of progesterone and estrogen receptors for endometrial dating. J Clin Endocrinol Metab 1988;67(1):80–7.
[100] McRae MA, Blasco L, Lyttle CR. Serum hormones and their receptors in women with normal and inadequate corpus luteum function. Fertil Steril 1984;42(1):58–63.
[101] Spirtos NJ, Yurewicz EC, Moghissi KS, Magyar DM, Sundareson AS, Bottoms SF. Pseudocorpus luteum insufficiency: a study of cytosol progesterone receptors in human endometrium. Obstet Gynecol 1985;65(4):535–40.
[102] Hirama Y, Ochiai K. Estrogen and progesterone receptors of the out-of-phase endometrium in female infertile patients. Fertil Steril 1995;63(5):984–8.
[103] Lessey BA, Damjanovich L, Coutifaris C, Castelbaum A, Albelda SM, Buck CA. Integrin adhesion molecules in the human endometrium. Correlation with the normal and abnormal menstrual cycle. J Clin Invest 1992;90(1):188–95.
[104] Ordi J, Creus M, Quinto L, Casamitjana R, Cardesa A, Balasch J. Within-subject between-cycle variability of histological dating, alpha v beta 3 integrin expression, and pinopod formation in the human endometrium. J Clin Endocrinol Metab 2003;88(5):2119–25.
[105] Ordi J, Creus M, Ferrer B, Fabregues F, Carmona F, Casamitjana R, et al. Midluteal endometrial biopsy and alphavbeta3 integrin expression in the evaluation of the endometrium in infertility: implications for fecundity. Int J Gynecol Pathol 2002;21(3):231–8.

ELSEVIER
SAUNDERS

Obstet Gynecol Clin N Am
31 (2004) 745–766

OBSTETRICS AND
GYNECOLOGY
CLINICS
OF NORTH AMERICA

Hormonal regulation of implantation

Pinar H. Kodaman, MD, PhD, Hugh S. Taylor, MD*

Division of Reproductive Endocrinology and Infertility, Department of Obstetrics, Gynecology, and Reproductive Sciences, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520, USA

Implantation requires synchronization between the developing embryo and endometrium. The dialog between embryo and endometrium and the receptivity of the latter is under the control of the sex steroids, estrogen and progesterone, as well as other hormones, such as prolactin, calcitonin, and human chorionic gonadotropin (hCG). Although the complex process of implantation remains to be characterized fully, numerous cellular and molecular markers of endometrial receptivity—many of which are regulated hormonally—have been defined. This article addresses the endocrine-mediated aspects of implantation as they pertain to normal reproduction and assisted reproductive technology (ART).

Normal implantation

Following fertilization in the fallopian tube 24 to 48 hours after ovulation, the zygote migrates through the fallopian tube until it reaches the uterine cavity at the morula stage on Day 18 of an ideal 28-day cycle [1,2]. On Day 19, the blastocyst forms, sheds its zona pellucida, superficially apposes, and adheres to the endometrium [3]. Although the initial apposition is unstable, adhesion involves increased physical interactions between embryo and uterine epithelium [4]. This is followed by trophoblast invasion through the endometrial epithelium and underlying stroma, the inner third of the myometrium, and the uterine vasculature, all of which ultimately result in placentation [5]. Implantation occurs only during the ''window of implantation,'' which corresponds to postovulatory Days 6 to 10 in humans [6]. The endometrium is one of the

* Corresponding author.
E-mail address: hugh.taylor@yale.edu (H.S. Taylor).

0889-8545/04/$ – see front matter
doi:10.1016/j.ogc.2004.08.008 obgyn.theclinics.com

few tissues in which implantation cannot take place except during this restricted, narrow time period [6].

In natural cycles, the implantation rate is difficult to determine because although ovulation can be confirmed, knowledge about successful fertilization and transport of the embryo to the uterine cavity is limited. The estimated rate of implantation in natural cycles—assuming the formation of only one embryo—is 15% to 30% [7]; the efficiency of human implantation is decreased compared with that of other species [8]. The implantation rate decreases with age in a nonlinear fashion until age 35, at which point there is an approximately 3% decrease per year [9].

In ART, and specifically with in vitro fertilization–embryo transfer (IVF-ET), implantation rates can be assessed more accurately. On average, the implantation rate (ie, the number of gestational sacs produced per number of healthy zygotes that are transferred into the uterine cavity) is only 10% to 15% [10,11]. Efforts to improve this rate have included allowing embryos to develop until the blastocyst stage (Day 5 versus Day 3 embryos) and using coculture techniques in which tubal, granulosa, endometrial, or other cell lines are incubated with the embryos [12].

Implicit in successful implantation is the concept of endometrial receptivity, which has been defined as "the temporally and spatially unique set of circumstances that allow for successful implantation of the embryo" [13]. Thus, a potential means of improving the implantation rate in natural and ART cycles involves the evaluation and potential manipulation of endometrial receptivity (see later discussion) which is under direct and indirect hormonal regulation.

The endometrium and the menstrual cycle

The endometrium—composed of the functionalis and basalis layers—undergoes a series of changes during each ovulatory cycle that render it temporarily amenable to implantation. The functionalis layer represents the upper two thirds of the endometrium and is the site of proliferation, secretion, and degradation, whereas the basalis layer comprises the lower one third and serves as a source for tissue regeneration. During the proliferative phase when ovarian follicular growth produces increased estrogen levels, the functionalis layer regenerates as a result of new growth of glands, stroma, and endothelial cells. Ciliogenesis—the appearance of ciliated cells around gland openings—also occurs in response to estradiol and begins on Day 7 or 8 of an ideal 28-day menstrual cycle [14]. The preovulatory increase in 17β-estradiol leads to further proliferation and differentiation of uterine epithelial cells [4].

With ovulation, the corpus luteum forms and secretes progesterone, which acts on the endometrium to promote active secretion of glycoproteins and peptides into the endometrial cavity. During this secretory phase, endometrial epithelial proliferation ceases, in part, because of progesterone-mediated blockade of es-

trogen receptor expression and stimulation of 17β-hydroxysteroid dehydrogenase and sulfotransferase activities, which metabolize the potent estradiol into estrone that is then excreted [15,16]. Approximately 7 days after the luteinizing hormone (LH) surge, peak secretory activity is reached, the endometrial stroma becomes extremely edematous, and vascular proliferation ensues in response to the sex steroids as well as local factors (eg, prostaglandins).

Decidualization, which begins late in the luteal phase under the influence of progesterone, involves increased mitosis and differentiation of stromal cells. Also associated with decidualization is the progesterone-dependent infiltration of specific leukocyte subsets into the endometrial stroma, including natural killer cells, T cells, and macrophages [17]. This steroid-mediated recruitment of leukocytes is indirect because these cells do not seem to possess estrogen or progesterone receptors [18]. In the absence of implantation, and therefore, trophoblast-derived hCG production, the transient corpus luteum undergoes regression which results in an abrupt decrease in estrogen and progesterone levels with subsequent shedding of the functionalis layer.

Mechanism of steroid hormone action

Steroid hormones act by way of their intracellular receptors to regulate gene expression of their downstream effectors, including peptide hormones, cytokines, and growth factors [4]. Unlike some steroid receptors, those for estrogen and progesterone are localized predominantly to the cell nucleus, although some nucleocytoplasmic shuttling does occur [19]. Binding of ligand to these steroid receptors leads to dimerization and subsequent binding of the steroid-receptor complexes to hormone responsive elements on DNA that results in transcriptional activation or repression of target genes [19].

Estrogen and progesterone have two receptor subtypes, α and β and A and B, respectively. Estrogen receptor (ER)-α is expressed by endometrial epithelial and stromal cells during the proliferative phase, but decreases during the secretory phase [20]. The cellular proliferation of the endometrial epithelium in response to estrogen is dependent upon stromal expression of ER-α [21]. There is little endometrial expression of ER-β; it is limited to glandular epithelial cells [22] and seems to modulate ER-α–mediated gene transcription in the uterus [23]. ER-α and -β can form homo- or heterodimers. The specific response of a cell to estrogen stimulation depends on the relative abundance of the ER subtype, the type of estrogen, and the targeted response element [19].

Similarly, the relative proportions of progesterone receptor (PR)-A and -B within a target cell determine if gene activation will occur upon hormonal stimulation because PR-A dominantly represses transcriptional activation by PR-B [24]. PR-A is expressed in the stroma and epithelium during the proliferative and secretory phases of the menstrual cycle; however, epithelial levels of PR-A gradually decrease during the secretory phase [25]. PR-B is present in glandular

and stromal nuclei only during the proliferative phase [26]. PR levels are increased by estrogens and growth factors and decrease in response to progesterone [27]. ER-β also seems to down-regulate PRs in the luminal epithelium [23]. The down-regulation of PR during the window of implantation is a prerequisite for endometrial receptivity (see later discussion) [28].

Endometrial receptivity and the luteal phase defect

Traditionally, endometrial receptivity has been assessed indirectly by the luteal phase endometrial biopsy with which a histologic determination is made regarding whether the degree of differentiation of the endometrial sample corresponds to the cycle day on which the biopsy was performed [29]. The luteal phase defect (ie, a greater than 2–3 day lag in endometrial maturation) implies a lack of endometrial receptivity. Yet, endometrial biopsies often are performed late in the luteal phase and thus, may not reflect directly on the window of implantation [13]. Furthermore, histologic endometrial maturation does not correlate necessarily with a functionally mature endometrium [30]. Recent studies suggested that two types of luteal phase defects may compromise endometrial receptivity. In the classical or type I defect, histologic endometrial maturation is delayed, whereas in the type II defect, endometrial histology is within normal limits; however, the expression of biochemical markers of maturation is impaired [31].

The type I luteal phase defect is a common condition even in fertile women; approximately one half of women who have normal cycles and who do not have diminished reproductive potential have an abnormal late luteal endometrial biopsy [32]. Furthermore, there is no statistically significant difference in the incidence of luteal phase defect between fertile and infertile women [33]. Because of the clear limitations of the endometrial biopsy and its lack of correlation with pregnancy, endometrial dating in the work-up of infertility has been discouraged [34].

The most compelling evidence for eliminating endometrial dating as part of the infertility evaluation comes from the Reproductive Medicine Network. This group reported the results of a recent large, prospective, multi-center, randomized trial at the 2002 Meeting of the American Society for Reproductive Medicine [35]. They enrolled 847 fertile and infertile women who were randomized to a mid- or late luteal endometrial biopsy. More fertile women had abnormal biopsies than did infertile women. Abnormalities were detected in 49% of fertile women and 43% of infertile women in the midluteal phase and in 35% and 23%, respectively, in the late luteal phase. These results demonstrated definitively that traditional endometrial dating is unlikely to be helpful in the most women who have infertility.

The evaluation of the endometrium for type II luteal phase defect may represent a more accurate means of assessing endometrial receptivity. Such an

evaluation would involve analysis of endometrial tissue for cellular and molecular markers that would predict successful implantation better.

Cellular/molecular markers and mechanisms underlying implantation

Implantation is a complex, hormonally-regulated process that requires synchronization between the developing embryo and differentiating endometrium. This is facilitated by molecular cross-talk between the embryo and endometrium [36]. Numerous studies have investigated potential markers of endometrial receptivity as predictors of successful implantation and, in doing so, have helped to define the cellular and molecular mechanisms by which implantation occurs. These markers include pinopodes, cell adhesion molecules, cytokines, homeobox (HOX) genes, growth factors, matrix metalloproteinases, and their inhibitors. Many clinical situations in which implantation is impaired (eg, hydrosalpinx) are associated with normal estrogen and progesterone levels; this implies that the downstream effectors of these hormones are dysregulated.

Pinopodes

With the onset of the secretory phase of the menstrual cycle, microvilli on the apical surface of the luminal endometrial epithelium fuse to form structures that are known as pinopodes [37]. The appearance of pinopodes coincides with increased progesterone levels and the down-regulation of PR-B during the window of implantation [25,38]. Although the exact function of pinopodes remains to be characterized fully, recent studies suggest that these progesterone-dependent structures extract fluid from the uterus, and thereby, facilitate closer contact between the blastocyst and endometrium [39]. The volume of uterine fluid is decreased during the window of implantation; this phenomenon is not seen following treatment with RU486, an antiprogestin [40].

Pinopodes last for only 1 or 2 days—usually Days 20 and 21 in an ideal cycle—although there is up to 5 days of variation in the timing of their appearance [37]. Furthermore, their numbers correlate with implantation [38,41]. Pinopodes form earlier in gonadotropin-stimulated cycles (Days 19–20) [42] and later in artificial, hormone replacement cycles for donor recipients (Days 21–22) [43]; this results in a loss of synchronization between the developing embryo and endometrium. Addressing this issue may represent a means of improving implantation rates in ART cycles. For example, it would be beneficial to postpone the window of implantation in women who are undergoing controlled ovarian hyperstimulation for IVF so that embryo maturation could catch up before embryo transfer [37]. Such a delay in endometrial development was accomplished in the rat with the use of the antiprogestin, RU-486, after ovulation [44].

Cell adhesion molecules

Numerous cell adhesion molecules (CAMs), including mucins [45,46] and trophinin [47], have been implicated in the attachment phase of implantation, during which they serve to tether the blastocyst to the endometrium as described by the receptor-mediated model of implantation [48]. Perhaps the best studied of the CAMs have been the integrins, which are heterodimeric glycoproteins that consist of noncovalently associated α and β subunits [49]. At least 20 types of integrin heterodimers have been defined, which form from 14 α and 9 β subunits [50]. Integrins are unusual cell surface receptors in that they bind with low affinity and are present in large numbers; this allows for ligand motility without loss of attachment.

Endometrial epithelial cells constitutively express certain integrins, whereas others are cycle-dependent [51]. Among the latter is $\alpha v\beta 3$, which is present on the apical surface of luminal endometrial cells and human embryos [52]. Osteopontin (OPN), one of the ligands for $\alpha v\beta 3$, is a glycoprotein that is secreted by the endometrium and likely serves as a bridging molecule between the embryo and endometrium [49,53]. Immunostaining for $\alpha v\beta 3$ and OPN corresponds to the endometrial pinopodes that form during the window of implantation [54].

During the secretory phase of the menstrual cycle, elevated progesterone levels increase OPN secretion [55] and result in a down-regulation of endometrial PRs [56]. The latter is associated with an increase in $\alpha v\beta 3$ expression which signals the onset of endometrial receptivity [28]. The significance of $\alpha v\beta 3$ is underscored by the finding that the loss of PR and the expression of $\alpha v\beta 3$ are delayed in infertile women who have type I luteal phase defects [28,57]. Furthermore, there is evidence that treatment of the condition that underlies the luteal phase defect or progesterone supplementation restores PR down-regulation and $\alpha v\beta 3$ expression [31,51].

Although antibodies that block $\alpha v\beta 3$ or the use of ligands that compete with OPN compromise implantation in rabbits [58], gene knock-out studies demonstrated that β_3-deficient mice are fertile. This implies that although $\alpha v\beta 3$ has a role in implantation, there is redundancy within this process [59].

Mucin 1 (MUC-1), another CAM, is a highly glycosylated glycoprotein that is present on the surface of endometrial epithelial cells, which, in response to progesterone combined with estrogen priming, is up-regulated during the window of implantation in humans [60]. Because of its extensively negatively charged nature, MUC-1 has been described as an antiadhesion molecule; it serves as such in other species where it is down-regulated during the window of implantation [61,62]. In humans, during the apposition phase of implantation, the embryo increases endometrial MUC-1 expression; this is followed by a selective decrease in MUC-1 expression, specifically at the implantation site during adhesion [63]. Thus, MUC-1 expression is regulated by steroid hormones and the implanting embryo. It was hypothesized that embryos of poor quality may not have the capacity to down-regulate MUC-1 adequately for successful implantation [63], whereas endometrial deficiency in MUC-1 may

allow for implantation of abnormal embryos that leads to recurrent pregnancy loss [64].

Cytokines

As with the CAMs, numerous cytokines have been implicated in implantation. Colony-stimulating factor (CSF)-1, for example, is expressed by human endometrium during the midproliferative and midsecretory phases [65]. Mice that have a null mutation in this gene have decreased implantation rates, which are improved with exogenous CSF-1 administration [66]. It was postulated that CSF-1 facilitates blastocyst attachment [13].

Similarly, the interleukins may facilitate the cross-talk between the embryo and endometrium. Interleukins are expressed abundantly by leukocytes that infiltrate the endometrium during progesterone-mediated decidualization [17]. Because these leukocytes do not possess steroid receptors, chemoattractant cytokines (chemokines), such as interleukin (IL)-8 and Monocyte Chemoattractan Protein-1 (MCP-1), seem to mediate the steroid-dependent recruitment of leukocytes to the endometrium [67]. Chemokines also result in the secondary induction of other cytokines, including leukemia inhibiting factor (LIF) and IL-1 and the growth factor heparin-binding epidermal growth factor (HB-EGF) [5]. IL-8 and MCP-1 are expressed by endometrial glandular and lumenal epithelial cells [68,69] where they are up-regulated by progesterone during the window of implantation [70]. This up-regulation is by way of an indirect mechanism that likely involves stromal cells or other endometrial cell types. Conversely, the embryo directly regulates endometrial IL-8 expression by increasing mRNA expression and translation, at least in vitro [70].

IL-1α, IL-1β, and the IL-1 receptor antagonist (IL-1RA) also are expressed by human endometrium [71]; levels of IL-1 receptor type 1 are maximal during the secretory phase [72]. A recent study showed that IL-1RA inhibits implantation by down-regulating the integrin subunits, α4, αv, and β3 [73]. Still, as with the integrins, there is redundancy with respect to the role that the IL-1 system plays in implantation because null mutations in the IL-1α and IL-1β genes have no appreciable effects on fertility [74].

LIF, a member of the IL-6 family, is a well-substantiated marker of implantation. This glycoprotein is expressed by human endometrium and decidua [75] where it is regulated by other cytokines and steroid hormones (eg, estrogen) [76]. There is little LIF expression in proliferative endometrium; however, levels increase during the secretory phase and reach a maximum between Days 19 and 25, which coincides with the implantation window [75]. The effects of LIF on cellular proliferation and differentiation are mediated by its receptors, LIF-R and glycoprotein 130, both of which are expressed constitutively by proliferative and secretory endometrium and trophoblasts [77]. The responsiveness of LIF-R to LIF, however, seems to be mediated by estradiol and progesterone [78]. LIF stimulates trophoblasts to increase fibronectin production, which facilitates anchoring [79] and differentiates these cells into an invasive phenotype [80].

Blastocyts cannot implant in mice that lack the LIF gene [81]. Conversely, blastocysts from LIF-deficient mice can implant into wild-type, pseudopregnant mice; this demonstrates conclusively that implantation requires maternal LIF expression [82].

That LIF is involved in human implantation is suggested by the findings that conditioned media from endometrial explants of women who have unexplained infertility have decreased levels of LIF compared with those of fertile women [83]; some infertile women have mutations in the coding region of the LIF gene [84]. Furthermore, antiprogestin treatment results in reduced LIF expression [85] and women who have unexplained infertility are more likely to have undetectable levels of LIF in their uterine flushings [86]. Similarly, women who have recurrent pregnancy loss have decreased endometrial secretion of LIF [87].

Homeobox genes

Another group of molecules that clearly are integral to implantation are the HOX genes, which encode a class of transcription factors. There are at least 39 Hox (mouse)/HOX (human) genes, all of which have a similar 183-base pair DNA sequence, the homeobox, that encodes a highly-conserved 61–amino acid domain that is known as the homeodomain [88]. Many of these transcription factors mediate embryonic development by determining regional body patterning along the anterior–posterior body axis, including that of the reproductive tract [89]. Specifically, Hoxa-9 is expressed in the developing oviduct, Hoxa-10 in the uterus, Hoxa-11 in the lower uterine segment and cervix, and Hoxa-13 in the upper vagina [90].

Unlike most Hox genes, which are expressed only during the embryonic period, those that are specific to the female reproductive tract continue to play a role in the adult [90]. For example, HOXA-10 and HOXA-11 are expressed by endometrial glands and stroma throughout the menstrual cycle [91,92]; their levels increase maximally during the midsecretory phase at the time of implantation [90].

HOXA-10 and HOXA-11 are up-regulated by 17β-estradiol and progesterone [91] and the effects of these steroids are a direct result of their receptors (ER or PR) binding to the regulatory regions of the Hoxa-10 or Hoxa-11 genes [92,93]. The continued expression of Hox/HOX genes in the female reproductive tract facilitates the growth and differentiation of the endometrium, and thereby allows for the retention of developmental plasticity, which is important for successful implantation.

One downstream target of HOXA-10 is Drosophia empty spiracles gene (EMX2) (human)/Emx2 (mouse) [94]. Emx2 is expressed in the developing brain and urogenital tract [95]; mice that lack this gene have severe urogenital malformations that result in death shortly after birth [96]. During the midluteal phase when HOXA-10 levels are maximal, EMX2 expression decline; this down-regulation occurs as a result of HOXA-10 binding to the regulatory region of the

EMX2 gene [94]. In women who have endometriosis, EMX2 expression is abnormally high during the peri-implantation period [97]; this dysregulation may be associated with the decreased implantation rates that are seen with this disease. Although the functional significance of EMX2 expression is unclear, further elucidation of the HOX system should help to define the role of EMX2 in endometrial development.

Other downstream targets for HOXA have been defined. For example, HOXA-10 binds to the β3-integrin gene and up-regulates its expression in endometrial cells; this demonstrates that HOXA-10 mediates integrin involvement in early embryo–endometrial interactions [98]. Similarly, a recent study showed that maternal Hoxa-10 expression is required for pinopode formation in the mouse [99]. Blockade of Hoxa-10 decreased pinopode number during the window of implantation in the mouse uterus, whereas overexpression of this gene increased pinopode number; this demonstrated that Hoxa-10 likely contributes to endometrial receptivity for blastocyst implantation [99]. Although there are no known human mutations in HOXA-10 or HOXA-11, women who have decreased expression of these two genes during the secretory phase have decreased implantation rates [100]. For example, endometrial HOXA-10 levels are decreased in patients who have polycystic ovarian syndrome (PCOS) [101] and in the presence of hydrosalpinx fluid [102]; the midluteal increase in HOXA-10 and HOXA-11 expression does not occur consistently in women who have endometriosis [100]. Targeted disruption of the Hoxa-10 gene in mice results in a transformation of the upper uterine segment into an oviduct-like structure and inhibits implantation, even when embryos are transferred to the grossly unaffected lower uterine segment [103,104]. Similarly, mice that have a homozygous mutation in the Hoxa-11 gene are infertile as a result of implantation defects [105] and have reduced expression of LIF [106]. Hoxa-10 and Hoxa-11 null mice produce normal numbers of embryos that are able to implant in wild-type surrogate mice, whereas wild-type embryos from surrogate mice cannot implant in the Hoxa-10 and Hoxa-11 deficient mice [103–105]. Thus, as with LIF, maternal expression of Hoxa-10 and Hoxa-11 by the endometrium is essential for implantation.

Selective alteration of endometrial Hoxa-10 expression in mice, through the use of liposome-mediated gene transfection, dramatically alters implantation, and again, demonstrates the importance of maternal Hoxa-10 for endometrial receptivity [107]. In this study, wild-type mice uteri were transfected on post-coital Day 2 with a Hoxa-10 antisense oligodeoxyribonucleotide that is designed to prevent Hoxa-10 expression. Hoxa-10 protein levels decreased as did the number of implanted embryos and the size of the resulting litters. In contrast, when the mice were transfected with Hoxa-10 cDNA, the number of implanted embryos and litter size increased significantly.

Although similar studies have not been performed in higher animal models or humans, transfection of a human endometrial adenocarcinoma cell line (Ishikawa cells) with a Hoxa-10 antisense oligodeoxyribonucleotide also resulted in decreased HOXA-10 expression. Furthermore, efficient transfection and expres-

sion of an Escherichia lacZ reporter gene was accomplished in intact human uteri ex vivo; this showed that gene transfer to the intact female reproductive tract is feasible. Thus, a gene therapy approach that involves the manipulation of HOX-10 expression may have a role in the enhancement of endometrial receptivity and implantation.

Growth factors

Growth factors are proteins that bind to specific receptors, and thereby, result in cellular differentiation or proliferation. Among the growth factors that are relevant to implantation are the HB-EGF [108,109] and amphiregulin [110]. In the mouse, HB-EGF expression is limited spatially and temporally to the site of blastocyst implantation [111], and therefore, is believed to play a role in blastocyst attachment. In women, HB-EGF also is expressed during the window of implantation [108,109], and this growth factor stimulates the growth and development of human [112] and mouse [111] blastocysts in vitro. It seems that HB-EGF also regulates endometrial $\alpha v\beta 3$ expression [113].

Like many other growth factors, endometrial HB-EGF expression is under the control of steroid hormones. For example, in the absence of estrogen, implantation in the mouse can be delayed indefinitely; however, when estrogen is provided, the blastocyst becomes activated and HB-EGF expression rapidly increases at the site of blastocyst apposition [111]. Although a role for amphiregulin in human implantation has not been defined, in the mouse, this growth factor—which is another member of the EGF family—is expressed during the period of maximal endometrial receptivity initially throughout the uterine epithelium and then, specifically at the sites of blastocyst implantation [110].

Other growth factors, such as transforming growth factor (TGF)-β, act as "maternal restraints" during implantation in that they limit trophoblast invasion [114]. TGF-β1 expression by endometrial glands and stroma increases during the secretory phase; it inhibits proliferation of cytotrophoblasts, stimulates them to differentiate into a noninvasive phenotype, and induces protease inhibitors (eg, plasminogen activator inhibitor [PAI] and tissue inhibitors of matrix metalloproteases [TIMP]-1]) that counteract extracellular matrix degradation by trophoblast-derived proteases [115].

The insulin-like growth factors (IGF)-I and -II are single-chain polypeptides that, like insulin, promote growth and differentiation of cells and also regulate cellular metabolism locally [19]. Insulin-like growth factor binding protein (IGFBP)-1, which is secreted by the secretory endometrium and decidua [116,117], serves as another restraint on trophoblast invasion by binding IGF-I and IGF-II, thereby blocking their actions. The latter growth factor is expressed in large amounts by cytotrophoblasts [117]; IGFBP-1 blocks the invasion of these cells into decidualized endometrial stromal cells in vitro [118]. The role of IGFBP-1 is not understood fully because it also was found to stimulate trophoblast invasion in other in vitro systems [119,120]. Furthermore, IGFBP-1 has been implicated in embryo recognition and the events that are associated with

early implantation because it interacts directly with integrins (eg, $\alpha5\beta1$) that are expressed by cytotrophoblasts [118,119].

Proteases and protease inhibitors

In addition to acting as a receptor for the embryo, $\alpha v\beta3$ also activates matrix metalloproteinases (MMP), such as MMP-2 [121], which degrade extracellular matrix proteins, and thereby, facilitate the invasive phase of implantation [122]. Other MMPs, including MMP-7 and MMP-11, are expressed in the endometrium during menses and the proliferative phase but are down-regulated by progesterone during the secretory phase [123]. Protease activity, and consequently, trophoblast invasion also are regulated by TIMP and other protease inhibitors, such as $\alpha2$-macroglobulin [13]. Among the TIMPs, TIMP-3 seems to be especially pertinent to implantation because it is expressed by murine decidua just adjacent to the sites of embryo implantation [124]. Furthermore, TIMP-3 also is expressed by human cytotrophoblasts [125] and decidualizing stromal cells where it is up-regulated by progesterone [126].

The invading cytotrophoblasts also express proteases (eg, MMP-9) and cathepsins B and L [127,128]. IL-1 increases MMP-9 expression by cytotrophoblasts [129]; elevated concentrations of this cytokine in embryo culture medium were correlated with successful pregnancy after IVF-ET [130].

Connexins

Connexins are a family of proteins that facilitate gap junctions between cells, and thereby, regulate cell–cell interactions. Progesterone inhibits endometrial expression of connexins, cx43 and cx26. This is believed to allow for trophoblast attachment and invasion [131].

Other endocrine mediators of implantation

Although the above discussion describes the regulation of the various markers of implantation by the sex steroids, prostaglandins and peptide hormones also play a role in implantation.

Prostaglandins

In addition to apposition, attachment, and invasion, successful implantation requires increased endometrial vascular permeability followed by angiogenesis—the generation of new blood vessels from pre-existing ones. The process of angiogenesis in the peri-implantational endometrium is not understood completely; however, it is likely that, as in other tissues, angiogenic factors (eg, vascular endothelial growth factor [VEGF]) [132] and the angiopoietins [133] are involved. Better characterized are the prostaglandins, which are arachidonic acid

metabolites that mediate a wide array of biologic processes, including angiogenesis, cellular proliferation, and differentiation. These compounds, which are generated by the cyclooxygenases (COX1 and COX2), facilitate increased vascular permeability in the endometrium during implantation [134].

Mice with null mutations in the inducible isoform of cyclooxygenase (COX2), have multifactorial reproductive failure, including impaired ovulation, fertilization, implantation, and decidualization, whereas mice that are deficient in the constitutive enzyme (COX1) are not affected in this regard. [135]. More recent studies that investigated the role of COX2 in implantation revealed that wild-type embryos are able to implant successfully in COX2-deficient mice, although there is a lag in decidualization following implantation [136].

Thus, although COX2-generated prostaglandins have a role in implantation, there, again, seems to be redundancy within this process. COX1, but not COX2 expression, is under the control of 17β-estradiol and progesterone. These steroids decrease the production of COX1, such that levels decrease drastically in the midluteal phase during the implantation window [137]. Conversely, COX2 expression is restricted to the site of implantation and is upregulated by IL-1 that is secreted by the blastocyst [129,135,137].

Calcitonin

Calcitonin is a peptide hormone that is secreted primarily by parafollicular C cells of the thyroid gland and is distributed widely throughout the body [138,139]. Although this hormone functions to decrease blood calcium by inhibiting bone osteoclast activity, it also has been implicated in the regulation of calcium flux across cell membranes [140]. Recently, calcitonin synthesis was identified in glandular epithelial cells of the rat uterus where it peaks transiently on the day before implantation [141,142]. Similarly, calcitonin is expressed by human glandular epithelial endometrium during the window of implantation where it is regulated by progesterone and inhibited by the antiprogestin, RU486 [143]. Estrogen has no direct effect on calcitonin expression, but antagonizes the effect of progesterone [142]. Administration of antisense oligodeoxynucleotides against calcitonin mRNA resulted in a significant reduction in the number of implanted embryos in the rat [144]; this implicated this peptide hormone as an important mediator of implantation. The mechanism of action may involve the dissolution of gap junctions between cells because a calcitonin-induced increase in intracellular calcium decreases endometrial cell expression of E-cadherin, a cell-surface glycoprotein that mediates cell–cell adhesion among epithelial cells [145]. Such increased permeability is hypothesized to facilitate implantation of the blastocyst [145].

Human chorionic gonadotropin

hCG, a glycoprotein hormone that is synthesized by syncytiotrophoblasts, principally serves to maintain corpus luteum progesterone function until the pla-

centa is able to take over at 60 to 70 days' gestation. The recent discovery of the chorionic gonadotropin (CG)/LH receptor in the human uterus, however, as well as LH receptor up-regulation during the period of endometrial receptivity led to much interest in the potential direct role of hCG in implantation [146]. Uterine infusion studies showed that hCG increased the secretion of several proteins from the endometrial epithelium (eg, VEGF, LIF, MMP-9), whereas it decreased IGFBP-1 and Macrophage Colony Stimulating Factor (M-CSF) expression [147]. hCG also induces the production of glycodelin, a major endometrial secretory protein that is associated with immunosuppression and epithelial cell differentiation [148]. In stromal cells, hCG promotes decidualization in the presence of estrogen and progesterone as determined by the increased transcription of prolactin, a marker of such differentiation [149].

Prolactin

Prolactin, another peptide hormone, is secreted by the endometrium during the late luteal phase and throughout pregnancy. This hormone is stimulated by progesterone and estrogen, enhances endometrial cell growth, and is requisite for implantation in mice [150]. Although the role of prolactin in human implantation is not understood fully, this hormone seems to mediate the production of macrophage activating factors (eg, interferon), and thus, may have a local immunomodulatory function [151].

Corticotropin-releasing hormone

Another hormone with a potential immunomodulatory role in implantation is corticotropin-releasing hormone (CRH), a 41–amino acid peptide that is a proinflammatory mediator and potent vasodilator. This peptide initiates the inflammatory response and stimulates leukocytes to produce IL-1 [152]. In rats, increased levels of CRH mRNA and protein have been reported at the site of implantation [153]. This peptide hormone is induced by prostaglandins and is down-regulated by estrogen and progesterone [154].

Effects of androgens on implantation

Elevated androgen levels are associated with infertility and increased miscarriage rates, in part, because of direct effects on the endometrium [155] by way of the androgen receptor, which is expressed throughout the menstrual cycle in endometrial stromal and epithelial cells [156]. Androgens seem to have pleiotropic effects on the endometrium. Although these steroids increase prolactin secretion by stromal cells in vitro [157], they negatively affect levels of glycodelin [155], a marker of endometrial secretory function. A recent study demonstrated that androstenedione inhibits endometrial cell growth and secretory activity [158]. In contrast, testosterone and dihydrotestosterone increase

endometrial concentrations of the receptor for epidermal growth factor, and thereby, promote endometrial hyperplasia, as often is seen in the setting of PCOS, a condition that is associated with hyperandrogenism [159]. In the endometrium of women who have PCOS, HOXA-10 expression is decreased markedly; similarly, testosterone decreases HOXA-10 expression in isolated endometrial cells [101]. Thus, it is not surprising that although the chronic anovulation that is associated with PCOS usually can be treated with ART, overall pregnancy rates are not high [160] and spontaneous miscarriages occur frequently [161], in part, because of the persistent effects of hyperandrogenemia on the endometrium.

Hormonal supplementation in assisted reproductive technology cycles

The increased levels of luteal phase estrogen that follow controlled ovarian hyperstimulation (COH) have a negative impact upon implantation. Such elevated levels of estrogens in the postovulatory period reflect the mechanism that is behind postcoital hormonal contraception [162]. For instance, estrogen inhibits 3β-hydroxysteroid dehydrogenase, thereby decreasing progesterone synthesis by the corpus luteum [163]. Although progesterone supplementation in animal models has been an effective means of increasing the implantation rate [164] and despite the fact that progesterone supplementation is used widely, randomized studies have not demonstrated the benefit of this practice in gonadotropin-induced cycles [165]. Conversely, in IVF-ET cycles in which prolonged GnRH analog administration is used for pituitary suppression, luteal phase serum levels of estradiol and progesterone are decreased and adversely affect implantation [166]. In this setting, luteal progesterone supplementation is beneficial [167].

Summary

Implantation is a complex, still incompletely understood process that involves the hormonally-regulated interplay between the embryo and a receptive endometrium. Although female sex steroids are the primary regulators of the cellular and molecular mediators of implantation, numerous other endocrine factors, including prostaglandins and peptide hormones, also play a role. The luteal phase endometrial biopsy is not useful for predicting endometrial receptivity, and therefore, should not be used routinely in the work-up of infertility. The analysis of cellular and molecular markers of endometrial function likely will predict successful implantation better, especially in clinical situations where estrogen and progesterone levels are within normal levels, but defects in their downstream effectors exist. Elevated androgen levels impair implantation by altering ovarian function and affecting the endometrium directly. Similarly, abnormally elevated estrogen levels in the setting of COH or postcoital contraception have detrimental effects on embryo implantation. Implanta-

tion rates in IVF-ET cycles in which GnRH agonists are used can be improved with progesterone supplementation.

References

[1] Hertig AT, Rock J, Adams EC. A description of 34 human ova within the first 17 days of development. Am J Anat 1956;98:435–91.

[2] Croxatto HB, Diaz S, Fuentealba BA, Croxatto HD, Carrilo D, Fabres C. Studies on the duration of egg transport in the human oviduct: The time interval between ovulation and egg recovery from the uterus in normal women. Fertil Steril 1972;23:447–58.

[3] Enders AC, Schlafke S. Cytological aspects of trophoblast-uterine interactions in early implantation. Am J Anat 1969;125:1–30.

[4] Norwitz ER, Schust DJ, Fisher SJ. Implantation and survival of the early pregnancy. N Engl J Med 2001;345(19):1400–8.

[5] Cross JC, Werb Z, Fischer SJ. Implantation and the placenta: key pieces of the development puzzle. Science 1994;266:1508–18.

[6] Fawcett DW. The development of mouse ova under the capsule of the kidney. Anat Rec 1950;108:71–91.

[7] Miller JF, Williamson E, Glue J, Gordon YB, Grudzinskas JG, Sykes A. Fetal losses after implantation. Lancet 1980;2(8194):554–6.

[8] Valbuena D, Jasper M, Remohi J, Pellicer A, Simon C. Ovarian stimulation and endometrial receptivity. Hum Reprod 1999;14(Suppl 2):107–11.

[9] Spandorfer SD, Chung PH, Kligman I, Liu HC, Davis OK, Rosenwaks Z. An analysis of the effect of age on implantation rates. J Assist Reprod Genet 2000;17(6):303–6.

[10] Paulson RJ, Sauer MV, Lobo RA. Embryo implantation after human in vitro fertilization: importance of endometrial receptivity. Fertil Steril 1990;53:870–4.

[11] Lopata A. Implantation of the human embryo. Hum Reprod 1996;11(Suppl 1):175–84.

[12] Menezo Y, Veiga A, Benkhalifa M. Improved methods for blastocyst formation and culture. Hum Reprod 1998;13(Suppl 4):256–65.

[13] Giudice LC. Potential biochemical markers of uterine receptivity. Hum Reprod 1999; 14(Suppl 2):3–16.

[14] Ludwig H, Spornitz UM. Microarchitecture of the human endometrium by scanning electron microscopy: menstrual desquamation and remodeling. In: Bulletti C, Gurpide E, editors. The primate endometrium. New York: The New York Academy of Sciences; 1991. p. 28–46.

[15] Gurpide E, Gusberg S, Tseng L. Estradiol binding and metabolism in human endometrial hyperplasia and adenocarcinoma. J Steroid Biochem 1976;7:891–6.

[16] Falany JL, Falany CN. Regulation of estrogen sulfotransferase in human endometrial adenocarcinoma cells by progesterone. Endocrinology 1996;137:1395.

[17] Bulmer JN, Morrison L, Longfellow M, Ritson A, Pace D. Granulated lymphocytes in human endometrium: histochemical and immunohistochemical studies. Hum Reprod 1991;6: 761–8.

[18] King A, Gardner L, Loke YW. Evaluation of oestrogen and progesterone receptor expression in uterine mucosal lymphocytes. Hum Reprod 1996;11:1079–82.

[19] Speroff L, Glass RH, Cage N. Clinical Gynecologic endocrinology and infertility. 6th edition. Philadelphia: Lipincott Williams & Wilkins; 1999.

[20] Lessey BA, Killam AP, Mezger DA, Haney AF, Greene GL, McCarthy KS. Immunohistochemical analysis of human uterine estrogen and progesterone receptors throughout the menstrual cycle and the menopause. J Endocrinol Metab 1988;67:334–40.

[21] Kurita T, Young P, Brody JR, Lydon JP, O'Malley BW, Cunha GR. Stromal progesterone receptors mediate the inhibitory effects of progesterone on estrogen-induced uterine epithelial cell deoxy-ribonucleic acid synthesis. Endocrinology 1998;139:4708–13.

[22] Matsuzaki S, Fukaya T, Suzuku T, Mukurami T, Sasano H, Yajima A. Estrogen receptor alpha and beta mRNA expression in human endometrium throughout the menstrual cycle. Mol Hum Reprod 1999;5:559–64.
[23] Wiehua Z, Saji S, Makinen S, Chens G, Jensen EV, Warner M, et al. Estrogen receptor (ER) beta, a modulator or ER alpha in the uterus. Proc Natl Acad Sci USA 2000;97:5936–41.
[24] Graham JD, Yeates C, Balleine RL, et al. Progesterone receptor A and B protein expression in human breast cancer. J Steroid Biochem Mol Biol 1996;56:93–8.
[25] Stavreus-Evers A, Nikas G, Sahlin L, Eriksson H, Landgren B-M. Formation of pinopodes in human endometrium is associated with the concentrations of progesterone and progesterone receptors. Fertil Steril 2001;76(4):782–91.
[26] Wang H, Critchley HOD, Kelly RW, Shen D, Baird DT. Progesterone receptor subtype B is differentially regulated in human endometrial stroma. Mol Hum Reprod 1998;4:407–12.
[27] Kraus WL, Katzenellenbogen BS. Regulation of progesterone receptor gene expression and growth in the rat uterus: modulation of estrogen actions by progesterone and sex steroid hormone antagonists. Endocrinology 1993;132:2371–9.
[28] Lessey BA, Yeh I, Castelbaum AJ, Fritz MA, Ilesanmi AO, Korzeniowski P, et al. Endometrial progesterone receptors and markers of uterine receptivity in the window of implantation. Fertil Steril 1996;65(3):477–83.
[29] Noyes RW, Hertig AT, Rock J. Dating and the endometrial biopsy. Fertil Steril 1950;1:3.
[30] Swiersz L, Giudice LC. Unexplained infertility and the role of uterine receptivity. Infertil Clinics N Am 1997;8:523–43.
[31] Lessey BA, Castlebaum AJ, Sawin SJ. Integrins as markers of uterine receptivity in women with primary unexplained infertility. Fertil Steril 1995;63:535–42.
[32] Davis OK, Berkeley AS, Naus GJ, Cholst IN, Freedman KS. The incidence of luteal phase defect in normal fertile women, determined by serial endometrial biopsies. Fertil Steril 1989; 51:582–6.
[33] Balasch J, Creus M, Marquez M, Burzaco I, Vanrell JA. The significance of luteal phase deficiency in fertility: A diagnostic and therapeutic approach. Hum Reprod 1986;1:145–7.
[34] Crosignani PG, Collins JA, Cook ID, Diczfalusy E, Rubin B. Recommendations of the ESHRE workshop on unexplained infertility. Hum Reprod 1993;8:977–80.
[35] The Reproductive Medicine Network. The endometrial biopsy as a diagnostic tool in the evaluation of the infertile patient. Fertil Steril 2002;3(Suppl 1):S2.
[36] Glasser SR, Mulholland J, Mani SK. Blastocyst endometrial relationships: reciprocal interactions between uterine epithelial and stromal cells and blastocysts. Trophoblast Res 1991;5: 225–80.
[37] Nikas G. Endometrial receptivity: changes in cell-surface morphology. Semin Reprod Med 2000;18:229–36.
[38] Nikas G. Cell surface morphological events relevant to human implantation. Hum Reprod 1999;14(Suppl. 2):37–44.
[39] Enders AC, Nelson DM. Pinocytotic activity in the uterus of the rat. Am J Anat 1973;138: 277–300.
[40] Gemzell-Danielsson K, Hamberg M. The effect of antiprogestin (RU486) and prostaglandin biosynthesis inhibitor (naproxen) on uterine fluid prostaglandin F2a concentrations. Hum Reprod 1994;9:1626–30.
[41] Nikas G. Pinopodes as markers of endometrial receptivity in clinical practice. Hum Reprod 1999;14(Suppl 2):99–106.
[42] Nikas G, Develioglu OH, Toner JP, Jones HW. Endometrial pinopods indicate a shift in the window of receptivity in IVF cycles. Hum Reprod 1999;14:787–92.
[43] Develioglu OH, Hsiu JG, Nikas G, Toner JP, Oehninger S, Jones HW. Endometrial estrogen and progesterone receptor and pinopod expression in stimulated cycles in oocyte donation. Fertil Steril 1999;71(6):1040–7.
[44] Sarantis L, Roche D, Psychoyos A. Displacement of receptivity for nidation in the rat by the progesterone antagonist RU 486: a scanning electron microscopy study. Hum Reprod 1988;3:251–5.

[45] Carson DD, Rohde LH, Surveyor G. Cell surface glycoconjugates as modulators of embryo attachment to uterine epithelial cells. Int J Biochem 1994;26:1269–77.
[46] Feinberg RF, Kliman HJ. MAG (mouse ascites Golgi) mucin in the endometrium; a potential marker of endometrial receptivity to implantation. In: Diamond MP, Osteen KG, editors. Endometrium and endometriosis. Malden (MA): Blackwell Science; 1997. p. 131–9.
[47] Fukuda MN, Sato T, Nakayama J, Klier G, Mikami M, Aoki D, et al. Trophinin and tastin: a novel adhesion molecule complex with potential involvement in embryo implantation. Genes Dev 1995;9(10):1199–210.
[48] Yoshinaga K. Receptor concept in implantation research. In: Yoshinaga K, Mori T, editors. Development of preimplantation embryos and their environment. New York: Alan Liss, Inc.; 1989. p. 379–87.
[49] Lessey BA, Castlebaum AJ. Integrins and implantation in the human. Rev Endocrinol Metab Disord 2002;3:107–17.
[50] Lindhard A, Bentin-Ley U, Ravn V, Islin H, Hviid T, Rex S, et al. Biochemical evaluation of endometrial function at the time of implantation. Fertil Steril 2002;78:221–33.
[51] Lessey BA, Damjanovich L, Coutifaris C, Castlebaum AJ, Albelda SM, Buck CA. Integrin adhesion molecules in the human endometrium. Correlation with the normal and abnormal menstrual cycle. J Clin Invest 1992;90:180–95.
[52] Campbell S, Swann HR, Seif MW, Kimber SJ, Aplin JD. Cell adhesion molecules on the oocyte and preimplantation human embryo. Hum Reprod 1995;10:1571–8.
[53] Apparao KBC, Zhang J, Truong P, Lessey BA. Osteopontin expression during the menstrual cycle in women: regulation and binding to the endometrial avb3 integrin. J Clin Endocrionol Metab 2001;86:4991–5000.
[54] Usadi RS, Murray MJ, Bagnell RC. Temporal and morphologic characteristics of pinopod expression across the secretory phase of the endometrial cycle in normally cycling women with proven fertility. Fertil Steril 2003;79(40):970–4.
[55] Young MF, Kerr JM, Termine JD, Wewer UM, Wang MG, McBride OW, et al. cDNA cloning, mRNA distribution and heterogeneity, chromosomal location and RFLP analysis of human osteopontin (OPN). Genomics 1990;7(4):491–502.
[56] Slayden OD, Zelinski-Wooten MB, Chwalisz K, Stouffer RL, Brenner RM. Chronic treatment of cycling rhesus monkeys with low doses of the antiprogestin ZK 137316: morphometric assessment of the uterus and oviduct. Hum Reprod 1998;13:269–77.
[57] Creus M, Balasch J, Ordi J, Fabregues F, Casamitjana R, Quinto L, et al. Integrin expression in normal and out-of-phase endometria. Hum Reprod 1998;13:3460–8.
[58] Illera MJ, Lorenzo PL, Gui L-T, Mohammad A, Appa-Rao KBC, Lessey BA. The role of avb3 integrin during implantation in the rabbit. Biol Reprod 2000;62:1285–90.
[59] Hodivala-Dilke KM, Kairbaan M, McHugh KP. b3 integrin deficient mice are a model for Glanzmann thrombasthenia showing placental defects and reduced survival. J Clin Invest 1999; 103:229–38.
[60] Hey NA, Graham RA, Seif MW, Aplin JD. The polymorphic epithelial mucin MUC1 is regulated with maximal expression in the implantation phase. J Clin Endocrinol Metab 1994; 78:337–42.
[61] Surveyor GA, Gendler SJ, Pemberton L, Das SK, Chakraborty I, Julian J, et al. Expression and steroid hormonal control of MUC1 in the mouse uterus. Endocrinology 1995;136: 3639–47.
[62] DeSouza MM, Mani SK, Julian J, Carson DD. Reduction of MUC1 expression during the receptive phase in the rat uterus. Biol Reprod 1998;58:1503–7.
[63] Meseguer M, Aplin JD, Caballero-Campo P, O'Connor JE, Martin JC, Remohi J, et al. Human endometrial mucin MUC1 is up-regulated by progesterone and down-regulated in vitro by human blastocyst. Biol Reprod 2001;64:181–92.
[64] Aplin JD. Adhesion molecules in implantation. Rev Reprod 1997;2:84–93.
[65] Bartocci A, Pollard JW, Stanley ER. Regulation of CSF-1 during pregnancy. J Exp Med 1986;164:956–61.
[66] Wiktor-Jedrzejczak W, Urbanowska E, Aukerman SL. Correction by CSF-1 of defects in

the osteopetrotic op/op mouse suggests local developmental and humoral requirements for this growth factor. Exp Hematol 1991;19:1049–54.
[67] Wood GW, Hausmann X, Choudhuri R. Relative role of CSF-1, MCP-1/JE and RANTES in macrophage recruitment during successful pregnancy. Mol Reprod Dev 1997;46:62–70.
[68] Arici A, Head JR, MacDonald PC, Casey ML. Regulation of interleukin-8 gene expression in human endometrial cells in culture. Mol Cell Endocrinol 1993;94:195–204.
[69] Arici A, MacDonald PC, Casey ML. Regulation of monocyte chemotactic protein-1 gene expression in human endometrial cells in cultures. Mol Cell Endocrinol 1995;107:189–97.
[70] Cabellero-Campo P, Dominguez F, Coloma J, Meseguer M, Remohi J, Pellicer A, et al. Hormonal and embryonic regulation of chemokines IL-8, MCP-1, and RANTES in the human endometrium during the window of implantation. Mol Hum Reprod 2002;8(4):375–84.
[71] Simon C, Frances A, Pellicer A. Cytokines in implantation. Semin Reprod Endocrinol 1995; 13:142–51.
[72] Simon C, Piquette GN, Frances A. Localization of interluekin-1 type 1 receptor and interleukin-1 beta in human endometrium throughout the menstrual cycle. J Clin Endocrinol Metab 1993; 77:549–55.
[73] Simon C, Valbuena D, Krussel J, Bernal A, Murphy CR, Shaw T, et al. Interleukin-1 receptor antagonist prevents embryonic implantation by a direct effect on the endometrial epithelium. Fertil Steril 1998;70(5):896–906.
[74] Yamada H, Mizumd S, Horai R. Protective role of interleukin-1 in mycobacterial infection in IL-1 alpha/beta double-knockout mice. US and Canadian Academy of Pathology 2000;80: 759–67.
[75] Charnock-Jones DS, Sharkey AM, Fenwick P, Smith SK. Leukemia inhibitory factor mRNA concentration peaks in human endometrium at the time of implantation and the blastocyst contains mRNA for the receptor at this time. J Reprod Fertil 1994;101:421–6.
[76] Arici A, Engin O, Attar E, Olive DL. Modulation of leukemia inhibitory factor gene expression and protein synthesis in human endometrium. J Clin Endocrinol Metab 1995;80:1908–15.
[77] Cullinan EB, Abbondanzo SJ, Anderson PS, Pollard JW, Lessey BA, Stewart CL. Leukemia inhibitory factor (LIF) and LIF receptor expression in human endometrium suggests a potential autocrine/paracrine function in regulating embryo implantation. Proc Natl Acad Sci USA 1996; 93:3115–200.
[78] Cheng J-G, Rodriguez CI, Stewart CL. Control of uterine receptivity and embryo implantation by steroid hormone regulation of LIF production and LIF receptor activity: towards a molecular understanding of the window of implantation. Rev Endocrinol Metab Disord 2002;3:119–26.
[79] Natchtigall MJ, Kliman HJ, Feinberg RF, Olive DL, Engin O, Arici A. The effect of leukemia inhibitory factor on trophoblast differentiation: a potential role in human implantation. J Clin Endocrinol Metab 1996;81(2):801–6.
[80] Bischof P, Haenggeli L, Campana A. Effect of leukemia inhibitory factor on human cytotrophoblast differentiation along the invasive pathway. Am J Reprod Immunol 1995;34:225–30.
[81] Stewart CL, Kaspar P, Brunet LJ, Bhatt H, Gadi I, Kontgen F, et al. Blastocyst implantation depends on maternal expression of leukemia inhibitory factor. Nature 1992;359:76–9.
[82] Stewart CL. Leukemia inhibitory factor and the regulation of pre-implantation development of the mammalian embryo. Mol Reprod Dev 1994;39:233–8.
[83] Hambartsoumian E. Endometrial leukemia inhibitory factor (LIF) as a possible cause of unexplained infertility and multiple failures of implantation. Am J Reprod Immunol 1998;39: 137–43.
[84] Giess R, Tanasescu I, Steck T, Sendtner M. Leukemia inhibitory factor gene mutations in infertile women. Mol Hum Reprod 1999;5:581–6.
[85] Danielsson KG, Swahn ML, Bygdeman M. The effect of various doses of mifepristone on endometrial leukemia inhibitory factor expression in the midluteal phase-an immunohistochemical study. Hum Reprod 1997;12:1293–7.
[86] Laird SM, Tuckerman EM, Dalton CF, Dunphy BC, Li TC, Zhang X. The production of leukaemia inhibitory factor by human endometrium: presence in uterine flushings and production by cells in culture. Hum Reprod 1997;12:569–74.

[87] Chaouat G, Menu E, Delage G, et al. Immuno-endocrine interactions in early pregnancy. Hum Reprod 1995;10:55–9.
[88] McGinnis W, Krumlauf R. Homeobox genes and axial patterning. Cell 1992;68:283–302.
[89] Taylor HS. The role of HOX genes in the development and function of the female reproductive tract. Semin Reprod Med 2000;18:81–9.
[90] Taylor HS, Vanden Heuvel GB, Igarahi P. A conserved Hox axis in the mouse and human female reproductive system: late establishment and persistent adult expression of the Hoxa cluster genes. Biol Reprod 1997;57:1338–45.
[91] Taylor HS, Arici A, Olive D, Igarashi P. Hoxa10 is expressed in response to sex steroids at the time of implantation. J Clin Invest 1998;101:1379–82.
[92] Taylor HS, Igarashi P, Olive D, Arici A. Sex steroids mediate HOXA11 expression in human peri-implantation endometrium. J Clin Endocrinol Metab 1999;84:1129–35.
[93] Ma L, Lim H, Dey SK, Maas RL. Abdominal B (AbdB) Hoxa genes: regulation in the adult uterus by estrogen and progesterone and repression in the mullerian duct by the synthetic estrogen diethylstilbestrol. Dev Biol 1998;197:141–54.
[94] Troy P, Daftary G, Bagot C, Taylor HS. Transcriptional repression of peri-implantation EMX2 expression in mammalian reproduction by HOXA10. Mol Cell Biol 2003;23(1):1–13.
[95] Simeone A, Acampora D, Gulisand M. Nested expression domains of four homeobox genes in developing rostral brain. Nature 1992;358:687–90.
[96] Pellegrini M, Mansouri A, Simeone A, Bocinelli E, Gruss P. Dentate gyrus formation requires Emx2. Development 1996;2:335–45.
[97] Daftary G, Taylor HS. EMX2 gene expression in the female reproductive tract and aberrant expression in the endometrium of patients with endometriosis. J Clin Endocrinol Metab 2004;89:2390–6.
[98] Daftary G, Troy P, Bagot C, Young SL, Taylor HS. Direct regulation of beta3-integrin subunit gene expression by HOXA10 in endometrial cells. Mol Endocrinol 2002;16(3):571–9.
[99] Bagot CN, Kliman HJ, Taylor HS. Maternal Hoxa10 is required for pinopod formation in the development of mouse uterine receptivity to embryo implantation. Dev Dyn 2001;222:538–44.
[100] Taylor HS, Bagot C, Kardana A, Olive D, Arici A. Hox gene expression is altered in the endometrium of women with endometriosis. Hum Reprod 1999;14:1328–31.
[101] Cermik D, Selam B, Taylor HS. Regulation of HOXA-10 expression by testosterone in vitro and in the endometrium of patients with polycystic ovary syndrome. J Clin Endocrinol Metab 2003;88(1):238–43.
[102] Daftary G, Taylor HS. Hydrosalpinx fluid diminishes endometrial cell HOXA10 expression. Fertil Steril 2002;78(3):577–80.
[103] Benson GV, Lim H, Parai BC. Mechanisms of reduced fertility in Hoxa-10 mutant mice: uterine homeosis and loss of maternal Hoxa-10 expression. Development 1996;58:337–47.
[104] Satokata I, Benson G, Maas R. Sexually dimorphic sterility phenotypes in Hoxa-10 deficient mice. Nature 1995;374:460–3.
[105] Hsieh-Li HM, Witte DP, Weinstein M. Hoxa11 structure, extensive antisense transcription, and function in male and female infertility. Development 1995;121:373–85.
[106] Gendron RL, Paradis H, Hsieh-Li HM, Lee DW, Potter SS, Markoff E. Abnormal uterine stromal and glandular function associated with maternal reproductive defects in Hoxa-11 null mice. Biol Reprod 1997;56:1097–105.
[107] Bagot C, Troy PJ, Taylor HS. Alteration of maternal Hoxa10 expression by in vivo gene transfection affects implantation. Gene Ther 2000;7:1378–84.
[108] Yoo HJ, Barlow DH, Mardon HJ. Temporal and spatial regulation of expression of heparin-binding epidermal growth factor-like growth factor in the human endometrium: a possible role in blastocyst implantation. Dev Genet 1997;21:102–8.
[109] Birdsall MA, Hopkisson JF, Grant KE, Barlow DH, Mardon HJ. Expression of heparin-binding epidermal growth factor messenger RNA in the human endometrium. Mol Hum Reprod 1996;2(1):31–4.
[110] Das SK, Chakraborty I, Paria BC, Wang XN, Plowman G, Dey SK. Amphiregulin is an

implantation-specific and progesterone regulated gene in the mouse uterus. Mol Endocrinol 1995;9:691–705.
[111] Das SK, Wang XN, Paria BC, Damm D, Abraham JA, Klagsburn M, et al. Heparin biding EGF-like growth factor gene is induced in the mouse uterus temporally by the blastocyst solely at the site of its apposition: a possible ligand for interaction with blastocyst EGF-receptor in implantation. Development 1994;120(5):1071–83.
[112] Martin KL, Barlow DH, Sargent IL. Heparin-binding epidermal growth factor significantly improves human blastocyst development and hatching in serum-free medium. Hum Reprod 1998;13:1645–52.
[113] Lessey BA, Gui Y, Apparao KB, Young SL, Mulholland J. Regulated expression of heparin-binding EGF-like growth factor (HB-EGF) in the human endometrium: a potential paracrine role during implantation. Mol Reprod Dev 2002;62(4):446–55.
[114] Graham CH, McCrae KR, Lala PK. Molecular mechanisms of controlling trophoblast invasion of the uterus. Trophoblast Res 1993;7:237–50.
[115] Graham CH, Lysiak JJ, McCrae KR, Lala PK. Localization of transforming growth factor beta at the human fetal-maternal interface: role in trophoblast growth and differentiation. Biol Reprod 1992;46(4):561–72.
[116] Zhou J, Bondy C. Insulin-like growth factor II and its binding proteins in placental development. Endocrinology 1992;131:1230–40.
[117] Han VK, Bassett N, Walton J, Challis JR. The expression of insulin-like growth factor (IGF) and IGF binding protein genes in the human placenta and membranes: evidence for IGF: IGFBP interactions at the feto-maternal interface. J Clin Endocrinol Metab 1996;81(7): 2680–93.
[118] Irwin JC, Giudice LC. IGFBP-1 binds to the a5b1 integrin in human cytotrophoblasts and inhibits their invasion into decidualized endometrial stromal cells in vitro. Growth Hormone and IGF Research 1998;8:21–31.
[119] Irving JA, Lala PK. Functional role of cell surface integrins on human trophoblast cell migration: regulation by TGF-beta, IGF-II, and IGFBP-1. Exp Cell Res 1995;217:419–27.
[120] Hamilton GS, Lysiak JJ, Han VKM, Lala PK. Autocrine-paracrine regulation of human trophoblast invasiveness by IGF-II and IGFBP-1. Exp Cell Res 1998;244:147–56.
[121] Brooks PC, Stromblad S, Sanders LC, von Schalscha TL, Aimes RT, Stetler-Stevenson WG, et al. Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin alpha v beta 3. Cell 1996;85:683–93.
[122] Turpeenniemi-Hujanen T, Feinberg RF, Kauppila A, Puistola U. Extracellular matrix interactions in early human embryos: implications for normal implantation events. Fertil Steril 1995;64:132–8.
[123] Osteen KG, Keller NR, Feltus FA, Meiner MH. Paracrine regulation of matrix metallo-proteinase expression in normal human endometrium. Gynecol Obstet Invest 1999;48(Suppl 1): 2–13.
[124] Harvey MB, Leco KJ, Arcellana-Panlilio MY, Zhang X, Edwards DR, Schultz GA. Proteinase expression in early mouse embryos is regulated by leukemia inhibitory factor and epidermal growth factor. Development 1995;121(4):1005–14.
[125] Bass KE, Li H, Hawkes SP, Howard E, Bullen E, Vu TK, et al. Tissue inhibitor of metalloproteinase-3 expression is upregulated during human cytotrophoblast invasion in vitro. Dev Genet 1997;21(1):61–7.
[126] Higuchi T, Kanzaki H, Nakayama H, Fujimoto M, Hatayama H, Kojima K, et al. Induction of tissue inhibitor of metalloproteinase 3 gene during in vitro decidualization of human endometrial stromal cells. Endocrinology 1995;136(11):4973–81.
[127] Librach CL, Werb Z, Fitzgerald ML, et al. Type IV collagenase mediates invasion of human cytotrophoblasts. J Cell Biol 1991;113:437–49.
[128] Afonso S, Romagnano L, Babiarz B. The expression and function of cystatin C and cathepsin B and cathepsin L during mouse embryo implantation and placentation. Development 1997; 124:3415–25.
[129] Sheth KV, Roca GL, Al-Sedairy ST, Parhar RS, Hamilton GS, al-Abdul Jabar F. Prediction

of successful embryo implantation by measuring interleukin-1-alpha and immunosuppressive factor(s) in preimplantation embryo culture fluid. Fertil Steril 1991;55:952–7.
[130] Librach CL, Feigenbaum SL, Bass KE, et al. Interleukin-1 beta regulates human cytotrophoblasat metalloproteinase activity and invasion in vitro. J Biol Chem 1994;269:17125–31.
[131] Grummer R, Winterhager E. Regulation of gap junction connexins in the endometrium during the early pregnancy. Cell Tissue Res 1998;293:189–94.
[132] Ma W, Tan J, Matsumoto H, Robert B, Abrahamson DR, Das SK, et al. Adult tissue angiogenesis: evidence for negative regulation by estrogen in the uterus. Mol Endocrinol 2001; 15(11):1983–92.
[133] Maisonpierre PC, Suri C, Jones PF, Barunkova S, Wiegand SJ, Radziejewski C, et al. Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. Science 1997;277: 55–60.
[134] Chakraborty I, Das SK, Wang J, Dey SK. Developmental expression of the cyclo-oxygenase 1 and cyclo-oxygenase 2 genes in the peri-implantational mouse uterus and their differential regulation by the blastocyst and ovarian steroids. J Mol Endocrinol 1996;16:107–22.
[135] Lim H, Paria BC, Das SK, Dinchuk JE, Langenbach R, Trzaskos JM, et al. Multiple female reproductive failures in cyclooxygenase-2 deficient mice. Cell 1997;91(2):197–208.
[136] Cheng J-G, Stewart CL. Loss of cyclooxygenase-2 retards decidual growth but does not inhibit embryo implantation or development to term. Biol Reprod 2002;68(2):401–4.
[137] Marions L, Danielsson KG. Expression of cyclo-oxygenase in human endometrium during the implantation period. Mol Hum Reprod 1999;5:961–5.
[138] Foster GV. Calcitonin (thyrocalcitonin). N Engl J Med 1968;279:349–60.
[139] Austin LA, Heath H. Calcitonin. N Engl J Med 1981;304:269–78.
[140] Copp DH. Modern view of the physiological role of calcitonin in vertebrates. In: Gennari C, Segre G, editors. The effects of calcitonin in man: proceedings of The First International Workshop. Florence (Italy): University of Florence; 1982. p. 3–12.
[141] Ding YQ, Zhu LJ, Bagchi MK, Bagchi IC. Progesterone stimulates calcitonin gene expression in the uterus during implantation. Endocrinol 1994;135:2265–74.
[142] Ding YQ, Bagchi MK, Bardin CW, Bagchi IC. Calcitonin gene expression in the rat uterus during pregnancy. Rec Progr Horm Res 1995;50:373–8.
[143] Kumar S, Zhu LZ, Polihronis M, et al. Calcitonin is a progesterone-regulated marker of uterine receptivity for implantation in the human. J Clin Endocrinol Metab 1998;83:4443–50.
[144] Zhu LJ, Bagchi MK, Bagchi IC. Attenuation of calcitonin gene expression in pregnant rat uterus leads to a block in embryonic implantation. Endocrinology 1998;139:330–9.
[145] Bagchi IC, Li Q, Cheon YP. Role of steroid hormone-regulated genes in implantation. Ann NY Acad Sci 2001;943:68–76.
[146] Reshef E, Lei ZM, Rao CV, Pridham DD, Chegini N, Luborsky JL. The presence of gonadotropin receptors in nonpregnant human uterus, placenta, fetal membranes, and decidua. J Clin Endocrinol Metab 1990;70:421.
[147] Licht P, Russu V, Wildt L. On the role of human chorionic gonadotropin (hCG) in the embryo-endometrial microenvironment: implications for differentiation and implantation. Semin Reprod Med 2001;19:37.
[148] Fazleabas AT, Donnelly KM, Srinivasan S, Fortman JD, Miller JB. Modulation of the baboon uterine endometrium by chorionic gonadotropin during the period of uterine receptivity. Proc Natl Acad Sci USA 1999;96:2543.
[149] Han SW, Lei ZM, Rao CV. Treatment of human endometrial stromal cells with chorionic gonadotropin promotes their morphological and functional differentiation into decidua. Mol Cell Endocrinol 1999;147:7.
[150] Saunder S, Lenton EA. Endocrinology of the peri-implantation period. Clin Obstet Gynecol 2000;14(5):789–800.
[151] Healy DL. Endometrial prolactin and implantation. Bailleres Clin Obstet Gynecol 1991;5(1): 95–105.
[152] Psychoyos A, Nikas G, Gravanis A. The role of prostaglandins in blastocyst implantation. Hum Reprod 1995;10(Suppl 2):30–44.

[153] Makrigiannakis A, Margioris AN, Le Goascogne C, et al. The corticotropin releasing hormone gene (CRH) is expressed at the implantation site in the early pregnant rat uterus. Life Sci 1995;57:1869–75.
[154] Zoumakis E, Makrigiannakis A, Margioris AN, et al. Endometrial corticotrophin releasing hormone. Its potential autocrine and paracrine actions. Ann N Y Acad Sci 1997;26:84–94.
[155] Okon MA, Laird SM, Tuckerman EM, Li TC. Serum androgen levels in women who suffer recurrent miscarriage and their correlation with markers of endometrial function. Fertil Steril 1998;69:682–90.
[156] Horie K, Takakura K, Imai K, Liao S, Mori T. Immunohistochemical localization of androgen receptor in the human endometrium, decidua, and placenta, and pathological conditions of the endometrium. Hum Reprod 1992;7:1461–6.
[157] Narukawa S, Kanzaki H, Inoue T, Imai K, Higuchi T, Hatayama H, et al. Androgens induce prolactin production by endometrial stromal cells in vitro. J Clin Endocrinol Metab 1994; 78:165–8.
[158] Tuckerman EM, Okon MA, Tin-Chiu L, Laird SM. Do androgens have a direct effect on endometrial function? An in vitro study. Fertil Steril 2000;74(4):771–9.
[159] Watson H, Franks S, Bonney RC. Regulation of epidermal growth factor receptor by androgens in human endometrial cells in culture. Hum Reprod 1998;13:2585–91.
[160] Homburg R, Aramar NA, Eshel A, Adams J, Jacobs HS. Influence of serum luteinizing hormone concentrations on ovulation, conception, and early pregnancy loss in polycystic ovary syndrome. BMJ 1988;297:1024–6.
[161] Tulppala M, Stenman UH, Cacciatore B, Ylikorlaka O. Polycystic ovaries and levels of gonadotropins and androgen in recurrent miscarriage: prospective study in 50 women. Br J Obstet Gynaecol 1993;100:348–52.
[162] Licciardi FL, Kwiatkowski A, Noyes NL, Berkeley AS, Krey LL, Grifo JA. Oral versus intramuscular progesterone for in vitro fertilization: a prospective, randomized study. Fertil Steril 1999;71:614–8.
[163] Vega M, Devoto L, Castro O, Kohen P. Progesterone synthesis by human luteal cells: modulation by estradiol. J Clin Endocrinol Metab 1994;79:466–9.
[164] Gidley-Baird AA, O'Neill C, Sinosich MJ, Porter RN, Pike IL, Saunders DM. Failure of implantation in human in vitro fertilization and embryo transfer patients: the effects of altered progesterone/estrogen ratios in humans and mice. Fertil Steril 1986;45:69–74.
[165] Soliman S, Daya S, Collins J, Hughes EG. The role of luteal phase support in infertility treatment: a meta-analysis of randomized controlled trials. Fertil Steril 1994;61:1068–76.
[166] Hutchinson-Williams KA, Lunenfeld B, Diamond MP, Lavy G, Boyers SP, Decherney AH. Human chorionic gonadotropin, estradiol, and progesterone profiles in conception and nonconception cycles in an in vitro fertilization program. Fertil Steril 1989;52:441–5.
[167] Artini PG, Volpe A, Angioni S, Galassi MC, Battaglia C, Genazzani AR. A comparative, randomized study of three different progesterone support of the luteal phase following IVF/ET program. J Endocrinol Invest 1995;18:51–6.

ELSEVIER
SAUNDERS

Obstet Gynecol Clin N Am
31 (2004) 767–778

OBSTETRICS AND
GYNECOLOGY
CLINICS
OF NORTH AMERICA

Hormonal monitoring of the first trimester of pregnancy

Engin Oral, MD[a,*], Mehmet R. Genç, MD, PhD[b]

[a]*Cerrahpasa Medical Faculty, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology, Istanbul University, Cerrahpasa PTT PK 31, 34301 Istanbul, Turkey*
[b]*Jinemed Hospital, Department of Obstetrics and Gynecology, Division of Perinatology, Nushetite Cad., Deryadil Sok. No: 1, Besiktas, Istanbul, Turkey*

The hormonal changes and maternal adaptations of human pregnancy are among the most remarkable phenomena in nature. Endocrinologic parameters in the early gestation period have been used to predict abnormal pregnancies and to identify fetuses that have chromosomal aberrations. This article focuses on the changes in hormones that are secreted by the maternal-fetal-placental unit that are unique for the first trimester of pregnancy and their impact on clinical outcome.

Progesterone and 17 α-hydroxyprogesterone

The principal source of progesterone during pregnancy is the placenta, although the corpus luteum is the major source during the first 6 to 8 weeks of gestation when progesterone is essential for the development of a secretory endometrium to receive and implant a blastocyst [1]. By 8 weeks' gestation, the developing trophoblasts take over as the principal producers of progesterone because removal of the corpus luteum before this time leads to abortion [2]. After 8 weeks' gestation, the corpus luteum contributes only a fraction of the progesterone that is secreted. Maternal progesterone plasma levels increase from 25 ng/mL during the late luteal phase to 40 ng/mL near the end of the first trimester to 150 ng/mL at term. Because progesterone has a shorter half-life than

* Corresponding author.
E-mail address: eoral@superonline.com (E. Oral).

0889-8545/04/$ – see front matter
doi:10.1016/j.ogc.2004.08.004 obgyn.theclinics.com

human chorionic gonadotropin (hCG), it is expected to reflect any change in the dynamics of the pregnancy more accurately.

17 α-Hydroxyprogesterone (17OHP) is a steroid that is secreted in parallel to progesterone from the corpus luteum. 17OHP may be a better marker of corpus luteum function in early pregnancy than progesterone because there is limited placental hydroxylation at this stage.

A single measurement of serum progesterone level has been used to distinguish a normal pregnancy from a nonviable or an ectopic one. A value of 25 ng/mL or more is associated with a normal intrauterine pregnancy 98% of the time, whereas a value of less than 5 ng/mL identifies a nonviable pregnancy, regardless of the location [3]. Most patients, however, have a progesterone level that is between 10 ng/mL and 20 ng/mL at presentation; this significantly limits the clinical usefulness of the progesterone measurement. The value of 25 ng/mL as an indicator of a normal intrauterine pregnancy was established in women who had spontaneous ovulation and pregnancies. The appropriate number for women who receive medication for the induction of ovulation is probably higher; this further limiting its use as a diagnostic tool in these cases. Therefore, a single measurement of serum progesterone level must be viewed as an adjunct to hCG levels and ultrasonography.

Estrogen

The corpus luteum of pregnancy is the principal source of estrogen during the first few weeks. Subsequently, nearly all of the estrogen is formed by the trophoblast of the placenta. Estrogen production and plasma estrogen level increase markedly and lead to a 1000-fold increase in urinary estriol.

Human chorionic gonadotropin

hCG is a glycoprotein with a molecular mass of 38 kd which consists of two noncovalently-linked subunits—α and β [4]. The α unit is shared by follicle-stimulating hormone and luteinizing hormone but the β subunit (β-hCG) is specific to hCG. β-hCG has been used extensively as a pregnancy test and can be detected in the serum as early as 6 to 8 days after ovulation. The most widely accepted theory regarding the role of hCG in pregnancy is the maintenance of the early corpus luteum to ensure continued progesterone and, possibly, relaxin secretion by the ovary until this function is taken over by the growing syncytiotrophoblasts.

hCG that is secreted by the syncytiotrophoblast of the placenta is released into the fetal and maternal circulation and is excreted in maternal urine. Human embryos secrete hCG into the culture medium 5 to 8 days after fertilization; pregnancy-specific β-hCG is detectable in peripheral maternal blood 1 week after conception. β-hCG can be detected in 5% of ovulatory cycles on the eighth

day; in 16% on the ninth day; in 53% on the 10th day; and in all cycles on the 11th day after the preovulatory luteinizing hormone surge [5]. Most commercially available monoclonal antibody-based urine pregnancy tests can detect β-hCG at a level that is greater than 25 IU/L; this corresponds to the 24th or 25th day of a regular 28-day cycle.

It was shown that maternal serum hCG levels double over 1.4 to 1.6 days from the time of first detection to the 35th day of pregnancy and then doubles over 2.0 to 2.7 days from the 35th to the 42nd day [6]. Plasma levels continue to increase rapidly during normal pregnancy and reach a peak between 60 and 90 days of gestation. Thereafter, the maternal serum hCG concentration plateaus and declines until delivery [7]. With the development of accurate and rapid quantitative measurements of β-hCG in serum, these tests have been used extensively to confirm a normally-developing embryo and trophoblasts. A doubling of the β-hCG levels at 48-hour intervals usually signifies a normal viable intrauterine pregnancy [8], whereas decreased or decreasing levels are associated with an inevitable abortion or an ectopic pregnancy [9].

Activin

Activins are homodimers that consist of βAβA (activin-A), βAβB (activin-AB), and βBβB (activin-B) subunits that are linked by disulphide bonds. The feto–placental unit is the main source of activin A in early pregnancy, although an ovarian contribution also was suggested [10]. Maternal serum activin-A that is measured in first trimester does not predict pregnancy outcome.

Carcinoembryonic antigen-125 (CA-125)

In normal pregnancies, maternal CA-125 serum values in the first trimester are elevated as compared with nonpregnant levels. In pregnant women who present with intact fetal heartbeat and vaginal bleeding, maternal CA-125 levels increase beyond those that are found in women who have uncomplicated pregnancies [11]. Similarly, women who have ectopic pregnancies—ruptured or unruptured—are more likely to have elevated levels of serum CA-125 compared with women who have intrauterine pregnancies [12]. An elevation in maternal CA-125 serum is dependent on the extent of decidual disruption. A single CA-125 measurement does not have a prognostic value in most cases; however, sequential determinations of increased maternal CA-125 measurements seem to be highly predictive for subsequent abortion [11]. Among aborters, CA-125 levels that are measured 5 to 7 days apart remain high or increased, whereas nonaborters have constantly low or steeply declining CA-125 measures.

Insulin growth factor binding protein–1

Insulin growth factor binding protein (IGFPB)-1—also known as placental protein 12—is one of the six proteins that specifically binds insulin like growth factors (IGFs) in body fluids and tissues [13]. IGFBP-1 contains 234 amino acids and has a molecular mass of 25 kd. The human IGFBP-1 gene is located on chromosome 7. IGFBP-1 is synthesized in large amounts by the decidua in early pregnancy [14] and its concentration is increased in the maternal circulation. It also is the predominant IGFBP in amniotic fluid and the major IGFBP in fetal plasma [15]. IGFBP-1 also is a local modulator of IGF action that regulates fetal growth and is able to interact independently with cytotrophoblast cells.

Pregnancy-associated plasma protein–a

Pregnancy-associated plasma protein–A (PAPP-A) is a 187-kd macromolecular glycoprotein that is produced by the trophoblast; its serum levels increase during pregnancy. This glycoprotein circulates as a complex with the proform of eosinophil major basic protein (pro-MBP) [16]. The pro-MBP also binds to the complement component, C3, and to angiotensinogen [17]. Because polyclonal antibodies against PAPP-A also recognize pro-MBP, these antibodies cross-react partially with C3 and angiotensinogen [18].

The biologic function of PAPP-A is not clear. PAPP-A is a protease for IGFBP-4 and -5 [19]. Following cleavage, the affinity of IGFBPs for IGF-I and -II is reduced. Variables that regulate the amount of proteolysis regulate the action of the IGFs [20]. Locally-synthesized IGFs promote cellular mitosis and differentiation and probably are important in embryogenesis and the regulation of fetal and placental growth [20,21]. At term, cord blood concentration of IGF-I correlates positively—whereas that of IGFBP-1 correlates inversely—with birth weight [22]. The level of PAPP-A in maternal serum might reflect the local level of PAPP-A and the availability of IGFs. Decreased levels of PAPP-A might indicate low levels of IGFs and poorer fetal or placental growth. It is not known whether poor fetal growth is a consequence of poor placental function or if fetal growth and placental function are poorer because of the influence of the same factors (eg, decreased levels of growth factors such as IGFs).

The clinical usefulness of PAPP-A measurements during pregnancy was investigated [23]. Several studies found an association between decreased maternal serum PAPP-A at 10 to 14 gestational weeks and the delivery of babies who are small for gestational age [24–26]. The predictive value of maternal PAPP-A levels seems to be weaker after adjustment for smoking [26]. A 15% reduction in the level of PAPP-A in smokers was reported [27,28]; the explanation was that smoking inhibits apoptosis of the syncytiotrophoblasts which results in disturbed feto-placental exchange [28]. Additionally, the direct influence of smoking on reduced PAPP-A production may cause decreased levels in maternal serum and, probably more importantly, in levels of intrauterine

PAPP-A. It is well-known that smoking negatively affects the placental vessels and nutrient supply to the fetus; it also affects PAPP-A production and decreases IGFs that may have synergistic effects on fetal growth.

In women who delivered babies who were large for gestational age (LGA), the PAPP-A level at 10 to 14 gestational weeks was significantly increased when compared with women who delivered babies who were appropriate for gestational age, after adjustment for free β-hCG, inhibin-A, nuchal translucency, maternal age, smoking, gravidity, and gestational diabetes mellitus (GDM) [26]. PAPP-A levels at 10 to 14 gestational weeks are decreased in women who have pre-existing diabetes mellitus or GDM [24,29], including those who delivered LGA babies [26]. It seems that PAPP-A is not responsible for increased fetal growth in the latter cases and increased fetal growth that is associated with diabetes is likely due to maternal hyperglycemia.

In ectopic pregnancies, the specificity of PAPP-A measurements has been the subject of debate. Some investigators reported that PAPP-A was depressed or even undetectable in ectopic pregnancies [30], whereas others found only slightly depressed PAPP-A levels [31]. In an on-going pregnancy, decreased PAPP-A levels—as a result of abnormal placentation or abnormal placental function—is a precedent to fetal death in utero. In a series of 5297 patients who had a miscarriage rate of 1%, Ong et al [24] found 20.4% of miscarriages were associated with PAPP-A levels that were less than the 10th percentile. Similarly, Ruge et al [32] found that 25% of miscarriages were associated with PAPP-A levels that were less than the 10th percentile. In first trimester pregnancies with an ultrasonically-proven live fetus, Kwik and Morris [33] confirmed the association between decreased maternal serum PAPP-A levels that were measured at 11–13 weeks' gestation, fetal death in utero, and birth weight that was less than the 10th percentile. PAPP-A levels had a 49% predictive value of fetal demise with a sensitivity of 89% [34]. The association between decreased PAPP-A level and miscarriage is not limited to pregnancies with fetal chromosomal aberrations. Yaron et al [25] reported a relative risk of 8.76 for subsequent spontaneous miscarriage with PAPP-A levels of less than 0.25 multiples of the median (MoM) that were measured between 10 and 13 weeks' gestation, after excluding fetuses that had chromosome aberrations or anomalies.

Other studies that examined the correlation between first trimester PAPP-A levels and adverse fetal outcomes have not produced consistent results. In one study, reduced circulating PAPP-A concentrations during the first trimester was associated with preterm labor and low birth weight [35]; however, this was not confirmed by other studies [36]. Yaron et al [25] reported that PAPP-A was diminished in women who had proteinuric pregnancy-induced hypertension (PIH), but not in those who had nonproteinuric PIH. Again, this finding was not confirmed by other investigators [26].

Discrepant findings about measurements of PAPP-A in normal and abnormal pregnancies were attributed, in part, to the cross-reaction between polyclonal antibodies against PAPP-A and pro-MBP [18], the immunologic heterogeneity of PAPP-A [37], and the use of different reagents by the various investigators

[38]. Recently, selected monoclonal antibodies that do not recognize pro-MBP, have been raised against PAPP-A and were evaluated for Down's syndrome screening [39]. This screening test had a significantly increased specificity and sensitivity over the methods that use polyclonal antibodies; however, the use of monoclonal antibodies to measure PAPP-A levels did not improve the performances of this test significantly in the diagnosis of pregnancy failure [40].

Inhibins

Inhibins are glycoproteins that belong to the transforming growth factor (TGF)-β superfamily [41]. They consist of an 18-kd α-subunit and a 32-kd β_A (inhibin-A) or a 14-kd β_B subunit (inhibin-B) that are linked by disulphide bonds. Only the dimeric forms of inhibin are bioactive, although the α-subunits circulate in vast excess as biologically-inert monomers.

Corpus luteum is the major site of inhibin production during the luteal phase in a normal menstrual cycle [42]. Immunoreactive inhibin concentrations increase after conception in early pregnancy [43]. Numerous studies have attempted to identify the predominant site of inhibin-A production during pregnancy. Lockwood et al [44] investigated the source of inhibins in early pregnancies that were conceived in vitro with and without corpus luteum function. Comparable inhibin-A concentrations were found in pregnancies that were conceived spontaneously or following in vitro fertilization that involved the transfer of fresh or frozen embryos. This suggested that the main source of dimeric inhibin-A in early pregnancy is the fetoplacental unit. In another study that used a donor-egg model in conjunction with ELISA specific for inhibin-A, the concentrations of inhibin-A in the first trimester of human pregnancy were not significantly different in the women who did or did not have corpora lutea; this again suggested a fetoplacental origin [45]. In contrast, other investigators suggested that the corpus luteum is a major source of circulating inhibin-A in early pregnancy, based on inhibin-A concentrations that were significantly decreased in women who conceived with donor oocyte as compared with women who conceived after ovarian stimulation. Furthermore, inhibin-A concentrations were not significantly different between singleton and multiple pregnancies in the ovarian stimulation protocol; this suggested that the size of the early trophoblast does not seem to influence the secretion of inhibin-A [46].

The physiologic role of inhibins in humans is unclear. Inhibin-A suppresses hCG release; this suppression is gestation-dependent with no effect in the first trimester [47]. Animal studies suggest a role for inhibin-A in the maintenance of luteal progesterone output [48]. It also is believed that inhibin-A plays a part in the cell signaling, and therefore, possibly in trophoblast invasion.

Inhibin-A concentrations increase throughout the first trimester, whereas inhibin-B concentrations in circulation are unaltered in early pregnancy [42,49]. After the completed 10 weeks gestation, inhibin-A levels start to decrease

with gestation [50]. Regulation of placental expression of inhibin-A is not clear; however, it was showed that hCG, prostaglandins, and epidermal growth factor stimulate—whereas activin and TGF-α suppress—placental inhibin production [50].

Inhibin-A has a shorter half-life than hCG or progesterone; therefore, it may be more sensitive to changes in the trophoblasts [51]. Decreased inhibin-A levels in early pregnancy have been associated with biochemical pregnancies and missed miscarriages [49]; however, inhibin-A levels were not decreased in women who were sampled 3 or more weeks before a miscarriage. For this reason, some investigators do not consider inhibin-A to be a useful marker to predict subsequent miscarriage in a currently viable pregnancy [52,53]. Al-Azemi et al [54] disagreed with this view. Women who had a history of recurrent spontaneous miscarriage (at least three previous consecutive first-trimester pregnancy losses) had consistently lower concentrations of inhibin-A in the serum as early as 6 weeks' gestation if the current pregnancy was destined to miscarry.

Inhibins also are elevated in pregnant women who have PIH [26,55]; however, the differences in inhibin-A levels between hypertensive and normotensive women are small and hinders its clinical usefulness. Petraglia [55] proposed that the trophoblast increases the production of inhibin-A as an adaptive response to pathologic conditions. Impaired placental perfusion or placental damage may be followed by a regenerative process with increased synthesis of placental products; however, the spillage into maternal circulation as a consequence of placental damage after impaired placentation also could be the reason for increased levels of markers [56].

Inhibin pro-αC

Inhibin pro-αC circulates as a functionally inactive monomer and as part of high-molecular weight functional dimers [49]. Inhibin pro-αC levels peak at 4 weeks of gestation and then decrease until 11 weeks of gestation. Inhibin pro-αC is believed to play a role as a paracrine and endocrine regulator of placental function. Maternal serum inhibin pro-αC concentrations are decreased in failed intrauterine pregnancies. Interruption of the hormonal activity of the corpus luteum by administration of mifepristone in women who underwent induced termination of pregnancy led to a decrease in pro-αC levels [13]. Elson et al [57] evaluated numerous biomarkers, including β-hCG, progesterone, 17OHP, IGFBP-1, inhibin-A, and inhibin pro-αC, for successful expectant management of incomplete miscarriage. Inhibin-A levels of less than 3.9 pmol/L, IGFBP-1 levels of greater than 15 mg/L, and inhibin pro-αC levels of less than 400 pmol/L were associated significantly with successful expectant management of miscarriage. Nevertheless, β-hCG was the best predictor for pregnancies that resolved spontaneously.

Leptin

Maternal leptin concentrations increase to levels that are twofold to threefold greater than nonpregnant concentrations; peak levels occur at approximately 28 weeks of gestation [58]. Results from clinical studies suggest that pregnancy-associated increases in maternal plasma leptin may result from an up-regulation of adipocyte leptin synthesis in the presence of increasing insulin resistance and hyperinsulinemia in the second half of pregnancy [59]. The studies that have been published on maternal leptin concentrations in pregnancies that are complicated by GDM reported conflicting results. Festa et al [60] reported that women who had mild GDM presented with relative hypoleptinaemia compared with women who had normal glucose tolerance. In contrast, Kautzky-Willer et al [61] reported that women who had GDM have increased plasma leptin concentrations during and after pregnancy. Elevated maternal serum leptin concentrations in early pregnancy may be a predictor of GDM later in pregnancy. In one study, women who had increased plasma leptin concentrations experienced a 4.7-fold increased risk of GDM as compared with women who had concentrations of 14.3 ng/mL or less [62].

Leptin also may be involved in the pathogenesis of preeclampsia. Increased maternal and cord leptin concentrations in pregnancies that were complicated by preeclampsia were reported [63,64]. It is not known whether increased maternal leptin concentrations that are measured in early pregnancy predict preeclampsia.

Macrophage migration inhibitory factor and macrophage inhibitory cytokine–1

Recent findings indicated possible roles for macrophage migration inhibitory factor (MIF) in a variety of reproductive phenomena (eg, ovulation, blastocyst implantation, and embryogenesis). MIF mRNA and protein have been detected in murine and human ovaries, human follicular fluid, and the murine early embryo [65]. Additionally, it was demonstrated that MIF is expressed in glandular epithelium, stromal and predecidualized stromal cells of the human endometrium, as well as in the decidua [66] and trophoblast [67] of first-trimester placenta. Yamada and colleagues [68] investigated serum concentrations of MIF in pregnant women who had had recurrent miscarriages. They compared serum MIF concentrations in women who had a subsequent miscarriage and normal fetal karyotype with those who had a miscarriage and abnormal karyotype or those who had live births. MIF concentrations were decreased significantly in the serum of women who had subsequent first-trimester miscarriage and normal karyotype compared with women who had pregnancies that ended in live birth. Macrophage inhibitory cytokine (MIC)-1 is a member of the TGF-β superfamily [69]. Maternal serum MIC-1 is decreased in women who miscarry. Furthermore, decreased concentrations of MIC-1 levels precede miscarriage by several weeks [70].

Summary

Measurement of hormonal biomarkers, such as PAPP-A and free β–hCG, between 10 and 14 weeks of gestation are the mainstay of screening strategies for fetal chromosomal anomalies. PAPP-A levels that are determined for such a screening also may be used to alert the obstetrician to potential adverse pregnancy outcomes (eg, poor fetal growth, miscarriage). Although progesterone, inhibin-A, IGFBP-1, inhibin pro-αC, and serial measurements of CA-125 may be used to predict poor fetal development, serial measurement of β-hCG to monitor doubling of its maternal serum level, in combination with ultrasound, remains the most effective strategy to distinguish failed or ectopic pregnancies from normal, early pregnancies. Studies on the association between early pregnancy levels of inhibin-A or PAPP-A and PIH has yielded inconsistent results; further research is warranted. Similarly, more work is needed to establish the role of leptin in GDM and to determine whether it can be clinically useful in the management of patients who have this condition. Efforts to understand early pregnancy endocrinology will shed light on pregnancy physiology and will provide us with tools to manage adverse outcomes more efficiently.

References

[1] Tulchinsky D, Hobel CJ. Plasma human chorionic gonadotropin, estrone, estradiol, estriol, progesterone and 17α-hydroxyprogesterone in human pregnancy III: early normal pregnancy. Am J Obstet Gynecol 1973;117(7):884–93.
[2] Csapo AL, Pulkkinen MO, Wiest WG. Effects of luteectomy and progesterone replacement in early pregnant patients. Am J Obstet Gynecol 1973;6(115):759–65.
[3] Stovall TG, Ling FW, Carson SA, Buster JE. Serum progesterone and uterine curettage in differential diagnosis of ectopic pregnancy. Fertil Steril 1992;57(2):456–7.
[4] Ren SG, Braunstein G-D. Human chorionic gonadotropin. Semin Reprod Endocrinol 1992; 10:95–105.
[5] Gerhard I, Runnebaum B. The endocrinology of pregnancy. In: Runnebaum B, Rabe T, editors. Gynecological endocrinology and reproductive medicine. Berlin: Springer-Verlag; 1997. p. 344–417.
[6] Pittaway D, Reish R, Colston Wentz A. Doubling times of human chorionic gonadotrophin increase in early viable intrauterine pregnancies. Am J Obstet Gynecol 1985;159(3):299–302.
[7] Saxena BB, Landesman R. Diagnosis and management of pregnancy by the radioimmunoassay of hCG. Am J Obstet Gynecol 1978;131(1):97–107.
[8] Ackerman R, Deutch S, Krunholz B. Levels of human chorionic gonadotropin in unruptured and ruptured ectopic pregnancy. Obstet Gynecol 1982;60(1):13–4.
[9] Hussa RO. Clinical utility of human chorionic gonadotropin and β -subunit measurements. Obstet Gynecol 1982;60(1):1–12.
[10] Muttukrishna S, Child TJ, Groome NP, Ledger WL. Source of circulating levels of inhibin A, pro alpha C-containing inhibins and activin A in early pregnancy. Hum Reprod 1997;12(5): 1089–93.
[11] Schmidt T, Rein DT, Foth D, Eibach HW, Kurbacher CM, Mallmann P, et al. Prognostic value of repeated serum CA 125 measurements in first trimester pregnancy. Eur J Obstet Gynecol Reprod Biol 2001;97(2):168–73.
[12] Sadovsky Y, Pineda J, Collins JL. Serum CA-125 levels in women with ectopic and intrauterine pregnancies. J Reprod Med 1991;36(12):875–8.

[13] Shimasaki S, Ling N. Identification and molecular characterisation of insulinlike growth factor binding proteins (IGFBP-1, -2, -3, -4, -5, -6). Prog Growth Factor Res 1992;3:243–66.
[14] Rutanen E-M. Insulin-like growth factor binding protein-1. Sem Reprod Endocrinol 1992;10: 154–62.
[15] Drop SL, Kortleve DJ, Guyda HJ, Posner BI. Immunoassay of a somatomedin binding protein from human amniotic fluid: levels in fetal, neonatal and adult sera. J Clin Endocrinol Metab 1984;59(5):908–15.
[16] Oxvig C, Sand O, Kristensen T, Gleich GJ, Sottrup-Jensen L. Circulating human pregnancy-associated plasma protein-A is disulphide-bridged to the pro-form of eosinophil major basic protein. J Biol Chem 1993;268(17):12243–6.
[17] Oxvig C, Haaning J, Kristensen L, Wagner JM, Rubin I, Stigbrand T, et al. Identification of angiotensinogen and complement C3dg as novel proteins binding the pro-form of eosinophil major basic protein in human pregnancy serum and plasma. J Biol Chem 1995;270(23):13645–51.
[18] Bersinger NA, Meisser A, Bessou T, Seguin P, Birkhaüser MH, Bischof P. Production and characterisation of monoclonal antibodies against pregnancy-associated plasma protein-A. Mol Hum Reprod 1999;5(7):675–81.
[19] Laursen LS, Overgaard MT, Soe R, Boldt HB, Sottrup-Jensen L, Giudice LC, et al. Pregnancy-associated plasma protein-A (PAPP-A) cleaves insulin-like growth factor binding protein (IGFBP)-5 independent of IGF: implications for the mechanism of IGFBP-4 proteolysis by PAPP-A. FEBS Lett 2001;504(1–2):36–40.
[20] Clemmons DR. Role of insulin-like growth factor binding proteins in controlling IGF actions. Mol Cell Endocrinol 1998;140(1–2):19–24.
[21] van Kleffens M, Groffen C, Lindenbergh-Kortleve DJ, van Neck JW, Gonzalez-Parra S, Dits N, et al. The IGF system during fetal-placental development of the mouse. Mol Cell Endocrinol 1998;140(1–2):129–35.
[22] Giudice LC, de Zegher F, Gargosky SE, Dsupin BA, de las Fuentes L, Crystal RA, et al. Insulin-like growth factors and their binding proteins in the term and preterm human fetus and neonate with normal and extremes of intrauterine growth. J Clin Endocrinol Metab 1995;80(5):1548–55.
[23] Stabile I, Grudzinskas JG, Chard T. Clinical applications of pregnancy protein estimations with particular reference to pregnancy-associated plasma protein-A (PAPP-A). Obstet Gynecol Surv 1988;42(2):73–82.
[24] Ong CY, Liao AW, Spencer K, Munim S, Nicolaides KH. First trimester maternal serum free beta human chorionic gonadotrophin and pregnancy associated plasma protein A as predictors of pregnancy complications. BJOG 2000;107(10):1265–70.
[25] Yaron Y, Heifetz S, Ochshorn Y, Lehavi O, Orr-Urtreger A. Decreased first trimester PAPP-A is a predictor of adverse pregnancy outcome. Prenat Diagn 2002;22(9):778–82.
[26] Tul N, Pusenjak S, Osredkar J, Spencer K, Novak-Antolic Z. Predicting complications of pregnancy with first-trimester maternal serum free-betahCG, PAPP-A and inhibin-A. Prenat Diagn 2003;23(12):990–6.
[27] Spencer K. The influence of smoking on maternal serum PAPP-A and free beta hCG levels in the first trimester of pregnancy. Prenat Diagn 1999;19(11):1065–6.
[28] de Graaf IM, Cuckle HS, Pajkrt E, Leschot NJ, Bleker OP, van Lith JM. Co-variables in first trimester maternal serum screening. Prenat Diagn 2000;20(3):186–9.
[29] Pedersen JF, Sorensen S, Molsted-Pedersen L. Serum levels of human placental lactogen, pregnancy-associated plasma protein A and endometrial secretory protein PP14 in first trimester of diabetic pregnancy. Acta Obstet Gynecol Scand 1998;77(2):155–8.
[30] Sjöberg J. Pregnancy-associated plasma protein-A in pregnancy related gynaecologic emergency. Hum Reprod 1987;2(7):615–6.
[31] Bischof P, Reyes H, Herrmann WL, Sizonenko PC. Circulating levels of pregnancy-associated plasma protein-A (PAPP-A) and human chorionic gonadotropin (hCG) in intrauterine and extrauterine pregnancies. BJOG 1983;90(2):323–5.
[32] Ruge S, Pedersen JF, Sorensen S, Lange AP. Can pregnancy-associated plasma protein A (PAPP-A) predict the outcome of pregnancy in women with threatened abortion and confirmed foetal viability? Acta Obstet Gynecol Scand 1990;69(7–8):589–95.

[33] Kwik M, Morris J. Association between first trimester maternal serum pregnancy associated plasma protein-A and adverse pregnancy outcome. Aust N Z J Obstet Gynaecol 2003;43(6): 438–42.
[34] Westergaard JG, Teisner B, Sinosich MJ, Madsen LT, Grudzinskas JG. Does ultrasound examination render biochemical tests obsolete in the prediction of early pregnancy failure? BJOG 1985;92(1):77–83.
[35] Johnson MR, Riddle AF, Grudzinskas JG, Sharma V, Collins WP, Nicolaides KH. Reduced circulating placental protein concentrations during the first trimester are associated with preterm labour and low birth weight. Hum Reprod 1993;8(11):1942–7.
[36] Morssink LP, Kornman LH, Hallahan TW, et al. Maternal serum levels of free β -hCG and PAPP-A in the first trimester of pregnancy are not associated with subsequent foetal growth retardation or preterm delivery. Prenat Diagn 1998;18(2):147–52.
[37] Bischof P, Meisser A. Immunological heterogeneity of pregnancy-associated plasma protein-A (PAPP-A) Effect on the radioimmunoassay of PAPP-A. Br J Obstet Gynaecol 1989;96(7): 8870–5.
[38] Seppala M, Purhonen M. The use of hCG and other pregnancy proteins in the diagnosis of ectopic pregnancy. Clin Obstet Gynaecol 1987;30(1):148–54.
[39] Bersinger NA, Meisser A, Bessou T, Seguin P, Birkhaüser MH, Bischof P. Production and characterisation of monoclonal antibodies against pregnancy-associated plasma protein-A. Mol Hum Reprod 1999;5(7):675–81.
[40] Dumps P, Meisser A, Pons D, Morales MA, Anguenot JL, Campana A, et al. Accuracy of single measurements of pregnancy-associated plasma protein-A, human chorionic gonadotropin and progesterone in the diagnosis of early pregnancy failure. Eur J Obstet Gynecol Reprod Biol 2002;100(2):174–80.
[41] Kingsley DM. The TGF-β superfamily: new members, new receptors and new genetic tests of function in different organisms. Genes Dev 1994;8(2):133–46.
[42] Illingworth PJ, Reddi K, Smith KB, Baird DT. The source of inhibin secretion during the human menstrual cycle. J Clin Endocrinol Metab 1991;73(3):667–73.
[43] Tovanabutra S, Illingworth PJ, Ledger WL, Glasier AF, Baird DT. The relationship between peripheral immunoactive inhibin, human chorionic gonadotrophin, oestradiol and progesterone during human pregnancy. Clin Endocrinol 1993;38(1):101–7.
[44] Lockwood GM, Ledger WL, Barlow DH, Groome NP, Muttukrishna S. Measurement of inhibin and activin in early pregnancy: demonstration of fetoplacental origin and role in prediction of early pregnancy outcome. Biol Reprod 1997;57(6):1490–4.
[45] Birdsall M, Ledger WL, Groome NP, Abdulla H, Muttukrishna S. Inhibin A and activin A in the first trimester of human pregnancy. J Clin Endocrinol Metab 1997;82(5):1557–60.
[46] Treetampinich C, O'Conner AE, MacLachen V, Groome NP, de Kretser DM. Maternal serum inhibin A concentrations in early pregnancy after IVF and embryo transfer reflect the corpus luteum contribution and pregnancy outcome. Hum Reprod 2000;15(9):2028–32.
[47] Mersol-Barg MS, Miller KF, Choi CM, Lee AC, Kim MH. Inhibin suppresses human chorionic gonadotropin secretion in term, but not first trimester, placenta. J Clin Endocrinol Metab 1990; 71(5):1294–8.
[48] Webley GE, Marsden PL, Knight PG. Differential control of immunoreactive alpha-inhibin and progesterone production by marmoset luteal cells in vitro: evidence for a paracrine action of alpha-inhibin on basal and gonadotrophin stimulated progesterone production. Biol Reprod 1994;50(6):1394–402.
[49] Muttukrishna S, Child TJ, Groome NP, Ledger WL. Source of circulating levels of inhibin A, pro alpha C-containing inhibins and activin A in early pregnancy. Hum Reprod 1997;12(5): 1089–93.
[50] Spencer K, Liao AW, Ong CY, Geerts L, Nicolaides KH. Maternal serum levels of dimeric inhibin A in pregnancies affected by trisomy 21 in the first trimester. Prenat Diagn 2001;21(6): 441–4.
[51] Jackson M, Dudley DJ. Endocrine assays to predict preterm delivery. Clin Perinatol 1998;25(4): 837–57.

[52] Wallace EM, Marjono B, Tyzack K, Tong S. First trimester levels of inhibins and activin A in normal and failing pregnancies. Clin Endocrinol 2004;60(4):484–90.
[53] France JT, Keelan J, Song L, Liddell H, Zanderigo A, Knox B. Serum concentrations of human chorionic gonadotrophin and immunoreactive inhibin in early pregnancy and recurrent miscarriage: a longitudinal study. Aust N Z J Obstet Gynaecol 1996;36(3):325–30.
[54] Al-Azemi M, Ledger WL, Diejomaoh M, Mousa M, Makhseed M, Omu A. Measurement of inhibin A and inhibin pro- pro-alphaC in early human pregnancy and their role in the prediction of pregnancy outcome in patients with recurrent pregnancy loss. Fertil Steril 2003; 80(6):1473–9.
[55] Petraglia F. Inhibin, activin and follistatin in the human placenta – a new family of regulatory proteins. Placenta 1997;18:3–8.
[56] Sebire NJ, Roberts L, Noble P, Wallace E, Nicolaides KH. Raised maternal serum inhibin A concentration at 10 to 14 weeks of gestation is associated with pre-eclampsia. BJOG 2000; 107(6):795–7.
[57] Elson J, Salim R, Lawrence A, Cassik P, Jurkovic D. The value of serum biochemistry in the expectant management of incomplete miscarriage. Ultrasound Obstet Gynecol 2003; 22(Suppl 1):53.
[58] Schubring C, Englaro P, Siebler T, Blum WF, Demirakca T, Kratzsch J, et al. Longitudinal analysis of maternal plasma leptin levels during pregnancy, at birth and up to six weeks after birth: relation to body mass index, skinfolds, sex steroids and umbilical cord blood leptin levels. Horm Res 1998;50(5):276–83.
[59] Laivuori H, Kaaja R, Koistinen H, Karonen S-L, Andersson S, Koivisto V, et al. Leptin during and after preeclamptic or normal pregnancy: its relation to serum insulin and insulin sensitivity. Metabolism 2000;49(2):259–63.
[60] Festa A, Shnawa N, Krugluger W, Hopmeier P, Schernthaner G, Haffner SM. Relative hypoleptinaemia in women with mild gestational diabetes mellitus. Diabetes Med 1999;16(8): 656–62.
[61] Kautzky-Willer A, Pacini G, Tura A, Bieglmayer CN, Schneider B, Ludvik B, et al. Increased plasma leptin in gestational diabetes. Diabetologia 2001;44(2):164–72.
[62] Qiu C, Williams MA, Vadachkoria S, Frederick IO, Luthy DA. Increased maternal plasma leptin in early pregnancy and risk of gestational diabetes mellitus. Obstet Gynecol 2004;103(3):519–25.
[63] McCarthy JF, Misra DN, Roberts JM. Maternal plasma leptin is increased in preeclampsia and positively correlates with foetal cord concentration. Am J Obstet Gynecol 1999;180(3 Pt 1):731–6.
[64] Teppa RJ, Ness RB, Crombleholme WR, Roberts JM. Free leptin is increased in normal pregnancy and further increased in preeclampsia. Metabolism 2000;49(8):1043–8.
[65] Nishihira J. Novel pathophysiological aspects of macrophage migration inhibitory factor. Int J Mol Med 1998;2(1):17–28.
[66] Arcuri F, Ricci C, Ietta F, Cintorino M, Tripodi SA, Cetin I, et al. Macrophage migration inhibitory factor in the human endometrium: expression and localization during the menstrual cycle and early pregnancy. Biol Reprod 2001;64(4):1200–5.
[67] Arcuri F, Battistini S, Hausknecht V, Cintorino M, Lockwood CJ, Schatz F. Expression of macrophage migration inhibitory factor transcript and protein by first-trimester human trophoblasts. Biol Reprod 1999;60(6):1299–303.
[68] Yamada H, Kato EH, Morikawa M, et al. Decreased serum levels of macrophage migration inhibition factor in miscarriages with normal chromosome karyotype. Hum Reprod 2003;18: 616–20.
[69] Bootcov MR, Bauskin AR, Valenzuela SM, Moore AG, Bansal M, He XY, et al. MIC-1, a novel macrophage inhibitory cytokine, is a divergent member of the TGF-beta superfamily. Proc Natl Acad Sci USA 1997;94:11514–9.
[70] Tong S, Marjono B, Brown DA, Mulvey S, Breit SN, Manuelpillai U, et al. Serum concentrations of macrophage inhibitory cytokine 1 (MIC 1) as a predictor of miscarriage. Lancet 2004;363(9403):129–30.

ELSEVIER
SAUNDERS

Obstet Gynecol Clin N Am
31 (2004) 779–794

OBSTETRICS AND
GYNECOLOGY
CLINICS
OF NORTH AMERICA

Endocrinology of ectopic pregnancy

Erkut Attar, MD

Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology, Istanbul University, Istanbul Medical School, Capa, Istanbul, 34093, Turkey

Ectopic pregnancy is the consequence of an abnormal implantation of the blastocyst—frequently (95%–98%) in the fallopian tube. Another 2%–2.5% of the ectopic pregnancies occur in the cornua of the uterus; the remainder are found in the ovary, cervix, or abdominal cavity. Because none of these anatomic sites can accommodate placental attachment or a growing embryo, the potential for rupture and hemorrhage exists. Ectopic pregnancy occurs in approximately 2% of all pregnancies in the United States and is the leading cause of maternal mortality in the first trimester. It accounts for 10% to 15% of all maternal deaths [1]. During the last 2 decades, the incidence of ectopic pregnancy has risen; this may be due, in part, to known risk factors, such as pelvic inflammatory disease (PID), the use of intrauterine devices (IUD), and smoking [2]. Based on hospital discharge data, the incidence of ectopic pregnancy increased from 4.5 cases per 1000 pregnancies in 1970 to 19.7 cases per 1000 pregnancies in 1992 [3,4]. Essentially, the increased incidence of ectopic pregnancy is due to improved diagnostic techniques [5]. Modern advances in ultrasound technology and the determination of serum β-subunit human chorionic gonadotropin (β-hCG) levels have made it easier to diagnose ectopic pregnancy. Some ectopic pregnancies that are detected today, for instance, would have resolved spontaneously without detection or intervention in the past. Nonetheless, the diagnosis still remains a challenge. This article reviews the etiology, diagnosis, and treatment of ectopic pregnancy from the viewpoint of endocrinology.

Etiology

Although a proportion of women who have ectopic pregnancy have no identifiable causal factors, the risk is increased by several factors, including a

E-mail address: attar@superonline.com

0889-8545/04/$ – see front matter
doi:10.1016/j.ogc.2004.08.011 obgyn.theclinics.com

history of infertility, previous ectopic pregnancy, tubal surgery, pelvic infection, Diethylstil-bestrol (DES) exposure, use of IUDs, and in vitro fertilization for tubal disease [6]. These risk factors may share common mechanisms that can be anatomic, functional, or both. Obviously, it is difficult to assess the cause of an ectopic implantation in the absence of detectable tubal abnormalities. Changes in the hormonal milieu of the reproductive tract may cause functional abnormalities in the fallopian tube. Altered paracrine and autocrine mediators in the reproductive tract and the embryo may play a role in the ectopic implantation of the embryo.

Normally, an egg is fertilized in the fallopian tube and travels down the tube to the implantation site. Any mechanism that interferes with the normal function of the fallopian tube during this process increases the risk of ectopic pregnancy. The endocrine origin of ectopic pregnancy revolves around certain endocrine events that modify reproductive tract function. Hormonal changes that may cause abnormalities in the transportation and establishment of communication between the ectopic implantation site and well-developed embryo result in ectopic pregnancy. The ectopic site of the implantation behaves as the endometrium itself.

Implantation window in tubal ectopic pregnancies

In humans, the period during which a blastocyst can implant is limited and depends almost exclusively on the establishment of an equilibrium between the hormonal milieu of the woman and the degree of development of the blastocyst and the signals that it sends to the maternal organism. An oviductal cycle is recognized and the ciliated and secretory cells are influenced by the changes of hormones during the menstrual cycle. An environment that is high in estrogen or progestogen can contribute to an increased risk of ectopic pregnancy. This observation stresses the importance of the proper hormonal balance rather than the absolute value of a single hormone in the normal implantation process [7]. A local action of estrogens on progesterone-primed tissues may be required for the release of crucial signals for the blastocyst activation or to make epithelial cells sensitive to the presence of the embryo [8]. Tissues from ectopic pregnancies were found to be positive for progesterone receptors. Implantation of trophoblast cells at extrauterine sites results in decidualization.

Implantation in humans is a complex process that involves a variety of molecules that is not unique; however, they play a unique role in the process of implantation. The overall situation has some features that are analogous to invasion by a tumor and the inflammatory response. In addition, and also in common with cancer and inflammation, there is a release of biologically-active molecules at the implantation site. The molecular dialog that occurs between the implanting conceptus and the implanted site involves cell–cell and cell–extracellular matrix interaction that is mediated by lectins, integrins, matrix-degrading cumulus and their inhibitors, prostaglandins and various growth factors, and cytokines and their receptors and modulator proteins (Fig. 1).

Blastocyst implantation can begin only during a brief phase of receptivity that is known as the ‘‘implantation window.’’ In this period of the cycle, morphologic

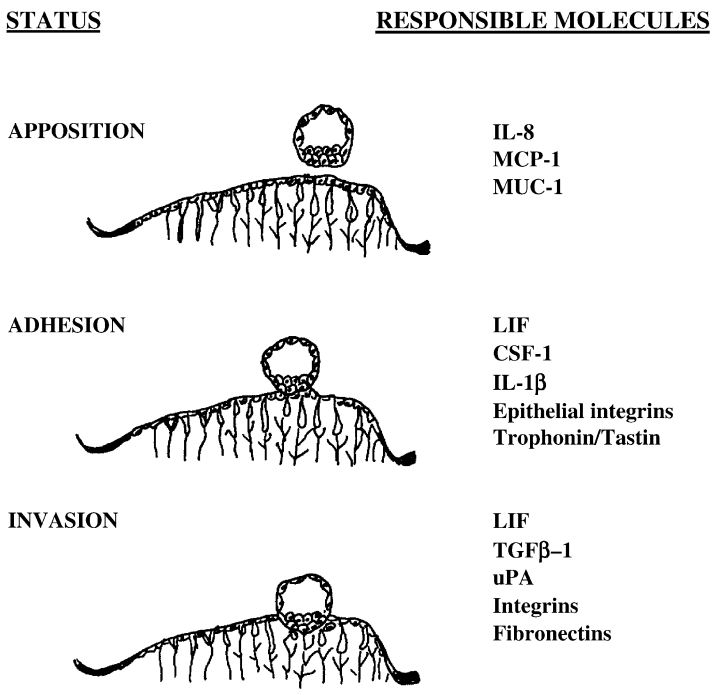


Fig. 1. Schematic diagram of the implantation process showing the responsible molecules at each step of implantation. CSF, Colony-Stimulating Factor; IL-1β, Interleukin-1β; IL-8, Interleukin-8; LIF, leukemia inhibitory factor; MCP-1, Monocyte Chemotactic Protein; MUC-1, Mucin; TGFβ1, transforming growth factor-β1; uPA, urokinase plasminogen activator.

and biochemical changes occur in the endometrium. These changes are characterized by the expression of certain type of endometrial cells, pinopodes and described specific molecules. These molecules act in a paracrine/autocrine manner and are driven by endocrine hormones. These systemic and local modifications can be considered to constitute ''the maternal recognition of pregnancy.'' Normal tubal epithelium may have an implantation window—at about the same time as the endometrium—that affords the opportunity for trophoblast attachment should a 5- to 7- day embryo be retained unduly in the tube [9].

Cytokines and tubal implantation

Cytokines are pleiotropic proteins; the release of any set of these factors initiates a cascade of local events with ensuing release of other cytokines and activation in surrounding cells. Moreover, they exhibit considerable overlap in their biologic effects on various target cells. Autocrine and paracrine actions of certain cytokines are directed to modulate cell proliferation and differentiation and to induce the expression of new antigenic epitopes and adhesion mole-

cules. These cytokines play an important role in the successful establishment of the pregnancy.

Leukemia inhibitory factor (LIF) is a cytokine with pleiotropic effects; it has a principal function in successful blastocyst implantation and growth regulation [10]. Female mice lacking a functional LIF gene are fertile, but their blastocysts fail to implant and develop. The blastocysts are viable; when transferred into a wild-type pseudopregnant recipient, they can implant and develop to term [11]. The regulation of LIF secretion was found to be abnormal in infertile women who presented with repeated failures of embryonic implantation or unexplained primary sterility [12]. LIF is expressed in the endometrium in a menstrual cycle–dependent manner and peaks in human endometrium between Days 19 and 25 of the menstrual cycle (mid- and late luteal phase of the cycle) which corresponds to the implantation window [13,14]. Similarly, in mice, LIF reaches its peak on Day 4 or 5 of the cycle that is the most receptive time of mouse endometrium [15]. Previously, we showed that LIF is expressed in the human fallopian tube with a slight variation during the menstrual cycle; however, it is elevated markedly in association with an ectopic pregnancy [16]. Steroid hormones (estradiol and progestins) did not show a stimulatory effect on LIF mRNA expression or protein production in endometrium and the human fallopian tube. Interleukin (IL)-1, tumor necrosis factor (TNF)-α, and transforming growth factor (TGF)-β are potent inducers of LIF expression in endometrial and fallopian tube stromal cells in culture. TGF-β is a protein that also is known to antagonize many of the functions of the epidermal growth factor receptor system and was localized immunohistochemically in unruptured ectopic pregnancies, that were removed by salpingectomy, and uterine decidua from ectopic pregnancies [17]. Elevated expression with ectopic implantation—as it occurs in the endometrium—suggests a link between LIF and ectopic pregnancies. Induction of LIF secretion in the fallopian tube epithelial and stromal cells by growth factors and cytokines suggests a role for inflammation in ectopic implantation.

Fallopian tube epithelial cells secrete LIF mRNA at increased constitutive levels compared with stromal cells; in contrast, LIF secretion is less regulated in these cells. It is possible that the increased constitutive levels of LIF that are expressed in the ampullary portion of the fallopian tube may play a role in gamete development and function [16,18]. The increased constitutive secretion of LIF may be important in the support of early pluripotent embryonic cells and may lead to greater viability of the early embryo [19]. The embryo is not yet ready for implantation in the distal part of the fallopian tube. The human fallopian tube usually is protected from ectopic implantation by transportation of the embryo to the endometrium before hatching. This finding implicates that the delay in transportation may cause ectopic implantation without triggering the LIF surge in the fallopian tube.

Although ectopic pregnancy is a common occurrence in humans, it occurs rarely in other species. It was shown that the presence of a factor in rabbit endosalpinx actively suppresses ectopic implantation in the oviduct [20]. Bronson and Cunnane [21] transferred the blastocyst that was retrieved from the uterine cavity to the oviduct of a mouse. They noted enlargement of the

blastocysts, loss of the zona pellucida, and adherence to the tubal epithelium; however, implantation did not occur at that level.

Inflammation and ectopic implantation

Optimal oviductal function is necessary to provide a proper environment for early human life. Changes in this environment from external or internal origins can lead to abnormalities in implantation, including ectopic pregnancy. The underlying tubal disease (chronic salpingitis, follicular salpingitis, or salpingitis isthmica nodosa) seems to be the major factor that is associated with, and probably the cause for, the recurrent tubal gestation. The rate of ectopic pregnancy is increased almost seven-fold after a single attack of PID [22].

PID affects approximately one million women in the USA every year and accounts for 250,000–300,000 hospitalizations. These numbers are actually thought to be significantly higher because of the many cases that cause no symptoms or are not recognized as PID. Undoubtedly, PID has been more common; increased risk of further attacks of PID reflects the chronic and subclinic behavior of the infection that may cause an inflammatory response. The underlying tubal disease implicates the role of inflammation in the ectopic implantation. Inflammation refers to a complex set of mechanisms by which tissues respond to injury and infection. Among the many soluble mediators that are associated with this process, cytokines are known to be crucial in regulating a variety of cellular and molecular events. TNF-α, originally identified as an inflammation-associated cytokine, is synthesized throughout the female reproductive tract as well as in the placenta and embryo [23]. TNF-α, IL-6, IL-11, and possibly other members of this cytokine family are the key mediators in various inflammatory processes (eg, acute-phase reaction, tissue damage, infection) [24,25]. These changes also can lead to abnormalities in implantation, including ectopic pregnancy, by altering the tubal environment.

Chlamydia trochomatis has become a more important cause of PID and subclinical tubal infection that produces plasma cell salpingitis commonly may underlie ectopic pregnancy. Elevated titers of *Chlamydia trochomatis* IgG antibody were found in patients who had tubal factor infertility [26]. It is highly probable that *Chlamydia* is one of the ''trigger'' bacteria that may induce inflammatory cytokines in the fallopian tube. Human chlamydial salpingitis is associated with at least a five-fold elevation of TNF-α in tubal fluid [27]; infection of human tubal explants with gonococci induces a greater than six-fold elevation in TNF-α concentrations. There also is evidence that the presence of a hydrosalpinx adversely affects early pregnancy events by altering the uterine and tubal environment; patients who had a hydrosalpinx had significantly greater rates of ectopic pregnancy [28]. Hydrosalpinx, even without sonographic evidence of dilated fallopian tubes, can lead to an increased incidence of ectopic pregnancies [29]. Reducing the incidence of ectopic pregnancy involves the prevention of gynecologic infections, and in particular, sexually transmitted diseases.

Immunologic factors that are associated with ectopic implantation

Leukocytes form a substantial proportion of the constituent cells of human endometrium. The endometrial leukocyte population primarily consists of granulated lymphocytes, macrophages, and T cells. Large granular lymphocytes, a form of natural killer cell, represent the most abundant lymphoid cell population in human endometrium around the time of implantation and early pregnancy. No differences were detected in the number or proportion of these decidual leukocytes in normal pregnancy compared with ectopic pregnancy. Endometrial granulated lymphocytes were the most abundant leukocyte population and macrophages and T cells made up the second and third major leukocyte subpopulations in the decidua from women who had ectopic pregnancy. These data support the hypothesis that decidual leukocyte recruitment or increase during pregnancy primarily are regulated hormonally; however, endometrial granulated lymphocytes were absent from the tubal mucosa away from the implantation site and the tubal implantation site [30,31]. T lymphocytes and macrophages formed the predominant leukocyte subpopulations in both tubal areas. Most of the leukocytes at the ectopic implantation site were HLA-DR positive macrophages and there was a small number of mature T lymphocytes (UCHT1 and Dako-T1 positive cells) [32]. The absence of endometrial granulated lymphocytes from the tubal implantation site suggests that the local presence of these cells is not essential for implantation and early placental development events. In addition to these findings, there is evidence that the immunoregulatory potential of the leukocyte population depends on the interaction of a viable trophoblast and the implantation site. It has been shown that the communication between the viable trophoblast and nonadherent suppressor cells appears to be a prerequisite for the trophoblast activation. Conversely, the contact between trophoblast and adherent decidual leukocytes is not necessary for the suppressive function of leucocytes; however, suppressive activity of leucocytes is much greater in ectopic implantation [33]. This finding implicates that the communication might be necessary for proper function of the adherent decidual leukocytes.

Trophoblast invasion in ectopic pregnancy

Earl et al [34] studied the reactivity of the various trophoblast populations that are found in ectopic fallopian tube pregnancy with established trophoblast-reactive markers and monoclonal antibodies to major histocompatibility (MHC) antigens. They demonstrated that the fetal trophoblast in an ectopic tubal pregnancy showed an identical reaction pattern as monoclonal antibodies to MHC antigens as in intrauterine pregnancy. This suggested that ectopic implantation is not related to an inherent immunologic abnormality of fetal trophoblast.

Several tissue remodeling events that require extracellular proteolysis are believed to be mediated by plasminogen activators (PAs) that convert the inactive proenzyme, plasminogen, to active plasmin. The involvement of PAs in many

biologic phenomena reflects the ubiquitous presence of plasminogen and the ability of numerous cell types to synthesize PA in a highly-regulated manner. The human hemochorial placenta arises primarily through proliferation, migration, and invasion of the endometrium and its vasculature by the embryonic trophoblast. The complex invasive processes that accompany implantation of the embryo are controlled at the embryo–maternal interface by factors from decidualized endometrium and the trophoblast itself. Studies in nonhuman systems proposed a critical role for trophoblast-secreted urokinase PAs (uPAs) and inhibitors (PAI) during implantation and placentation. They control invasion of the maternal decidua by the trophoblast during human implantation. The receptor for uPA (uPAR) is a key molecule in cell surface–directed plasminogen activation that binds PA, and thereby, focuses plasminogen activation on the cell surface. uPAR, PAI-1, and PAI-2 were localized immunohistochemically in early human implantation sites in unruptured ectopic pregnancies; this indicated that the ectopic site also behaves as endometrium. In tubal ectopic placental tissues, an increased expression of uPAR was seen in the trophoblast and was associated with deposits of fibrin-type fibrinoid [35].

Hormonal changes and tubal motility

Hormonal changes also can affect the tubal motility and transportation of the embryo in the fallopian tube that may result in ectopic pregnancy. The rapid transportation of gametes involves a complex reorganization of the oviductal smooth muscle electrical activity that precedes the mechanical activity. The transport of the embryo in the oviductal fluid depends on ciliary beat (generally regarded as the leading factor) and on muscle contractions, mainly in the ampullary-isthmic and in the utero-tubal junction, where a sphincter action is stimulated by estrogens and relaxed by progesterone. Other substances (catecholamines, prostaglandins, oxytocin) are believed to be involved in ovum transport, although their role is unclear [8]. The human fallopian tube usually is protected from ectopic implantation by transport of the embryo to the endometrium before hatching.

Environmental factors that are associated with ectopic implantation

Ectopic pregnancy also was found to be associated with maternal smoking. It was shown that external factors (eg, smoking) that may interfere with the transportation of the embryo in the fallopian tube also can cause ectopic implantation. Transport of preimplantation embryos through the hamster oviduct was retarded in females who inhaled doses of mainstream or sidestream smoke that produced serum cotinine levels within the range reported for women who actively or passively smoke during pregnancy. Preimplantation embryo transport and muscle contraction were more sensitive to sidestream smoke. These data

demonstrate that inhalation of doses of mainstream and sidestream cigarette smoke that are similar to those received by active and passive human smokers adversely affects functioning of the oviduct and may explain the increased incidence of ectopic pregnancies in women who smoke [36–38].

Sperm and genetic defects in ectopic pregnancy

Abnormal early embryo development that may arise from chromosomal defects leads to ectopic implantation. The involvement of the male in the ectopic pregnancy also was proposed. Sperm defects may be associated with the expression of paternal genes that cause abnormal early embryo development and predispose the embryos to interact inappropriately with the genital tract epithelium, and thus, increase the risk of ectopic implantation. DNA aneuploidy in spermatozoa was associated with tubal implantation; abnormal embryogenesis may contribute significantly to the occurrence of ectopic implantation [39]. It also was discussed whether there was an increased risk of ectopic pregnancy when spermatozoa from the male partner were used rather than donor spermatozoa; however, no significant association was found between ectopic pregnancy and the origin of the spermatozoa [40]. Dotters et al [41] determined the sex assignment in 80 cases of ectopic pregnancy; however, there did not appear to be any predilection for ectopic implantation by either sex. Karyotype analyses of the ectopic conceptuses had a rate of abnormality that was similar to that reported for intrauterine gestations. Block et al [42] studied the relationship between the clinical findings and karyotype in ectopic pregnancies; usually, the karyotype was normal when a gestational sac or fetal pole was identified by ultrasound.

Diagnosis of ectopic pregnancy

The most common clinical presentations of ectopic pregnancy are pelvic pain, vaginal spotting, and amenorrhea. These usually present after 5 to 9 weeks of amenorrhea. Other classic symptoms are dizziness, pregnancy symptoms, and vaginal passage of tissue. Pain usually results from the stretching of the peritoneum over the tube. After the tube ruptures, pain usually decreases or disappears. The diagnosis can be difficult unless the condition is suspected; it may be confused with miscarriage, an ovarian accident, or PID [43]. The presence of known risk factors can increase suspicion, but any sexually active woman who presents with abdominal pain and vaginal bleeding after an interval of amenorrhea has an ectopic pregnancy until proven otherwise.

The most common classic finding on physical examination is adnexal tenderness. The presence of an adnexal mass also has been described. Other typical signs include abdominal tenderness, uterine enlargement, and orthostatic changes. If the tube has ruptured, the patient may present in shock with tachycardia and hypotension. Shoulder pain from diaphragmatic irritation is a late sign and seldom is seen.

Although these classic signs and symptoms still serve as useful indicators of possible ectopic pregnancy, it must be remembered that they were used before the development of reliable and accurate techniques for evaluating patients who have risk factors. Today, sensitive β-hCG assays and transvaginal ultrasound can lead to the diagnosis of ectopic pregnancy before symptoms and conservative treatment for preservation of the fallopian tube is possible [44,45].

A proper history and physical examination remain the foundation for initiating an appropriate work-up that will result in the accurate and timely diagnosis of an ectopic pregnancy. Pretreatment testing includes serum hormonal assays and transvaginal ultrasound. Ectopic pregnancies produce decreased concentrations of hCG compared with normal pregnancies; however, the change in concentrations provides more information [46]. β-hCG is an important hormone to follow serially. A subnormal increase in serum β-hCG in early pregnancy (<66% in 48 hours) suggests that the pregnancy is not viable [47]. An absence of this increase is suggestive of an ectopic pregnancy, although it also can be associated with early pregnancy failure. Free hCG is considered to be more specific than the other isoforms in diagnosing ectopic pregnancy [48]. Transvaginal ultrasonography should be able to identify the presence of an intrauterine gestation in nearly 100% of normal pregnancies when β-hCG exceeds 2400 mIU/mL in serum [49]. Serum progesterone concentrations also may be helpful as an adjunct to β-hCG in the evaluation of ectopic pregnancy. A progesterone concentration of greater than 25 ng/mL is associated with an intrauterine pregnancy in 97.5% of cases [50]. This value only can be used in cycles in patients who conceived without ovulation induction. Conversely, progesterone levels of less than 5.0 ng/mL indicate a nonviable pregnancy, regardless of location [51]. Although progesterone concentrations that are less than 5.0 ng/mL do not identify the location of the pregnancy, they do suggest that the pregnancy is nonviable. For progesterone concentrations that are between 5 ng/mL and 25 ng/mL, viability should be ruled out by ultrasound or serial measurements of β-hCG. Transvaginal ultrasound is a valuable diagnostic tool. The presence of an intrauterine pregnancy generally excludes ectopic pregnancy, although other ultrasound findings have to be considered, especially if symptoms are atypical, severe, or persistent. The presence or absence of an intrauterine sac must be documented. The discriminatory zone is the level of serum β-hCG that is greater than 2400 mIU/mL. A gestational sac can be visualized consistently at the serum concentrations above the discriminatory zone; however, the hCG discriminatory concentration is dependent on the hCG standard that is used in any given laboratory. With transabdominal sonography, this value is 6500 mIU/mL. In addition, ultrasonic detection of adnexal cardiac activity is useful in determining the appropriate therapy for ectopic pregnancy [52]. Uterine curettage can be used to determine the presence or absence of chorionic villi when the β-hCG level is decreasing or has not doubled in 48 hours and a nonviable pregnancy is suspected, regardless of its location. Histologic evidence of chorionic villi will confirm an intrauterine pregnancy, whereas the absence of villi strongly suggests ectopic pregnancy. A decline in β-hCG that is greater than

15%, 8 to 12 hours following curettage, suggests a diagnosis of complete abortion [53]. An ectopic pregnancy can be suspected when the β-hCG level fails to decline by at least 15% in 12 hours or the histologic findings do not include chorionic villi [54]. A plateau or increase in β-hCG suggests the continued presence of trophoblast. In evaluating β-hCG, ultrasound, and histologic data in women who conceived following superovulation, the possibility of combined intra- and extrauterine pregnancies must be considered. In patients who are minimally symptomatic or hemodynamically stable, serial β-hCG levels can be followed. Even at extremely low levels, the β-hCG measurement should double every 2 days. The lack of a 48-hour doubling indicates the presence of an abnormal pregnancy but does not indicate the location of the failing pregnancy [55].

Other placental markers can be used to diagnose an ectopic pregnancy—serum creatine kinase (CK) levels, pregnancy-specific β(1)-glycoprotein (SP1), human placental lactogen (HPL), and pregnancy-associated plasma protein (PAPP)-A. Neither increased serum CK nor decreased serum SP-1, PAPP-A, and HPL concentrations is helpful to exclude the ectopic pregnancy from early pregnancy loss [56,57]. The combination of three independent markers (triple marker test) in the formula (VEGF: Vascular Endothelial Growth Factor, P: Progesterone):

$$\mathrm{VEGF}/(\mathrm{PAPP}-\mathrm{A}\times\mathrm{P})$$

largely was superior to the measure of any single marker [58]. Serum IL-8, IL-6, and TNF-α concentrations also were increased in women who had ectopic pregnancy compared with those who had miscarriage or normal pregnancy. Develioglu et al [59] revealed that single serum CK measurements may help to discriminate ruptured from unruptured ectopic pregnancies. Similarly, elevated serum Cancer Antigen 125 (CA125) concentrations may be a valuable parameter in early pregnancy failure, but are not specific for ectopic implantation [60].

Today, culdocentesis is performed rarely because ultrasonography can reveal the presence of any free fluid. Thus, the procedure is used primarily when ultrasonography is not readily available. A culdocentesis that is positive for nonclotting bloody fluid strongly suggests the presence of a bleeding ectopic pregnancy. The finding of yellow or straw-colored fluid is more consistent with a ruptured ovarian cyst.

Management

Many options are available to the clinician in the treatment of tubal pregnancy. These include surgical treatment, which can be performed radically or conservatively, either laparoscopically or by an open surgical procedure; medical treatment, with a variety of drugs that can be administered systemically or locally by different routes (transvaginally under sonographic guidance or under laparos-

copic guidance); and expectant management. The choice of a treatment modality should be based on short-term (primary treatment success and reinterventions for clinical symptoms or persistent trophoblast) and long-term outcome measures (tubal patency and future fertility).

Expectant management

Some ectopic pregnancies resolve spontaneously and expectant management is possible in selected cases. This may be an option for the patient who is willing and able to comply with close follow-up. Expectant management is possible for patients who are selected according to precise criteria. It avoids therapeutic escalation, if there is a doubt as to whether it is a miscarriage or an ectopic pregnancy or for asymptomatic patients who have spontaneously decreased hCG levels. Thus, the dimensions of ectopic gestational tissue (less than 3.5 cm in greatest dimension) and decreased, declining β-hCG values are the medical selection criteria that are used for expectant management. It is important, therefore, to monitor serum titers of hCG serially in patients who are being managed expectantly. The higher the serum concentration, the more likely it is that expectant management will fail. Overall, if the initial serum concentration of hCG is less than 1000 IU/L, expectant management is successful in up to 88% of patients [61,62].

Medical therapy

Earlier diagnosis has made the medical management of ectopic pregnancy an option. The potential advantages are the avoidance of surgery and its concomitant hazards, the preservation of tubal patency and function, and a lower cost. Chemical agents that have been investigated include hyperosmolar glucose, urea, cytotoxic agents (eg, methotrexate and actinomycin), prostaglandins, and mifeproston (RU486) [63,64].

Methotrexate, a folic acid antagonist and potent inducer of apoptosis in trophoblastic tissue, is used for medical management in patients before rupture who are hemodynamically stable. It can be given intramuscularly or injected into the ectopic pregnancy—a route that delivers high concentrations locally with smaller systemic distribution; however, rates of successful treatment are not significantly different than with systemic methotrexate and it requires a laparoscopic or ultrasound-guided needle procedure. The efficacy of local transvaginal methotrexate injection is greater in patients with stable levels of hCG or levels that are greater than 2000 mIU/mL [65]. Single-dose regimens have had a success rate of 71%. The success rate increases to between 84% and 94% with the addition of a second dose [66,67]. A recent meta-analysis showed that the single dose was used much more commonly; however, the multi-dose regimen is more effective in the treatment of ectopic pregnancy [68]. Although the potential for serious toxic effects exists, the low dosages of methotrexate that are used generally cause mild, self-limited reactions. Common side effects include nausea

Box 1. Absolute and relative requirements of methotrexate administration

Absolute requirements

Hemodynamic stability
Ultrasound findings consistent with an ectopic pregnancy
Willingness of the patient to adhere to close follow-up
No contraindications to methotrexate therapy

Relative requirements

Unruptured ectopic mass less than 3.5 cm in greatest dimension
No fetal cardiac motion detected
β-subunit hCG level that does not exceed 5,000 mIU/L

and vomiting, urinary frequency, and mild diarrhea. Thus, when the diagnosis is certain and an ectopic mass is less than 3.5 cm in greatest dimension, methotrexate therapy is an option. Criteria for the use of methotrexate are summarized in Box 1. Overlooking any of these criteria may cause failure in treatment [69]. High serum hCG concentrations and fetal cardiac activity were the most important factors that were associated with the failure of treatment with methotrexate in cervical pregnancies [70]. Close follow-up with serial measurements of serum concentrations of hCG is required. Fan and Long [71] revealed that measurement of serum progesterone concentrations also can be used as an index for selecting patients for methotrexate treatment and is a good indicator for assessing the therapeutic effect after treatment. In this study, the recommended critical serum progesterone level for assessing the effect of methotrexate treatment was 11 ng/mL; the time for serum progesterone to decrease to less than normal levels was significantly shorter than for β-hCG. A second course of treatment may be necessary and some patients may require surgical intervention. Methotrexate treatment may produce significant side effects.

Surgery

Surgical treatments may be radical (salpingectomy) or conservative (usually salpingotomy) and they may be performed by laparoscopy or laparotomy. Laparotomy is the preferred technique when the patient is hemodynamically unstable, the surgeon has not been trained in laparoscopy, physical facilities and supplies to perform laparoscopic surgery are lacking, or technical barriers to laparoscopy are present. In many cases, these patients require salpingectomy because of extensive tubal damage; occasionally, a salpingotomy can be performed. Rarely, an accompanying oophorectomy is indicated. Hemodynamically

stable patients who have an unruptured ectopic pregnancy and in whom a salpingotomy is considered may have this performed by laparoscopic techniques. Laparoscopic salpingotomy is indicated in patients who have an unruptured ectopic pregnancy that is no greater than 5 cm in transverse diameter that is visualized completely through the laparoscope. Linear salpingotomy by laparoscopy or laparotomy is performed to remove an unruptured ectopic pregnancy by incising the antimesenteric surface of the tube with pinpoint cautery, scissors, or laser. Injection of dilute pitressin may be used to improve hemostasis. The ectopic gestation is expressed gently through the incision and bleeding points are cauterized. Excessive cautery inside the lumen may predispose to subsequent occlusion and should be avoided. Healing by secondary intention or closure with fine sutures yields similar results. Linear salpingotomy is best suited for patients who have an ampullary site of implantation. These ectopics are less likely to have trophoblastic invasion into the muscularis than isthmic implantations. The patient who has an isthmic implantation may benefit more from segmental resection and later anastomosis [72]; however, if diagnosis is made early, even isthmic sites may be amenable to salpingotomy. In an ectopic pregnancy that is located at the fimbrial ends, a ''milking'' technique allows the trophoblastic tissue to pass through the fimbria. Generally, laparoscopy permits fewer hospital days and less time lost from employment than laparotomy. Laparoscopic partial salpingectomy and total salpingectomy may be indicated in patients who have pre-existing tubal disease and known risk factors that predispose to ectopic pregnancy.

The most frequent complications of surgery are recurrence of ectopic pregnancy (5%–20%) and incomplete removal of trophoblastic tissue. It was suggested that a single dose of methotrexate should be given postoperatively as a prophylactic measure in very high-risk patients [73].

References

[1] Nederlof KP, Lawson HW, Saftlas AF, et al. Ectopic pregnancy surveillance, United States, 1970–1987. MMWR CDC Surveill Summ 1990;39(4):9–17.
[2] Pisarska MD, Carson SA. Incidence and risk factors for ectopic pregnancy. Clin Obstet Gynecol 1999;42(1):2–8; quiz 55–6.
[3] Leads from the MMWR. Ectopic pregnancy–United States, 1984 and 1985. JAMA 1988; 260(18):2637–40.
[4] Goldner TE, Lawson HW, Xia Z, et al. Surveillance for ectopic pregnancy–United States, 1970–1989. MMWR CDC Surveill Summ 1993;42(6):73–85.
[5] Ong S, Wingfield M. Increasing incidence of ectopic pregnancy: is it iatrogenic? Ir Med J 1999; 92(5):364–5.
[6] Bouyer J, Coste T, Shojaei JL, et al. Risk factors for ectopic pregnancy: a comprehensive analysis based on a large case-control, population-based study in France. Am J Epidemiol 2003;157(3):185–94.
[7] Lavy G, DeCherney AH. The hormonal basis of ectopic pregnancy. Clin Obstet Gynecol 1987; 30(1):217–24.
[8] De Cecco L, Capitanio GL, Croce S, et al. Biology of nidation and ectopic implantation. Acta Eur Fertil 1984;15(5):347–55.

[9] Sulz L, Valenzuela AM, Salvatierra ME, et al. The expression of alpha(v) and beta3 integrin subunits in the normal human Fallopian tube epithelium suggests the occurrence of a tubal implantation window. Hum Reprod 1998;13(10):2916–20.
[10] Senturk LM, Arici A. Leukemia inhibitory factor in human reproduction. Am J Reprod Immunol 1998;39(2):144–51.
[11] Stewart CL, Kaspar P, Brunet LJ, et al. Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. Nature 1992;359(6390):76–9.
[12] Delage G, Moreau JL, Taupin E, et al. Modulation in vitro de la production de la cytokine HILDA/LIF secretee pardes explants d'endometre humain: resultats preliminaires. [In vitro modulation of the production of the cytokine HILDA/LIF, secreted by human endometrial explants: preliminary results]. Contracept Fertil Sex 1995;23(10):622–5 [in French].
[13] Vogiagis D, Marsh MM, Fry RC, et al. Leukaemia inhibitory factor in human endometrium throughout the menstrual cycle. J Endocrinol 1996;148(1):95–102.
[14] Arici A, Engin O, Attar E, et al. Modulation of leukemia inhibitory factor gene expression and protein biosynthesis in human endometrium. J Clin Endocrinol Metab 1995;80(6):1908–15.
[15] Bhatt H, Brunet LJ, Stewart CL. Uterine expression of leukemia inhibitory factor coincides with the onset of blastocyst implantation. Proc Natl Acad Sci USA 1991;88(24):11408–12.
[16] Keltz MD, Attar E, Buradagunta S, et al. Modulation of leukemia inhibitory factor gene expression and protein biosynthesis in the human fallopian tube. Am J Obstet Gynecol 1996; 175(6):1611–9.
[17] Selick CE, Horowitz GM, Gratch M, et al. Immunohistochemical localization of transforming growth factor-beta in human implantation sites. J Clin Endocrinol Metab 1994;78(3):592–6.
[18] Attar E, Ozsait B, Bulgurcuoglu S, et al. Effect of leukaemia inhibitory factor on long-term sperm motility and survival. Reprod Biomed Online 2003;7(1):71–4.
[19] Fry RC. The effect of leukaemia inhibitory factor (LIF) on embryogenesis. Reprod Fertil Dev 1992;4(4):449–58.
[20] Pauerstein CJ, Eddy CA, Koong MK, et al. Rabbit endosalpinx suppresses ectopic implantation. Fertil Steril 1990;54(3):522–6.
[21] Bronson R, Cunnane M. Transfer of uterine implantation blastocysts to the oviduct in mice. Fertil Steril 1975;26(5):455–9.
[22] Stock RJ. Histopathology of fallopian tubes with recurrent tubal pregnancy. Obstet Gynecol 1990;75(1):9–14.
[23] Terranova PF, Hunter VJ, Roby KF, et al. Tumor necrosis factor-alpha in the female reproductive tract. Proc Soc Exp Biol Med 1995;209(4):325–42.
[24] Mannel DN, Echtenacher B. TNF in the inflammatory response. Chem Immunol 2000;74: 141–61.
[25] Gadient RA, Patterson PH. Leukemia inhibitory factor, interleukin 6, and other cytokines using the GP130 transducing receptor: roles in inflammation and injury. Stem Cells 1999; 17(3):127–37.
[26] Brunham RC, Binns B, McDoweli J, et al. *Chlamydia trachomatis* infection in women with ectopic pregnancy. Obstet Gynecol 1986;67(5):722–6.
[27] Toth M, Jeremias J, Ledger WJ, et al. In vivo tumor necrosis factor production in women with salpingitis. Surg Gynecol Obstet 1992;174(5):359–62.
[28] Cohen MA, Lindheim SR, Sauer MV. Hydrosalpinges adversely affect implantation in donor oocyte cycles. Hum Reprod 1999;14(4):1087–9.
[29] Barmat LI, Rauch E, Spandorfer S, et al. The effect of hydrosalpinges on IVF-ET outcome. J Assist Reprod Genet 1999;16(7):350–4.
[30] Vassiliadou N, Bulmer JN. Characterization of tubal and decidual leukocyte populations in ectopic pregnancy: evidence that endometrial granulated lymphocytes are absent from the tubal implantation site. Fertil Steril 1998;69(4):760–7.
[31] Stewart-Akers AM, Krasnow JS, DeLoia JA. Decidual leukocyte populations in ectopic pregnancies. Fertil Steril 1997;68(6):1103–7
[32] Earl U, Lunny DP, Bulmer JN. Leucocyte populations in ectopic tubal pregnancy. J Clin Pathol 1987;40(8):901–5.

[33] Petrovic O, Gudelj L, Rubesa G, et al. Decidual-trophoblast interactions: decidual lymphoid cell function in normal, anembryonic, missed abortion and ectopic human pregnancy. J Reprod Immunol 1994;26(3):217–31.
[34] Earl U, Wells M, Bulmer JN. The expression of major histocompatibility complex antigens by trophoblast in ectopic tubal pregnancy. J Reprod Immunol 1985;8(1):13–24.
[35] Hofmann GE, Glatstein I, Schatz F, et al. Immunohistochemical localization of urokinase-type plasminogen activator and the plasminogen activator inhibitors 1 and 2 in early human implantation sites. Am J Obstet Gynecol 1994;170(2):671–6.
[36] DiCarlantonio G, Talbot P. Inhalation of mainstream and sidestream cigarette smoke retards embryo transport and slows muscle contraction in oviducts of hamsters (*Mesocricetus auratus*). Biol Reprod 1999;61(3):651–6.
[37] Castles A, Adams EK, Melvin CL, et al. Effects of smoking during pregnancy. Five meta-analyses. Am J Prev Med 1999;16(3):208–15.
[38] Bouyer J, Coste J, Fernandez H, et al. Tabac et grossesse extra-uterine. Arguments en farveur d'unerelation causale. [Tobacco and ectopic pregnancy. Arguments in favor of a causal relation]. Rev Epidemiol Sante Publique 1998;46(2):93–9 [in French].
[39] Karikoski R, Aine R, Heinonen PK. Abnormal embryogenesis in the etiology of ectopic pregnancy. Gynecol Obstet Invest 1993;36(3):158–62.
[40] Warnes GM, Petrucco OM, Seamark RF, et al. Is the male involved in the aetiology of ectopic pregnancy? Hum Reprod 1998;13(12):3505–10.
[41] Dotters D, Davis JR, Christian CD. Sex ratio in ectopic gestations. Fertil Steril 1984;41(5): 778–80.
[42] Block Jr WA, Wolf GC, Best RG. Chromosomal abnormalities in ectopic pregnancy chorionic villi. J Soc Gynecol Investig 1998;5(6):324–6.
[43] Kimata P, Amar N, Benifla JL, et al. Diagnostic des grossesses extra-uterines. [Diagnosis of ectopic pregnancy]. Rev Prat 2002;52(16):1781–4 [in French].
[44] Davies S, Byrn F, Cole LA. Human chorionic gonadotropin testing for early pregnancy viability and complications. Clin Lab Med 2003;23(2):257–64.
[45] Della-Giustina D, Denny M. Ectopic pregnancy. Emerg Med Clin North Am 2003;21(3): 565–84.
[46] Pittaway DE, Reish RL, Wentz AC. Doubling times of human chorionic gonadotropin increase in early viable intrauterine pregnancies. Am J Obstet Gynecol 1985;152(3):299–302.
[47] Kadar N, Romero R. Observations on the log human chorionic gonadotropin-time relationship in early pregnancy and its practical implications. Am J Obstet Gynecol 1987;157(1):73–8.
[48] Borrelli PT, Butler SA, Docherty SM, et al. Human chorionic gonadotropin isoforms in the diagnosis of ectopic pregnancy. Clin Chem 2003;49(12):2045–9.
[49] Fossum GT, Davajan V, Kletzky OA. Early detection of pregnancy with transvaginal ultrasound. Fertil Steril 1988;49(5):788–91.
[50] Stovall TG, Ling FW, Cope BJ, et al. Preventing ruptured ectopic pregnancy with a single serum progesterone. Am J Obstet Gynecol 1989;160(6):1425–8 discussion 1428–31.
[51] Dart R, Ramanujam P, Dart L. Progesterone as a predictor of ectopic pregnancy when the ultrasound is indeterminate. Am J Emerg Med 2002;20(7):575–9.
[52] Sauer MV, Gorrill MJ, Rodi IA, et al. Nonsurgical management of unruptured ectopic pregnancy: an extended clinical trial. Fertil Steril 1987;48(5):752–5.
[53] Stovall TG, Ling FW, Carson SA, et al. Nonsurgical diagnosis and treatment of tubal pregnancy. Fertil Steril 1990;54(3):537–8.
[54] Pisarska MD, Carson SA, Buster JE. Ectopic pregnancy. Lancet 1998;351(9109):1115–20.
[55] Ankum WM, Van der Veen F, Hamerlynck JV, et al. Suspected ectopic pregnancy. What to do when human chorionic gonadotropin levels are below the discriminatory zone. J Reprod Med 1995;40(7):525–8.
[56] Johnson MR, Riddle AF, Irvine R, et al. Corpus luteum failure in ectopic pregnancy. Hum Reprod 1993;8(9):1491–5.
[57] Dumps P, Meisser A, Pons D, et al. Accuracy of single measurements of pregnancy-associated

plasma protein-A, human chorionic gonadotropin and progesterone in the diagnosis of early pregnancy failure. Eur J Obstet Gynecol Reprod Biol 2002;100(2):174–80.
[58] Mueller MD, Raio L, Spoerri S, et al. Novel placental and nonplacental serum markers in ectopic versus normal intrauterine pregnancy. Fertil Steril 2004;81(4):1106–11.
[59] Develioglu OH, Askalli C, Uncu G, et al. Evaluation of serum creatine kinase in ectopic pregnancy with reference to tubal status and histopathology. BJOG 2002;109(2):121–8.
[60] Fiegler P, Kaminski K, Wegrzyn P. Zachowanie sie stezenia antygenu CA125–nowe aspekty przydatnosci Klinicznej. [Serum CA125 variability–new aspects of clinical usefulness]. Ginekol Pol 2003;74(5):412–8 [in Polish].
[61] Shalev E, Peleg D, Tsabari A, et al. Spontaneous resolution of ectopic tubal pregnancy: natural history. Fertil Steril 1995;63(1):15–9.
[62] Trio D, Strobelt N, Picciolo C, et al. Prognostic factors for successful expectant management of ectopic pregnancy. Fertil Steril 1995;63(3):469–72.
[63] Strohmer H, Obruca A, Lehner R, et al. Successful treatment of a heterotopic pregnancy by sonographically guided instillation of hyperosmolar glucose. Fertil Steril 1998;69(1):149–51.
[64] Anandakumar C, Choolani MA, Adaikan PG, et al. Combined chemotherapy in the medical management of tubal pregnancy. Aust N Z J Obstet Gynaecol 1995;35(4):437–40.
[65] Gervaise A, Fernandez H. Le methotrexate dans le traitement medical des grossesses extra-uterines non rompues. Etude prospective: injection intra-musculaire versus injection in situ sous controle echographique. [Methotrexate administration in the treatment of unruptured tubal pregnancy. Prospective non-randomized study: intramuscular injection versus transvaginal sonography-guided injection]. J Gynecol Obstet Biol Reprod (Paris) 2003;32(5):420–5 [in French].
[66] Stovall TG, Ling FW. Single-dose methotrexate: an expanded clinical trial. Am J Obstet Gynecol 1993;168(6 Pt 1):1759–62 [discussion 1762–5].
[67] Parker J, Bisits A, Proietto AM. A systematic review of single-dose intramuscular methotrexate for the treatment of ectopic pregnancy. Aust N Z J Obstet Gynaecol 1998;38(2):145–50.
[68] Barnhart KT, Gosman G, Ashby R, et al. The medical management of ectopic pregnancy: a meta-analysis comparing "single dose" and "multidose" regimens. Obstet Gynecol 2003;101(4): 778–84.
[69] Potter MB, Lepine LA, Jamieson DJ. Predictors of success with methotrexate treatment of tubal ectopic pregnancy at Grady Memorial Hospital. Am J Obstet Gynecol 2003;188(5): 1192–4.
[70] Bai SW, Lee JS, Park JH, et al. Failed methotrexate treatment of cervical pregnancy. Predictive factors. J Reprod Med 2002;47(6):483–8.
[71] Fan JT, Long FY. Value of serum progesterone for ectopic pregnancy in therapeutic effect monitoring. Di Yi Jun Yi Da Xue Xue Bao 2003;23(2):170–4.
[72] DeCherney AH, Boyers SP. Isthmic ectopic pregnancy: segmental resection as the treatment of choice. Fertil Steril 1985;44(3):307–12.
[73] Hajenius PJ, Engelsbel S, Mol BW, et al. Randomised trial of systemic methotrexate versus laparoscopic salpingostomy in tubal pregnancy. Lancet 1997;350(9080):774–9.

ELSEVIER
SAUNDERS

Obstet Gynecol Clin N Am
31 (2004) 795–816

OBSTETRICS AND
GYNECOLOGY
CLINICS
OF NORTH AMERICA

Steroid hormone synthesis in pregnancy

Caleb B. Kallen, MD, PhD

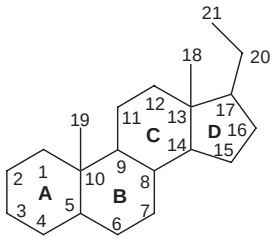
Department of Obstetrics and Gynecology, Section of Reproductive Endocrinology and Infertility, Yale University School of Medicine, 333 Cedar Street, P.O. Box 208063, New Haven, CT 06520, USA

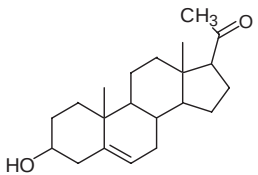
The establishment and maintenance of pregnancy in humans requires a highly regulated series of events that involves dynamic features of maternal, placental, and embryonic physiology. The essential roles of steroid hormones in producing many of these adaptations are well-recognized. The profound consequences of defects in the steroid metabolic pathways are testimony to the importance of steroid hormones in the establishment and development of normal pregnancies. Fundamental to understanding steroid hormone metabolism in pregnancy is the observation that, during much of gestation, neither the placenta nor the developing fetal adrenal glands is capable of producing sufficient quantities of the full complement of steroid hormones (ie, glucocorticoids, mineralocorticoids, progestins, androgens, estrogens) independently [1–5]. Thus, although the specifics and timing of steroidogenic metabolism in the human placenta, the fetal adrenals, the fetal membranes, and the maternal decidualized endometrium continue to be explored, the underlying principle remains that the process is cooperative for much of gestation. Steroidogenic substrates flow from mother and fetus to placenta, and in reverse; this permits a complex array of metabolic pathways that dictate the placental hormonal milieu of pregnancy. This observation led to the description of a unique and polyglandular endocrine system that was coined "the fetoplacental unit" [6], now also termed "the maternofetoplacental unit."

Steroid hormones participate in essential reproductive processes, including oocyte maturation and ovulation [7–11]; the function and preparedness of cervix, fallopian tubes, and endometrium for oocyte and sperm interaction during their transit, fertilization, and subsequent implantation as newly-formed blastocysts; the widespread changes in maternal physiology that are needed to optimize

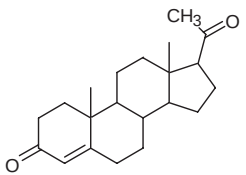
E-mail address: caleb.kallen@yale.edu

0889-8545/04/$ – see front matter
doi:10.1016/j.ogc.2004.08.009 obgyn.theclinics.com

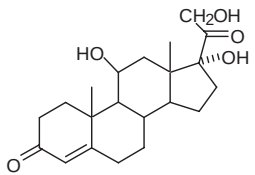




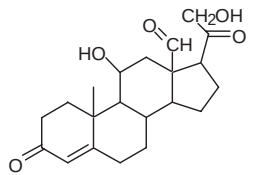
pregnenolone
3β-hydroxy-5-pregnen-20-one



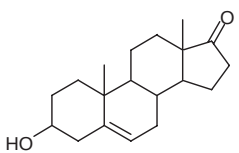
progesterone
4-pregnene-3,20-dione



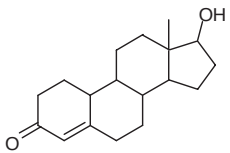
cortisol
11β,17α,21-trihydroxy-4-pregenene-3,20-dione



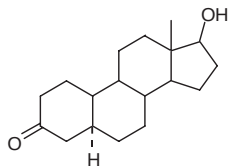
aldosterone
11β,21-dihydroxy-3,20-dioxopregn-4-en-18-al



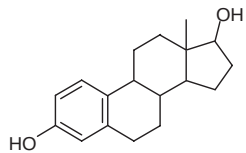
dehydroepiandrosterone
3β -hydroxy-5-androsten-17-one



testosterone
17β-hydroxy-4-androsten-3-one



5α-dihydrotestosterone
17β-hydroxy-5α-androstan-3-one



estradiol
1,3,5(10)-estratriene-3, 17β-diol

nourishment of, and elimination of waste from the developing conceptus; the sheltering of this allograft (ie, the fetus) from maternal immune surveillance [12–20]; the appropriate development of the growing fetus and maturation of fetal endocrine systems near term; and the correct timing of parturition (for comprehensive review see [16]). The unique steroid hormone requirements of pregnancy have been met by a complex interplay that involves steroidogenic tissues of the mother and those of the developing placenta and fetus. Essential products of these interactions are abundant quantities of placental estrogens and progesterone which engender many of the aforementioned adaptations in maternal and fetal physiology.

Important effects of progesterone include preparation of the endometrium for implantation [21,22], modulation of the maternal immune response to tolerate the fetal allograft, maintenance of myometrial quiescence [23–27], and preparation of the breasts for lactation. Estrogens appear to influence uterine blood flow [28–33] and neovascularization [16,34–37], increase the expression of critical proteins that are involved in progesterone production and steroid metabolism (ie, the receptor for the cholesterol-rich low-density lipoprotein [LDL-R] and the P450 side chain cleavage enzyme [P450scc/CYP11A1]), and participate in preparation of the breasts for lactation [16]. The goal of this article is to summarize what is known about the pathways of steroid hormone synthesis and metabolism in human pregnancy. Emphasis is placed on the distinctions between steroidogenic pathways in adults and those that are operative during human pregnancy.

Substrate for steroid hormone synthesis

The precursor for all steroid hormone synthesis is the 27 carbon-containing cholesterol molecule, a lipid that is composed of four fused rings (the 17 carbon-containing cyclopentanoperhydrophenanthrene backbone) with associated side chains (Fig. 1). Cholesterol substrate, a major component of cell membranes, can be obtained from cholesterol-laden lipoproteins in human plasma, de novo cholesterol synthesis in steroidogenic tissues, and intracellular lipid droplets that store cholesterol esters [38]. Steroid hormones are produced from the cholesterol precursor through modifications of bonds within the four fused rings (altering the locations of single and double bonds between carbon atoms) and by way of additional modifications (ie, oxidations and reductions) at locations along the steroid backbone and at side-chains that lie above (β) and below (α) the plane of the ringed molecule.

The cytochrome P450s (CYPs) catalyze major alterations in the sterol skeleton, including cleavage of the side chain, hydroxylation, and aromatization of the A ring (see Fig. 1) [39]. The hydroxysteroid dehydrogenases (HSD) oxidize

Fig. 1. The chemical structure and carbon numbering of the cyclopentanoperhydrophenanthrene steroid backbone, as well as the structures (with systematic and common nomenclatures) for selected steroid hormones.

hydroxyl groups and reduce ketone groups. They play an important role in determining the levels of bioactive hormone that are available to steroid hormone receptors by activating or inactivating selected steroid hormones [40]. Steroid reductases, sulfotransferases, and sulfatases further regulate the function of steroid hormones at target tissues. Biologically-active metabolites include the of 21 carbon pregnanes (ie, progesterone), 19 carbon androstanes (ie, testosterone), and 18 carbon estranes (ie, estradiol). The numbering of the carbons for the three cyclohexane and one cyclopentane rings that are fused to make up the steroid backbone as well as the structures of selected steroid hormones are depicted (see Fig. 1).

Progesterone is indispensable for the maintenance of pregnancy and is produced first by the corpus luteum of the ovary in response to the mid-cycle luteinizing hormone (LH) surge and then placental human chorionic gonadotropin for the initial 6 to 8 weeks of human gestation [41,42]. For the remainder of human pregnancy, the placenta is the essential source of progesterone synthesis. The principal source of cholesterol for placental steroid hormone synthesis is the low-density lipoprotein (LDL) that is derived from the maternal circulation [43–46] and internalized by way of the placental receptor for LDL apolipoprotein B receptor (LDL-R/ApoB-R) [47]. Additionally, cholesterol may be internalized by way of the very low-density lipoprotein receptor (VLDL-R) [48] or the LDL receptor-related protein (LRP) [49] and may be synthesized de novo—although in uncertain quantities—during early primate gestation [50,51]. Fetal adrenal glands use LDL cholesterol [52,53] and cholesterol can be synthesized de novo in the fetal liver and adrenals [54–56]. Although some in vitro data suggest minimal use of high-density lipoprotein (HDL) as a source of cholesterol for steroid hormone synthesis in the human fetal adrenal glands [52], the receptor for HDL is present and its role in vivo is unclear.

Estrogens and human chorionic gonadotropin (hCG) up-regulate the expression of the cell surface receptor for LDL in primates [57,58] and human placental cells [59–61]. This is a critical component of the receptor-mediated uptake of circulating lipoproteins that involve clathrin-coated pits that are located on the plasma membrane [62]. The LDL receptor mediates the uptake of lipoproteins by a mechanism of endocytosis, delivering them to intracellular lysosomes where apolipoproteins are degraded and lipoprotein cholesterol esters are subsequently hydrolyzed (by an acid lipase) leading to the release of free cholesterol. It is notable that pregnancies in which there is no maternal LDL (hypobetalipoproteinemia) demonstrate reduced levels of progesterone in the luteal phase, and in pregnancy, although steroid hormone levels remain sufficient to sustain pregnancies to term [63]. One can conclude that in the absence of circulating maternal LDL, cholesterol synthesis and acquisition by way of alternative sources are sufficient to sustain the steroidogenic needs of the placenta and developing fetus.

The possibility of low levels of de novo cholesterol synthesis in the primate placenta, including the human, has been suggested [50,51]. Additional sources of placental cholesterol likely include very low-density lipoprotein (VLDL) by way of its receptors (VLDL-R/ApoE-R, LRP) [48,64] and cholesterol from

acetylated or oxidized LDL that is recovered by way of the scavenger receptor class A, types I and II [65]. The relative contribution of HDL cholesterol to placental steroidogenesis in humans is unclear. The binding of HDL through its receptor, scavenger receptor class B, type I CD36 and LIMPII analogue (SR-BI/HDL-R/CLA-1), has been observed for the human placenta [66] and for human placental cell lines [52]; the in vivo use of this cholesterol source in humans seems likely [67]. Supporting a role for SR-BI in human placental steroidogenesis is up-regulation of its expression by the transcription factor steroidogenic factor-1 (SF-1/AD4BP/NR5A1) Ad4 binding protein (AD4BP) = SF-1 and in the steriod nomenclature is now also known as Nuclear Receptor subfamily 5, group A1 (NR5A1) in the gonads and adrenals [66]. SF-1 is a master regulator of multiple genes that are involved in adrenal and gonadal development and steroid hormone biosynthesis [68,69] but does not seem to be present in human placenta [70]. Alternative transcription factors may substitute for SF-1 in the regulation of expression of the steroidogenic machinery in the human placenta [71]. SR-BI gene expression also is regulated by estrogen, hCG, and corticotropin [72–74].

Recent observations demonstrate that lipid mobilization from HDL works differently than for LDL. The entire HDL particle does not need to be internalized for efficient transfer of lipid to the recipient cell [75]. Rather, it occurs by way of the association of HDL with dimerized SR-BI—all within microvillar channels that are located on the plasma membrane–from whence lipid transfer to the plasma membrane is facilitated by uncertain mechanisms [76] and is followed by hydrolysis of cholesterol esters by a neutral hydrolase to produce free cholesterol [77].

Overview of steroidogenesis in pregnancy

Measurement of steroid hormone production in pregnancy demonstrates a placenta that produces vast quantities of progesterone (250 mg/d at term [78]) and estriol (35–45 mg/d [79–81]) and lesser amounts of estrone, estradiol, and estetrol (Fig. 2) [16,82]. The secretion of all placental estrogens increases throughout gestation [83]. The relative concentrations of estrogens in the maternal serum (estradiol > estriol) do not reflect placental production; this is reflected better by the maternal urinary excretion of these estrogens (roughly 90% of maternal urinary estrogen is estriol conjugated to sulfate and glucuronide moieties). This is explained by the observation that the excretion of estriol is more efficient than for estradiol; estriol is conjugated efficiently and does not bind to sex hormone-binding globulin with high affinity [83–86].

Meanwhile, the fetal adrenal gland produces increased levels of dehydroepiandrosterone sulfate (DHEAS) (100–200 mg/d, see [5] and references therein) and pregnenolone sulfate [87] and produces some measure of cortisol early and late in fetal development (see the article by Rainey et al elsewhere in this issue).

The ‘‘incomplete’’ steroidogenic organs of pregnancy (ie, the fetal adrenal and the placenta) are described classically as a fetal adrenal that lacks the enzyme,

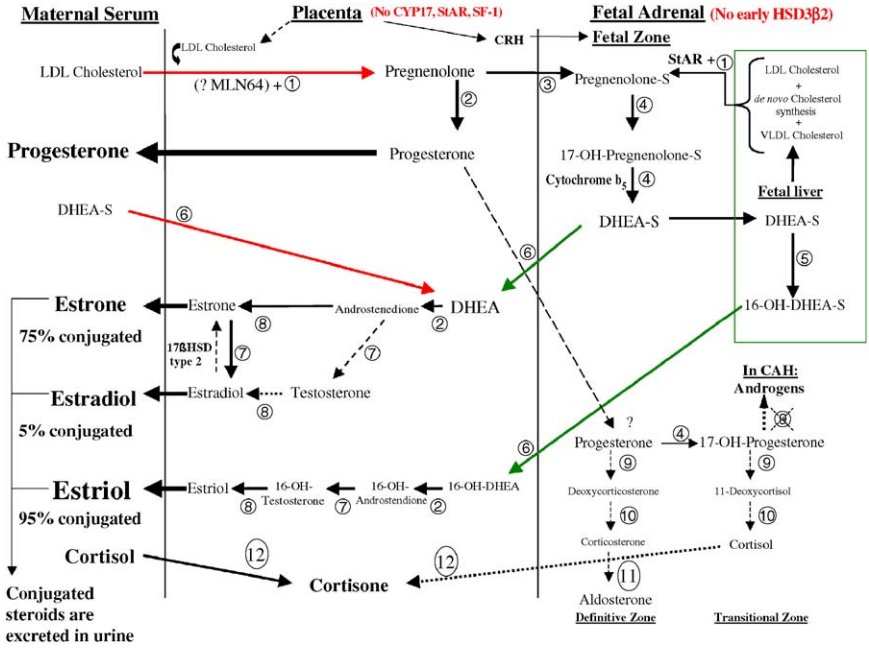


Fig. 2. The steroidogenic pathways of the materno-fetoplacental unit. Pathways that are represented with dotted arrows are proposed but are not established firmly in humans. Gene names and common names are provided below. CAH, congenital adrenal hyperplasia; DHEAS: dehydroepiandrosterone-sulfate; SF-1: steroidogenic factor-1 (NR5A1); StAR: steroidogenic acute regulatory protein. 1) CYP11A1: P450 Cholesterol side-chain cleavage/desmolase 2) HSD3β1: Placental 3β-Hydroxysteroid dehydrogenase/Δ 4-5 Isomerase type I - the type II enzyme, HSD3β2, is present in gonads and adrenals 3) SULT2A1: Fetal Adrenal Steroid Sulfotransferase 4) CYP17: P450c17 or 17α-Hydroxylase/17,20-Lyase 5) CYP3A7: Fetal hepatic 16α-Hydroxylase -to a lesser extent there may exist fetal adrenal 16α-Hydroxylase activity 6) STS: Placental Steroid Sulfatase 7) 17ßHSD1: 17β-Hydroxysteroid dehydrogenase type I enzyme -note that 17ßHSD type II (17ßHSD2) is detected in placental capillary endothelial cells 8) CYP19: P450-Aromatase 9) CYP21B: P450c21, or 21-Hydroxylase 10) CYP11β1: 11β-Hydroxylase 11) CYP11β2: 11β -Hydroxylase/18-Hydroxylase, or Aldosterone Synthase 12) 11ßHSD2: 11β -Hydroxysteroid dehydrogenase, type 2 -the placenta also expresses the type 1 enzyme (11ßHSD1).

3β-hydroxysteroid dehydrogenase/Δ5-Δ4 isomerase (HSD3β2), and a placenta that lacks the enzyme, 17-hydroxylase/17,20-lyase (CYP17) (see Fig. 2) [6,88,89]. The implications of these findings are that: (1) placental steroidogenesis from cholesterol substrate stops after two enzymatic reactions and culminates in the synthesis of progesterone; (2) the androgen substrate for placental estrogen production (occurring by way of placental aromatase activity) is of fetal adrenal and maternal origin; and (3) the fetal adrenal cannot convert Δ5 to Δ4 steroids, and therefore, is incapable of producing glucocorticoids or mineralocorticoids de novo. These findings are in contrast to the complete repertoire of steroidogenic enzymes of the adult adrenals and gonads. In the adult steroidogenic organs there is no requirement for steroid precursors to complete the appropriate pathways of

steroid hormone production (with the caveat that there can be further metabolism of selected steroids in peripheral tissues by enzymes [eg, 5-α-reductase, which converts testosterone to dihydrotestosterone in skin, and aromatase, which converts testosterone to estradiol in adipose tissue and breast]).

With the discovery of the steroidogenic acute regulatory protein (StAR) in 1994 [90], the limitations of placental steroidogenesis were extended to describe the surprising absence of StAR from this highly steroidogenic organ. StAR was identified in the gonads and adrenal glands as the mediator of cholesterol transport from the outer to the inner mitochondrial membranes. The p450 side chain cleavage enzyme system (P450scc/CYP11A1) (CYP11A1=P450scc=P450 cholesterol side-chain cleavage/desmolase enzyme) resides on the cholesterol-poor, matrix side of the inner mitochondrial membrane and uses cholesterol substrate in the first committed step of steroid hormone synthesis—the conversion of cholesterol to pregnenolone [91,92]. Cholesterol availability to the P450scc within the mitochondrion, which is mediated by StAR in the gonads and adrenals, is the rate-limiting factor in steroid hormone synthesis.

The important questions that are raised by these admittedly oversimplified observations are: (1) without StAR, how does the placenta deliver cholesterol to the P450scc enzyme system for conversion to pregnenolone, which, in turn, is further metabolized to progesterone? (2) without CYP17, how does the placenta make such copious quantities of estrogens? and (3) without 3β-HSD, how does the fetal adrenal make cortisol? The problems that are raised by these questions are reconciled by the elucidation of the cooperative endocrine system that is termed the "maternofetoplacetal unit."

Cholesterol availability to p450 side chain cleavage system

The acute stimulation of steroidogenesis in the adrenals and gonads is triggered by trophic hormone-induced generation of cyclic adenosine monophosphate (cAMP), with subsequent activation of phosphorylation and gene transcription by the cAMP-dependent protein kinase A. The first committed step in steroid hormone synthesis is the side-chain cleavage of cholesterol to form pregnenolone, a step that is catalyzed by the side-chain cleavage enzyme system (P450scc/CYP11A1), which is located on the matrix side of the inner mitochondrial membrane [93]. The translocation of cholesterol from the cholesterol-rich outer mitochondrial membrane to the cholesterol-poor inner membrane is the true rate-limiting step in the acute control of steroidogenesis [94]. This is a process that can be circumvented by using freely diffusible cholesterol substrates (hydroxysterols) [95,96] and one that depends upon de novo synthesis of proteins [97,98]. Collectively, these observations suggested a model in which trophic hormone, through the intermediacy of cAMP, promotes the de novo synthesis of a labile or short-acting protein which functions to enhance transport of cholesterol from the outer to the inner mitochondrial membrane where it serves as substrate for the P450scc enzyme system.

Using two-dimensional gel electrophoresis of metabolically-labeled, steroid-producing cells, Pon and colleagues [99] demonstrated a family of 37-, 32-, and 30-kd phosphoproteins that were induced rapidly in response to trophic hormone. Clark and colleagues [90] purified murine pp30 from MA-10 Leydig cells and cloned the cDNA that encodes what they designated StAR. Expression of the StAR cDNA in MA-10 cells was sufficient to promote steroidogenesis in the absence of stimulation by trophic hormone. Using an in vitro transcription and translation system, they demonstrated that the 37-kd StAR protein that was expressed by the cDNA was imported and processed by isolated mitochondria to produce the mature 30-kd protein.

The importance of the StAR protein in regulating steroidogenesis was soon confirmed soon by the cloning of the human StAR gene [100,101] and demonstration that lack of functional StAR causes congenital lipoid adrenal hyperplasia (lipoid CAH) [102]. This autosomal recessive disease is characterized by severe impairment of steroidogenesis in the gonads and adrenals and is manifested by male pseudohermaphroditism and postnatal adrenal insufficiency that requires hormone replacement therapy for life [103]. The pathophysiology of lipoid CAH includes insufficient movement of cholesterol to the side-chain cleavage enzyme which results in minimal production of steroid hormones, as well as massive accumulation of unmetabolized cholesterol in cytoplasmic lipid droplets which compress the cytoplasmic organelles and autoxidize leading to peroxidative damage of the cells. Engineering of a StAR null mouse that recapitulates the human disease confirmed the essential role of StAR in steroidogenesis [104].

The precise mechanism of StAR function is not certain. It was found to bind to cholesterol [105] by way of a conserved domain (termed StAR-related lipid transfer domain, or "START") and is able to function on the outside of mitochondria, without importation [106,107]. StAR was shown to transfer sterols between protein-free lipid vesicles; this suggested an intrinsic property of the protein to promote cholesterol transport without obvious need for cofactor binding [108,109].

Although StAR gene expression occurs in the human fetal adrenal gland [110,111]—consistent with the regulation of fetal adrenal steroidogenesis by ACTH [5]—the protein is absent from the human placenta [110]. This finding is consistent with the phenotype of fetuses that are affected by lipoid CAH; sufficient placental progesterone synthesis occurs to permit ongoing gestation, even after the luteo-placental shift in steroid hormone production. One factor that may subserve the function of StAR in the human placenta is a protein, Metastatic Lymph Node 64 (MLN64) [112,113]. MLN64 contains high homology to StAR, particularly in the START domain, and is capable of promoting steroidogenesis in a model cell system [114,115].

It is notable that mice that are functionally null for MLN64 are viable and fertile [116], unlike humans and mice that are null for the StAR protein, both of whom require steroid hormone replacement therapy for life. The steroidogenic potential of the rodent placenta, however, may not reflect that which is found in the human. The rodent placenta, for example, expresses StAR [71]. Moreover, the rodent

ovary sustains an important steroidogenic role throughout much of gestation [117], unlike the human corpus luteum, which loses steroidogenic function early within the first trimester of human gestation. Thus, the available data do not exclude a role for MLN64 in human placental steroid hormone synthesis.

The steroidogenic pathways in human pregnancy

The current understanding of steroid hormone synthesis during human pregnancy is depicted (see Fig. 2). The placental syncytiotrophoblast readily converts cholesterol substrate (largely, but not exclusively, derived from maternal LDL) to pregnenolone by way of the P450scc enzyme [118–120]. Required for this reaction are two cofactors: a flavoprotein reductase (ferredoxin/adrenodoxin reductase) and an iron sulfoprotein (ferredoxin/adrenodoxin); together, these ensure reducing equivalents (transferred from nicotinamide adenine dinucleotide phosphate) for the reaction of the side-chain cleavage enzyme [121]. A cAMP-mediated signal transduction cascade stimulates the expression of the genes for P450scc, adrenodoxin, and adrenodoxin reductase, although the gene promoters that are used behave in species- and cell type–specific fashion [122–124]. In primates, rabbits, and human placental cells in vitro, estrogens enhanced P450scc enzyme activity [125–129].

A complete loss of function of the P450scc enzyme would be expected to be incompatible with embryonic development. It should result in deficient placental production of progesterone at the time of the steroidogenic shift from the corpus luteum to the placenta (6–8 weeks of gestation). Complete deficiency of P450scc enzyme has not been reported; however, relative deficiencies of the enzyme have been reported and can produce adrenal insufficiency and XY sex reversal in affected males [130,131]. P450scc-null mice likely depend upon maternal ovarian steroidogenesis in pregnancy, affected male embryos do not undergo normal sexual development, and both sexes develop deficiencies in all steroid hormones postnatally that lead to adrenal insufficiency [132].

The placenta-specific 3β-hydroxysteroid dehydrogenase/Δ5-4 isomerase protein, type I (HSD3β1)(the adrenals and gonads express HSD3β2), readily converts pregnenolone to progesterone (which is secreted preferentially into the maternal circulation) [16,133]. The enzyme has two catalytic functions: (1) the oxidation (dehydrogenase) of Δ5-ene-3β-hydroxysterols to yield 3–keto-steroids, and (2) steroid Δ5 to Δ4 isomerase activities, which entail the movement of the Δ5 double bond in the B ring to the Δ4 position in the A ring of the steroid skeleton. The enzyme is expressed principally in the syncytiotrophoblast and intermediate cytotrophoblast cells but also is detected in trophoblast cells of the chorion and decidua of the fetal membranes [134].

Placentally-derived pregnenolone also may travel to the fetal adrenal gland where it is promptly sulfurylated by the DHEA-sulfotransferase enzyme, SULT2A1 [135,136]. SULTs catalyze the transfer of the sulfate group (SO_3-) from the 3′-phosphoadenosine 5′-phosphosulfate to the hydroxyl group or

amine group of the acceptor (ie, sulfate group transferred to the 3β-ol of DHEA to form DHEA-3–sulfate or DHEAS). In general, the water soluble sulfurylated product is excreted more readily in the urine. Because it binds with high affinity to albumin, the sulfoconjugate is cleared less readily by the liver, however, the primary site for clearance for circulating steroid hormones. In contrast, steroid hormone glucuronates bind weakly to albumin and are cleared rapidly by the liver. Conjugated steroids often are biologically less active/inactive.

SULT2A1 is expressed highly in the liver and adrenal glands (including fetal adrenal) and catalyzes the sulfate conjugation of DHEA, pregnenolone, and 17α-hydroxypregnenolone [137]. The sulfurylated products, pregnenolone-S and 17α-hydroxypregnenolone–S, are metabolized less efficiently by the CYP17 enzyme's 17,20-lyase activity than are the nonsulfurylated substrates; this may account for the significant fetal adrenal secretion of these products [138]. In contrast, the human placenta expresses increased concentrations of the more substrate-selective sulfotransferase isoforms, SULT2B1a (which sulfurylates pregnenolone) and SULT2B1b (which sulfurylates cholesterol). These represent splice variants from the same gene [139]. The human placenta also expresses the estrogen sulfotransferase (SULT1E1/EST) which is believed to play an important role in protecting the fetus from excessive exposure to biologically-active estrogens [140]. Meanwhile, placental steroid sulfatase (STS) removes the sulfate groups from the DHEAS and from additional fetal precursors of the placental estrogens (i.e. 16α-hydroxyDHEAS). These unconjugated steroids may serve as renewed substrates for fetal steroidogenesis. Thus, it is likely that there exists a complex balance and possibly a recycling of active and inactive steroid precursors between the fetoplacental unit.

Pregnenolone sulfate also may be produced de novo, within the fetus, using cholesterol substrate from the fetal liver and adrenal glands [54–56]. The pregnenolone sulfate is metabolized by fetal adrenal 17α-hydroxylase/17,20-lyase (CYP17) to form 17α-hydroxypregnenolone sulfate and then DHEAS. The 17α-hydroxylase/17,20-lyase enzyme is another example of a bifunctional enzyme; it first performs hydroxylation at the 17 carbon position, followed by 17,20 lyase reaction that produces a 17-keto steroid (the C19 androgens). The 17α-hydroxylase function of the enzyme is all that is required for continued synthesis of glucocorticoid and mineralocorticoid. Thus, the regulation of the second function, the scission of the 17-20 bond (lyase), is important because it commits the substrate toward the pathway of sex steroid production. Factors that were shown to enhance the lyase activity of the enzyme include phosphorylation at selected serine or threonine residues on the protein as well as the allosteric interaction with protein partner cytochrome b_5 [138,141–143]. Complete deficiency of one or both enzymatic activities has been described in humans, resulting from a plethora of gene mutations and producing a wide array of phenotypes [144–147].

The overwhelming preponderance of evidence suggests that the human placenta is unable to perform the functions of 17α-hydroxylase/17,20-lyase because of a lack of significant expression of this gene [2,89,148]. The relative

absence of CYP17 in the human placenta mandates that androgen substrates for production of estrogens be derived from the fetus (DHEAS, 16α-hydroxy-DHEAS, 15α,16α-dihydroxyDHEAS) or mother (DHEAS). The process of DHEAS synthesis in the fetus often proceeds directly from steroid sulfate precursor to steroid sulfate product; this process is not observed in adult steroidogenic tissues where sulfurylated substrates are metabolized poorly by the adult steroidogenic machinery [149].

DHEAS is the most abundant steroid hormone product of the fetal adrenal (100–200 mg/d) and is made principally in the fetal zone of the developing adrenals, which makes up 80% to 90% of the fetal adrenal cortex [5]. The fetal zone is destined to involute immediately postpartum. Two additional zones of the fetal adrenal gland have been identified and likely represent the precursors to the adult adrenocortical tissues: the definitive zone (destined to become the zona glomerulosa) and the transitional zone (destined to become the zona fasciculata) [5]. The steroidogenic activities of the definitive and transitional zones during human gestation are controversial and are discussed below.

DHEAS that is produced in the fetal zone or of maternal origin has two potential fates. When supplied directly to the placenta, it serves as substrate for the production of estrone and estradiol and when metabolized by the fetal liver, it serves ultimately as substrate for placental estriol synthesis (see Fig. 2). In the first instance, DHEAS is transported into the placenta [150] and is acted upon by the placental STS [151] to produce DHEA. Steroid sulfatase deficiency results in decreased production of fetoplacental estrogens and is associated with delayed parturition and recessive X-chromosome-linked ichthyosis after birth [152–158]. DHEA is metabolized further by placental HSD3β1 to produce androstenedione. Androstenedione may be metabolized by placental 17βHSD1 to produce testosterone which is aromatized to 17β-estradiol [159]. Alternatively, androstenedione is aromatized directly to estrone [160].

In the second and more common fate, DHEAS is metabolized in the fetal liver (and possibly to a lesser extent, by the fetal adrenal itself) by hepatic 16α-hydroxylase enzyme (CYP3A7) to form 16α-hydroxy-DHEAS [161–163]. This substrate is acted upon by a placental sulfatase to produce16α-hydroxy-DHEA, which is metabolized further to estriol within the placenta [149]. Similarly, through the intermediacy of a fetal hepatic15α-hydroxylase activity, fetal 15α,16α-dihydroxy-DHEAS substrate can be used by the placenta to produce estetrol.

Placental progesterone production can proceed independently of maternal or fetal adrenal steroid substrate (depending chiefly on maternal and fetal supplies of cholesterol). Conditions of intrauterine fetal demise often are associated with normal progesterone production from the placenta. Meanwhile, several conditions have been associated with diminished placental estrogen production (eg, anencephalic fetuses which produce minimal corticotropin, exhibit minimal development of fetal zone of the adrenals, and produce scant quantities of DHEAS [164–166]; also, conditions of placental sulfatase deficiency or aromatase deficiency, both of which produce hypoestrogenemia in the mother). In

many of these cases, placental progesterone levels remain sufficient to sustain the pregnancies. The principal determinants of placental progesterone production seem to be the expression of the steroidogenic enzymes, CYP11A1 and HSD3β1, in the placental trophoblast cells [167]. Factors that may be involved in the regulation of these genes include estrogens, placental LH-releasing hormone, and hCG.

Questions and controversies regarding human steroid hormone production in pregnancy

From the above-mentioned physiology, one can explain the integrated process that results in placental production of progesterone, estrone, estradiol, and estriol. Several important issues warrant further investigation.

When and how does the fetal adrenal first produce glucocorticoids and mineralocorticoids? Prevailing theory has supported the notion that fetal deficiency of 3β-HSD ensures increased production of fetal DHEAS (the substrate for placental estrogens) and precludes significant adrenal production of mineralocorticoids and glucocorticoids. This was believed to be operative until near term, when the awakening of the fetal hypothalamic–pituitary–adrenal axis occurred before parturition [168]. This theory has had to be reconciled with the phenotype of congenital adrenal hyperplasia (CAH) that results from—for example—21-hydroxylase (CYP21B/P450c21) enzyme deficiency. In this condition, virilization of affected female embryos occurs from excessive fetal adrenal androgen production in utero. This was believed to result from insufficient feedback inhibition of fetal corticotropin in the setting of cortisol deficiency (ie, CAH dictates that fetal adrenal cortisol synthesis occurs during the time of sexual differentiation, which is approximately 7–10 weeks of gestation) (see Fig. 2).

Conflicting data regarding the first detectable expression of 3β-HSD might be explained by differences in methodologies (eg, measuring in vivo versus in vitro tissues, treated or not treated with trophic hormone, human versus nonhuman, mRNA levels versus protein levels) [111,168–172]. Further research is needed to clarify the ontogeny of expression of 3β-HSD protein and to define the adrenal zones in which it may be operative in early gestation. Some of the data suggest early and late expression of the enzyme (biphasic expression), with a nadir between 10 and 23 weeks' gestation, followed by detectable expression thereafter. Additional data seek to explain the excess androgen production in CAH by a pathway in which placental progesterone can serve as substrate for early cortisol production (see Fig. 2) [173].

Similarly, there is controversy regarding whether the human placenta expresses the CYP17 enzyme. One group reported detecting mRNA for CYP17 using quantitative reverse transcription polymerase chain reaction in human placental preparations [171]. An assay of CYP17 activity on human placental tissues in vitro also reported measurable production of DHEA from cholesterol [174]. The significance of detecting minute quantities (more than 100-fold less

abundant than in adrenal or testis) of mRNA or of assaying for enzymatic function in tissues placed in vitro, is unclear [171]. The possibility remains that low levels of de novo estrogen production occur in the human placenta independently of fetal adrenal and maternal substrate. Such a function would have to be modest, but might explain the detectable levels of estrogens in selected conditions (ie, those with decreased estrogen production in pregnancies that are affected by placental sulfatase deficiency, anencephaly, and fetal adrenal suppression by exogenous glucocorticoid therapy). The observed maternal hypoestrogenemia in these circumstances is testimony to the important contribution of the fetal adrenals to placental estrogen synthesis.

What roles are fulfilled by the pregnancy hormones that are produced by the maternofetoplacental unit? Although great work in a multitude of animal models has revealed important functions of the pregnancy-associated steroid hormones, many questions remain. The mechanisms whereby, for example, endometrial receptivity, uterine quiescence, and immune-tolerance of the fetus are accomplished remain incompletely defined. The minimal essential steroidogenic requirements for successful pregnancy, fetal development, and parturition have not been fully characterized and the individual roles of each steroid hormone have not been determined.

Which factors regulate steroid hormone production in pregnancy and how is target-tissue responsiveness to these hormones regulated? To this end, the regulated expression of steroidogenic enzymes that are unique to pregnancy has not been fully described. What distinguishes the placenta-specific isoform of 3β-HSD from the one that is found classically in the adrenals and gonads?

What placental factors substitute for the transcription factor SF-1 in regulating the genes for the steroidogenic enzymes? Is mLN64 truly the StAR protein for placental cholesterol mobilization in the mitochondrion? What determines the balance of expression between placental 11β-HSD1 (which produces biologically-active cortisol from inactive cortisone) and 11β-HSD2 (which does the opposite)? This balance is likely to have profound effects on processes, such as maturation of the fetal hypothalamic–pituitary–adrenal axis, fetal growth, and the timing of parturition [175]. These are some of the important questions that await further study.

Acknowledgments

The author is deeply grateful to Dr. Richard Hochberg for the critical review of this manuscript and for assistance in the preparation of the figures.

References

[1] Siiteri P, MacDonald PC. The utilization of circulating dehydroisoandrosterone sulfate for estrogen synthesis during human pregnancy. Steroids 1963;2:713–30.

[2] Siiteri PK, MacDonald PC. Placental estrogen biosynthesis during human pregnancy. J Clin Endocrinol Metab 1966;26:751–61.
[3] Ryan KJ, Hopper BR. Placental biosynthesis and metabolism of steroid hormones in primates. Contrib Primatol 1974;3:258–83.
[4] Ryan KJ, Meigs R, Petro Z. The formation of progesterone by the human placenta. Am J Obstet Gynecol 1966;96:676–86.
[5] Mesiano S, Jaffe RB. Developmental and functional biology of the primate fetal adrenal cortex. Endocr Rev 1997;18:378–403.
[6] Diczfalusy E. Steroid metabolism in the human foeto-placental unit. Acta Endocrinol (Copenh) 1969;61:649–64.
[7] Richards JS, Russell DL, Ochsner S, Hsieh M, Doyle KH, Falender AE, et al. Novel signaling pathways that control ovarian follicular development, ovulation, and luteinization. Recent Prog Horm Res 2002;57:195–220.
[8] Britt KL, Stanton PG, Misso M, Simpson ER, Findlay JK. The effects of estrogen on the expression of genes underlying the differentiation of somatic cells in the murine gonad. Endocrinology 2004;145:3950–60.
[9] Stouffer RL. Progesterone as a mediator of gonadotrophin action in the *corpus luteum*: beyond steroidogenesis. Hum Reprod Update 2003;9:99–117.
[10] Findlay JK, Britt K, Kerr JB, O'Donnell L, Jones ME, Drummond AE, et al. The road to ovulation: the role of oestrogens. Reprod Fertil Dev 2001;13:543–7.
[11] Dupont S, Krust A, Gansmuller A, Dierich A, Chambon P, Mark M. Effect of single and compound knockouts of estrogen receptors alpha (ERalpha) and beta (ERbeta) on mouse reproductive phenotypes. Development 2000;127:4277–91.
[12] Siiteri PK, Febres F, Clemens LE, Chang RJ, Gondos B, Stites D. Progesterone and maintenance of pregnancy: is progesterone nature's immunosuppressant? Ann N Y Acad Sci 1977;286:384–97.
[13] Feinberg BB, Anderson DJ, Steller MA, Fulop V, Berkowitz RS, Hill JA. Cytokine regulation of trophoblast steroidogenesis. J Clin Endocrinol Metab 1994;78:586–91.
[14] Stites DP, Bugbee S, Siiteri PK. Differential actions of progesterone and cortisol on lymphocyte and monocyte interaction during lymphocyte activation–relevance to immunosuppression in pregnancy. J Reprod Immunol 1983;5:215–28.
[15] Stites DP, Siiteri PK. Steroids as immunosuppressants in pregnancy. Immunol Rev 1983; 75:117–38.
[16] Pepe GJ, Albrecht ED. Actions of placental and fetal adrenal steroid hormones in primate pregnancy. Endocr Rev 1995;16:608–48.
[17] Szekeres-Bartho J. Immunological relationship between the mother and the fetus. Int Rev Immunol 2002;21:471–95.
[18] Thellin O, Coumans B, Zorzi W, Igout A, Heinen E. Tolerance to the foeto-placental 'graft': ten ways to support a child for nine months. Curr Opin Immunol 2000;12:731–7.
[19] Herrler A, von Rango U, Beier HM. Embryo-maternal signalling: how the embryo starts talking to its mother to accomplish implantation. Reprod Biomed Online 2003;6:244–56.
[20] Beagley KW, Gockel CM. Regulation of innate and adaptive immunity by the female sex hormones oestradiol and progesterone. FEMS Immunol Med Microbiol 2003;38:13–22.
[21] Okada H, Nakajima T, Yoshimura T, Yasuda K, Kanzaki H. Microarray analysis of genes controlled by progesterone in human endometrial stromal cells in vitro. Gynecol Endocrinol 2003;17:271–80.
[22] Itskovitz J, Hodgen GD. Endocrine basis for the initiation, maintenance and termination of pregnancy in humans. Psychoneuroendocrinology 1988;13:155–70.
[23] Wray S. Uterine contraction and physiological mechanisms of modulation. Am J Physiol 1993;264:C1–18.
[24] Wray S, Kupittayanant S, Shmygol A, Smith RD, Burdyga T. The physiological basis of uterine contractility: a short review. Exp Physiol 2001;86:239–46.
[25] Hertelendy F, Zakar T. Regulation of myometrial smooth muscle functions. Curr Pharm Des 2004;10:2499–517.

[26] Astle S, Khan RN, Thornton S. The effects of a progesterone metabolite, 5 beta-dihydroprogesterone, on oxytocin receptor binding in human myometrial membranes. BJOG 2003; 110:589–92.
[27] Astle S, Slater DM, Thornton S. The involvement of progesterone in the onset of human labour. Eur J Obstet Gynecol Reprod Biol 2003;108:177–81.
[28] Rosenfeld CR, Chen C, Roy T, Liu X. Estrogen selectively up-regulates eNOS and nNOS in reproductive arteries by transcriptional mechanisms. J Soc Gynecol Investig 2003;10: 205–15.
[29] Rosenfeld CR, Roy T, Cox BE. Mechanisms modulating estrogen-induced uterine vasodilation. Vascul Pharmacol 2002;38:115–25.
[30] Rosenfeld CR, Cox BE, Roy T, Magness RR. Nitric oxide contributes to estrogen-induced vasodilation of the ovine uterine circulation. J Clin Invest 1996;98:2158–66.
[31] Magness RR, Parker Jr CR, Rosenfeld CR. Systemic and uterine responses to chronic infusion of estradiol-17 beta. Am J Physiol 1993;265:E690–8.
[32] Rosenfeld CR, Rivera R. Circulatory responses to systemic infusions of estrone and estradiol-17alpha in nonpregnant, oophorectomized ewes. Am J Obstet Gynecol 1978;132:442–8.
[33] Longo LD. Maternal blood volume and cardiac output during pregnancy: a hypothesis of endocrinologic control. Am J Physiol 1983;245:R720–9.
[34] Merviel P, Evain-Brion D, Challier JC, Salat-Baroux J, Uzan S. The molecular basis of embryo implantation in humans. Zentralbl Gynakol 2001;123:328–39.
[35] Presta M. Sex hormones modulate the synthesis of basic fibroblast growth factor in human endometrial adenocarcinoma cells: implications for the neovascularization of normal and neoplastic endometrium. J Cell Physiol 1988;137:593–7.
[36] Charnock-Jones DS, Sharkey AM, Rajput-Williams J, Burch D, Schofield JP, Fountain SA, et al. Identification and localization of alternately spliced mRNAs for vascular endothelial growth factor in human uterus and estrogen regulation in endometrial carcinoma cell lines. Biol Reprod 1993;48:1120–8.
[37] Cullinan-Bove K, Koos RD. Vascular endothelial growth factor/vascular permeability factor expression in the rat uterus: rapid stimulation by estrogen correlates with estrogen-induced increases in uterine capillary permeability and growth. Endocrinology 1993;133:829–37.
[38] Soccio RE, Breslow JL. Intracellular cholesterol transport. Arterioscler Thromb Vasc Biol 2004;24:1150–60.
[39] Hall PF. Cytochromes P-450 and the regulation of steroid synthesis. Steroids 1986;48:131–96.
[40] Penning TM. Molecular endocrinology of hydroxysteroid dehydrogenases. Endocr Rev 1997;18:281–305.
[41] Csapo AI, Pulkkinen M. Indispensability of the human corpus luteum in the maintenance of early pregnancy. Luteectomy evidence. Obstet Gynecol Surv 1978;33:69–81.
[42] Csapo AI, Pulkkinen MO, Kaihola HL. The relationship between the timing of luteectomy and the incidence of complete abortions. Am J Obstet Gynecol 1974;118:985–9.
[43] Winkel CA, Snyder JM, MacDonald PC, Simpson ER. Regulation of cholesterol and progesterone synthesis in human placental cells in culture by serum lipoproteins. Endocrinology 1980;106:1054–60.
[44] Winkel CA, MacDonald PC, Simpson ER. The role of receptor-mediated low-density lipoprotein uptake and degradation in the regulation of progesterone biosynthesis and cholesterol metabolism by human trophoblasts. Placenta Suppl 1981;3:133–43.
[45] Hellig H, Gattereau D, Lefebvre Y, Bolte E. Steroid production from plasma cholesterol. I. Conversion of plasma cholesterol to placental progesterone in humans. J Clin Endocrinol Metab 1970;30:624–31.
[46] Gwynne JT, Strauss III JF. The role of lipoproteins in steroidogenesis and cholesterol metabolism in steroidogenic glands. Endocr Rev 1982;3:299–329.
[47] Cummings SW, Hatley W, Simpson ER, Ohashi M. The binding of high and low density lipoproteins to human placental membrane fractions. J Clin Endocrinol Metab 1982;54:903–8.
[48] Wittmaack FM, Gafvels ME, Bronner M, Matsuo H, McCrae KR, Tomaszewski JE, et al.

Localization and regulation of the human very low density lipoprotein/apolipoprotein-E receptor: trophoblast expression predicts a role for the receptor in placental lipid transport. Endocrinology 1995;136:340–8.
[49] Shi W, Swan KF, Henson MC. Expression of placental low-density lipoprotein receptor-related protein and scavenger receptors AI/AII transcripts in the baboon. Am J Obstet Gynecol 2003; 188:1211–2.
[50] Shi W, Swan KF, Lear SR, O'Neil JS, Erickson SK, Henson MC. Regulation of pathways determining cholesterol availability in the baboon placenta with advancing gestation. Biol Reprod 1999;61:1499–505.
[51] Van Leusden H, Villee CA. The de novo synthesis of sterols and steroids from acetate by preparations of human term placenta. Steroids 1965;6:31–45.
[52] Carr BR, Parker Jr CR, MacDonald PC, Simpson ER. Metabolism of high density lipoprotein by human fetal adrenal tissue. Endocrinology 1980;107:1849–54.
[53] Parker Jr CR, Carr BR, Winkel CA, Casey ML, Simpson ER, MacDonald PC. Hypercholesterolemia due to elevated low density lipoprotein-cholesterol in newborns with anencephaly and adrenal atrophy. J Clin Endocrinol Metab 1983;57:37–43.
[54] Carr BR, Simpson ER. Lipoprotein utilization and cholesterol synthesis by the human fetal adrenal gland. Endocr Rev 1981;2:306–26.
[55] Carr BR, Ohashi M, Simpson ER. Low density lipoprotein binding and de novo synthesis of cholesterol in the neocortex and fetal zones of the human fetal adrenal gland. Endocrinology 1982;110:1994–8.
[56] Carr BR, Simpson ER. Cholesterol synthesis by human fetal hepatocytes: effect of lipoproteins. Am J Obstet Gynecol 1984;150:551–7.
[57] Henson MC, Pepe GJ, Albrecht ED. Regulation of placental low-density lipoprotein uptake in baboons by estrogen: dose-dependent effects of the anti-estrogen ethamoxytriphetol (MER-25). Biol Reprod 1991;45:43–8.
[58] Albrecht ED, Henson MC, Pepe GJ. Regulation of placental low density lipoprotein uptake in baboons by estrogen. Endocrinology 1991;128:450–8.
[59] Grimes RW, Pepe GJ, Albrecht ED. Regulation of human placental trophoblast low-density lipoprotein uptake in vitro by estrogen. J Clin Endocrinol Metab 1996;81:2675–9.
[60] Schmid KE, Davidson WS, Myatt L, Woollett LA. Transport of cholesterol across a BeWo cell monolayer: implications for net transport of sterol from maternal to fetal circulation. J Lipid Res 2003;44:1909–18.
[61] Shanker YG, Shetty UP, Rao AJ. Regulation of low density lipoprotein receptor mRNA levels by estradiol 17beta and chorionic gonadotropin in human placenta. Mol Cell Biochem 1998; 187:133–9.
[62] Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. Science 1986;232:34–47.
[63] Parker Jr CR, Illingworth DR, Bissonnette J, Carr BR. Endocrine changes during pregnancy in a patient with homozygous familial hypobetalipoproteinemia. N Engl J Med 1986;314: 557–60.
[64] Gafvels ME, Coukos G, Sayegh R, Coutifaris C, Strickland DK, Strauss III JF. Regulated expression of the trophoblast alpha 2-macroglobulin receptor/low density lipoprotein receptor-related protein. Differentiation and cAMP modulate protein and mRNA levels. J Biol Chem 1992;267:21230–4.
[65] Bonet B, Chait A, Gown AM, Knopp RH. Metabolism of modified LDL by cultured human placental cells. Atherosclerosis 1995;112:125–36.
[66] Cao G, Garcia CK, Wyne KL, Schultz RA, Parker KL, Hobbs HH. Structure and localization of the human gene encoding SR-BI/CLA-1. Evidence for transcriptional control by steroidogenic factor 1. J Biol Chem 1997;272:33068–76.
[67] Wadsack C, Hammer A, Levak-Frank S, Desoye G, Kozarsky KF, Hirschmugl B, et al. Selective cholesteryl ester uptake from high density lipoprotein by human first trimester and term villous trophoblast cells. Placenta 2003;24:131–43.

[68] Parker KL, Rice DA, Lala DS, Ikeda Y, Luo X, Wong M, et al. Steroidogenic factor 1: an essential mediator of endocrine development. Recent Prog Horm Res 2002;57:19–36.
[69] Parker KL, Schimmer BP. Steroidogenic factor 1: a key determinant of endocrine development and function. Endocr Rev 1997;18:361–77.
[70] Ramayya MS, Zhou J, Kino T, Segars JH, Bondy CA, Chrousos GP. Steroidogenic factor 1 messenger ribonucleic acid expression in steroidogenic and nonsteroidogenic human tissues: northern blot and in situ hybridization studies. J Clin Endocrinol Metab 1997;82:1799–806.
[71] Ben-Zimra M, Koler M, Melamed-Book N, Arensburg J, Payne AH, Orly J. Uterine and placental expression of steroidogenic genes during rodent pregnancy. Mol Cell Endocrinol 2002;187:223–31.
[72] Lafond J, Charest MC, Alain JF, Brissette L, Masse A, Robidoux J, et al. Presence of CLA-1 and HDL binding sites on syncytiotrophoblast brush border and basal plasma membranes of human placenta. Placenta 1999;20:583–90.
[73] Lopez D, Sanchez MD, Shea-Eaton W, McLean MP. Estrogen activates the high-density lipoprotein receptor gene via binding to estrogen response elements and interaction with sterol regulatory element binding protein-1A. Endocrinology 2002;143:2155–68.
[74] Stangl H, Graf GA, Yu L, Cao G, Wyne K. Effect of estrogen on scavenger receptor BI expression in the rat. J Endocrinol 2002;175:663–72.
[75] Gu X, Trigatti B, Xu S, Acton S, Babitt J, Krieger M. The efficient cellular uptake of high density lipoprotein lipids via scavenger receptor class B type I requires not only receptor-mediated surface binding but also receptor-specific lipid transfer mediated by its extracellular domain. J Biol Chem 1998;273:26338–48.
[76] Reaven E, Cortez Y, Leers-Sucheta S, Nomoto A, Azhar S. Dimerization of the scavenger receptor class B type I: formation, function, and localization in diverse cells and tissues. J Lipid Res 2004;45:513–28.
[77] Connelly MA, Williams DL. SR-BI and cholesterol uptake into steroidogenic cells. Trends Endocrinol Metab 2003;14:467–72.
[78] Johansson ED. Plasma levels of progesterone in pregnancy measured by a rapid competitive protein binding technique. Acta Endocrinol (Copenh) 1969;61:607–17.
[79] Frandsen VA, Stakemann G. The clinical significance of oestriol estimations in late pregnancy. Acta Endocrinol (Copenh) 1963;44:183–95.
[80] Frandsen VA, Stakemann G. The urinary excretion of oestriol during the early months of pregnancy.normal and pathological cases. Acta Endocrinol (Copenh) 1963;44:196–208.
[81] Frandsen VA, Stakemann G. Urinary excretion of estriol during normal pregnancy. Dan Med Bull 1960;7:95–8.
[82] Frandsen VA, Lundwall F. The excretion of oestrone-oestradiol and oestriol in normal human pregnancy. Acta Endocrinol (Copenh) 1966;53:93–100.
[83] Oakey RE. The progressive increase in estrogen production in human pregnancy: an appraisal of the factors responsible. Vitam Horm 1970;28:1–36.
[84] Moutsatsou V, Oakey RE. Oestriol and non-protein-bound oestriol concentrations in human peripheral plasma before labour and delivery. J Endocrinol 1986;108:75–80.
[85] Moutsatsou V, Oakey RE. Oestriol binding to plasma proteins. J Steroid Biochem 1988;29: 319–23.
[86] Penney LL. Ratio of unconjugated to total estriol in uncomplicated third-trimester pregnancy. J Reprod Med 1980;25:259–62.
[87] Seron-Ferre M, Lawrence CC, Siiteri PK, Jaffe RB. Steroid production by definitive and fetal zones of the human fetal adrenal gland. J Clin Endocrinol Metab 1978;47:603–9.
[88] Simpson ER, MacDonald PC. Endocrine physiology of the placenta. Annu Rev Physiol 1981; 43:163–88.
[89] Voutilainen R, Miller WL. Developmental expression of genes for the stereoidogenic enzymes P450scc (20,22-desmolase), P450c17 (17 alpha-hydroxylase/17,20-lyase), and P450c21 (21-hydroxylase) in the human fetus. J Clin Endocrinol Metab 1986;63:1145–50.
[90] Clark BJ, Wells J, King SR, Stocco DM. The purification, cloning, and expression of a novel luteinizing hormone-induced mitochondrial protein in MA-10 mouse Leydig tumor cells.

Characterization of the steroidogenic acute regulatory protein (StAR). J Biol Chem 1994; 269:28314–22.
[91] Kallen CB, Arakane F, Christenson LK, Watari H, Devoto L, Strauss III JF. Unveiling the mechanism of action and regulation of the steroidogenic acute regulatory protein. Mol Cell Endocrinol 1998;145:39–45.
[92] Strauss III JF, Kallen CB, Christenson LK, Watari H, Devoto L, Arakane F, et al. The steroidogenic acute regulatory protein (StAR): a window into the complexities of intracellular cholesterol trafficking. Recent Prog Horm Res 1999;54:369–94 [discussion 394–5].
[93] Stone D, Hechter O. Studies on ACTH action in perfused bovine adrenals: site of action of ACTH corticosteroidogenesis. Arch Biochem Biophys 1954;51:457–69.
[94] Privalle CT, Crivello JF, Jefcoate CR. Regulation of intramitochondrial cholesterol transfer to side-chain cleavage cytochrome P-450 in rat adrenal gland. Proc Natl Acad Sci USA 1983;80:702–6.
[95] Lambeth JD, Kitchen SE, Farooqui AA, Tuckey R, Kamin H. Cytochrome P-450scc-substrate interactions. Studies of binding and catalytic activity using hydroxycholesterols. J Biol Chem 1982;257:1876–84.
[96] Tuckey RC, Atkinson HC. Pregnenolone synthesis from cholesterol and hydroxycholesterols by mitochondria from ovaries following the stimulation of immature rats with pregnant mare's serum gonadotropin and human choriogonadotropin. Eur J Biochem 1989;186:255–9.
[97] Garren LD, Ney RL, Davis WW. Studies on the role of protein synthesis in the regulation of corticosterone production by adrenocorticotropic hormone in vivo. Proc Natl Acad Sci USA 1965;53:1443–50.
[98] Ferguson JJ. Protein synthesis and adrenocorticotropin responsiveness. J Biol Chem 1963;238:2754–9.
[99] Pon LA, Hartigan JA, Orme-Johnson NR. Acute ACTH regulation of adrenal corticosteroid biosynthesis. Rapid accumulation of a phosphoprotein. J Biol Chem 1986;261:13309–16.
[100] Sugawara T, Holt JA, Driscoll D, Strauss III JF, Lin D, Miller WL, et al. Human steroidogenic acute regulatory protein: functional activity in COS-1 cells, tissue-specific expression, and mapping of the structural gene to 8p11.2 and a pseudogene to chromosome 13. Proc Natl Acad Sci USA 1995;92:4778–82.
[101] Sugawara T, Lin D, Holt JA, Martin KO, Javitt NB, Miller WL, et al. Structure of the human steroidogenic acute regulatory protein (StAR) gene: StAR stimulates mitochondrial cholesterol 27-hydroxylase activity. Biochemistry 1995;34:12506–12.
[102] Lin D, Sugawara T, Strauss III JF, Clark BJ, Stocco DM, Saenger P, et al. Role of steroidogenic acute regulatory protein in adrenal and gonadal steroidogenesis. Science 1995;267:1828–31.
[103] Bose HS, Sugawara T, Strauss III JF, Miller WL. The pathophysiology and genetics of congenital lipoid adrenal hyperplasia. International Congenital Lipoid Adrenal Hyperplasia Consortium. N Engl J Med 1996;335:1870–8.
[104] Caron KM, Soo SC, Wetsel WC, Stocco DM, Clark BJ, Parker KL. Targeted disruption of the mouse gene encoding steroidogenic acute regulatory protein provides insights into congenital lipoid adrenal hyperplasia. Proc Natl Acad Sci USA 1997;94:11540–5.
[105] Tsujishita Y, Hurley JH. Structure and lipid transport mechanism of a StAR-related domain. Nat Struct Biol 2000;7:408–14.
[106] Arakane F, Kallen CB, Watari H, Stayrook SE, Lewis M, Strauss III JF. Steroidogenic acute regulatory protein (StAR) acts on the outside of mitochondria to stimulate steroidogenesis. Endocr Res 1998;24:463–8.
[107] Arakane F, Kallen CB, Watari H, Foster JA, Sepuri NB, Pain D, et al. The mechanism of action of steroidogenic acute regulatory protein (StAR). StAR acts on the outside of mitochondria to stimulate steroidogenesis. J Biol Chem 1998;273:16339–45.
[108] Kallen CB, Billheimer JT, Summers SA, Stayrook SE, Lewis M, Strauss 3rd JF. Steroidogenic acute regulatory protein (StAR) is a sterol transfer protein. J Biol Chem 1998;273:26285–8.
[109] Bose HS, Baldwin MA, Miller WL. Evidence that StAR and MLN64 act on the outer mitochondrial membrane as molten globules. Endocr Res 2000;26:629–37.

[110] Pollack SE, Furth EE, Kallen CB, Arakane F, Kiriakidou M, Kozarsky KF, et al. Localization of the steroidogenic acute regulatory protein in human tissues. J Clin Endocrinol Metab 1997; 82:4243–51.
[111] Narasaka T, Suzuki T, Moriya T, Sasano H. Temporal and spatial distribution of cortico-steroidogenic enzymes immunoreactivity in developing human adrenal. Mol Cell Endocrinol 2001;174:111–20.
[112] Moog-Lutz C, Tomasetto C, Regnier CH, Wendling C, Lutz Y, Muller D, et al. MLN64 exhibits homology with the steroidogenic acute regulatory protein (STAR) and is over-expressed in human breast carcinomas. Int J Cancer 1997;71:183–91.
[113] Alpy F, Stoeckel ME, Dierich A, Escola JM, Wendling C, Chenard MP, et al. The steroidogenic acute regulatory protein homolog MLN64, a late endosomal cholesterol-binding protein. J Biol Chem 2001;276:4261–9.
[114] Watari H, Arakane F, Moog-Lutz C, Kallen CB, Tomasetto C, Gerton GL, et al. MLN64 contains a domain with homology to the steroidogenic acute regulatory protein (StAR) that stimulates steroidogenesis. Proc Natl Acad Sci USA 1997;94:8462–7.
[115] Strauss III JF, Kishida T, Christenson LK, Fujimoto T, Hiroi H. START domain proteins and the intracellular trafficking of cholesterol in steroidogenic cells. Mol Cell Endocrinol 2003;202:59–65.
[116] Tatsuro K, Kostetskii I, Zhang Z, Martinez F, Liu P, Walkley SU, et al. Targeted mutation of the MLN64 START domain causes only modest alterations in cellular sterol metabolism. J Biol Chem 2004;279:19276–85.
[117] Pepe GJ, Rothchild I. Serum progesterone levels in ovariectomized rats injected with progesterone and estrone: relation to pregnancy maintenance and growth of decidual tissue. Endocrinology 1973;93:1193–9.
[118] Miller WL. Molecular biology of steroid hormone synthesis. Endocr Rev 1988;9:295–318.
[119] Chung BC, Matteson KJ, Voutilainen R, Mohandas TK, Miller WL. Human cholesterol side-chain cleavage enzyme, P450scc: cDNA cloning, assignment of the gene to chromosome 15, and expression in the placenta. Proc Natl Acad Sci USA 1986;83:8962–6.
[120] Pasanen M, Pelkonen O. Purification and immunological characterization of human placental mitochondrial cytochrome P-450. J Steroid Biochem 1986;24:669–75.
[121] Sparkes RS, Klisak I, Miller WL. Regional mapping of genes encoding human steroidogenic enzymes: P450scc to 15q23-q24, adrenodoxin to 11q22; adrenodoxin reductase to 17q24-q25; and P450c17 to 10q24-q25. DNA Cell Biol 1991;10:359–65.
[122] Hum DW, Miller WL. Transcriptional regulation of human genes for steroidogenic enzymes. Clin Chem 1993;39:333–40.
[123] Hum DW, Aza-Blanc P, Miller WL. Characterization of placental transcriptional activation of the human gene for P450scc. DNA Cell Biol 1995;14:451–63.
[124] Rodriguez H, Hum DW, Staels B, Miller WL. Transcription of the human genes for cytochrome P450scc and P450c17 is regulated differently in human adrenal NCI-H295 cells than in mouse adrenal Y1 cells. J Clin Endocrinol Metab 1997;82:365–71.
[125] Pepe GJ, Albrecht ED. Central integrative role of oestrogen in the regulation of placental steroidogenic maturation and the development of the fetal pituitary-adrenocortical axis in the baboon. Hum Reprod Update 1998;4:406–19.
[126] Babischkin JS, Pepe GJ, Albrecht ED. Estrogen regulation of placental P-450 cholesterol side-chain cleavage enzyme messenger ribonucleic acid levels and activity during baboon pregnancy. Endocrinology 1997;138:452–5.
[127] Babischkin JS, Grimes RW, Pepe GJ, Albrecht ED. Estrogen stimulation of P450 cholesterol side-chain cleavage activity in cultures of human placental syncytiotrophoblasts. Biol Reprod 1997;56:272–8.
[128] Babischkin JS, Pepe GJ, Albrecht ED. Regulation of progesterone biosynthesis by estrogen during baboon pregnancy: placental mitochondrial cholesterol side-chain cleavage activity in antiestrogen (ethamoxytriphetol, MER-25)-treated baboons. Endocrinology 1989;124: 1638–45.

[129] Holt JA, Wittmaack FM, Schreiber JR, Ghosh DK, Menon KM. Estrogen increases precursor for pregnenolone synthesis with temperature-sensitive occupancy of P-450scc in mitochondria of rabbit corpus luteum. Endocrinology 1988;122:1948–57.
[130] Katsumata N, Ohtake M, Hojo T, Ogawa E, Hara T, Sato N, et al. Compound heterozygous mutations in the cholesterol side-chain cleavage enzyme gene (CYP11A) cause congenital adrenal insufficiency in humans. J Clin Endocrinol Metab 2002;87:3808–13.
[131] Tajima T, Fujieda K, Kouda N, Nakae J, Miller WL. Heterozygous mutation in the cholesterol side chain cleavage enzyme (p450scc) gene in a patient with 46,XY sex reversal and adrenal insufficiency. J Clin Endocrinol Metab 2001;86:3820–5.
[132] Hu MC, Hsu NC, El Hadj NB, Pai CI, Chu HP, Wang CK, et al. Steroid deficiency syndromes in mice with targeted disruption of Cyp11a1. Mol Endocrinol 2002;16:1943–50.
[133] Luu-The V, Lachance Y, Labrie C, Leblanc G, Thomas JL, Strickler RC, et al. Full length cDNA structure and deduced amino acid sequence of human 3 beta-hydroxy-5-ene steroid dehydrogenase. Mol Endocrinol 1989;3:1310–2.
[134] Riley SC, Dupont E, Walton JC, Luu-The V, Labrie F, Pelletier G, et al. Immunohistochemical localization of 3 beta-hydroxy-5-ene-steroid dehydrogenase/delta 5-delta 4 isomerase in human placenta and fetal membranes throughout gestation. J Clin Endocrinol Metab 1992;75:956–61.
[135] Falany CN, Vazquez ME, Kalb JM. Purification and characterization of human liver dehydroepiandrosterone sulphotransferase. Biochem J 1989;260:641–6.
[136] Falany CN, Comer KA, Dooley TP, Glatt H. Human dehydroepiandrosterone sulfotransferase. Purification, molecular cloning, and characterization. Ann N Y Acad Sci 1995;774:59–72.
[137] Falany CN, Wheeler J, Oh TS, Falany JL. Steroid sulfation by expressed human cytosolic sulfotransferases. J Steroid Biochem Mol Biol 1994;48:369–75.
[138] Rainey WE, Carr BR, Sasano H, Suzuki T, Mason JI. Dissecting human adrenal androgen production. Trends Endocrinol Metab 2002;13:234–9.
[139] Meloche CA, Falany CN. Expression and characterization of the human 3 beta-hydroxysteroid sulfotransferases (SULT2B1a and SULT2B1b). J Steroid Biochem Mol Biol 2001;77:261–9.
[140] Miki Y, Nakata T, Suzuki T, Darnel AD, Moriya T, Kaneko C, et al. Systemic distribution of steroid sulfatase and estrogen sulfotransferase in human adult and fetal tissues. J Clin Endocrinol Metab 2002;87:5760–8.
[141] Auchus RJ, Lee TC, Miller WL. Cytochrome b5 augments the 17,20-lyase activity of human P450c17 without direct electron transfer. J Biol Chem 1998;273:3158–65.
[142] Kominami S, Ogawa N, Morimune R, De-Ying H, Takemori S. The role of cytochrome b5 in adrenal microsomal steroidogenesis. J Steroid Biochem Mol Biol 1992;42:57–64.
[143] Geller DH, Auchus RJ, Miller WL. P450c17 mutations R347H and R358Q selectively disrupt 17,20-lyase activity by disrupting interactions with P450 oxidoreductase and cytochrome b5. Mol Endocrinol 1999;13:167–75.
[144] Auchus RJ. The genetics, pathophysiology, and management of human deficiencies of P450c17. Endocrinol Metab Clin North Am 2001;30:101–19.
[145] Geller DH, Auchus RJ, Mendonca BB, Miller WL. The genetic and functional basis of isolated 17,20-lyase deficiency. Nat Genet 1997;17:201–5.
[146] Costa-Santos M, Kater CE, Dias EP, Auchus RJ. Two intronic mutations cause 17-hydroxylase deficiency by disrupting splice acceptor sites: direct demonstration of aberrant splicing and absent enzyme activity by expression of the entire CYP17 gene in HEK-293 cells. J Clin Endocrinol Metab 2004;89:43–8.
[147] Costa-Santos M, Kater CE, Auchus RJ. Two prevalent CYP17 mutations and genotype-phenotype correlations in 24 Brazilian patients with 17-hydroxylase deficiency. J Clin Endocrinol Metab 2004;89:49–60.
[148] Pion R, Jaffe R, Eriksson G, Wiqvist N, Diczfalusy E. Studies on the metabolism of C-21 steroids in the human foeto-placental unit. I. Formation of a beta-unsaturated 3-ketones in midterm placentas perfused in situ with pregnenolone and 17-alpha-hydroxypregnenolone. Acta Endocrinol (Copenh) 1965;48:234–48.
[149] Strauss III JF, Barbieri RL. Yen and Jaffe's reproductive endocrinology. Fifth Edition. Philadelphia: Elsevier Saunders; 2004. p. 125–54, 327–66.

[150] Ugele B, St-Pierre MV, Pihusch M, Bahn A, Hantschmann P. Characterization and identification of steroid sulfate transporters of human placenta. Am J Physiol Endocrinol Metab 2003;284:E390–8.
[151] Bonifas JM, Epstein Jr EH. Detection of carriers for X-linked ichthyosis by southern blot analysis and identification of one family with a de novo mutation. J Invest Dermatol 1990;95: 16–9.
[152] Bradshaw KD, Carr BR. Placental sulfatase deficiency: maternal and fetal expression of steroid sulfatase deficiency and X-linked ichthyosis. Obstet Gynecol Surv 1986;41:401–13.
[153] Bonifas JM, Morley BJ, Oakey RE, Kan YW, Epstein Jr EH. Cloning of a cDNA for steroid sulfatase: frequent occurrence of gene deletions in patients with recessive X chromosome-linked ichthyosis. Proc Natl Acad Sci USA 1987;84:9248–51.
[154] Epstein Jr EH, Bonifas JM. Recessive X-linked ichthyosis: lack of immunologically detectable steroid sulfatase enzyme protein. Hum Genet 1985;71:201–5.
[155] Epstein Jr EH, Bonifas JM, Barber TC, Haynes M. Cholesterol sulfotransferase of newborn mouse epidermis. J Invest Dermatol 1984;83:332–5.
[156] Elias PM, Williams ML, Maloney ME, Bonifas JA, Brown BE, Grayson S, et al. Stratum corneum lipids in disorders of cornification. Steroid sulfatase and cholesterol sulfate in normal desquamation and the pathogenesis of recessive X-linked ichthyosis. J Clin Invest 1984; 74:1414–21.
[157] Epstein Jr EH, Bonifas JM. Glucocorticoid receptors of normal human epidermis. J Invest Dermatol 1982;78:144–6.
[158] Kashork CD, Sutton VR, Fonda Allen JS, Schmidt DE, Likhite ML, Potocki L, et al. Low or absent unconjugated estriol in pregnancy: an indicator for steroid sulfatase deficiency detectable by fluorescence in situ hybridization and biochemical analysis. Prenat Diagn 2002; 22:1028–32.
[159] Kragie L. Aromatase in primate pregnancy: a review. Endocr Res 2002;28:121–8.
[160] Cantineau R, Kremers P, De Graeve J, Gielen JE, Lambotte R. Aromatization of 15 alpha and 16 alpha hydroxylated androgens in the human placental using [1,2–3H]-substrates. J Steroid Biochem 1982;16:157–63.
[161] Cantineau R, Kremers P, De Graeve J, Gielen JE, Lambotte R. 15- and 16-hydroxylations of androgens and estrogens in the human fetal liver: a critical step in estetrol biosynthesis. J Steroid Biochem 1985;22:195–201.
[162] Kitada M, Kamataki T, Itahashi K, Rikihisa T, Kanakubo Y. P-450 HFLa, a form of cytochrome P-450 purified from human fetal livers, is the 16 alpha-hydroxylase of dehydroepiandrosterone 3-sulfate. J Biol Chem 1987;262:13534–7.
[163] Lacroix D, Sonnier M, Moncion A, Cheron G, Cresteil T. Expression of CYP3A in the human liver–evidence that the shift between CYP3A7 and CYP3A4 occurs immediately after birth. Eur J Biochem 1997;247:625–34.
[164] Sucheston ME, Cannon MS. Microscopic comparison of the normal and anencephalic human adrenal gland with emphasis on the transient-zone. Obstet Gynecol 1970;35:544–53.
[165] Benirschke K. Adrenals in anencephaly and hydrocephaly. Obstet Gynecol 1956;8:412–25.
[166] Gray ES, Abramovich DR. Morphologic features of the anencephalic adrenal gland in early pregnancy. Am J Obstet Gynecol 1980;137:491–5.
[167] DeGroot LJ, Jameson JL, text editors. Endocrinology. In: Strauss III JF, Parry S, series editors. Placental hormones. Philadelphia: W.B. Saunders; 2000.
[168] Coulter CL, Goldsmith PC, Mesiano S, Voytek CC, Martin MC, Mason JI, et al. Functional maturation of the primate fetal adrenal in vivo. II. Ontogeny of corticosteroid synthesis is dependent upon specific zonal expression of 3 beta-hydroxysteroid dehydrogenase/isomerase. Endocrinology 1996;137:4953–9.
[169] Parker Jr CR, Faye-Petersen O, Stankovic AK, Mason JI, Grizzle WE. Immunohistochemical evaluation of the cellular localization and ontogeny of 3 beta-hydroxysteroid dehydrogenase/delta 5–4 isomerase in the human fetal adrenal gland. Endocr Res 1995;21:69–80.
[170] Mason JI, Ushijima K, Doody KM, Nagai K, Naville D, Head JR, et al. Regulation of

expression of the 3 beta-hydroxysteroid dehydrogenases of human placenta and fetal adrenal. J Steroid Biochem Mol Biol 1993;47:151–9.
[171] Pezzi V, Mathis JM, Rainey WE, Carr BR. Profiling transcript levels for steroidogenic enzymes in fetal tissues. J Steroid Biochem Mol Biol 2003;87:181–9.
[172] Goto M, Brickwood S, Wilson DI, Wood PJ, Mason JI, Hanley NA. Steroidogenic enzyme expression within the adrenal cortex during early human gestation. Endocr Res 2002;28:641–5.
[173] Macnaughton MC, Taylor T, McNally EM, Coutts JR. The effect of synthetic ACTH on the metabolism of [4–14C]-progesterone by the previable human fetus. J Steroid Biochem 1977;8:499–504.
[174] Loganath A, Peh KL, Wong PC. Evidence for the biosynthesis of DHEA from cholesterol by first-trimester human placental tissue: source of androgens. Horm Metab Res 2002;34: 116–20.
[175] Michael AE, Thurston LM, Rae MT. Glucocorticoid metabolism and reproduction: a tale of two enzymes. Reproduction 2003;126:425–41.

ELSEVIER
SAUNDERS

Obstet Gynecol Clin N Am
31 (2004) 817–835

OBSTETRICS AND
GYNECOLOGY
CLINICS
OF NORTH AMERICA

Fetal and maternal adrenals in human pregnancy

William E. Rainey, PhD*, Khurram S. Rehman, MD, Bruce R. Carr, MD

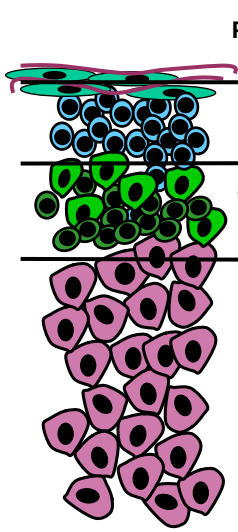
Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75235-9032, USA

Structure of the fetal adrenal

The human fetal adrenal gland is structurally quite different from its adult counterpart. The human fetal adrenal's most distinctive feature, the histologically unique fetal zone, was described first by Elliott and Armour [1] in 1911. After the first trimester, the centrally located fetal zone accounts for most of the fetal adrenal mass and acts to provide steroid precursors that are used by the placenta to produce estrogens (Fig. 1). The outer zone of the fetal adrenal is called the "definitive zone" or neocortex; this zone likely gives rise to the adult adrenal glomerulosa. A third zone called the "transitional zone" recently was identified using immunohistochemistry. It lies just between the neocortex and fetal zone and is believed to develop into the cortisol-producing zona fasciculata [2,3]. The definitive and transitional zones become active only late in gestation and then play a critical role in the neonate as the glomerulosa and fasciculata zones of the postnatal adrenal. Conversely, the fetal zone begins a process of involution that commences immediately after birth. The weight of the adrenal glands decreases strikingly during the first few weeks of life; the size that is attained by the fetal glands just before birth is not achieved again until late in adolescence (Fig. 2).

* Corresponding author.
E-mail address: william.rainey@utsouthwestern.edu (W.E. Rainey).

0889-8545/04/$ – see front matter
doi:10.1016/j.ogc.2004.08.006 obgyn.theclinics.com



Fetal adrenal zones	Steroid products	Fetal functions
Definitive zone	Aldosterone	Initiated late in gestation to control mineral balance in the neonate
Transitional zone	Cortisol	Increases in late gestation to promote organ maturation and assist in timing of labor
Fetal zone	DHEA-S and Preg-S	Precursor for placental estrogen production. Late in gestation the increasing production of DHEA-S acts to influence the estrogen to progesterone ratio.

Fig. 1. Human fetal adrenal functional zonation. Shown are the three putative fetal adrenal zones, which have been characterized based on immunohistochemical analysis, with their primary steroid products and potential fetal functions. Dehydroepiandrosterone-sulfate (DHEA-S). Pregnenolone-sulfate (Preg-S).

Development of the fetal adrenal

The fetal adrenal cortex develops from common adrenogonadal stem cells that also give rise to the steroid-producing cells of the gonads. The cells that make up the early adrenal cortex can be identified in the 8-mm embryo. The fetal adrenal gland undergoes significant growth as the fetal zone increases in size such that by 18 weeks' gestation the gland is approaching the size of the kidney (see Fig. 2). The fetal zone continues to enlarge, particularly during the last 6 weeks of gestation. This results in a highly active steroidogenic organ that can produce as much as 200 mg of steroid per day; this is considerably more than an adult adrenal at rest can produce. The large proportional size of the adrenal rapidly changes after birth with involution of the transient fetal zone, such that more than 50% of the gland's size is lost over the first months of neonatal life (see Fig. 2).

Steroidogenic enzymes in the fetal adrenal

Within the fetal adrenal, steroidogenic function and zonation are different from the adult (see Fig. 1). The principal difference is the presence of a histologically distinct fetal zone [4,5] which accounts for the bulk of the human fetal adrenal gland (85%) during the second and third trimesters and is the source for steroid precursors that are used by the placenta to produce estrogens. The transient fetal zone is not present in most mammals; it seems to be unique to humans and a few nonhuman primates [4,5]. Functionally, the fetal zone is similar in many ways to

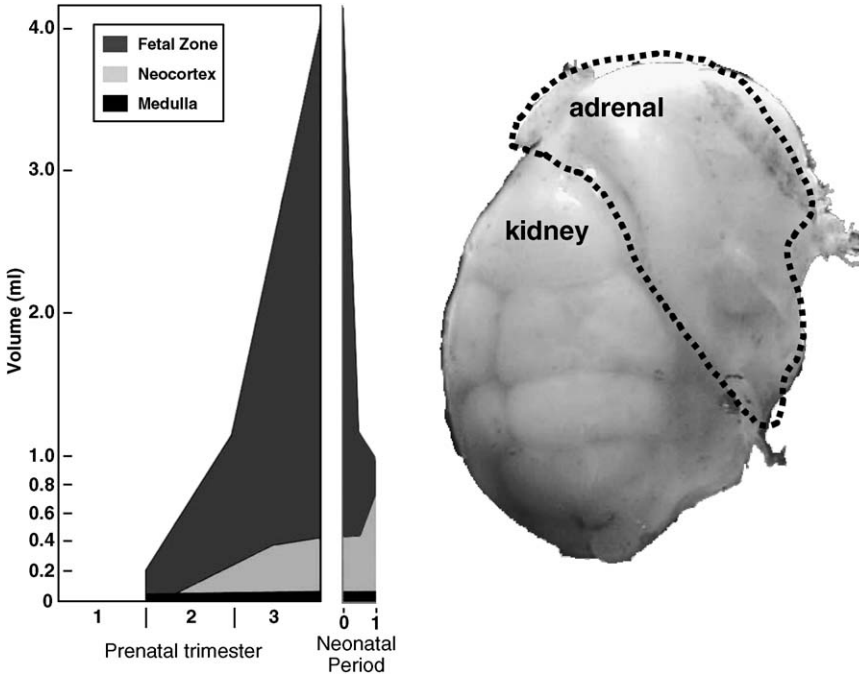


Fig. 2. (*Left panel*) Size of the adrenal gland and its component parts in utero, during infancy, and during childhood. (*Adapted from* Bethune JE. The adrenal cortex: a scope monograph. Kalamazoo (MI): Upjohn; 1974. p. 11). (*Right Panel*) Photograph of an 18-week fetal kidney with adrenal.

the adult zona reticularis. The outer zone—the definitive zone (or neocortex)—is believed to give rise to the postnatal adrenal glomerulosa. A third transitional zone, between the fetal zone and neocortex, is believed to give rise to the postnatal zona fasciculata.

Recent studies that used immunohistochemistry defined zonal differences in fetal adrenal expression of steroidogenic enzymes [2,3,6]. These patterns of enzyme expression provided important clues into the role of each zone in fetal adrenal steroidogenesis (Fig. 3). The definitive zone expresses the enzymes that are needed for the production of aldosterone but does not express 17α-hydroxylase or dehydroepiandrosterone (DHEA)-sulfotransferase (SULT2A1). During the second half of gestation, the transitional zone expresses 17α-hydroxylase and 3β-hydroxysteroid dehydrogenase type II (HSD3B2) which allows production of cortisol. HSD3B2 is the enzyme that is needed for the conversion of Δ5 to Δ4 steroids, specifically pregnenolone and 17α-hydroxypregnenolone to progesterone and 17α-hydroxyprogesterone, respectively. During most of gestation, the fetal adrenal lacks HSD3B2; this prevents cortisol and aldosterone synthesis and directs steroid production toward dehydroepiandrosterone-sulfate (DHEA-S) production [7,8]. Increased expression of SULT2A1 in the fetal zone accounts for sulfonation of most of the Δ5 steroids that are produced, including DHEA

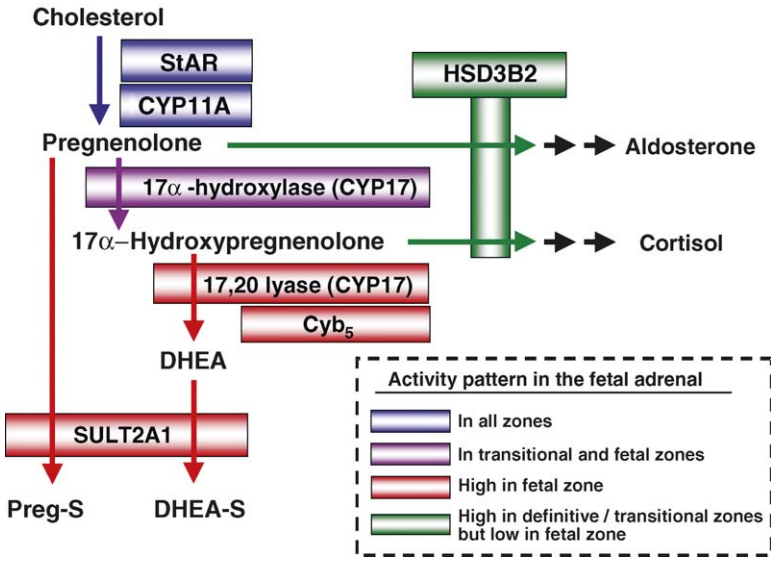


Fig. 3. Variations in the expression of steroidogenic enzymes (or enzymatic activity for 17α-hydroxylase 17,20 lyase) within the fetal adrenal zones and the impact on steroids produced. The production of all adrenal steroids relies on steroidogenic acute regulatory (StAR) protein and cholesterol side-chain cleavage enzyme (CYP11A), which are expressed throughout the fetal adrenal gland. Production of cortisol and mineralocorticoids requires the expression of HSD3B2; this enzymes is found in the definitive and transitional zones of the fetal adrenal. Steroid 17α-hydroxylase (CYP17) is required for DHEA and cortisol biosynthesis and its activity is found in the transitional zone and the fetal zone. The 17,20-lyase activity of CYP17 is higher in the fetal zone cells as is DHEA-sulfotransferase (SULT2A1). Also positively impacting DHEAS biosynthesis is cytochrome b_5, which enhances the 17,20-lyase activity of CYP17. Negatively impacting the production of DHEA-S is HSD3B2, which is absent in the fetal zone. 16-OH DHEA-S, 16α-hydroxydehydroepiandrosterone–sulfate.

and pregnenolone. These observations point to the regulation of steroidogenic enzymes which are critical determinants in modulating the biosynthetic capacity and the steroid products of the fetal adrenal gland.

As is the case for all steroid hormones, the precursor for fetal adrenal steroid production is cholesterol. To provide the necessary cholesterol, the fetal adrenal glands have a highly developed ability to bind and use cholesterol that is carried by low-density lipoproteins (LDL). Use of LDL for steroidogenesis by the fetal adrenal would represent a quarter of the total daily LDL cholesterol turnover in adults. The fetal adrenal glands can synthesize cholesterol from two-carbon fragments (ie, acetate). All enzymes that are involved in cholesterol biosynthesis are elevated compared with the adult adrenal gland [9]. Thus, the rate of de novo cholesterol synthesis by fetal adrenal tissue is extremely high; however, it is insufficient to account for the steroids that are produced by these glands. Therefore, the fetal adrenal relies on both cholesterol from the fetal circulation and produced within the gland.

Role of adrenocorticotropic hormone in fetal adrenal regulation

Corticotropin plays a primary role in the development and function of the fetal zone of the human fetal adrenal. Corticotropin is known to promote hypertrophy and production of DHEA-S and cortisol by fetal adrenal cells in vitro. Further, Mesiano and colleagues [3] showed that fetal adrenal cells respond to corticotropin by secreting growth factors that seem to stimulate hyperplasia of the fetal zone.

Taken together, these data indicate that corticotropin is needed for fetal adrenal DHEA production. It is likely, however that other factors are necessary to stimulate the massive amount of fetal adrenal steroidogenesis, particularly during the last months of gestation. The fact that corticotropin levels do not increase significantly during the last trimester of gestation [10] makes it likely that growth and differentiation of the fetal adrenal glands are influenced by placenta-derived factors during this time. This hypothesis is supported by the observation that the fetal zone of the adrenal undergoes rapid involution immediately after birth when placenta-derived factors are no longer available (see Fig. 2).

Role of placental corticotropin-releasing hormone in fetal adrenal regulation

There is now evidence that placental corticotropin-releasing hormone (CRH) may regulate the increase in fetal adrenal steroidogenesis during the last weeks of gestation. The ability of CRH to stimulate human fetal adrenal cells directly to produce cortisol and DHEA-S is well-documented [11–14]. The concentrations that are needed to increase fetal adrenal cell steroid production are within the physiologic range that is seen in the fetal circulation [11–14]. The 41–amino acid peptide, CRH, was the first hypothalamic-releasing factor to be characterized; it controls corticotropin release by the anterior pituitary, and thus, cortisol and DHEA-S secretion by the adrenal cortex. By reflecting the ‘‘set point’’ of glucocorticoid negative feedback at the hypothalamic level, CRH secretion defines the canonic endocrine negative feedback loop of the hypothalamic–pituitary–adrenal axis for glucocorticoid production. The circulating CRH that is seen in pregnancy is identical to maternal and fetal hypothalamic CRH but is synthesized in the placenta in large amounts [15,16]. Unlike hypothalamic CRH, which is under the control of glucocorticoid negative feedback, cortisol stimulated placental CRH production in vitro and in vivo in humans and other primates [17–19]. The ability of cortisol to stimulate placental CRH makes it possible to create a feed-forward endocrine cascade that does not end until separation of the fetus from the placenta at delivery (Fig. 4). This positive feedback cascade was proposed to drive the increase in CRH as well as fetal adrenal steroidogenesis in late gestation.

Maternal plasma CRH levels are decreased in the first trimester and increase from midgestation to term. In the last 12 weeks of gestation, CRH plasma levels increase considerably, peak during labor, and decrease precipitously after delivery [20,21]. Umbilical cord blood levels and amniotic fluid levels of CRH are increased similarly in late gestation [22]. Fetal CRH levels are lower than those in

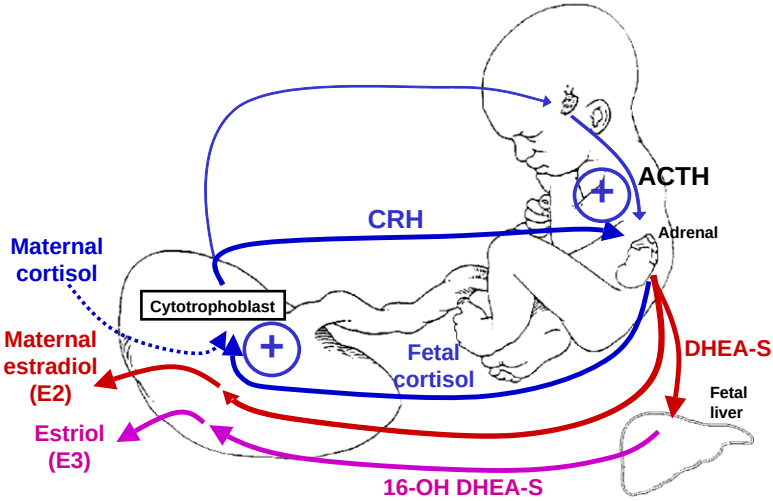


Fig. 4. The placental/fetal adrenal endocrine cascade.

maternal circulation (50 versus 1000 picomolar (pM)), but are substantial compared with levels in men and nonpregnant women. CRH is the only trophic hormone/releasing factor to have a specific serum-binding protein. During most of pregnancy, that CRH-binding protein (CRH-BP) seems to bind most of the circulating CRH in the fetal and maternal compartment; this likely serves to control the activity of placental CRH tightly [23]. During the latter stages of pregnancy, CRH-BP levels decrease in maternal plasma and in amniotic fluid, whereas CRH levels increase; this gives rise to markedly increased levels of bioavailable CRH [24].

In pregnancies in which the fetus can be considered to be stressed as a result of various complications, the concentrations of CRH in fetal plasma, amniotic fluid, and in maternal plasma are increased over those seen in a normal gestation [25–28]. The placenta is the likely source for stress-associated increases in CRH; the placental content of CRH was four-fold higher in placentas from women who had preeclampsia compared with those from normal pregnancies. Moreover, the biologic impact of increased CRH levels is likely to be amplified in such instances as a result of subnormal levels of CRH-BP [29,30]. Such increases in placental CRH production during normal gestation and the excessive secretion of placental CRH in complicated pregnancies may play a role in the normal gestational increases in fetal adrenal cortisol synthesis [31] and the supranormal levels of cortisol in umbilical cord blood that occurs in stressed infants [25,32,33].

Fetal adrenal contributions to placental estrogen biosynthesis

It has long been known that women who are pregnant with an anencephalic fetus have much lower levels of circulating estrogens, particularly estriol. This,

together with the finding of increased levels of DHEA-S in cord blood of normal newborns, suggested that the fetal adrenal cortex is the principal source of placental estrogen precursors. This concept of a adrenal/placental endocrine axis was further confirmed when it was shown that radiolabeled DHEA-S could be converted to estradiol when perfused through the placenta [34,35]. Near term, about half of the estradiol that is produced in the placenta arises from maternal plasma DHEA-S, whereas half comes from fetal plasma DHEA-S [36]. Taken with more recent studies, the steroidogenic pathways that are involved in fetal adrenal contribution of substrate and placental metabolism to estrogens is now established (Fig. 5).

Estrogen products that are released from the placenta are dependent on the nature of the substrates that are available. Estradiol is but one placental estrogen secretory product and is the primary estrogen that circulates at term. In addition, however, significant levels of estriol and estetrol are found in the maternal circulation and increase particularly late in gestation. The role of the different estrogens in the physiology of pregnancy are not clear; however, the possibility of selective effects on the two known estrogen receptors has been suggested.

The hydroxylated forms of estrogen (estriol and estetrol) are produced in the placenta by using substrates that are produced by the combined efforts of the fetal adrenal gland and liver (see Fig. 5). Some 40 years ago, it was demonstrated that the placenta could synthesize estriol from α-hydroxylated C_{19}-steroids, particularly 16α-hydroxydehydroepiandrosterone [37,38]. Circulating levels of these hydroxylated steroids is increased [39] and explains why there is a disproportionate increase in estriol formation during pregnancy; as the fetal adrenal

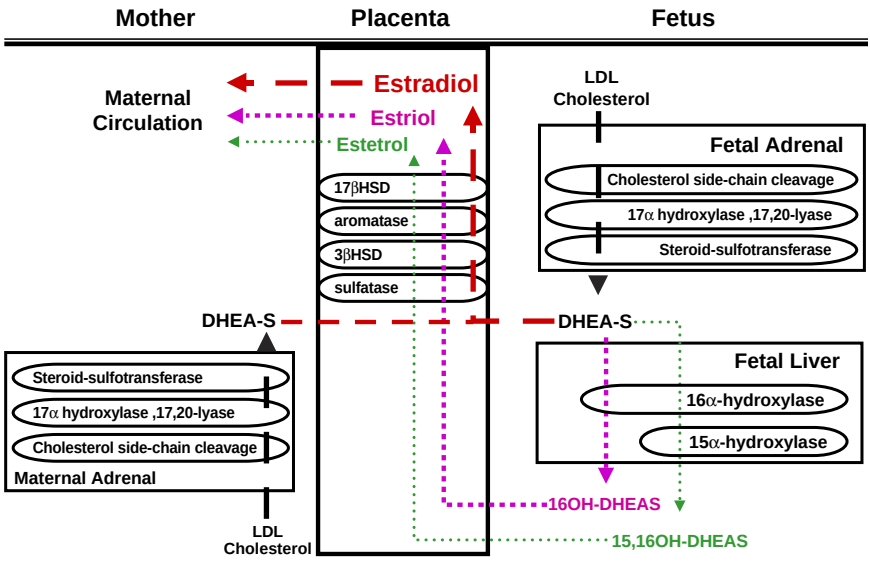


Fig. 5. Biosynthesis of estrogen in the human placenta.

enlarges, it provides the unique substrate for placental metabolism (see Fig. 5). The fetus, but not the mother, also produces significant amounts of 15α,16α-dihydroxyDHEA–S, which can be used by trophoblasts to produce estetrol. Because maternal estriol and estetrol are produced by steroid precursors that are produced almost solely in the fetus, measurements of these steroids have been used as indicators of fetal well-being.

Fetal adrenal/placental steroids as regulators of parturition

The origin for the signals that control the onset of human labor remains controversial, in contrast to animal models (eg, sheep). In humans, there are two potential contributions of the fetal adrenal to the timing of parturition. First, the initiation of fetal adrenal cortisol, which occurs late in gestation, may act in the feed-forward cascade that involves placental CRH. Some investigators proposed that the increasing level of CRH at the end of gestation reflects a ''fetal/placental clock'' (ie, that the human placenta and fetus, through endocrine events, determine the timing of parturition at the end of the normal gestational term) [40,41]. Second, the increasing levels of fetal adrenal production of DHEA-S (and its conversion to estrogens) were proposed to help time parturition. The increasing estrogen levels are believed to induce the expression of contraction-associated proteins within the myometrium, thus preparing this tissue for labor. This hypothesis is supported by the observation that women who have steroid sulfatase deficiency, which blocks placental use of sulfated precursors, have prolonged pregnancies [42].

Defects that affect the fetal adrenal/placental endocrine system

Congenital adrenal hypoplasia

Fetal adrenal cortical hypoplasia is rare and occurs in 1 in 12,500 births [43]. There seem to be several forms of adrenal hypoplasia. The miniature adult form is thus named because of the small cortical zone that is seen on histologic examination. Most of these cases result from anencephaly or abnormal pituitary function. In the absence of corticotropin, as in anencephaly, the fetal zone of the adrenal cortex is severely underdeveloped and the rate of formation of placental estrogens (especially estriol) is severely limited because of diminished availability of C_{19}-steroid precursors. Verification of the reduced precursor levels in anencephalic fetuses was provided by the finding of decreased levels of DHEA-S in cord blood of such newborns [44]. Therefore, almost all of the estrogens that are produced in women who are pregnant with an anencephalic fetus arise by the placental use of maternal plasma DHEA-S. Furthermore, in such pregnancies, the production of estrogens can be increased by the maternal administration of corticotropin, which stimulates the rate of DHEA-S secretion by the maternal adrenal. corticotropin does not cross the placenta, and thus, there

is no fetal adrenal stimulation. Finally, placental estrogen production is decreased in women who are pregnant with an anencephalic fetus when a potent glucocorticoid is given to the mother. This suppresses corticotropin secretion, and thus, decreases the rate of DHEA-S secretion from the maternal adrenal cortex [38,45]. Estriol formation is decreased disproportionately in pregnancies with an anencephalic fetus because the fetal adrenal, at term, normally provides 90% of placental estriol precursor. A similar phenotype seems to occur if the fetus has inactivating mutations in the corticotropin receptor (termed MC2R) [46].

The cytomegalic form of adrenal hypoplasia is so-called because these adrenals, although without the normal fetal adrenal structure, have nodular formation of eosinophilic fetal zone cells. Estrogen formation, particularly estriol, in pregnancies with such a fetus is limited, and suggests the absence of fetal adrenal C_{19}-precursors for placental estrogen formation. The cytomegalic form results from disruptive mutations in the gene that is known as DAX-1 (dosage-sensitive sex reversal-adrenal hypoplasia congenita critical region on the X chromosome, gene 1) [43].

Congenital adrenal hyperplasia

Congenital adrenal hyperplasia is the most frequent cause of ambiguous genitalia and adrenal insufficiency in newborn infants (see the article by Sobel and Imperato-McGinley elsewhere in this issue). These disorders result from inherited enzyme defects of cortisol biosynthesis. In these disorders, the enzymatic block results in decreased circulating levels of cortisol and, as a result, excessive fetal secretion of corticotropin. Increases in corticotropin secretion result in further stimulation of adrenal steroidogenesis and excessive outpouring of adrenal androgens and mineralocorticoids. This leads to an increase in size of the fetal adrenal gland, hence the name ‘‘congenital adrenal hyperplasia.’’ Congenital adrenal hyperplasia may result from the deficiency of steroidogenic acute regulatory (StAR) protein, cholesterol side chain cleavage (CYP11A), HSD3B2, 17α-hydroxylase (CYP17), 21-hydroxylase (CYP21), or 11-hydroxylase (CYP11B1). In the female fetus, deficiency of HSD3B2, CYP21, and CYP11B1 are associated with excessive adrenal androgen production and masculinization of the external genitalia and lead to congenital sexual ambiguity. In addition, these deficiencies can be life-threatening after birth, particularly if associated with salt wasting. Enzyme deficiencies that result in congenital adrenal hyperplasia are autosomal recessive disorders. In each offspring, there is a 25% chance of having the condition and a 50% chance of being a carrier.

Of the various enzymatic deficiencies, CYP21 deficiency is the most common enzyme defect; it accounts for more than 90% of patients who have classic adrenal hyperplasia. The enzyme block results in a decrease in production of 11-deoxycortisol and cortisol, an increased level of corticotropin, and secondary increases in the precursor steroid hormone levels before the block. This causes an increased production of adrenal androgens, which leads to masculinization of the external genitalia. The diagnosis is made by measurement of 17-hydroxypro-

gesterone in blood of the newborn infant and treatment consists of glucocorticoids. Recently, it was shown that if the diagnosis of adrenal hyperplasia due to CYP21 deficiency is confirmed early in fetal life, prenatal dexamethasone treatment of the mother can prevent the development of fetal adrenal hyperplasia by inhibiting fetal corticotropin and preventing sexual ambiguity. If a previous sibling was affected with CYP21 deficiency, early genetic testing by chorionic villus sampling is indicated. Glucocorticoid therapy is started as soon as diagnosed. If the chorionic villus sampling suggests that the infant is a female and CYP21 deficiency is confirmed by DNA testing, then dexamethasone therapy is continued throughout the remainder of pregnancy to prevent masculinization. If the fetus is a male (even if affected), dexamethasone can be discontinued during pregnancy. After birth, in addition to glucocorticoid steroid therapy, mineralocorticoid replacement therapy may be indicated in newborns that have CYP21 deficiency with coexistent salt-wasting. Newborn female infants who are not treated with dexamethasone in utero who virilize will require surgical reconstruction of the genitalia. After surgery, these individuals will experience normal vaginal intercourse and can ovulate and conceive. The earlier that therapy is initiated, the more likely the patient will be ovulatory and fertile. CYP21 deficiency is an autosomal recessive condition that is linked to the HLA complex. The gene for CYP21 is located in the major HLA complex on the short arm of chromosome 6; 85% of the cases of CYP21 deficiency result from genetic recombination at this locus [47].

In addition to CYP21 deficiency, female infants who have CYP11B1 deficiency and HSD3B2 deficiency are born with varying degrees of sexual ambiguity, depending on the severity of the enzymatic defect [48,49]. A CYP17 deficiency usually results in a female phenotype in both sexes, because there is an absence of androgen secretion from the adrenal as well as gonadal secretion. Finally, lipoid adrenal hyperplasia results from gene mutations that block cholesterol conversion to pregnenolone. In this disease, there is no steroid production by gonads or adrenals; thus, all effected individuals are born with a female phenotype and have salt-wasting. Mutations in StAR protein and cholesterol-side chain cleavage caused lipoid congenital adrenal hyperplasia [50].

Placental enzymatic deficiencies

In a normal pregnancy, there is no apparent rate-limiting enzymatic reaction in the placental pathway from C_{19}-steroids to estrogen biosynthesis (see Fig. 5); however, mutations in steroid sulfatase or aromatase (CYP19) can block metabolism of fetal adrenal-derived steroid precursors and cause decreased placental synthesis of estrogens. There are a few well-documented examples of aromatase deficiency [51]. Fetal adrenal DHEA-S is converted by the placenta to androstenedione in its metabolic pathway to estrogen (see Fig. 5); however, in cases of placental aromatase deficiency, androstenedione cannot be converted to estradiol but is secreted into the maternal or fetal circulations and causes virilization of the mother and the female fetus [52,53]. Pregnancies with a male

fetus that has aromatase deficiency can proceed normally; however, the estrogen-deficient males have delayed epiphyseal closure during puberty [54].

France and Liggins [55] were the first to establish steroid sulfatase deficiency as a cause of decreased estrogen levels in otherwise normal pregnancies. Sulfatase deficiency precludes the hydrolysis of C_{19}-steroid sulfates, the first enzymatic step in the placental use of these circulating precursors for estrogen biosynthesis (see Fig. 5). As expected, maternal plasma estriol levels are reduced severely, with a lesser reduction in estradiol levels. This is an X-linked recessive disorder and all affected fetuses are male. It occurs at an estimated frequency of 1 in 2000 to 5000 births and has been associated with delayed onset of labor. It also is associated with the development of ichthyosis—a scaling skin disorder—in affected males later in life [42]. The pathologic scaling is associated with accumulation of abnormal quantities of cholesterol sulfate in the outer epidermis that is due to abnormal expression of steroid sulfatase enzyme in the skin. Thus, the original description of ''placental sulfatase deficiency'' is a misnomer.

The maternal adrenal gland

Morphologic alterations

The human fetal adrenal gland undergoes marked morphologic changes during pregnancy and postpartum, as well as alterations in pathways of steroidogenesis. In marked contrast, the maternal adrenal gland undergoes minimal changes during the course of pregnancy. Thus, there is minimal change in weight and size of the adult adrenal gland; however, a modest increase in the size of the zona fasciculata was reported. This suggested that adrenocortical secretions may play a role in the physiologic changes that occur in women during pregnancy [37]. Although there are minimal changes in the structural parts of the adrenal gland, there are changes in maternal levels of glucocorticoids and mineralocorticoid blood levels during pregnancy.

Glucocorticoid hormones

During pregnancy, there is a three-fold to eight-fold increase in the levels of total cortisol (Fig. 6). The levels of cortisol are believed to increase primarily as a result of an increase in the corticosteroid-binding globulin (CBG) [56,57]. CBG is the primary carrier of cortisol in blood and is a protein that is synthesized in the liver and influenced by estrogen levels. A similar increase in CBG is seen in women who are on estrogen therapy, particularly oral contraceptive pills [58]. A modest increase in free cortisol (nonprotein-bound) concentration takes place in pregnancy and parallels the increase in total cortisol (see Fig. 6; Fig. 7) [59,60]. Although total and free cortisol levels are increased to concentrations that are observed in nonpregnant women who have Cushing's syndrome, there is little

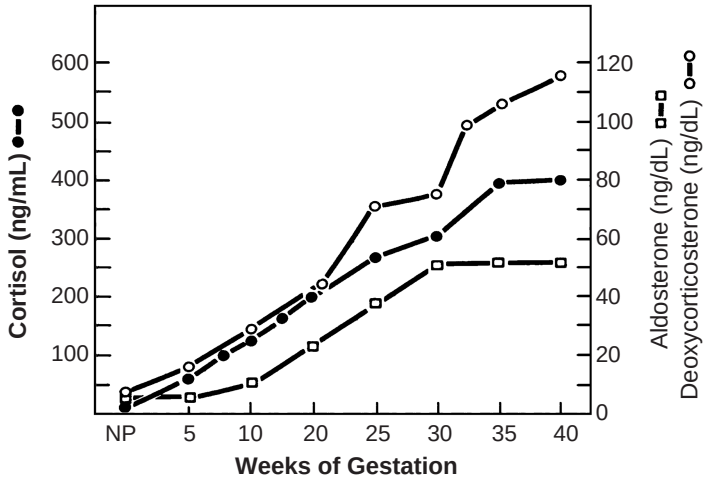


Fig. 6. Mean concentrations of cortisol, aldosterone, and deoxycorticosterone in sera of women throughout normal pregnancy. NP, nonpregnant value. (*From* Carr BR. The endocrinology of pregnancy: the maternal–fetal placental unit. In: Becker KL, editor. Principles and practice of endocrinology and metabolism. Philadelphia: JB Lippincott; 1990. p. 887; with permission.)

clinical evidence for hypercortisolism during pregnancy, nor is there a significant change in the diurnal rhythm of cortisol [61].

If one considers the marked changes in maternal cortisol levels during pregnancy, one would anticipate that corticotropin levels would be suppressed; however, corticotropin levels increase in maternal plasma throughout gestation

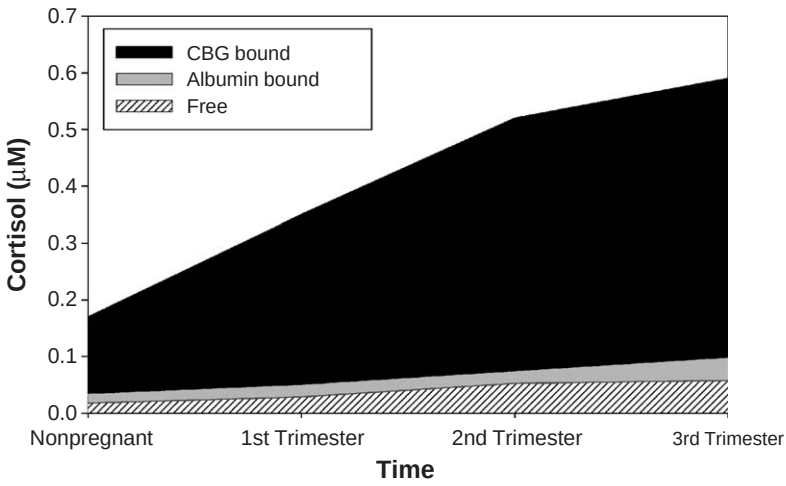


Fig. 7. Absolute distribution of bound and free cortisol in pregnant plasma. (*Adapted from* Rosenthal HE, Slaunwhite WR, Sandberg AA. Transcortin: a corticosteroid-binding protein of plasma. J Clin Endocrinol Metab 1969; 29:352; with permission.)

[62,63]. The relationship to maternal corticotropin and cortisol levels also may be related to CRH, which is synthesized by the placenta.

Mineralocorticoids

As is true for glucocorticoids, mineralocorticoid concentrations increase during pregnancy (see Fig. 6) [64]. Aldosterone increases markedly during pregnancy as a result of an increase in the activation of the renin-angiotensin system [57]. Renin substrate (angiotensinogen), which is secreted by the liver, is enhanced by the elevated levels of estrogen during pregnancy, as is true for CBG. This results in an increase of plasma renin activity that promotes increased angiotensin II levels, which stimulate aldosterone secretion by the maternal adrenal gland. As was true for cortisol, despite the large increase in aldosterone during gestation, women do not exhibit hyperaldosteronism or associated symptoms, including hyperkalemia, hyponatremia, or hypertension. The reason for this is believed to be the marked increase in levels of progesterone from the placenta which are antagonistic to the effects of aldosterone [65].

In addition to the increase in aldosterone levels, there also is a marked increased in deoxycorticosterone (DOC) levels during pregnancy (see Fig. 6) [66]. Current evidence suggests that corticotropin is not the mechanism that regulates DOC. Most of the DOC that is produced during human pregnancy is derived from extra-adrenal 21-hydroxylation of circulating progesterone [67]. In addition, despite the high circulating concentrations of DOC in pregnancy, it is unknown why all pregnant women are not hypertensive.

Adrenal androgens

During pregnancy, the secretion and production of C_{19} steroids are doubled but the metabolic clearance rates are increased; this results in a paradoxic decline in plasma DHEA levels near term [68,69]. Maternal DHEA and DHEA-S are transferred to the placenta where they serve as precursors for estrogen production. Most estrogen, however, seems to come from fetal adrenal precursors. Thus, fetal C_{19} steroids account for 90% of the estriol that is formed and 30% of estrone and estradiol [37]. The fact that androgen precursors are converted to estrogens decreases the chance of maternal androgen excess; this prevents virilization of the mother and the female fetus in utero.

Disorders of the maternal adrenal

In human pregnancy, disorders of the adrenal gland are extremely rare. This is because these disorders, including Cushing's syndrome and adrenal insufficiency as well as tumors, usually are associated with infertility that is due to chronic anovulation.

Adrenal insufficiency

Adrenal cortical insufficiency (Addison's disease) is rare in pregnancy because of an association of the disorder with infertility that results from chronic anovulation [70,71]. Addison's disease (also known as primary adrenal failure) is characterized by varying degrees of adrenal atrophy and decreased cortisol secretion. Secondary failure may result from reduced corticotropin secretion or inhibition by chronic steroid use. The use of glucocorticoids in pregnant women for maternal pulmonary disease or to prevent fetal virilization from CYP21 deficiency can result in oversuppression and secondary adrenal failure [72,73]. Most cases of Addison's disease are idiopathic, although some may be related to polyglandular failure with adrenal antibodies and thyroid deficiency [71]. In the rare cases where adrenal insufficiency is not diagnosed during pregnancy, the mortality rate can be high; however, with the use of glucocorticoid treatment, the risk has dropped essentially to zero. In women who have Addison's disease that is diagnosed before pregnancy, the replacement of glucocorticoids allows normal ovulation and pregnancy to occur without complications. One of the problems with diagnosis of Addison's disease during pregnancy is that the clinical picture and symptoms are similar to some of the common symptoms of pregnancy (eg, nausea, vomiting, tiredness). In some cases with excessive electrolyte loss, fludrocortisone, 0.05 mg/d to 0.1 mg/d, is added [71]. In addition, a glucocorticoid intravenous bolus should be given at the time of labor, approximately 100–200 mg of hydrocortisone or equivalent dose of synthetic glucocorticoid. Oral glucocorticoid replacement can be resumed postoperatively. Infants who are born to mothers who have adrenal insufficiency appear to be normal.

Cushing's syndrome

Only a few cases of Cushing's syndrome have developed during pregnancy [74]. Cushing's syndrome, in general, is due to an excess secretion of cortisol, most commonly due to small pituitary microadenomas that also are known as Cushing's disease. The clinical picture of Cushing's syndrome during pregnancy is complicated because the signs and symptoms are similar to those that are normal during pregnancy including (eg, weight gain, striae or stretch marks, tiredness). In the most severe cases, hypertension and central nervous system symptoms may develop. The evaluation of Cushing's syndrome in pregnancy by laboratory testing is difficult. The primary diagnostic test is measurement of 24-hour urinary free cortisol. Urinary free cortisol levels in pregnancy are greater than in nonpregnant women; however, in women who have Cushing's disease the levels are increased to well above that of normal pregnancy. In pregnant women, a level that is greater than 250 mg per 24 hours is diagnostic of Cushing's syndrome [75]. In addition, the loss of the diurnal variation of Cortisol is observed in pregnant women who have Cushing's disease. Elevated plasma corticotropin suggests a pituitary tumor, but this may be difficult to confirm. The use of MRI of the pituitary often is diagnostic. If the diagnosis of Cushing's

syndrome is made during pregnancy, it is critical that treatment be considered because a large number of pregnancies end in fetal loss, premature labor, or both [71]. If excess cortisol is due to Cushing's disease, the treatment options include transsphenoidal pituitary surgery. Medical therapy also is being considered for this disorder.

Pheochromocytomas

Pheochromocytomas are tumors of the adrenal medulla that are capable of secreting large amounts of catecholamines, including adrenaline and noradrenalin. Although this disorder is rare, the diagnosis often is missed which can lead to significant maternal morbidity and mortality [76–78]. In addition, 10% of these tumors are malignant and 10% are bilateral; tumors also can be found in various sites (eg, sympathetic ganglia). The primary disorder needs to be differentiated from pregnancy-induced hypertension or preeclampsia. Careful management, including the blockade of the circulatory action of catecholamines on the α- and β-adrenergic systems, is required. After medical control, surgical removal often is possible and can be delayed to the time of delivery by caesarean section.

Summary

The fetal adrenal glands undergo profound physiologic changes during human gestation; these changes are unparalleled in any other human endocrine organ. Their steroid products are important for fetal organ maturation. Together with the placenta and the maternal adrenal, they form a unique maternal/fetal endocrine system. Activation of the fetal adrenal as part of this endocrine system may be an important control of the timing of human labor. Disorders of the fetal adrenal gland are common; however, prenatal treatment is possible for some cases of congenital adrenal hyperplasia. In contrast, disorders of the maternal adrenal are diagnosed rarely during pregnancy. When they do occur, their diagnosis may be made more difficult as a result of the physiologic changes of pregnancy or to common signs and symptoms that occur during pregnancy.

References

[1] Elliott JR, Armour RG. The development of the cortex in the human suprarenal gland and its condition in hemicephaly. J Pathol 1911;15:481–96.

[2] Suzuki T, Sasano H, Takeyama J, Kaneko C, Freije WA, Carr BR, et al. Developmental changes in steroidogenic enzymes in human postnatal adrenal cortex: immunohistochemical studies. Clin Endocrinol (Oxf) 2000;53:739–47.

[3] Mesiano S, Coulter CL, Jaffe RB. Localization of cytochrome P450 cholesterol side-chain cleavage, cytochrome P450 17-α-hydroxylase/17,20-lyase, and 3-β-hydroxysteroid dehydrogenase isomerase steroidogenic enzymes in human and rhesus monkey fetal adrenal glands: reappraisal of functional zonation. J Clin Endocrinol Metab 1993;77:1184–9.

[4] Mesiano S, Jaffe RB. Developmental and functional biology of the primate fetal adrenal cortex. Endocr Rev 1997;18:378–403.
[5] Pepe GJ, Albrecht ED. Regulation of the primate fetal adrenal cortex. Endocr Rev 1990;11: 151–76.
[6] Coulter CL, Goldsmith PC, Mesiano S, Voytek CC, Martin MC, Mason JI, et al. Functional maturation of the primate fetal adrenal in vivo. II. Ontogeny of corticosteroid synthesis is dependent upon specific zonal expression of 3β-hydroxysteroid dehydrogenase/isomerase. Endocrinology 1996;137:4953–9.
[7] Doody KM, Carr BR, Rainey WE, Byrd W, Murry BA, Strickler RC, et al. 3β-hydroxysteroid dehydrogenase/isomerase in the fetal zone and neocortex of the human fetal adrenal gland. Endocrinology 1990;126:2487–92.
[8] Rehman KS, Carr BR, Rainey WE. Profiling the steroidogenic pathway in human fetal and adult adrenals. J Soc Gynecol Investig 2003;10:372–80.
[9] Rainey WE, Carr BR, Wang ZN, Parker Jr CR. Gene profiling of human fetal and adult adrenals. J Endocrinol 2001;171:209–15.
[10] Parker Jr CR, Stankovic AM, Goland RS. Corticotropin-releasing hormone stimulates steroidogenesis in cultured human adrenal cells. Mol Cell Endocrinol 1999;155:19–25.
[11] Hillhouse EW, Grammatopoulos DK. Role of stress peptides during human pregnancy and labour. Reproduction 2002;124:323–9.
[12] McGrath S, Smith R. Corticotrophin-releasing hormone and parturition. Clin Endocrinol (Oxf) 2001;55:593–5.
[13] Rehman KS, Sirianni R, Carr B, Rainey WE, Parker CR. Endocrine controls of human parturition: corticotropin-releasing hormone (CRH) directly stimulates cortisol and the cortisol biosynthetic pathway in the human fetal adrenal [abstract]. J Soc Gynecol Investig 2004;11:80A.
[14] McLean M, Bisits A, Davies J, Walters W, Hackshaw A, De Voss K, et al. Predicting risk of preterm delivery by second-trimester measurement of maternal plasma corticotropin-releasing hormone and α-fetoprotein concentrations. Am J Obstet Gynecol 1999;181:207–15.
[15] Campbell EA, Linton EA, Wolfe CD, Scraggs PR, Jones MT, Lowry PJ. Plasma corticotropin-releasing hormone concentrations during pregnancy and parturition. J Clin Endocrinol Metab 1987;64:1054–9.
[16] Petraglia F, Sawchenko P, Rivier J, Vale W. Evidence for local stimulation of ACTH by corticotropin-releasing hormone in human placenta. Nature 1987;328:717–9.
[17] Robinson BG, Emanuel RL, Frim DM, Majzoub JA. Glucocorticoid stimulates expression of corticotropin-releasing hormone gene in human placenta. Proc Natl Acad Sci USA 1988; 85:5244–8.
[18] Jones SA, Brooks AN, Challis JR. Steroids modulate corticotropin-releasing hormone production in human fetal membranes and placenta. J Clin Endocrinol Metab 1989;68:825–30.
[19] Karalis K, Majzoub JA. Regulation of placental corticotropin-releasing hormone by steroids. Possible implications in labor initiation. Ann N Y Acad Sci 1995;771:551–5.
[20] Goland RS, Wardlaw SL, Stark RI, Brown Jr LS, Frantz AG. High levels of corticotropin-releasing hormone immunoactivity in maternal and fetal plasma during pregnancy. J Clin Endocrinol Metab 1986;63:1199–203.
[21] Stalla GK, Bost H, Stalla J, Kaliebe T, Dorr HG, Pfeiffer D, et al. Human corticotropin-releasing hormone during pregnancy. Gynecol Endocrinol 1989;3:1–10.
[22] Lockwood CJ, Radunovic N, Nastic D, Petkovic S, Aigner S, Berkowitz GS. Corticotropin-releasing hormone and related pituitary-adrenal axis hormones in fetal and maternal blood during the second half of pregnancy. J Perinat Med 1996;24:243–51.
[23] Perkins AV, Wolfe CD, Eben F, Soothill P, Linton EA. Corticotrophin-releasing hormone-binding protein in human fetal plasma. J Endocrinol 1995;146:395–401.
[24] Florio P, Woods RJ, Genazzani AR, Lowry PJ, Petraglia F. Changes in amniotic fluid immunoreactive corticotropin-releasing factor (CRF) and CRF-binding protein levels in pregnant women at term and during labor. J Clin Endocrinol Metab 1997;82:835–8.
[25] Goland RS, Jozak S, Warren WB, Conwell IM, Stark RI, Tropper PJ. Elevated levels

of umbilical cord plasma corticotropin-releasing hormone in growth-retarded fetuses. J Clin Endocrinol Metab 1993;77:1174–9.
[26] Goland RS, Tropper PJ, Warren WB, Stark RI, Jozak SM, Conwell IM. Concentrations of corticotrophin-releasing hormone in the umbilical-cord blood of pregnancies complicated by pre-eclampsia. Reprod Fertil Dev 1995;7:1227–30.
[27] Petraglia F, Aguzzoli L, Florio P, Baumann P, Genazzani AD, Di Carlo C, et al. Maternal plasma and placental immunoreactive corticotrophin-releasing factor concentrations in infection-associated term and pre-term delivery. Placenta 1995;16:157–64.
[28] Ruth V, Hallman M, Laatikainen T. Corticotropin-releasing hormone and cortisol in cord plasma in relation to gestational age, labor, and fetal distress. Am J Perinatol 1993;10:115–8.
[29] Petraglia F, Florio P, Benedetto C, Gallo C, Woods RJ, Genazzani AR, et al. High levels of corticotropin-releasing factor (CRF) are inversely correlated with low levels of maternal CRF-binding protein in pregnant women with pregnancy-induced hypertension. J Clin Endocrinol Metab 1996;81:852–6.
[30] Hobel CJ, Arora CP, Korst LM. Corticotrophin-releasing hormone and CRH-binding protein. Differences between patients at risk for preterm birth and hypertension. Ann N Y Acad Sci 1999;897:54–65.
[31] Murphy BE. Human fetal serum cortisol levels related to gestational age: evidence of a midgestational fall and a steep late gestational rise, independent of sex or mode of delivery. Am J Obstet Gynecol 1982;144:276–82.
[32] Goland RS, Jozak S, Conwell I. Placental corticotropin-releasing hormone and the hypercortisolism of pregnancy. Am J Obstet Gynecol 1994;171:1287–91.
[33] Falkenberg ER, Davis RO, DuBard M, Parker Jr CR. Effects of maternal infections on fetal adrenal steroid production. Endocr Res 1999;25:239–49.
[34] Bolté E, Wiqvist N, Dicsfalusy E. Metabolism of dehydroepiandrosterone and dehydroepiandrosterone sulphate by the human foetus at midpregnancy. Acta Endocrinol 1966;52:583–97.
[35] Bolté E, Gattereau D, Joly M, et al. Perfusion of human term placenta in situ with C-19 and C-18 steroids. Acta Endocrinol 1969;61:307–19.
[36] Siiteri PK, MacDonald PC. Placental estrogen biosynthesis during human pregnancy. J Clin Endocrinol Metab 1966;26:751–61.
[37] Ryan KJ. Metabolism of C-16-oxygenated steroids by human placenta: the formation of estriol. J Biol Chem 1959;234:2006–8.
[38] MacDonald PC, Siiteri PK. Origin of estrogen in women pregnant with an anencephalic fetus. J Clin Invest 1965;44:465–74.
[39] Colas A, Heinrichs WL, Tatum HJ. Pettenkofer chromogens in the maternal and fetal circulations: detection of 3β, 16α-dihydroxyandrost-5-en-17-one in umbilical cord blood. Steroids 1964;3:417–34.
[40] Challis Jr CRH. A placental clock and preterm labour. Nat Med 1995;1:416.
[41] Fencl MD, Stillman RJ, Cohen J, Tulchinsky D. Direct evidence of sudden rise in fetal corticoids late in human gestation. Nature 1980;287:225–6.
[42] Bradshaw KD, Carr BR. Placental sulfatase deficiency: maternal and fetal expression of steroid sulfatase deficiency and X-linked ichthyosis. Obstet Gynecol Surv 1986;41:401–13.
[43] McCabe ERB. Adrenal hypoplasias and aplasias. In: Scriver CR, Beaudet AL, Sly WE, Valle D, Childs B, Kinzler KW, et al, editors. The metabolic and molecular bases of inherited disease. New York: McGraw-Hill; 2001. p. 4263–74.
[44] Nichols J, Lescure OL, Migeon CJ. Levels of 17-hydroxycorticosteroids and 17-ketosteroids in maternal and cord plasma in term anencephaly. J Clin Endocrinol 1958;18:444.
[45] MacDonald PC. Placental steroidogenesis. In: Wynn RM, editor. Fetal homeostasis. vol. I. New York: New York Academy of Sciences; 1965. p. 265.
[46] Clark AJ, Metherell L, Swords FM, Elias LL. The molecular pathogenesis of ACTH insensitivity syndromes. Ann Endocrinol (Paris) 2001;62:207–11.
[47] White PC, Speiser PW. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Endocr Rev 2000;21:245–91.

[48] Simard J, Moisan AM, Morel Y. Congenital adrenal hyperplasia due to 3β -hydroxysteroid dehydrogenase/Delta(5)-Delta(4) isomerase deficiency. Semin Reprod Med 2002;20:255–76.
[49] White PC. Steroid 11β -hydroxylase deficiency and related disorders. Endocrinol Metab Clin North Am 2001;30:61–79.
[50] Fujieda K, Okuhara K, Abe S, Tajima T, Mukai T, Nakae J. Molecular pathogenesis of lipoid adrenal hyperplasia and adrenal hypoplasia congenita. J Steroid Biochem Mol Biol 2003;85: 483–9.
[51] Simpson ER. Genetic mutations resulting in loss of aromatase activity in humans and mice. J Soc Gynecol Investig 2000;7:S18–21.
[52] Shozu M, Akasofu K, Harada T, Kubota Y. A new cause of female pseudohermaphroditism: placental aromatase deficiency. J Clin Endocrinol Metab 1991;72:560–6.
[53] Harada N, Ogawa H, Shozu M, Yamada K, Suhara K, Nishida E, et al. Biochemical and molecular genetic analyses on placental aromatase (P-450AROM) deficiency. J Biol Chem 1992;267:4781–5.
[54] Morishima A, Grumbach MM, Simpson ER, Fisher C, Qin K. Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. J Clin Endocrinol Metab 1995;80:3689–98.
[55] France JT, Liggins GC. Placental sulfatase deficiency. J Clin Endocrinol Metab 1969;29: 138–41.
[56] Carr BR, Parker Jr CR, Madden JD, MacDonald PC, Porter JC. Maternal plasma adrenocorticotropin and cortisol relationships throughout human pregnancy. Am J Obstet Gynecol 1981;139:416–22.
[57] Carr BR, Parker Jr CR, Milewich L, Porter JC, MacDonald PC, Simpson ER. The role of low density, high density, and very low density lipoproteins in steroidogenesis by the human fetal adrenal gland. Endocrinology 1980;106:1854–60.
[58] Burke CW, Roulet F. Increased exposure of tissues to cortisol in late pregnancy. BMJ 1970; 1:657–9.
[59] Nolten WE, Lindheimer MD, Oparil S, Ehrlich EN. Desoxycorticosterone in normal pregnancy. I. Sequential studies of the secretory patterns of desoxycorticosterone, aldosterone, and cortisol. Am J Obstet Gynecol 1978;132:414–20.
[60] Nolten WE, Rueckert PA. Elevated free cortisol index in pregnancy: possible regulatory mechanisms. Am J Obstet Gynecol 1981;139:492–8.
[61] Burke CW. Biologically active cortisol in plasma of oestrogen-treated and normal subjects. BMJ 1969;2:798–800.
[62] Carr BR, Parker Jr CR, Madden JD, MacDonald PC, Porter JC. Maternal plasma adrenocorticotropin and cortisol relationships throughout human pregnancy. Am J Obstet Gynecol 1981;139:416–22.
[63] Sasaki A, Shinkawa O, Margioris AN, Liotta AS, Sato S, Murakami O, et al. Immunoreactive corticotropin-releasing hormone in human plasma during pregnancy, labor, and delivery. J Clin Endocrinol Metab 1987;64:224–9.
[64] Dorr HG, Heller A, Versmold HT, Sippell WG, Herrmann M, Bidlingmaier F, et al. Longitudinal study of progestins, mineralocorticoids, and glucocorticoids throughout human pregnancy. J Clin Endocrinol Metab 1989;68:863–8.
[65] Landau RL, Lugibihl K. The catabolic and natriuretic effects of progesterone in man. Recent Prog Horm Res 1961;17:249–92.
[66] Parker Jr CR, Cutrer S, Casey ML, MacDonald PC. Concentrations of deoxycorticosterone, deoxycorticosterone sulfate, and progesterone in maternal venous serum and umbilical arterial and venous sera. Am J Obstet Gynecol 1983;145:427–32.
[67] Casey ML, MacDonald PC. Extraadrenal formation of a mineralocorticosteroid: deoxycorticosterone and deoxycorticosterone sulfate biosynthesis and metabolism. Endocr Rev 1982;3: 396–403.
[68] Baulieu EE, Dray F. Conversion of ^{3}H-dehydroepiandrosterone (3β-hydroxy-Δ^{5}-androstene-17-one) sulfate to ^{3}H-estrogens in normal pregnant women. J Clin Endocrinol 1963;23:1298.

[69] Belisle S, Schiff I, Tulchinsky D. The use of constant infusion of unlabeled dehydroepiandrosterone for the assessment of its metabolic clearance rate, its half-life, and its conversion into estrogens. J Clin Endocrinol Metab 1980;50:117–21.
[70] Bongiovanni AM, McPadden AJ. Steroids during pregnancy and possible fetal consequences. Fertil Steril 1960;11:181–6.
[71] Sipes SL, Malee MP. Endocrine disorders in pregnancy. Obstet Gynecol Clin North Am 1992; 19:655–77.
[72] McKenna DS, Wittber GM, Nagaraja HN, Samuels P. The effects of repeat doses of antenatal corticosteroids on maternal adrenal function. Am J Obstet Gynecol 2000;183:669–73.
[73] Ogueh O, Johnson MR. The metabolic effect of antenatal corticosteroid therapy. Hum Reprod Update 2000;6:169–76.
[74] Chandraharan E, Arulkumaran S. Pituitary and adrenal disorders complicating pregnancy. Curr Opin Obstet Gynecol 2003;15:101–6.
[75] Buescher MA, McClamrock HD, Adashi EY. Cushing syndrome in pregnancy. Obstet Gynecol 1992;79:130–7.
[76] Kalra JK, Jain V, Bagga R, Gopalan S, Bhansali AK, Behera A, et al. Pheochromocytoma associated with pregnancy. J Obstet Gynaecol Res 2003;29:305–8.
[77] Mannelli M, Bemporad D. Diagnosis and management of pheochromocytoma during pregnancy. J Endocrinol Invest 2002;25:567–71.
[78] Ahlawat SK, Jain S, Kumari S, Varma S, Sharma BK. Pheochromocytoma associated with pregnancy: case report and review of the literature. Obstet Gynecol Surv 1999;54:728–37.

ELSEVIER
SAUNDERS

Obstet Gynecol Clin N Am
31 (2004) 837–856

OBSTETRICS AND
GYNECOLOGY
CLINICS
OF NORTH AMERICA

Fetal hormones and sexual differentiation

Vivian Sobel, MD, Yuan-Shan Zhu, MD, PhD,
Julianne Imperato-McGinley, MD*

Department of Medicine, Weill Medical College of Cornell University, 525 East 68th Street, F-2006, New York, NY 10021, USA

The process of fetal sexual development is under intricate genetic and hormonal control; many factors that are involved in the process largely escape our understanding. In the 1950s, in his landmark experiments, Jost [1] proposed that fetal sexual differentiation proceeds along female lines in the absence of any hormonal stimulation and that two hormonal factors that are produced by the male testes—one of which induces regression of the müllerian ducts (precursors to the female internal reproductive tract) and one of which induces differentiation of the Wolffian ducts (precursors of the male internal reproductive tract)—are necessary for male sexual differentiation. These experiments form the basis of our current understanding of sexual differentiation.

Sexual differentiation can be divided into three discrete phases: (1) genetic sex is established by the presence of an XX (female) or an XY (male) genotype; (2) depending on the genetic sex, the bipotential gonad differentiates into ovaries or testes and establishes the gonadal se*x* of the fetus; and (3) phenotypic sex, defined by the internal and external sexual characteristics that an individual possesses, evolves as male or female.

Perturbations may occur at any of the phases of fetal sexual differentiation and development. Syndromes of abnormal sexual differentiation in humans and other animals have shed light on the process of normal sexual differentiation and defined the roles of various genetic, gonadal, and hormonal factors. This article discusses normal and abnormal fetal sexual differentiation and the factors that are known to be involved in the development of genetic, gonadal, and phenotypic sex.

* Corresponding author.
E-mail address: jimperat@med.cornell.edu (J. Imperato-McGinley).

0889-8545/04/$ – see front matter
doi:10.1016/j.ogc.2004.08.005 obgyn.theclinics.com

Formation of the bipotential gonad

Development of the undifferentiated gonad, which is present in male and female fetuses, begins during the fifth week of intrauterine life. An area of the coelomic epithelium that is located on the medial aspect of the mesonephros thickens to form the germinal epithelium [2,3]. Proliferation of the germinal epithelium and the underlying mesenchyme produces a protrusion on the medial side of the mesonephros which is called the "gonadal ridge." Chords of cells begin to proliferate from the epithelium into the mesenchyme to form the primary sex chords; these become the seminiferous tubules that are composed of Sertoli cells in the male and primary ovarian follicles in the female [2–4].

The urogenital ridge forms from the intermediate mesoderm under the influence of several factors. Three genes have been described that encode proteins which are critical for the formation of the urogenital ridge: Wilms' tumor-suppressor–1 (*Wt-1*) [5], steroidogenic factor–1 (SF-1) [6], and dosage-sensitive sex reversal–adrenal hypoplasia congenita on the X chromosome, gene 1 (*DAX-1*) [7,8]. Mutations of each of these genes in humans are associated with syndromes that are characterized by gonadal dysgenesis and malformation of other organs, such as the kidneys and adrenals [9–11].

Several other genes are crucial in early gonadal development (eg, *Lim1*, *Lhx9*, *Emx2*). Mice that are homozygous for a null mutation in these genes fail to form genital ridges [12–16]. These gene mutations also result in malformation of other organs that are derived from the intermediate mesoderm (eg, kidneys) [17]. Mutations in these genes have not been defined in humans.

Primordial germ cell migration

Primordial germ cells are first visible during the third week of fetal life along the endodermal somatic cells of the yolk sac near the origin of the allantois (Fig. 1) [3,18–20]. The yolk sac is incorporated partially into the embryo when the embryo folds. Folding of the embryo leads to passive migration of primordial germ cells from the yolk sac epithelium through the hindgut and gut mesentery during the fourth week of life [21,22]. In the fifth week of life, the germ cells actively migrate into the underlying mesenchyme to their destination in the bipotential gonad, which is formed by the end of the sixth week. At this stage, primordial germ cells still are undifferentiated sexually [2,3,23–25].

Migration of the germ cells is directed, at least partially, by the expression of the genes, *stella* and *fragilis*, which are expressed exclusively in differentiating germ cells [26]. Although the precise function of these genes is not clear, their expression is necessary for germ cell development. Their products also interact with bone morphogenetic protein (BMP)-4.

Most primordial germ cells reach the final destination of the gonad [27]. Thus far, the process of germ cell migration has been characterized best in *Drosophila*, in which two genes, *wunen* and *columbus*, are involved in directing the path of

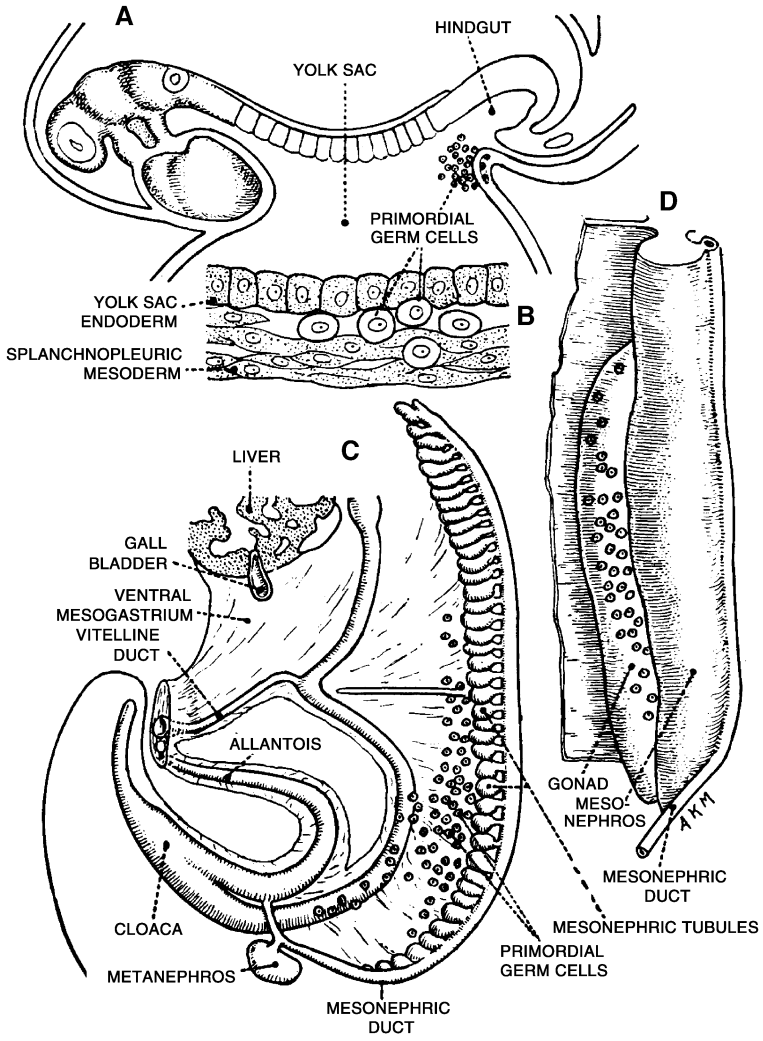


Fig. 1. Migration of primordial germ cells from the endoderm of the yolk sac. (*A*) 16 somite human embryo; (*B*) primordial germ cells between the yolk sac endoderm and the splanchnopleuric mesoderm; (*C*) 4.2 mm human embryo; (*D*) germ cells in the developing gonad. (*From* Hamilton WJ, Mossman HW. Human Embryology. Fourth Edition. Cambridge (UK): Williams and Wilkins; 1972. p. 402).

germ cells [27]. *Wunen* provides a repulsive signal that repels migrating cells from inappropriate places, whereas *columbus* is required to attract germ cells to the gonad. Other genes, RNAs, and proteins that are involved in migration of germ cells to the gonad have been described in different classes of animals [27]. Somatic and germline cells cooperate in forming the indifferent gonad; neither is a passive participant in germ cell migration.

Testicular differentiation

Embryology of testis formation

Germ cell differentiation in the testis begins at approximately 6 weeks' postconception [3,19,28,29]. The male primordial germ cells, or prespermatogonia, and the Sertoli cells become enclosed in the newly formed testicular chords [30]. Furthermore, the Sertoli cells come into contact with one another and begin to engulf the germ cells. At 7 weeks' gestation, mesonephric stromal cells penetrate the gonad and differentiate into Leydig cells [31]. The ends of the testicular chords form a network of chords—the rete testes—which is in contact with the mesonephric tubules. At 3 months' gestation, Leydig cells completely fill the interstitial spaces. By the sixth month, the ends of the rete testes develop a lumen that is continuous with the mesonephric tubules; these later develop into the ductuli efferentes [3].

Early differences in male and female sexual development are evident in the proliferation patterns of the germ cells. Male (XY) germ cells undergo mitosis during their migration; however, their growth is arrested in the G0 (quiescent) phase of the cell cycle after they reach the gonad. The prespermatogonia in the fetal gonads divide by mitosis within the chords without entering meiosis. After birth, the male germ cells resume the cell cycle and undergo meiosis to produce haploid germ cells (spermatogonia). Spermatogenesis is completed at puberty under the influence of the pituitary hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Sertoli cells sustain the germ cells by producing cytokines, Müllerian inhibiting substance (MIS), inhibin, activin, and insulin like growth factor–I [11]. Spermatogenic cells depend upon the Sertoli cells for maturation [32].

Genetics of testis formation

Sex determining region Y (SRY)

Testis differentiation is triggered by the presence of *SRY*, a gene that is located on the distal short arm of the Y chromosome [11,33]. The gene consists of a single exon that encodes a 204–amino acid protein [34]. Outside of its highly conserved, 79– amino acid homeobox or DNA-binding domain, the *SRY* protein is conserved poorly among mammals. *SRY* seems to function as a transcriptional regulator. Two pieces of evidence have implicated *SRY* as the testis-determining factor: (1) mutations in the homeobox region of *SRY* were found in a subgroup of 46,XY sex-reversed females who had pure gonadal dysgenesis [35] and (2) phenotypic males were produced following introduction of an *SRY* trans gene into XX mice [36]. Conversely, mutations of *SRY* have been identified in only 15% to 20% of XY females who have complete or partial gonadal dysgenesis [37].

In man, *SRY* mRNA expression begins at Day 41 postcoitum, peaks at Day 44 postcoitum at the time of testicular differentiation, and then persists throughout

and after embryogenesis [38]. Neither the biologic targets nor the mechanism of action of the *SRY* gene is known.

Sox9

Other autosomes are known to be involved in determining testicular differentiation and development [17,39–41]. Sox9, an *SRY*-related homeobox (HMG) box gene that is found on an autosome, is a candidate *SRY* target. SOX9, a transcription factor, was first isolated in studies of patients who had campomelic dysplasia, a bone developmental defect that is associated with sex reversal in 75% of XY individuals [42,43]. High expression of Sox9 correlates with testis differentiation, independent of *SRY*. Abnormal upregulation of Sox9 expression in XX individuals is associated with sex reversal in humans and mice [44,45]. One potential target of Sox9 could be Fgf9. The Sox9 and Fgf9 genes clearly play a critical role in the formation of the testis chords, the first step in testicular differentiation [17,46].

Steroidogenic factor–1 (Sf-1) and Wilms' tumor suppressor–1 (Wt-1)

The *Sf-1* and *Wt-1* genes also play a specific role in male differentiation [17]. *Sf-1* is involved in different steps of the testis-determining pathway, including regulation of Müllerian inhibiting substance (MIS) gene expression and of multiple steroidogenic enzymes [47,48]. Absence of a functional *Sf-1* gene in mice, caused by targeted disruption, causes death early in postnatal life. These mice lack adrenals, gonads, and have female internal and external genitalia, regardless of sex chromosomal complement [16]. Heterozygous mutations in the *Sf-1* gene have been associated with 46,XY sex-reversal and adrenal failure in humans [9].

Wt-1 is a genetic regulator of *SRY* [5]. *Wt-1* knockout mice die in utero, lacking kidneys, adrenals, and gonads; they have a female phenotype with an XY karyotype [15]. Three syndromes in humans are associated with mutations in *Wt-1*: Wilms' tumor-aniridia (WAGR) syndrome [49], Denys-Drash syndrome [50], and the Frasier syndrome [5,11].

Dosage-sensitive sex reversal–adrenal hypoplasia congenita on the X chromosome, gene 1 (Dax-1)

Dax1 encodes an atypical member of the nuclear hormone receptor superfamily that is located on Xp21.3. Mutations in *Dax1* in humans lead to the syndrome of adrenal hypoplasia congenita, in which patients have defects in adrenal cortex function, hypogonadotrophic hypogonadism, testicular germ cell absence, and Sertoli cell immaturity [51]. In mice, *Dax1* deletions lead to progressive germinal epithelium damage in males [8,52]. This gene may be responsible for a sex reversal syndrome in humans (see Genetics of ovarian differentiation).

Ovarian differentiation

Embryology of ovarian differentiation

Female (XX) germ cells also undergo mitosis during migration to the genital ridge. After the cells enter the ovary, they proceed through the initial stages of the first meiotic division until they arrest at prophase 1 at birth. This is in contrast to male (XY) germ cells, which are arrested in mitosis upon gonadal entry. It was proposed that ovarian fate is dependent upon the presence of meiotic germ cells (ie, autonomous entry of germ cells into meiosis dictates ovarian differentiation). This pathway is antagonized by the presence of *SRY* in males, which leads to formation of testis cords that, in turn, sequester germ cells and cause mitotic arrest [52].

During the ninth week postfertilization, primordial germ cells begin to differentiate into oogonia. Initially, the primordial germ cells are difficult to distinguish from oogonia; the latter have higher mitotic activity, a smooth and regular outline, and a tendency to form clusters of dividing cells that are joined by intercellular bridges [53]. Upon their arrival at the gonad, the germ cells begin to interact with surrounding somatic cells. Ovarian epithelial components form groups of cells (‘‘medullary chords’) that envelop primitive granulosa cells and oogonia. During Weeks 12 and 13 postfertilization, proliferating oogonia begin to differentiate into oocytes [53]. At this stage, meiosis begins through the first meiotic prophase. Upon meiotic arrest, the female germ cells become surrounded by a single layer of somatic granulosa cells that form primordial follicles. At the time of puberty, these primordial follicles are stimulated by FSH to grow into primary, secondary, and preovulatory follicles [54]. Furthermore, oocyte-derived growth and differentiation factor 9; BMP-15; zona pellucida proteins 1, 2, and 3; and other granulosa cell products are secreted to maintain oocytes and control ovulation [55]. The granulosa cells in the developing female maintain germ cells that are analogous to Sertoli cells in the developing male [11].

Genetics of ovarian differentiation

Absence of the Y chromosome or the *SRY* gene leads to the development of ovaries. Ovarian development, however, will not be maintained unless a 46,XX karyotype is present [56]. Deletion studies of the X chromosome demonstrated that the long and the short arm are necessary for gonadal development. Deletion of portions of the short arm leads to a syndrome that mimics Turner’s syndrome, with streak gonads and other skeletal and somatic anomalies. Long arm deletions are associated with streak gonads without any of the other stigmata of Turner’s syndrome [57].

Dax-1 has been proposed as a potential mediator of ovarian development because 46-XY sex reversal, termed ‘‘dosage sensitive sex reversal,’’ has been described in some genetic males who have two copies of a 160-kilobase (kb) region of Xp21 that contain the *Dax-1* gene [58,59]. Humans who have

duplication of the critical region of Xp21 have normal female or ambiguous external genitalia and differing degrees of gonadal dysgenesis that range from incompletely differentiated testes to the presence of one or two streak gonads. XY mice that carry extra copies of mouse *Dax1* have delayed testicular development but no sex reversal [59]. *Dax1* does cause sex reversal in some mice when tested against weak alleles of Sry; this leads to normal female internal reproductive tracts with ovaries that have diminished numbers of follicles or to hermaphroditism with mixed male/female reproductive tracts and different degrees of ovarian and testicular development. This phenomenon suggests that sex reversal that is caused by *Dax1* is dose- and time-dependent [59]. *Dax-1* inhibited SF-1 transcriptional activity in vitro [7]. The loss of *Dax-1* in female knockout mice affects neither ovarian development nor fertility [52].

Follicular development begins at 16 weeks' gestation, whereas primordial follicles with granulosa cells develop by 20 weeks' gestation. The ovaries contain 6 to 7 million oocytes at 20 weeks' gestation; this declines to 2 million by term [60–62]. Until recently, the reproductive biologic dogma held that postnatal mammalian ovaries are devoid of germline stem cells and that a mammalian female is born with a finite pool of follicles which becomes exhausted at the time of menopause. This doctrine recently was refuted in mice; studies of atretic follicles in mouse ovaries showed that the rate of follicular atresia (or degeneration) far outpaces the rate of follicular depletion; follicular renewal from germline stem cells is on-going, at least through early adulthood in the mouse ovary. Candidate germline stem cells were identified near the surface of the adult mouse ovary by Johnson et al [63] in 2004.

Formation of the internal and external genital tracts

Internal genital differentiation in the male

The Wolffian and Müllerian ducts are present in the fetuses of both sexes early in gestation (Fig. 2). The Wolffian ducts appear during the fourth week, whereas the müllerian ducts appear during Weeks 6 to 7. In the male, the müllerian ducts regress at 8.5 weeks' gestation as a result of secretion of MIS by fetal Sertoli cells [1,4]. MIS is the earliest marker of testicular differentiation to be secreted by the gonad. Regression of the müllerian ducts occurs in a craniocaudal fashion, corresponding to a concentration gradient of MIS. The persistent müllerian duct syndrome can occur in males if they have testes that are incapable of secreting MIS or if they have inactivating mutations of the MIS receptor [64].

Secretion and action of Müllerian inhibiting substance

MIS is a 140-kd glycoprotein member of the transforming growth factor (TGF)-β superfamily that is composed of two identical subunits. The *MIS* gene, located at 19p13.3, contains 275 base pairs and five exons [65]. In the testes, *MIS*

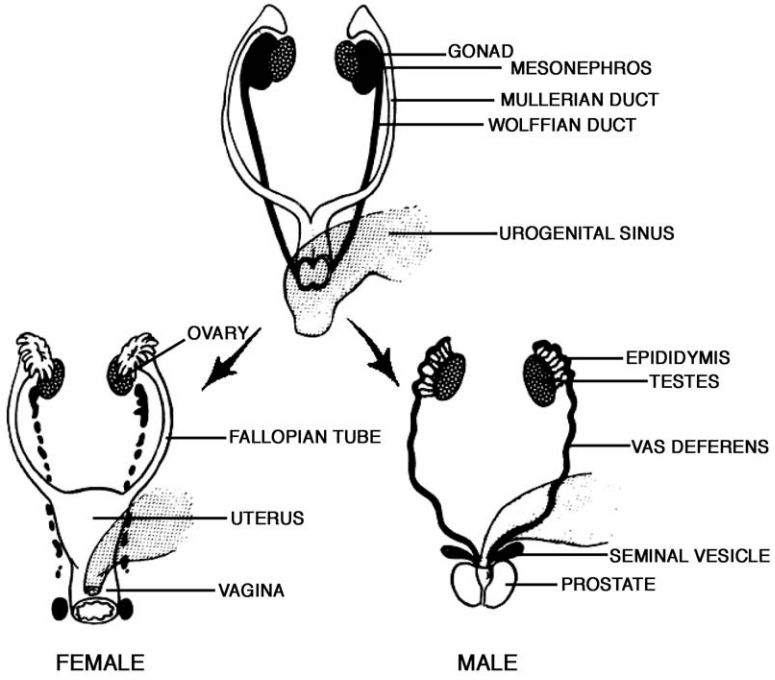


Fig. 2. Differentiation of the male and female internal genital tracts from the Wolffian ducts and the Mullerian ducts, respectively.

secretion begins at the time of testicular differentiation and continues until puberty. In contrast, *MIS* is not secreted by the granulosa cells of the ovaries until after birth [66,67].

There are two *MIS* receptors; however, only the type II receptor was identified as being important for the actions of *MIS* [66]. This is a serine-threonine receptor with one transmembrane domain. It is encoded by a gene on 19p13.3 with 275 base pairs and five exons. *MIS* binds to its receptor; it induces receptor phosphorylation and activation of an intracytoplasmic second messenger, which, in turn, causes activation and tyrosine phosphorylation of cytoplasmic proteins that are called Smads. The Smads bind to Smad4, which ultimately carries the activated second messenger to the nucleus, where it binds to target gene promoters and causes gene transcription [66]. Coactivator molecules also are involved in this process [68].

Other genes have been described that interact with the *MIS* gene. *Sox9* was implicated clearly in activating transcription of the gene that encodes *MIS*. Endogenous expression of MIS in early postnatal rat Sertoli cells also requires multiple *Sf-1* and *GATA*-4–binding sites [69,70]. The regulation of *MIS* expression is not well-understood, but it seems to be stimulated initially by *Sox-9* and then by *Sf-1*, *Wt-1*, and *GATA*-4 [71,72].

Secretion of testosterone

Testosterone secretion by fetal Leydig cells begins at approximately 8 weeks' gestation and leads to differentiation of the Wolffian ducts to form the epididymis, vas deferens, seminal vesicles, and ejaculatory ducts. This process of internal sexual differentiation in the male fetus is complete at 12 weeks' gestation. The secretion of testosterone in the fetal testes is believed to be regulated by chorionic gonadotropin, which is secreted by the placenta and binds to the LH receptor [73]. LH secretion in the fetus does not begin until 10 weeks' gestation—well after genital differentiation has begun—and it does not become pulsatile until 11 to 12 weeks' gestation [74]. Serum testosterone levels in the male fetus peak at 11 to 17 weeks' gestation.

Enzymes involved in testosterone synthesis

Six enzymatic reactions involving five genes lead to the biosynthesis of testosterone (Fig. 3). Genetic defects that lead to intrauterine deficiencies in any of the following enzymes can lead to incomplete Wolffian differentiation and external genital masculinization in the male fetus and ambiguous genitalia in the male newborn. Defects in the steroid acute regulatory (StAR) protein, which is involved in cholesterol transport, also were reported as a cause of failure of masculinization [4]. Recently, mutations in P450 oxidoreductase, the flavoprotein that donates electrons to all microsomal P450 enzymes, including the steroidogenic enzymes, P450c17 and P450c21, were described in four individuals. This resulted in disordered steroidogenesis and genital ambiguity in the affected male [75].

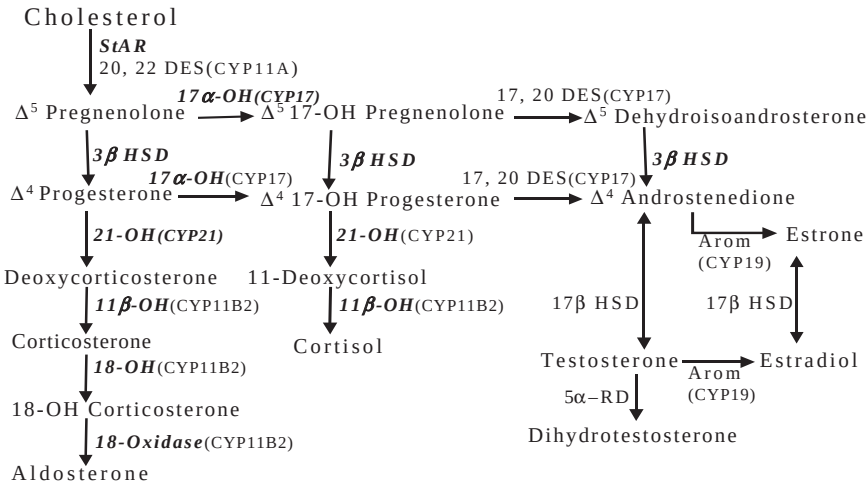


Fig. 3. Synthesis of testosterone/dihydrotestosterone from cholesterol.

Steroid acute regulatory protein

Testosterone synthesis, like the synthesis of all steroid hormones, begins with cholesterol (Fig. 3). Cholesterol, which is located on the outer mitochondrial membrane, must be delivered to the inner mitochondrial membrane to be available for steroid synthesis. Transport across the inner mitochondrial membrane of the Leydig cell seems to be regulated by *StAR* protein [76], which is encoded by a gene at 8p11.2 [77]. Mutations in the *StAR* gene cause lipoid adrenal hyperplasia that results in severe impairment of all steroidogenesis [76,78,79]. In this condition, cholesterol accumulates in adrenocortical and Leydig cells as large lipid droplets [71,80]. Infants who are born with *StAR* defects lack gonadal and adrenal androgens; this results in female external genital formation in affected males. The defect in cortisol or aldosterone synthesis leads to adrenal insufficiency shortly after birth. Patients who are not treated immediately do not survive the perinatal period [80].

Cholesterol 20,22-desmolase (P450scc)

Cholesterol within the mitochondria is converted to pregnenolone—the rate-limiting step in all steroid biosynthesis—by the cholesterol side chain cleavage enzyme (P450scc or CYP11A) that is encoded by a 20-kb gene at 15q23-q24 [81]. This enzyme actually catalyzes three separate reactions: 20α-hydroxylation, 22-hydroxylation, and side chain cleavage of cholesterol. Thus, P450scc is a mixed-function oxidase. Mutations in the enzyme have not been described.

P450C17

This enzyme, which is encoded by a gene at 10q24.3 [81], has 17α-hydroxylase and 17,20-lyase activity. 17α-Hydroxylase converts pregnenolone to 17α-hydroxypregnenolone and progesterone to 17α-hydroxyprogesterone in the adrenals and gonads. 17,20-Lyase or -desmolase activity converts 17α-hydroxypregnenolone to Dehydroepiandrosterone (DHEA) and 17α-hydroxyprogesterone to androstenedione. Defects in the gene that encode P450C17 cause combined 17α-hydroxylase/17,20-lyase deficiency; this results in defects in the synthesis of cortisol and sex steroids. Sex steroid deficiency in the adrenals and gonads results in a female phenotype in both genetic sexes as well as hypertension and hypokalemia that is due to excess of the mineralocorticoid, desoxycorticosterone [82]. Genetic defects also were reported that preferentially affect the 17,20-lyase activity of this enzyme. Isolated 17,20-lyase deficiency was reported as a cause of genital ambiguity of varying degrees in the absence of hypertension in genetic males [83,84].

3β-Hydroxysteroid dehydrogenase

Two genes that encode two isoforms of this enzyme have been identified. They are located on chromosome 1 at locus p11-p13 [85,86]. The genes that encode 3β-hydroxysteroid dehydrogenase (*3β-HSD*) I and II contain four exons; they encode proteins of 371 and 372 amino acids, respectively. The enzyme

converts pregnenolone to progesterone, 17-hydroxypregnenolone to 17-hydroxyprogesterone, DHEA to androstenedione, and androstenediol to testosterone. Type I is expressed in tissues, such as the liver and placenta, whereas type II is expressed in the adrenals and gonads. Mutations in *3β-HSD* II are found in humans and lead to males who are born with ambiguous genitalia [87]. Cortisol, testosterone, and aldosterone production are decreased in these patients. Severe defects can result in death in early infancy from adrenocortical insufficiency and salt wasting unless the patients are treated; however, milder enzymatic deficiencies were reported that were diagnosed later in childhood. The genital ambiguity in genetic males who have this condition is dependent on the severity of the enzymatic defect; however, it often is characterized by varying degrees of hypospadias with a bifid scrotum [80].

17β-Hydroxysteroid dehydrogenase-3

Seven different 17β-hydroxysteroid dehydrogenase (*17βHSD*) isoenzymes have been described; each one has a unique pattern of tissue expression [88–92]. The isoenzymes share minimal DNA sequence homology, differ in their chromosomal and subcellular localizations, and have different substrate and catalytic preferences [88]. In general, these enzymes catalyze the reversible synthesis and inactivation of testosterone and estradiol, and thus, control the steady state levels of these hormones.

The isozyme, *17βHSD-3*, which is found almost exclusively in the testes, converts the inactive androgen, androstenedione, to testosterone [88,93,94]. Defects in the human *17βHSD-3* gene, which is located on chromosome 9q22 and encodes a 310– amino acid protein, cause severely ambiguous external genitalia in males who are homozygous for genetic mutations in the enzyme [91].

46XY infants who are born with *17βHSD-3* deficiency can be designated as female at birth and raised as girls; however, at puberty they undergo virilization secondary to an increase in testosterone and dihydrotestosterone levels—mainly due to the increased peripheral conversion of androstenedione to testosterone by other *17βHSD* isoenzymes—and many change gender role from female to male. They develop phallic enlargement, and frequently, increased muscle mass. Individuals who have *17βHSD-3* deficiency have substantial growth of body and facial hair at puberty and can develop temporal hairline recession. Also, about half of affected subjects experience some degree of gynecomastia, which probably results from an elevated ratio of estrogen to testosterone [91,93,95].

Internal genital differentiation in the female

In the female, Wolffian ducts regress at 11 weeks' gestation in the absence of testosterone and the müllerian ducts differentiate to form the fallopian tubes, uterus, and upper portion of the vagina (in the absence of MIS) (see Fig. 3).

External genital differentiation in the male and female

The external male and female genital tracts share the same anlage (ie, the urogenital tubercle, urogenital folds, and urogenital swellings) (Fig. 4) [3,4,96]. In the male, the urogenital tubercle becomes the glans penis, whereas in the female it becomes the clitoris. The urogenital folds form the penile shaft in the male, whereas in the female they form the labia minora. In the male, the urogenital swellings form the scrotum; in the female they form the labia majora [97]. In the male fetus, the urogenital sinus forms the prostate, bulbourethral glands, and the prostatic and membranous urethra. The prostate arises from the endodermal buds in the urethral lining at 10 weeks' gestation and grows into the mesenchyme, which forms the muscular and connective tissue components.

Female external genital differentiation is complete by 11 weeks' gestation, whereas male external genital differentiation is complete by 14 weeks' gestation; however, phallic growth continues throughout the last two trimesters of pregnancy [98]. The migration of the testes from the abdominal cavity to the scrotum begins at 10 weeks' gestation and generally is completed by 25 to 35 weeks' gestation. The ovaries, like the testes, descend from the posterior abdominal wall to the pelvis [71].

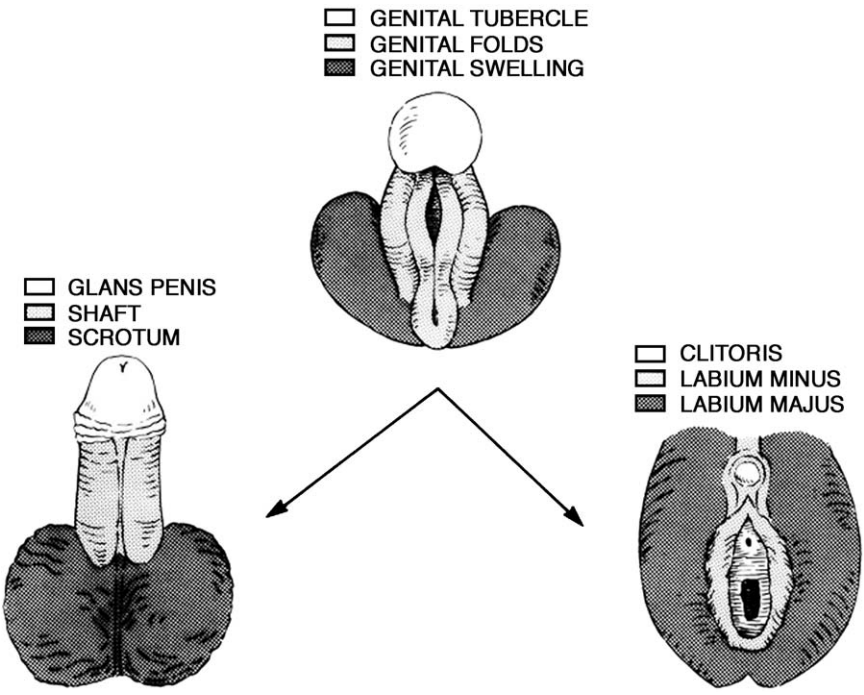


Fig. 4. Differentiation of the male and female external genitalia from common precursors.

Dihydrotestosterone and external sexual differentiation

Dihydrotestosterone, the 5α-reduced metabolite of testosterone, is produced in the external genital anlage and induces formation of the male external genitalia and prostate. In female fetuses, the lack of testes to produce testosterone as a substrate for 5α-reductase means that dihydrotestosterone (DHT) levels are insufficient for external genital masculinization; therefore, external genitalia differentiate along female lines. DHT is formed from testosterone by the microsomal 5α-reductase enzyme, which reduces the double bond at the 4–5 position of C19 steroids [99,100]. Testosterone and dihydrotestosterone bind to the same androgen receptor. DHT, however, binds to the androgen receptor with higher affinity than does testosterone [101]. Furthermore, the DHT-receptor complex binds DNA more efficiently than does the testosterone-receptor complex [102–104].

Two isoenzymatic forms of 5α-reductase have been identified, 5α-reductase–1 and 5α-reductase–2 (Table 1). Both 5α-reductase genes have five exons and four introns and encode highly hydrophobic 254– and 259–amino acid proteins, respectively. 5α-Reductase–2 (*5αRD-2*) is mapped to the short arm of chromosome 2p23, whereas 5α-reductase–1 is mapped to chromosome 5p15 [105]. *5αRD-2* is found primarily in the skin of the external genitalia and is critical for the formation of the male external genitalia. Mutations of the *5αRD-2* isozyme lead to incomplete formation of male external genitalia and prostate (see Fig. 4). This usually results in severe genital ambiguity. At birth, infants who have *5αRD-2* deficiency often are believed to be female and are raised as girls. Despite their severe genital ambiguity, individuals who have *5αRD-2* deficiency experience a definite male puberty and often undergo gender role change. In contrast to individuals who have *17βHSD-3* deficiency, they have decreased facial and body hair, do not develop male pattern balding, and have small prostates; this underscores the important role of DHT in these processes [4,99,106,107].

Table 1
Comparison of the 5α-reductase-1 and -2 enzymes

Characteristic	Type I	Type II
Gene structure	5 exons, 4 introns	5 exons, 4 introns
Gene, chromosome location	SRD5A1, 5p15	SRD5A2, 2p23
Size	259 amino acids, MW = 29,462	254 amino acids, MW = 28,398
Tissue distribution	liver, non-genital skin, prostate, brain, ovary, testis	prostate, epididymis, seminal vesicle, genital skin, liver, uterus, breast, hair, follicle, placenta, testis
$_{p}$H optima	neutral to basic	acidic or neutral
Prostate level	low	high
Activity in 5α-reductase deficiency	normal	deficient
Finasteride inhibition	Ki ≥300 nM	Ki ≥3–5 nM

5α-Androstane–3α,17β-diol is a product of DHT that is formed in the pouch young of some marsupial species by the enzyme, 3α-hydroxysteroid dehydrogenase. In marsupials, this steroid seems to play a role in prostatic development; however, the role of 3α-hydroxysteroid dehydrogenase in human fetal development is unknown, although it has been detected in human tissues [71,108].

Actions of testosterone and dihydrotestosterone

The androgen receptor

The androgen receptor (AR) is a member of the nuclear receptor superfamily. It resides in the cytoplasm and nucleus of all cells, but is present at higher concentrations in tissues of the external genitalia and other androgen target tissues. The bound form of the receptor is almost exclusively nuclear. It is a 98-kD protein of 910–919 amino acids and is encoded by a gene with eight exons at xq11.2-q12 [109,110].

When testosterone or DHT attaches to the ligand-binding domain, nuclear chaperone proteins (eg, heat shock proteins HSP90 and HSP70) dissociate, induce a conformational change in the receptor, and increase phosphorylation [111–114]. Dimerization between AR molecules occurs and the complex binds to androgen-response elements on DNA. This leads to mRNA transcription and new protein synthesis [109,115]. Gene transcription also is regulated by coactivator and corepressor proteins (eg, *Src-1* and *Ara70*) [116–118]. Mutations that block androgen responsiveness in the AR lead to the syndrome of complete androgen insensitivity, which is characterized by phenotypic females with testes that secrete male levels of testosterone. Affected individuals have female external genitalia but lack a uterus, fallopian tubes, and the upper two thirds of the vagina; this is caused by normal secretion of MIS by the testes. Breast development occurs at puberty that is due to unopposed estrogen that is secreted by testes and formed from peripheral conversion of testosterone by aromatase. Incomplete forms of this syndrome that are due to varying degrees of AR unresponsiveness have been described; diverse phenotypes range from women who have clitoromegaly to men who have infertility [119–121].

In summary, normal male fetal development is dependent on the presence of three functional hormones—testosterone, DHT, and MIS. A defect in the production or action of any of these hormones in an XY individual will prevent normal male sexual differentiation.

Summary

Sexual differentiation and development in the fetus is a complex evolution from undifferentiated gonad to testes or ovaries; from Wolffian and Müllerian ducts to male and female genital tracts, respectively; and from common anlagen to male and female external genitalia. Unraveling the function of these genes is in

its early phases and many genetic factors that regulate sexual differentiation remain to be uncovered.

The hormonal regulation of sexual differentiation is equally complex. Specifically, MIS, testosterone, and dihydrotestosterone are necessary for complete male sexual differentiation. The absence of these factors leads to female sexual differentiation. In general, the process of female sexual differentiation seems to be independent of hormonal stimulation. The field of sexual differentiation continues to be an exciting area of research, in light of the many unanswered questions.

References

[1] Jost A. A new look at the mechanism controlling sex differentiation in mammals. Johns Hopkins Med J 1972;130:38–53.
[2] Arey L. Development anatomy: a textbook and laboratory manual of embryology. Philadelphia: Saunders; 1965. p. 315–42.
[3] Imperato-McGinley J. Sexual differentiation: normal and abnormal. Curr Top Exp Endocrinol 1983;5:232–306.
[4] Imperato-McGinley J, Zhu Y-S. Gender and behavior in subjects with genetic defects in male sexual differentiation. In: Pfaff DW, Arnold AP, Etgen AM, et al, editors. Hormones, brain and behavior, vol. 5. San Diego (CA): Elsevier Science; 2002. p. 303–45.
[5] Hammes A, Guo JK, Lutsch G, Leheste JR, Landrock D, Ziegler U, et al. Two splice variants of the Wilms' tumor 1 gene have distinct functions during sex determination and nephron formation. Cell 2001;106:319–29.
[6] Parker KL, Schedl A, Schimmer BP. Gene interactions in gonadal development. Annu Rev Physiol 1999;61:417–33.
[7] Ito M, Yu R, Jameson JL. DAX-1 inhibits SF-1 mediated transactivation via a carboxy-terminal domain that is deleted in adrenal hypoplasia congenital. Mol Cell Biol 1997;17:1476–83.
[8] Meeks JJ, Crawford SE, Russell TA, Morohashi K, Weiss J, Jameson JL. Dax1 regulates testis cord organization during gonadal differentiation. Development 2003;130:1029–36.
[9] Achermann JC, Ito M, Hindmarsh PC, Jameson JL. A mutation in the gene encoding steroidogenic factor-1 causes XY sex reversal and adrenal failure in humans. Nat Genet 1999;22:125–6.
[10] Parker KL, Schimmer BP, Schedl A. Genes essential for early events in gonadal development. Cell Mol Life Sci 1999;55:831–8.
[11] MacLaughlin DT, Donahoe PK. Sex determination and differentiation. N Engl J Med 2004; 350:367–78.
[12] Shawlot W, Behringer RR. Requirement for Lim1 in head-organizer function. Nature 1995; 346:240–4.
[13] Birk OS, Casiano DE, Wassif CA, Cogliati T, Zhao L, Zhao Y, et al. The LIM homeobox gene Lhx9 is essential for mouse gonad formation. Nature 2000;403:909–13.
[14] Miyamoto N, Yoshida M, Kuratani S, Matsuo I, Aizawa S. Defects of urogenital development in mice lacking Emx2. Development 1997;124:1653–64.
[15] Kreidberg JA, Sariola H, Loring JM, Maeda M, Pelletier J, Housman D, et al. WT-1 is required for early kidney development. Cell 1993;74:679–91.
[16] Luo X, Ikeda Y, Parker KL. A cell-specific nuclear receptor is essential for adrenal and gonadal development and sexual differentiation. Cell 1994;77:481–90.
[17] Cotinot C, Pailhoux E, Jaubert F, Fellous M. Molecular genetics of sex determination. Semin Reprod Med 2002;20:157–67.

[18] Everett NB. Observation and experimental evidences relating to origin and differentiation of definitive germ cells in mice. J Exp Zool 1943;92:49–91.
[19] Witschi E. Migration of the germ cells of human embryos from the yolk sac to the primitive gonadal folds. Contrib Embryol 1948;32:67–80.
[20] Mintz B, Russell ES. Gene-induced embryological modifications of primordial germ cells in the mouse. J Exp Zool 1957;134:207–37.
[21] Makabe S, Motta PM. Migration of human germ cells and their relationship with the developing ovary: ultrastructural aspects. In: Motta PM, editor. Developments in ultrastructure of reproduction. New York: Alan R. Liss; 1989. p. 41–54.
[22] Fujimoto T, Miyayama T, Fujita M. The origin, migration and fine morphology of human primordial germ cells. Anat Rec 1985;211:271–8.
[23] Motta PM, Nottola SA, Makabe S. Natural history of the female germ cell from its origin to full maturation through prenatal ovarian development. Eur J Obstet Gynecol Repro Biol 1997; 75:5–10.
[24] Makabe S, Nottola SA, Motta PM. Life history of the human female germ cell: ultrastructural aspects. In: Van Blerkom J, Motta PM, editors. Ultrastructure of human gametogenesis and early embryogenesis. Boston: Kluwer Academic; 1989. p. 33–60.
[25] Zuckerman S, Baker TG. The development of the ovary and the process of oogenesis. In: Zuckerman S, Weir BJ, editors. The ovary. 2nd edition, vol. 1. New York: Academic Press; 1977. p. 41–67.
[26] Saitou M, Barton SC, Surani MA. A molecular programme for the specification of germ cell fate in mice. Nature 2002;418:293–300.
[27] Matova N, Cooley L. Comparative aspects of animal oogenesis. Dev Biol 2001;231:291–320.
[28] Wartenberg H. Development of the early human ovary and role of the mesonephros in the differentiation of the cortex. Anat Embryol (Berl) 1982;165:253–80.
[29] Lovell-Badge R, Robertson E. XY female mice resulting from a heritable mutation in the primary testis-determining gene, Tdy. Development 1990;109:635–46.
[30] Bendsen E, Byskov AG, Laursen SB, Larsen HPE, Andersen CY, Westergaard LG. Number of germ cells and somatic cells in human fetal testes during the first weeks after sexual differentiation. Hum Reprod 2003;18:13–8.
[31] DeKrester DM, Kerr JB. The cytology of the testis. In: Knobil E, Neill JD, editors. The physiology of reproduction. New York: Raven Press; 1994. p. 1177–290.
[32] Sharpe RM, Miller M, McKinnell C. Relative roles of testosterone and the germ cell complement in determining stage-dependent changes in protein secretion by isolated rat seminiferous tubules. Int J Androl 1993;16:71–81.
[33] Simpson E, Chandler P, Goulmy E, Disteche CM, Ferguson-Smith MA, Page DC. Separation of genetic loci for the H-Y antigen and for testis determination on the human Y chromosome. Nature 1987;326:876–8.
[34] Sinclair AH, Berta P, Palmer MS, Hawkins JR, Griffiths BL, Smith MJ, et al. A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif. Nature 1990;346:240–4.
[35] McElreavey K, Vilain E, Abbas N, Costa JM, Souleyreau N, Kucheria K, et al. XY sex reversal associated with a deletion 5′ to the SRY "HMG box" in the testis determining region. Proc Natl Acad Sci USA 1992;89:11016–20.
[36] Koopman P, Munsterberg A, Capel B, Vivian N, Lovell-Badge R. Expression of a candidate sex determining gene during mouse testis differentiation. Nature 1990;348:450–2.
[37] McElreavey K, Vilain E, Barbeaux S, Fuqua JS, Fechner PY, Souleyreau N, et al. Loss of sequences 3′ to the testis-determining gene, SRY, including the Y pseudoautosomal boundary associated with partial testicular determination. Proc Natl Acad Sci USA 1996;93:8590–4.
[38] Hanley NA, Hagen DM, Clement-Jones M, et al. SRY, Sox9, and DAX1 expression patterns during human sex determination and gonadal development. Mech Dev 2000;91:403–7.
[39] Jameson JL, Achermann JC, Osizik G, et al. Battle of the sexes: new insights into genetic pathways of gonadal development. Trans Am Clin Climatol Assoc 2003;114:51–63.

[40] Parker KL, Schimmer BP. Genes essential for early events in gonadal development. Ann Med 2002;34:171–8.
[41] Tilmann C, Capel B. Cellular and molecular pathways regulating mammalian sex determination. Recent Prog Horm Res 2002;57:1–18.
[42] Foster JW, Dominguez-Steglich MA, Guioli S, et al. Campomelic dysplasia and autosomal sex reversal caused by mutations in an SRY related gene. Nature 1994;372:525–30.
[43] Wagner T, Wirth J, Meyer J, et al. Autosomal sex reversal and campomelic dysplasia are caused by mutations in and around the SRY-related gene SOX9. Cell 1994;79:1111–20.
[44] Huang B, Wang S, Ning Y, Lamb AN, Bartley J. Autosomal XX sex reversal caused by duplication of Sox9. Am J Med Genet 1999;87:349–53.
[45] Vidal VP, Chaboissier MC, de Rooij DG, Schedl A. A Sox9-induced testis development in XX transgenic mice. Nat Genet 2001;28:216–7.
[46] Colvin JS, Green RP, Schmahl J, Capel B, Ornitz DM. Male-to-female sex reversal in mice lacking fibroblast growth factor 9. Cell 2001;104:875–89.
[47] Lala DS, Rice DA, Parker KL. Steroidogenic factor I, a key regulator of steroidogenic enzyme expression, is the mouse homolog of fushi tarazu-factor I. Mol Endocrinol 1992;6:1249–58.
[48] Morohashi K, Honda S, Inomata Y, Handa H, Omura T. A common trans-acting factor, Ad4-binding protein, to the promoters of steroidogenic P-450's. J Biol Chem 1992;267: 17913–9.
[49] Miller RW, Fraumeni JF, Manning MD. Association of Wilms' tumor with aniridia, hemihypertrophy and other congenital malformations. N Engl J Med 1964;270:922–7.
[50] Little M, Holmes G, Bickmore W, van Heyningen V, Hastie N, Wainwright B. DNA binding capacity of the WT1 protein is abolished by Denys-Drash syndrome WT1 point mutations. Hum Mol Genet 1995;4:351–8.
[51] Fujieda K, Nakae J, Abe S, Shinohara N, Murashita M, Sato K. Analysis of hypothalamic-pituitary-testicular axis in a patient with X-linked adrenal hypoplasia congenital [abstract]. Presented at the Endocrine Society Meeting, June 24–27, 1998, New Orleans, 1998.
[52] Yu RN, Ito M, Saunders TL, Camper SA, Jameson JL. Role of Ahch in gonadal development and gametogenesis. Nat Genet 1998;20:353–7.
[53] Yao HH, Tilmann C, Zhao GQ, Capel B. The battle of the sexes: opposing pathways in sex determination. Novartis Found Symp 2002;244:187–98.
[54] Matzuk MM, Burns KH, Viveiros MM, Eppig JJ. Intercellular communication in the mammalian ovary: oocytes carry the conversation. Science 2002;296:2178–80.
[55] Prasad SV, Skinner SM, Carino C, Wang N, Cartwright J, Dunbar BS. Structure and function of the proteins of the mammalian zona pellucida. Cells Tissues Organs 2000;166:148–64.
[56] Jones HW, Ferguson-Smith MA, Heller RH. The pathology and cytogenetics of gonadal agenesis. Am J Obstet Gynecol 1963;87:578–600.
[57] Ferguson-Smith MA. Karyotype-phenotype correlations in gonadal dysgenesis and their bearing on the pathogenesis of malformations. J Med Genet 1965;2:142.
[58] Bardoni B, Zanaria E, Giuoli S, Floridia G, Worley K, Tonini G, et al. A dosage sensitive locus at chromosome Xp21 is involved in male-to-female sex reversal. Nat Genet 1994;7: 497–501.
[59] Swain A, Narvaez V, Burgoyne P, Camerino G, Lovell-Badge R. Dax1 antagonizes Sry action in mammalian sex determination. Nature 1998;391:761–7.
[60] Block E. Quantitative morphological investigations of the follicular system in women; variations at different ages. Acta Anat 1952;14:108.
[61] Block E. A quantitative morphological investigation of the follicular system in newborn female infants. Acta Anat 1953;17:201.
[62] Baker TG. A quantitative and cytological study of germ cells in human ovaries. Proc R Soc London Ser B 1963;158:417.
[63] Johnson J, Canning J, Kaneko T, Pru JK, Tilly J. Germline stem cells and follicular renewal in the postnatal mammalian ovary. Nature 2004;428:145–50.
[64] Imbeaud S, Belville C, Messika-Zeitoun L, et al. A 27 base-pair deletion of the anti-Mullerian

type II receptor gene is the most common cause of the persistent Mullerian duct syndrome. Hum Mol Genet 1996;5:1269–77.
[65] Cohen-Hagenauer O, Picard JY, Mattei MG, et al. Mapping of the gene for anti-Mullerian hormone to the short arm of human chromosome 19. Cytogenet Cell Genet 1987;44:2–6.
[66] Josso N, di Clemente N, Gouedard L. Anti-Mullerian hormone and its receptors. Mol Cell Endocrinol 2001;179:25–32.
[67] Rey R, Lukas-Criosier C, Lasala C, Bedecarras P. AMH/MIS: what we know about the gene, protein, and its regulation. Mol Cell Endocrinol 2003;211:21–31.
[68] Arango NA, Lovell-Badge R, Behringer RR. Targeted mutagenesis of the endogenous mouse MIS gene promoter: in vivo definition of genetic pathways of vertebrate sexual development. Cell 1999;99:409–19.
[69] Watanabe K, Clarke TR, Lane AH, Wang X, Donahoe PK. Endogenous expression of Mullerian inhibiting substance in early postnatal rat Sertoli cells requires multiple steroidogenic factor-1 and GATA-4 binding sites. Proc Natl Acad Sci USA 2000;97:1624–9.
[70] DeSanta BP, Bonneaud N, Boizet B, et al. Direct interaction of SRY-related protein SOX9 and steroidogenic factor 1 regulates transcription of the human anti Mullerian hormone gene. Mol Cell Biol 1998;18:6653–65.
[71] Warne GL, Kanumakala S. Molecular endocrinology of sex differentiation. Semin Reprod Med 2002;20:169–79.
[72] Tremblay JJ, Viger RS. Transcription factor GATA-4 enhances Mullerian inhibitory substance gene transcription through a direct interaction with the nuclear receptor SF-1. Mol Endocrinol 1999;99:409–19.
[73] Clements J, Reyes E, Winter JSD, Faiman C. Studies on human sexual development. III. Fetal pituitary and serum, and amniotic fluid concentration of LH, CG, and FSH. J Clin Endocrinol Metab 1976;42:9–19.
[74] Kaplan SL, Grumbach MM, Aubert ML. The ontogenesis of pituitary hormones and hypothalamic factors in the human fetus: maturation of the central nervous system regulation of anterior pituitary function. Recent Prog Horm Res 1976;32:161–243.
[75] Fluck CE, Tajima T, Pandey AV, Arlt W, Okuhara K, Verge CF, et al. Mutant P450 oxidoreductase causes disordered steroidogenesis with and without Antley-Bixler syndrome. Nat Genet 2004;36:228–30.
[76] Stocco DM. The role of the StAR protein in steroidogenesis: challenges for the future. J Endocrinol 2000;164:247–53.
[77] Clark BJ, Wells J, King SR, Stocco DM. The purification, cloning and expression of a novel luteinizing-hormone induced mitochondrial protein in MA-10 mouse Leydig tumor cells. Characterization of the steroidogenic acute regulatory protein (StAR). J Biol Chem 1994; 269:28314–22.
[78] Prader A, Siebenmann RE. Adrenal insufficiency in congenital lipoid hyperplasia. Helv Pediatr Acta 1957;12:569–95.
[79] Lin D, Sugawara T, Strauss III JF, Clark BJ, Stocco DM, Saenger P, et al. Role of steroid acute regulatory protein in adrenal and gonadal steroidogenesis. Science 1995;267: 1828–31.
[80] Peterson R, Imperato-McGinley J. Male pseudohermaphroditism due to inherited deficiencies of testosterone biosynthesis. In: Serio M, editor. Sexual differentiation: basic and clinical aspects. New York: Raven Press; 1984. p. 301–19.
[81] Sparkes RS, Klisak I, Miller WL. Regional mapping of genes encoding human steroidogenic enzymes: P450scc to 15q23–24; andrenoxin to 11q22; andrenoxin reductase to 17q24–q25; and P450c17 to 10q24–q25. DNA Cell Biol 1991;10:359–65.
[82] Kater CE, Biglieri EG. Disorders of steroid 17α-hydroxylase deficiency. Endocrinol Metab Clin North Am 1994;23:341–57.
[83] Biason-Lauber A, Leiberman E, Zachman M. A single amino acid substitution in the putative redox partner-binding site of P450c17 as cause of isolated 17,20-lyase deficiency. J Clin Endocrinol Metab 1997;82:3807–12.

[84] Sherbet DP, Tiosano D, Kwist KM, Hochberg Z, Auchus RJ. Cyp17 mutation E305G causes isolated 17,20-lyase deficiency by selectively altering substrate binding. J Biol Chem 2003; 278:48563–9.
[85] Lorence MC, Corbin CJ, Kamimura N, et al. Structural analysis of the gene encoding the human 3β-hydroxysteroid dehydrogenase. Mol Endocrinol 1990;4:1850–5.
[86] Berube D, Luu-The V, Lachance Y, et al. Assignment f the human 3β-hydroxysteroid dehydrogenase gene to the p13 band of chromosome 1. Cytogenet Cell Genet 1989;52: 199–200.
[87] Bongiovanni AM. Unusual steroid pattern in congenital adrenal hyperplasia: deficiency of 3β-hydroxy dehydrogenase. J Clin Endocrinol Metab 1961;21:860–2.
[88] Andersson S, Russell DW, Wilson JD. 17β-hydroxysteroid dehydrogenase 3 deficiency. Trends Endocrinol Metab 1996;7:121–6.
[89] Luu-The V, Labrie C, Zhao HF, et al. Purification, cloning, complementary DNA structure, and predicted amino acid sequence of human estradiol 17β-dehydrogenase. Ann N Y Acad Sci 1989;595:40–52.
[90] Casey ML, MacDonald PC, Andersson S. l7β-hydroxysteroid dehydrogenase type 2: chromosomal assignment and progestin regulation of gene expression in human endometrium. J Clin Invest 1994;94:2135–41.
[91] Geissler WM, Davis DL, Wu L, et al. Male pseudohermaphroditism caused by mutations of testicular 17β-hydroxysteroid dehydrogenase 3. Nat Genet 1994;7:34–9.
[92] Adamski J, Normand T, Leenders F, et al. Molecular cloning of a novel widely expressed human 80 kDa 17β-hydroxysteroid dehydrogenase IV. Biochem J 1994;311:437–43.
[93] Andersson S, Geissler WM, Wu L, et al. Molecular genetics and pathophysiology of 17β-hydroxysteroid dehydrogenase 3 deficiency. J Clin Endocrinol Metab 1996;81:130–6.
[94] Boehmer ALM, Brinkman AO, Lodewijk AS, et al. 17β-hydroxysteroid dehydrogenase-3 deficiency: diagnosis, phenotypic variability, population genetics, and worldwide distribution of ancient and de novo mutations. J Clin Endocrinol Metab 1999;84:4713–21.
[95] Rosler A, Kohn G. Male pseudohermaphroditism due to 17β-hydroxysteroid dehydrogenase deficiency: studies on the natural history of the defect and effect of androgens on gender role. J Steroid Biochem 1983;19:663–74.
[96] Wilson JD. The role of androgens in male gender role behavior. Endocr Rev 1999;20: 726–37.
[97] Federman DD. In: Abnormal sexual development. A genetic and endocrine approach to differential diagnosis. Philadelphia: Saunders; 1967. p. 1–14.
[98] Wilson JD. Sexual differentiation. Annu Rev Physiol 1978;40:270–306.
[99] Imperato-McGinley J, Gerrero L, Gautier T, Peterson RE. Steroid 5α-reductase deficiency in man: an inherited form of male pseudohermaphroditism. Science 1974;186:1213–5.
[100] Walsh PC, Madden JD, Harrod MJ. Familial incomplete male pseudohermaphroditism, type 2. Decreased dihydrotestosterone formation in pseudovaginal perineoscrotal hypospadias. N Engl J Med 1974;291:944–9.
[101] Wilbert DM, Griffin JE, Wilson ID. Characterization of the cytosol androgen receptor of the human prostate. J Clin Endocrinol Metab 1983;56:113–20.
[102] Kovacs WJ, Griffin JE, Weaver DD, et al. A mutation that causes lability of the androgen receptor under conditions that normally promote transformation to the DNA binding state. J Clin Invest 1984;73:1095–104.
[103] Grino RB, Griffin JE, Wilson JD. Testosterone at high concentrations interacts with the human androgen receptor similarly to dihydrotestosterone. Endocrinology 1990;126:1165–72.
[104] Fratianni CM, lmperato-McGinley J. The syndrome of 5α-reductase deficiency. Endocrinologist 1994;4:302–14.
[105] Russell DW, Wilson JD. Steroid 5alpha-reductase: two genes/two enzymes. Annu Rev Biochem 1994;63:25–61.
[106] Imperato-McGinley J, Peterson RE, Gautier T, et al. Male pseudohermaphroditism secondary to 5α-reductase deficiency- a model for the role of androgens in both the development of

the male phenotype and the evolution of a male gender identity. J Steroid Biochem 1979;11: 637–45.
[107] Imperato-McGinley J, Peterson RE, Gautier T, et al. Androgens and the evolution of male-gender identity among male pseudohermaphrodites with 5α-reductase deficiency. N Engl J Med 1979;300:1233–7.
[108] Chetyrkin SV, Hu J, Gough WH, Dumaual N, Kedishvili NY. Further characterization of human microsomal 3α-hydroxysteroid dehydrogenase. Arch Biochem Biophys 2001;386:1–10.
[109] Beato M. Gene regulation by steroid hormones. Cell 1989;56:335–44.
[110] Quigley CA, De Bellis A, Marschke KB, el-Awady MK, Wilson EM, French FS. Androgen receptor defects: history, clinical and molecular perspectives. Endocr Rev 1995;16:271–321.
[111] Adachi H, Katsumo M, Minamiyama M, Sang C, Pagoulatos G, Angelidis C, et al. Heat shock protein 70 chaperone overexpression ameliorates phenotypes of the spinal and bulbar muscular atrophy transgenic mouse model by reducing nuclear-localized mutant androgen receptor protein. J Neurosci 2003;23:2203–11.
[112] Georget V, Terouanne B, Nicolas JC, Sultan C. Mechanism of antiandrogen action: key role of HSP90 in conformational change and transcriptional activity of the androgen receptor. Biochemistry 2002;41:11824–31.
[113] Marivoet S, Van Dijck P, Verhoeven G, Heyns W. Interaction of the 90-kDa heat shock protein with native and in vitro translated androgen receptor and receptor fragments. Mol Cell Endocrinol 1992;88:165–74.
[114] Haendler B, Schuttke I, Schleuning WD. Androgen receptor signalling: comparative analysis of androgen response elements and implication of heat shock protein 90 and 14–3-3eta. Mol Cell Endocrinol 2001;173:63–73.
[115] O'Malley BW, Means AR. Female steroid hormones and target cell nuclei. Science 1974;183: 610–20.
[116] Onate SA, Tsai SY, Tsai MJ, O'Malley BW. Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. Science 1995;270:1354–7.
[117] Yeh S, Chang C. Cloning and characterization of a specific coactivator, ARA70, for the androgen receptor in human prostate cells. Proc Natl Acad Sci USA 1996;93:5517–21.
[118] Glass CK, Rose DW, Rosenfeld MG. Nuclear receptor coactivators. Curr Opin Cell Biol 1997; 9:222–32.
[119] Sultan C, Lumbroso S, Paris F, et al. Disorders of androgen action. Sem Repro Med 2002;20: 217–27.
[120] Rodien P, Mebarki F, Mowszowicz I, et al. Different phenotypes in a family with androgen insensitivity caused by the same M780I point mutation in the androgen receptor gene. J Clin Endocrinol Metab 1996;81:2994–8.
[121] Melo KFS, Mendonca BB, Billerbeck AEC, et al. Clinical, hormonal, behavioral and genetic characteristics of androgen insensitivity syndrome in a Brazilian cohort: five novel mutations in the androgen receptor gene. J Clin Endocrinol Metab 2003;88:3241–50.

ELSEVIER
SAUNDERS

Obstet Gynecol Clin N Am
31 (2004) 857–872

OBSTETRICS AND
GYNECOLOGY
CLINICS
OF NORTH AMERICA

Biochemical screening for congenital defects

Ray O. Bahado-Singh, MD[a,*], Jaime Sutton-Riley, PhD[b]

[a]*Department of Obstetrics & Gynecology, University of Cincinnati College of Medicine, 231 Albert Sabin Way, Cincinnati, OH 45267, USA*
[b]*Department of Obstetrics and Gynecology, University of New Mexico, M.S.C. 105580, Albuquerque, NM 87131-0001, USA*

Midtrimester Down syndrome serum markers

Merkatz et al [1] were the first to report an observation that linked maternal serum α-fetoprotein (AFP) levels and fetal chromosome abnormalities. The combination of serum AFP and maternal age [2] resulted in a significant improvement in case detection compared with age alone. Numerous subsequent studies showed a correlation between low maternal serum AFP (MSAFP) and Down syndrome. In a meta-analysis of 38 studies with a total of 1328 cases of Down syndrome, the median MSAFP value was 0.75 multiples of the median (MOM) (95% confidence interval [CI], 0.72–0.78) [3].

Subsequently, Bogart et al [4] found elevated maternal serum levels of human chorionic gonadotropin (hCG) in affected pregnancies in the midtrimester. Meta-analysis of 28 studies with a total of 907 cases of Down syndrome revealed a median MOM hCG value of 2.06 (95% CI, 1.95–2.17) [3]. Maternal unconjugated serum estriol (uE_3) also is reduced in Down syndrome pregnancies and became the third analyte to be used for screening [5]. In a meta-analysis of 21 studies (15–22 weeks' gestation) that consisted of 733 cases of Down syndrome, a median uE_3 MOM value of 0.72 (95% CI, 0.68–0.75) was determined [3]. When each individual marker is combined with maternal age, the following detection rates are achieved—AFP, 36%; uE_3, 41%; and total hCG, 49%. Multiple marker screening that combines all three markers plus maternal age has been the standard midtrimester screening tool in the United States

* Corresponding author.
E-mail address: bahadoro@ucmail.uc.edu (R.O. Bahado-Singh).

doi:10.1016/j.ogc.2004.08.010

for approximately 15 years. Mathematically-modeled estimates of diagnostic accuracy for midtrimester triple serum screening was performed for the U.S. population. Using a 1:270 risk threshold in the second trimester [6] for the overall pregnant population in the United States, the estimated detection and false positive values were 73% and 9%, respectively.

Inhibin-A

Inhibin is a dimer that consists of an α subunit and one of two different β subunits. These are dimeric inhibin-A (α-βA) and inhibin-B (α-βB). Inhibin-A is measurable in the serum of pregnant women and may play a role in hCG secretion.

Cuckle et al [7] compared inhibin-A levels in 19 midtrimester Down syndrome and 95 matched controls. Median serum levels in the affected group was 1.6 MOM; this was statistically significantly elevated compared with normals. In a study that consisted of 58 Down syndrome and 38 normal serum specimens between 7 and 18 weeks' gestation, Aitken et al [8] observed a median inhibin-A level of 2.6 MOM in Down syndrome cases. This compared with median values of 2.09 MOM for free β-hCG and 1.82 MOM for intact hCG. Between 15 and 18 weeks' gestation, the median value for inhibin-A was 2.24 MOM compared with normal. Combined with maternal age, inhibin-A had a 48% detection rate and a 5% false positive rate for Down syndrome detection.

Spencer et al [9] found a median value for inhibin A of 1.77 MOM when 157 Down syndrome cases were compared with 367 controls at between 14 and 20 weeks' gestation. By itself, dimeric inhibin-A had a 37% detection rate; when combined with maternal age it had a 48% detection rate for Down syndrome. Among 56 Down syndrome and 280 unaffected cases between 13 and 21 weeks' gestation, the median inhibin-A level was 1.62 MOM (95% CI, 1.34–1.96) in the affected cases in a study that was performed by Cuckle et al [10].

Wallace et al [11] reported a median inhibin-A level of 2.6 MOM (95% CI, 2.25–3.57) when they compared cases of Down syndrome with controls; they calculated a 62% detection rate and a 5.3% false positive rate. Wenstrom et al [12] reported the results of 33 cases of Down syndrome and 313 normals between 14 and 20 weeks' gestation. Mean inhibin-A level was elevated significantly in Down syndrome compared with a normal group (2.84 versus 1.22 MOM, $P = .0001$). A 42% detection rate and 6% false positive rate were observed. A median inhibin-A level for Down syndrome of 1.62 MOM was reported for Asian women [13]. The study included 49 Down syndrome pregnancies and 341 controls. This yielded a 26% detection rate and a 5% false positive rate for inhibin-A [13]. Wald et al [14] estimated that the Down syndrome sensitivity of inhibin-A as an isolated marker was 44% to 45% with a 5% false positive rate. The same group performed a meta-analysis of six studies that consisted of 375 midtrimester cases of Down syndrome. These included most of the studies that were discussed above. Overall, the median inhibin-A level was 1.92 MOM (95%

CI, 1.75–2.15) [3]. Inhibin-A seems to be a sensitive midtrimester serum marker for Down syndrome.

Inhibin-A is largely uncorrelated with AFP and uE_3 levels, although there is a modest correlation with total hCG. An increasing number of centers in the United States now offer quadruple marker screening, including inhibin-A along with the three other midtrimester serum analytes. Projected diagnostic performance of the four-marker test in the U.S. population showed a moderate improvement over the triple test [6]. For women who are younger than 35 years of age, 67% sensitivity and 5.5% false positive rates are expected, whereas for women who are older than 35 years of age, the projected rates are 92% and 23.6%, respectively. For the overall population, the values are 79% and 7.8% respectively. At a 5% false positive rate threshold, a 59% detection rate with a 1 in 65 positive predictive value was reported for the triple test compared with 69% detection rate and a 1 in 55 positive predictive value for the quadruple test [3]. The addition of inhibin-A to the midtrimester triple screen increases the Down syndrome detection rate by 5% to 10%.

Free β-human chorionic gonadotropin

Since the association between a midtrimester Down syndrome fetus and elevated intact hCG levels was first reported [4], this analyte has become a mainstay of Down syndrome biochemical screening in the midtrimester. Although intact hCG remains the most commonly used form of this molecule, free β-hCG was shown to be elevated in midtrimester maternal serum in Down syndrome pregnancies [15]. Studies evaluated the usefulness of the two component subunits of intact hCG (ie, free α- and free β-subunits. Macri et al [16] compared maternal serum free β-hCG levels in 29 cases of trisomy 21 and 450 controls. Free β-hCG was elevated in cases of Down syndrome and had a 58.6% detection rate and a 5% false positive rate between 14 and 22 weeks' gestation. In cases of less than 17 weeks' gestation, the detection rate for Down syndrome increased to 66.7%. In a follow-up study that used stored serum specimens, 23 singleton pregnancies with Down syndrome were compared with 8 matched controls each. The gestational ages were 16 and 17 weeks [17]. The median value for free β-hCG was 2.05 MOM in the affected group. Overall, a 65% detection rate for Down syndrome was achieved with a 5% false positive rate. Spencer et al [18] compared free β-hCG to total hCG. In this study of 90 Down syndrome and 2800 normal cases, the median free β-hCG in Down syndrome pregnancies was elevated significantly ($P < .001$) compared with normals, at 2.41 MOM. For intact hCG, the value was 2.03 MOM, which also was elevated significantly compared with normals. Free β-hCG had a 41.1% detection rate and a 3.9% false positive rate compared with 32.2% and 3.7%, respectively, for intact hCG.

Ryall et al [19] compared free β-hCG to intact hCG in 57 Down syndrome pregnancies and 171 mid-trimester controls. The median free β-hCG value in the

affected group was 2.36 MOM compared with 2.12 MOM for intact hCG. This confirmed the apparent superiority of the free subunit for discriminating affected from normal cases. When combined with maternal age, the modeled detection and false positive values for free β-subunit and intact hCG for the general South Australia population were 40.6% and 8.44% and 35.3% and 9.75% respectively. Norgaard-Pederson et al [20] found a median value for free β-hCG of 2.31 MOM in their Down syndrome group compared with 2.11 MOM for intact hCG. When free β-hCG or intact hCG were combined with serum AFP and maternal age, a modest, but not significant, improvement in detection rate was noted. Down syndrome detection values of 59.0% and 50.5% and false positive rates of 4.7% and 4.9%, were found respectively, for free β-hCG, AFP and age versus intact hCG, AFP and age. In one of the few population-based screening studies at that time, Spencer [21] evaluated the free β-hCG levels in 67,904 midtrimester pregnancies; 107 had Down syndrome. The group that had Down syndrome had a median free β-hCG level of 2.53 MOM compared with 1.00 MOM in the normals. In a meta-analysis of 12 studies that consisted of 562 cases of Down syndrome in the midtrimester, the median free β-hCG value was 2.20 MOM (95% CI, 2.07–2.33) [15]. Overall, therefore, multiple studies indicate a small, but consistent, improvement in Down syndrome detection when free β-hCG, rather than the intact molecule, is measured in the midtrimester. Because the improvement in the detection rate is small, most screening algorithms continue to use intact hCG in the midtrimester algorithm.

Other serum markers

Cancer antigen 125

Cancer antigen (CA) 125 is an antigenic site on a glycoprotein. Its role is undetermined. CA 125 is expressed by areas of the genital tract (eg, fallopian tube, endometrium, cervical canal) in addition to the pleural and pericardial lining. Pathologic elevations are noted in ovarian cancer. In pregnancy, increased levels are observed in the decidual amniotic fluid and amnion. Check et al reported a potential association between abnormal early pregnancy serum levels and chromosomal anomalies [22]. Van Blerk et al [23] compared midtrimester serum levels of CA 125 in 19 cases of Down syndrome and 187 controls. Some of the specimens were from stored sera. Maternal serum CA 125 values were lower in cases of Down syndrome, with median values 0.72 MOM's in the Down syndrome group. This difference did not achieve statistical significance. Similarly, no significant association was found when amniotic fluid values were compared.

Spencer [24] reported no significant variations in serum levels in normal pregnancies between 16 and 20 weeks' gestation. There also was no significant difference in CA 125 levels between Down syndrome and normal midtrimester pregnancies. Van Lith et al [25] also found no significant difference in maternal

serum levels between 29 Down syndrome and 265 first and second trimester controls; they concluded that this was not a useful serum marker for the detection of Down syndrome. In a meta-analysis of five published studies that included 81 cases of Down syndrome, Wald et al [3] reported that median levels of this serum analyte was slightly less than 1.0 MOM in affected cases compared with the normal group. Based on these analyte studies, CA 125 does not have significant value in the detection of Down syndrome.

Urea-resistant neutrophil alkaline phosphatase

Urea-resistant neutrophil alkaline phosphatase (URNAP) activity level is reportedly elevated in individuals who have Down syndrome. Increased serum levels also were reported in nonpregnant women who previously had given birth to a Down syndrome fetus. Cuckle et al [26] used a semiquantitative technique for measuring the activity of this phosphatase in 72 cases of Down syndrome cases (three were in the first trimester) compared with 156 normals from the first and second trimester. The median activity level was 1.65 MOM (95% CI, 1.56–1.74), which was significantly greater than normal ($P < .0001$). Using a threshold value of at least 1.5 MOM, a detection rate of 68% was obtained with a false positive rate of 1% for first - and midtrimester cases [27]. Tafas et al [28] used an automated image analysis method to compare the measurement of neutrophil alkaline phosphatase (NAP) with URNAP in 15 Down syndrome cases and 25 unaffected controls. Median URNAP levels were 112 and 51 in affected cases versus controls, whereas NAP values were 86.1 and 51.5, respectively. The median ratios of URNAP:NAP was elevated significantly for midtrimester cases of Down syndrome. Peleg et al [29] did not find clinically significant differences in heat stable neutrophil-derived placental alkaline phosphatase or URNAP when they compared 13 normal and 13 affected samples. Although URNAP may be elevated moderately in Down syndrome pregnancies, large-scale screening seems to have limited practical value.

The use of invasive trophoblast antigen to detect gestational Down syndrome

Invasive trophoblast antigen (ITA)—also known as ''hyperglycosylated hCG''—is an hCG glycosylation variant that is produced at the time of implantation of pregnancy. ITA shares the same peptide sequence with regular hCG; however, the ITA molecule has a much greater carbohydrate content and has a different secretion pattern throughout early pregnancy [30,31]. ITA is produced only by the invasive cytotrophoblasts, instead of the differentiated syncytiotrophoblasts that produce regular hCG [32]. Moreover, ITA seems to have an independent function from hCG; ITA promotes cell invasion and likely is a necessary component of proper blastocyst implantation [33].

It was shown that Down syndrome pregnancy samples have an increased proportion of ITA compared with samples from normal pregnancies [34,35].

Later, this increased ITA expression was explained when it was shown that Down syndrome fetuses show a defect in differentiation of the cytotrophoblasts into syncytiotrophoblasts; this results in an accumulation of these ITA-producing cells [36–38].

Using an hCG preparation named C5, which was 100% hyperglycosylated, an ITA-specific antibody was generated (B152). This was followed by the development of a microtiter plate immunometric assay using B152 as the capture antibody and a commercial peroxidase-labeled anti-hCGβ as the tracer [35,39,40]. Recently, Nichols Institute Diagnostics (San Clemente, California) developed an automated assay for the Nichols Advantage immunoassay platform that uses the B152 antibody and a chemiluminescent monoclonal anti-hCGβ as the tracer [41]. This automated assay will be released soon in the United States and already is available in Europe.

Clinical trials began in 1997 to collect serum and urine samples from women who were undergoing amniocentesis during their second trimester because of advanced maternal age. In this blind study, using MOM statistics, urine samples that were taken from patients who were carrying Down syndrome fetuses (confirmed by karyotype analysis) had ITA levels that were 9.5-fold higher than the range that was given for urine samples that were taken from patients who had normal pregnancies. Receiver operating characteristic curve analysis indicated that a single-measurement test for ITA would detect 80% of cases of Down syndrome with a 5% false positive rate. Using the serum samples that were collected, a triple-screen test (hCG + unconjugated estriol + AFP) gave a detection rate of 64% with a 5% false positive rate. Finally, a multivariate analysis showed that using the urine ITA results in combination with the serum triple screen provided a test that achieved 96% detection with a 5% false positive rate [35].

Preliminary results from a study that used 10 serum samples from women in their second trimester showed elevated ITA levels in Down syndrome (confirmed by karyotype analysis) to a lesser degree than in urine [42]. Using MOM statistics, serum samples from Down syndrome pregnancies showed ITA levels that were 3.9-fold greater than levels from normal pregnancies. Serum ITA detected 60% of the Down syndrome cases with a 5% false positive rate [42]. In a further study that evaluated serum samples that were shipped at ambient temperatures and frozen at −20°C for 8 years, ITA serum measurements detected only 55% of the cases of Down syndrome with a 5% false positive rate [43]. When ITA serum measurements were used in combination with a quadruple-screen test (hCG + unconjugated estriol + AFP + inhibin), 95% of cases of Down syndrome were detected with a 5% false positive rate [43]. Large clinical trials are ongoing to continue to test the efficacy of ITA measurements in serum samples and fresh urine samples, as well as the use of ITA as part of a combination marker.

The value of ITA in the first trimester as a Down syndrome screen test is less well established. One study showed that serum ITA levels—from patients in their first trimester—could detect 37% of cases of Down syndrome with a 5% false

positive rate [44]. Another study showed that although combination serum measurements (hCG + free-β + pregnancy-associated plasma protein [PAPP]-A) detected 60% of the cases of Down syndrome with a 5% false positive rate, using urine ITA measurements with serum hCG free-β and PAPP-A detected 81% of cases of Down syndrome with a 5% false positive rate [45].

Most recently, it was shown that the increase in ITA in Down syndrome samples is due to a sialic acid–deficient ITA molecule [46]. This phenomena might explain the decreased efficacy of ITA in serum samples compared with urine samples, because sialic acid–deficient ITA—with its exposed liver galactose receptors—would be cleared from the circulation more rapidly than normal ITA [46]. The discovery of this sialic acid–deficient form of ITA—shown to be limited strictly to Down syndrome samples—might provide a more discriminatory screening tool in the future. Studies are underway with a goal to develop an assay that specifically recognizes the sialic acid–deficient form of ITA. This may improve greatly the discrimination between cases of Down syndrome and corresponding controls.

ITA is an unstable dimer and dissociates into subunits 10 times faster than regular hCG [47]. It has also been shown that with Down syndrome samples there is an increased risk for aggregation with multiple/slow freezing and thawing of the samples [48]. Therefore great lengths should be taken to properly store and handle Down syndrome samples, particularly when looking at ITA. Ideally an assay for detecting ITA would detect the β subunit of the ITA molecule and not the dimer itself. ITA appears to be an applicable tool in screening for gestational Down syndrome in early pregnancy, although the optimal gestational timing of the ITA measurement, as well as beneficial hormonal markers with which to combine it, remains to be elucidated.

First trimester serum screening for Down syndrome

In the last decade there has been a gradual shift away from the midtrimester screening toward first trimester–screening. The purported advantages of first trimester–screening include early reassurance with normal results, early diagnosis of fetal disorder, and—based on the standard algorithms that are used currently—improved diagnostic accuracy.

Since the midtrimester serum markers were the first ones used in Down syndrome screening, the logical question was whether these markers were also of value in first trimester–screening. Among the first studies to look at this issue was one by Cuckle et al [49]. Reduced levels of AFP and uE_3 was found in the first trimester. Since that time, extensive studies of AFP, intact hCG, and uE_3 have been performed in the first trimester. Wald et al [50] performed a multicenter, multi-national, case control trial of first trimester serum markers. A total of 77 stored Down syndrome pregnancy samples between 8 and 14 weeks' gestation were used. Each case was matched with five controls. The traditional midtrimester serum markers performed poorly in the first trimester compared

with the midtrimester. The median AFP level in first trimester Down syndrome was 0.87 MOM. Corresponding values for uE_3 and intact hCG were 0.96 MOM and 1.23 MOM, respectively. Individual sensitivities for Down syndrome—when an analyte was combined with maternal age—was 32% for AFP, 32% for intact hCG, and 30% for uE_3 in the first trimester; all values used a 5% threshold false positive rate.

In a meta-analysis of 26 studies from 1966 to 1999 in which 243 cases of first trimester Down syndrome were analyzed, the mean level of AFP was 0.79 MOM [51]. A meta-analysis of 8 series comprising 210 first trimester cases of Down syndrome found a median serum uE_3 (MOM) value of 0.71 (95% CI, 0.59–0.86) [52] for intact hCG. An analysis of 14 series that consisted of 352 affected cases that were evaluated between 1988 and 1995 reported an intact hCG level of 1.29 MOM (95% CI, 1.16–144). Thus, there is a statistically significant reduction in AFP and uE_3 in first trimester–Down syndrome pregnancies and an elevation of intact hCG levels. All of these values mirror changes that are observed in the midtrimester. Overall, however, these two markers are not considered to sufficiently sensitive for first trimester screening.

Free β–human chorionic gonadotropin

Free β-hCG has emerged as one of the most important biochemical markers in first trimester–Down syndrome screening. Extensive studies have been performed. It seems that this marker may have an advantage over the intact hCG molecule in detecting Down syndrome in the first trimester. In the multi-center, case control study of Wald et al [53], which contained 72 first trimester cases of Down syndrome, the median free β-hCG level was 1.79 MOM (95% CI, 1.49–2.16). The average free β-hCG value was greater than intact hCG in the affected cases. At a fixed false positive rate of 5%, the detection rate was 38% for free β-hCG and 32% for intact hCG.

A systematic review of 12 first trimester pregnancies (10–14 weeks' gestation) between 1990 and 1996, which consisted of 12 studies with 308 cases of Down syndrome, found a median free β-hCG level of 1.83 MOM (95% CI, 1.65–2.03). Corresponding values for intact hCG in 14 first trimester studies with a total of 352 cases of Down syndrome was 1.29 MOM (95% CI, 1.16–1.44) [3]. A meta-analysis of 17 first trimester series by Cuckle and van Lith [51] consisted of 579 cases of Down syndrome that were less than 14 weeks' gestation. The average free β-hCG level was 1.98 MOM (95% CI, 1.83–2.10 MOM) in the latter group. Recently, a large trial consisted of 40,387 singleton first trimester samples; 85 cases of Down syndrome were included [54]. A comparison was made of the median levels of intact and free β-hCG at intervals from 10 to 13 completed weeks of gestation. At 10 weeks' gestation, median MOM values for Down syndrome pregnancies were 0.96 and 1.94, respectively. The corresponding values for 11 completed weeks' gestation were 1.24 and 1.61, respectively; 1.45 and 2.22, respectively, for 12 completed weeks' gestation, and 2.07 and

2.50 MOMs, respectively for 13 completed weeks' gestation.. Based on the preponderance of published literature, it seems safe to conclude that between 10 and 14 weeks' gestation, free β-hCG offers an advantage over intact hCG as a screening marker for Down syndrome. The last referenced study [53] reported significant variation with gestational age in the first trimester detection rate that was achieved with free β-hCG. At a 5% false positive rate (excluding maternal age), the detection rate for Down syndrome, using free β-hCG, was 19% at 10 weeks' gestation, 28% at 11 weeks' gestation, 35% at 12 weeks' gestation, and 44% at 13 completed weeks' gestation. Corresponding values for intact hCG were 5%, 16%, 26%, and 41%, respectively. This information emphasizes the important point that the improvement in detection rate that is achieved by using free β-hCG, rather than the intact molecule, is affected significantly by the gestational age at which screening occurs.

Canick and Kellner [55], in a systematic review of 19 articles that were published between 1990 and 1999 and included 518 cases of first trimester Down syndrome, reported an overall median of free β-hCG of 1.91 MOM.

Pregnancy-associated plasma protein–A

PAPP-A represents the second of the two biochemical markers that have found wide usage in the screening of first trimester Down syndrome. This molecule is another glycoprotein product of the cytotrophoblast. Earlier studies by Brambati et al [56] and Wald et al [57] found significant depression of PAPP-A levels in the maternal serum of chromosomally-abnormal pregnancies. Brambati et al [58] evaluated 14 first-trimester cases from a population of 522 women who were between 7 and 11 weeks' gestation, before chorionic villus sampling. The median maternal serum PAPP-A level was 0.27 MOM compared with 1.01 MOM ($P < .00001$) in Down syndrome compared to normal pregnancies respectively.

PAPP-A was estimated to have a 58% detection rate for Down syndrome. In the first-trimester screening study of Wald et al [59], a median PAPP-A level of 0.43 MOM (95% CI, 0.34–0.53) was found among the 77 cases of Down syndrome. A meta-analysis of 12 published series between 1992 and 1996, including 297 cases of Down syndrome, found a median first-trimester PAPP-A level of 0.38 MOM (95% CI, 0.33–0.43) [52]. Cuckle [60] reported a meta-analysis of 341 first-trimester cases of Down syndrome that were obtained from 18 published series. Gestation age at screening had a significant impact on the median PAPP-A levels in cases of Down syndrome. Between 6 and 8 weeks' gestation, the average PAPP-A level in affected cases was 0.35 MOM; it was 0.40 MOM between 9 and 11 weeks' gestation and 0.62 MOM between 12 and 14 weeks' gestation. Thus, although free β-hCG has an improved detection rate with increasing gestation, the opposite is seen with first-trimester maternal serum PAPP-A measurements. This information was confirmed in the Serum, Urine, and Ultrasound screening (SURRUS) trial of 40,387 first-trimester singleton pregnancies [54]. Median first-trimester PAPP-A values in Down syndrome cases

were 0.42 MOM at 10 weeks' gestation, 0.38 at 11 weeks' gestation, 0.44 at 12 weeks' gestation, and 0.60 MOM at 13 weeks' gestation. By itself (ie, excluding maternal age), PAPP-A had 58%, 45%, 35%, and 27% detection rates at the aforementioned gestational ages.

Combined free β–human chorionic gonadotropin and pregnancy-associated plasma protein–A for Down syndrome screening

Several studies have modeled a combination of the two serum markers with maternal age for first-trimester Down syndrome screening. Canick and Kellner [55] reviewed five series that were published from 1995 to 1998. The detection rates varied from 55% to 63%; the average detection rate was 60% with a 5% false positive rate for the 226 cases of Down syndrome that were reviewed. Using a Down syndrome risk threshold of 1 in 250 at term, Cuckle [60] modeled the screening performance of PAPP-A combined with free β-hCG in the first trimester. A 72% detection rate was estimated with a 5.2% false positive rate; other less frequently used combinations of first-trimester biochemical markers also were modeled. When AFP was added to the above two analytes, a 73.9% detection rate with a 5.1% false positive rate was observed. PAPP-A, free β-hCG, and uE_3 had a 75.7% detection rate with a 4.4% false positive rate. These values suggest that additional first trimester–markers (beyond PAPP-A and free β-hCG) are likely to produce only incremental improvements in detection and false positive rates, and therefore, may not be cost effective. In the first large population-based trial in the United States that has been published, Wapner et al [61] reported a 67.2% detection rate with a 5% false positive rate in the first trimester when serum PAPP-A, free β-hCG, and maternal age were combined.

Combined first and second trimester biochemical markers

Recently, Wald et al [54] reported the combined measurement of first and second trimester maternal serum-only markers for Down syndrome detection. At a fixed 5% false positive rate using PAPP-A as the first trimester marker, sensitivity values of between 83% and 90% were observed, depending on the midtrimester serum marker that was used. For example, when first trimester PAPP-A was combined with midtrimester AFP and intact hCG, an 83% detection rate was achieved. A five-marker test that consisted of first trimester PAPP-A and midtrimester AFP, uE_3, free β-hCG, and inhibin-A had a 90% detection rate for Down syndrome. Using mathematical modeling, the same investigators calculated 80% sensitivity with a 5% false positive rate for a combined test that consisted of first trimester PAPP-A and midtrimester AFP, uE_3, and hCG [62].

The concern that has been raised about combining first and second trimester analytes for screening purposes is the practical impediment of having patients

wait for 2 to 4 weeks to complete a previously initiated test [63]. We believe this may be regarded as an undesirable option for many patients who are undergoing routine screening.

Trisomy 18

Trisomy 18 is the second most common aneuploidy that is diagnosed in the midtrimester. Because of its greatly reduced frequency compared with Down syndrome, much less attention has been invested in the detection of this disorder.

The serum biochemical markers that are used commonly for Down syndrome screening were found to be altered even more significantly in trisomy 18. Maratz et al [1] were the first to report that maternal serum AFP—used for neural tube defect screening—was reduced in trisomy 18 pregnancies. Subsequently, Staples et al [64] compared midtrimester serum analyte levels in 12 cases of trisomy 18 with 390 controls. Markers that were evaluated included uE_3, free α-hCG, free β-subunit hCG, estradiol, and human placental lactogen (hPL). Median MOM levels in cases of trisomy 18 were 0.68 for AFP, 0.31 for free β-hCG, and 0.55 for uE_3. Multiple-marker screening that combined maternal age, uE_3, free α-hCG, free β-hCG, estradiol, and hPL yielded a detection rate of 83.3% and a 2.6% false positive rate. In a larger study set, Palomaki et al [65] evaluated 89 midtrimester trisomy 18 pregnancies that were not complicated by abdominal wall or open neural tube defects. Median levels for AFP, uE_3, and hCG were 0.65, 0.43, and 0.36 MOM of the normal cases, respectively. A triple-marker algorithm that used a risk threshold of 1 in 200 yielded 60% sensitivity and a 0.2% false positive rate. Benn et al [66] reported a 75.9% detection rate and a 0.84% false positive rate when a triple analyte (AFP, hCG, and uE_3), risk-based screening test that incorporated maternal age was mathematically modeled for their screened population. This was superior to the diagnostic accuracy that was achieved with fixed risk thresholds (AFP $\leq$ 0.75 MOM; hCG $\leq$ 0.55 MOM; $uE_3 \leq$ 0.60 MOM). The latter yielded a 34% detection rate and a 0.18% false positive rate. Comparing data from two participating laboratories with 35 cases and 33 cases of trisomy, Sancken et al [67] reported a median hCG of 0.21 MOM, uE_3 of 0.31 MOM, and AFP of 0.80 MOM in one laboratory and corresponding values of 0.37 MOM, 0.44 MOM, and 0.80 MOM, respectively in another, after excluding cases of open neural tube or abdominal wall defects. Triple marker screens that used the same analytes yielded 83.3% and 75% detection rates for trisomy 18 with a 1% false positive rate; this demonstrates a good degree of consistency in screening performance. More recently, Benn et al [68] modeled the performance of the midtrimester triple marker trisomy 18 screenings for a population with age distribution that was similar to that of the United States (1998 natality figures). For the overall population, a 69.7% detection rate with a 0.17% false positive rate was expected. If, however, the screening performance of Down syndrome and trisomy 18 protocols were considered together (ie, if the

primary objective of the screening program was to detect trisomy 21 and 18 cases) and not completely independent of each other, then a 79.3% detection rate for Down syndrome and a simultaneous 77.8% detection rate for trisomy 18 would be achieved; overall, 7.5% of the normal population would have a false positive result. A 7.5% false positive rate would be considered to be unacceptable in the general population. Greater risk thresholds would need to be used to achieve false positive rates that were closer to 5%. The result would be a simultaneous reduction in the detection rates for both aneuploidies.

High diagnostic accuracies also were reported with the use of first trimester serum free β-hCG and PAPP-A for trisomy 18 detection. Both analyte levels are known to be reduced in this aneuploidy. In the previously discussed study of Wapner et al [61] in a U.S. screened population, an 81.8% detection rate of trisomy 18 with a false positive rate of 3.3% was reported.

Open neural tube and abdominal wall defect

Maternal serum screening for open spina bifida and is a well-established precept in obstetric care. The principal sites of production of AFP are the yolk sac and the fetal liver. Increased production rates of this protein lead to elevated levels in fetal serum and in the amniotic fluid into which it is excreted by the fetal kidney. AFP reaches the maternal circulation mainly by transplacental passage and from the amniotic fluid across the membranes. Disruption of the fetal structures leads to increased transudation into the amniotic fluid with ultimate passage into the maternal circulation.

The optimal time for screening is between 15 and 18 weeks' gestation. From a laboratory perspective, median values of maternal serum AFP are established for each week of gestation. Approximately 100 normal cases are used for each weekly interval to establish the normal weekly median values based on a regression equation [69]. Published Gaussian curves give the overlapping distribution of AFP (MOM) values for the normal and neural tube defect populations. The distribution of AFP MOM values for the neural tube defect population is to the right of that of a normal population indicating higher serum levels in the former group. A MOM threshold value that maximizes the proportion of neural tube defect (NTD) cases with AFP values that are at or greater than the threshold, while minimizing the percentage of normals that have values that are greater than the threshold, is chosen by the laboratory. These proportions correspond to the sensitivity and false positive rate values. Frequently, a threshold of at least 2.0 is used. Alternatively, a screening laboratory might use a threshold of at least 2.5 MOM AFP. The threshold value that is used depends on the desire to balance detection and false positive rates.

An important principle that should be understood is that the threshold value is inversely proportional to the number of cases of neural tube defect (NTD) that will be detected; if a smaller threshold value is used, more normal cases will be

falsely identified as being at risk. The practical consequence is more exposures to noninvasive (eg, ultrasound) and invasive testing (eg, amniocentesis).

The greater the disruption of the fetal integument, the greater will be the sensitivity of maternal serum AFP screening. A Scandinavian multi-center screening study was reported by Jorgensen et al [70]. Using midtrimester serum screening in a population of 10,264 patients, the overall detection rate for all NTDs was 75%. The detection rate was 100% for anencephaly, 0% for encephalocele, and 66.7% for spina bifida.

In addition to NTDs, fetal abdominal wall defects present with elevated MSAFP levels. Jorgensen et al [68] reported a sensitivity of 100% for the detection of abdominal wall defects using midtrimester serum screening. Milunsky et al [71] reported 90.9% sensitivity, a 4% false positive rate, a 1.9% positive predictive value, and 99.99% negative predictive when screening with elevated MSAFP. Corresponding values for anencephaly were 100%, 96.0%, 1.7%, and 100%, respectively. When other major congenital defects were considered, the sensitivity of elevated MSAFP was 16.7% with a 4% false positive rate. Positive and negative predictive values were 2.5% and 99.5%, respectively.

References

[1] Merkatz IR, Nitowsky HM, Macri JN, Johnson WE. An association between low maternal serum alpha-feto protein and fetal chromosomal abnormalities. Am J Obstet Gynecol 1984;148: 886–94.
[2] Baumgarten A. AFP screening and Down syndrome. Lancet 1985 ;I:751.
[3] Wald NJ, Kennard A, Hackenshaw A, McGuire A. Antenatal screening for Down's syndrome. Health Technol Assess (Rockv) 1998;2:9–32.
[4] Bogart MH, Pandian MR, Jones OW. Abnormal maternal serum chorionic gonadotropin levels in pregnancies with fetal chromosome abnormalities. Prenat Diagn 1987;7:623–30.
[5] Canick JA, Knight GJ, Palomaki GE, Haddow JE, Cuckle HS, Wald NJ. Low second trimester maternal serum unconjugated oestriol in pregnancies with Down syndrome. Br J Obstet Gynecol 1988;95:330–3.
[6] Benn PA. Advances in prenatal screening for Down syndrome: I. General principles and second trimester testing. Clinica Chimica Acta 2002;323:1–16.
[7] Cuckle HS, Holding S, Jones R, Wallace EM, Groome NP. Maternal serum dimeric inhibin A in second-trimester Down's syndrome pregnancies. Prenat Diagn 1995;15:385–92.
[8] Aitken DA, Wallace EM, Crossley JA, Swanston IA, van Pereren Y, van Maarle M, et al. Dimeric inhibin A as a marker for Down's syndrome in early pregnancy. N Engl J Med 1996; 334:1231–6.
[9] Spencer K, Wallace EM, Ritoe S. Second trimester dimeric inhibin-A in Down's syndrome screening. Prenat Diagn 1996;16:1101–10.
[10] Cuckle H, Holding S, Jones R, et al. Combining inhibin-A with existing second-trimester markers in maternal serum screening for Down's syndrome. Prenat Diagn 1996;16:1095–100.
[11] Wallace EM, Swanston IA, McNeilly AS, Ashby JP, Blundell G, Calder AA, et al. Second trimester screening for Down's syndrome using maternal serum dimeric inhibin-A. Clin Endocrinol 1996;44:17–21.
[12] Wenstrom KD, Owen J, Chu DC, Boots L. Elevated second-trimester dimeric inhibin-A levels identify Down's syndrome pregnancies. Am J Obstet Gynecol 1997;177:992–6.
[13] Lam YH, Tang HY. Second-trimester maternal serum inhibin-A screening for fetal Down's syndrome in Asian women. Prenat Diagn 1999;19:463–7.

[14] Wald NJ, Densem JW, George L, Muttukrishna S, Knight PG, Watt H, et al. Inhibin-A in Down's syndrome pregnancies: revised estimate of standard deviation. Prenat Diagn 1997;17: 285–90.
[15] Stone S, Henely R, Reynolds T, John R. A comparison of total and free β-hCG assays in Down syndrome screening. Prenat Diagn 1993;13:535–7.
[16] Macri JN, Kasturi RV, Kreintz DA, Cook EJ, Moore ND, Young JA, et al. Maternal serum Down's syndrome screening: free β-protein is a more effective marker than human chorionic gonadotropin. Am J Obstet Gynecol 1990;163:1248–53.
[17] Spencer K, Macri JN. Early detection of Down's syndrome using free beta human choriogonadotropin. Ann Clin Biochem 1992;29:349–50.
[18] Spencer K, Coombes EJ, Mallard AS, Ward AM. Free beta human choriogonadotropin in Down's syndrome screening: a multicenter study of its role compared with other biochemical markers. Ann Clin Biochem 1992;29:506–18.
[19] Ryall RG, Staples AJ, Robertson EF, Pollard AC. Improved performance in a prenatal screening programme for Down's syndrome incorporating serum-free hCG subunit analyses. Prenat Diagn 1992;12:251–61.
[20] Norgaard-Pederson B, Alfthan H, Arends J, Hogdall CK, Larsen SO, Pettersson K, et al. A new simple and rapid dual assay for AFP and free β-hCG in screening for Down's syndrome. Clin Genet 1994;45:1–4.
[21] Spencer K. Second trimester prenatal screening for Down's syndrome using alpha-fetoprotein and free beta hCG: a seven year review. Br J Obstet Gynecol 1999;106:1287–93.
[22] Check JH, Kosrow N, Vaze M, Wapner R, Seefried L. Very hi gh CA 125 levels during early first trimester in three cases of spontaneous abortion with chromosomal abnormalities. Am J Obstet Gynecol 1990;162:674–5.
[23] Van Blerk M, Smitz J, de Catte L, Kimps C, Van der Elst J, Van Steirteghem AC. Second-trimester cancer antigen 125 and Down's Syndrome. Prenat Diagn 1992;12:1062–6.
[24] Spencer K. Maternal serum CA 125 is not a second trimester marker for Down's Syndrome. Ann Clin Biochem 1991;28:299–300.
[25] Van Lith JMM, Mantingh A, de Bruijn HWA, Dutch Working Party on Prenatal Diagnosis. Maternal serum CA125 levels in pregnancies with chromosomally-normal and abnormal fetuses. Prenat Diagn 1993;13:1123–31.
[26] Cuckle HS, Wald NJ, Goodburn SF, Sneddon J, Amess JAL. Measurement of activity of urea resistant neutrophil alkaline phosphatase as an antenatal screening test for Down's Syndrome. Br Med J 1990;301:1024–6.
[27] Grozdea J, Maret A, Vergnes H, et al. Cytochemical and biochemical studies on neutrophil alkaline phosphatase in parents of trisomy 21 children. Hum Genet 1984;67:313–6.
[28] Tafas T, Cuckle HS, Nasr S, Krivchenia EL, Resvani E, Evans MI. An automated image analysis method for the measurement of neutrophil alkaline phosphatase in the prenatal screening of Down Syndrome. Fetal Diagn Ther 1996;11:254–9.
[29] Peleg L, Ries L, Getslev V, Lusky A, Chaki R, Lipitz S, et al. Heat stable and urea resistant alkaline phosphataase in maternal neurophils from normal and Down syndrome pregnancy. Prenatal Diagn 1999;19:224–8.
[30] Amano J, Nishimura R, Mochizuki M, Kobata A. Comparative study of the mucin-type sugar chains of human chorionic gonadotropin present in the urine of patients with trophoblastic diseases and healthy pregnant women. J Biol Chem 1988;263:1157–65.
[31] Elliott MM, Kardana A, Lustbader JW, Cole LA. Carbohydrate and peptide structure of the α- and β-subunits of human chorionic gonadotropin from normal and aberrant pregnancy and choriocarcinoma. Endocrine 1997;7:15–32.
[32] Kovaleskaya G, Genbacev O, Fisher SJ, Caceres E, O'Connor JF. Trophoblast origin of hCG isoforms: cytotrophoblasts are the primary source or choriocarcinoma-like hCG. Mol Cell Endocrinol 2002;94:147–55.
[33] Lei ZM, Taylor DD, Gercel-Taylor C, Rao CV. Human chorionic gonadotropin promotes tumorigenesis of choriocarcinoma JAR cells. Troph Res 1999;13:147–59.

[34] Cole LA, Shahabi S, Rinne KM, Oz UA, Bahado-Singh RO, Mahoney MJ. Urinary screening tests for fetal Down sndrome: II. Hyperglycosylated hCG. Prenat Diagn 1999;19:351–9.
[35] Cole LA, Shahabi S, Oz UA, Bahado-Singh RO, Mahoney MJ. Hyperglycosylated human chorionic gonadotropin (invasive trophoblast antigen) immunoassay: a new basis for gestational Down syndrome screening. Clin Chem 1999;45:2109–19.
[36] Massin N, Frendo JL, Luton D, Govagrandi Y, Muller F, Vidaud M, et al. Defect of syncytiotrophoblast formation and hCG expression in Down's syndrome. Placenta 2001;22: S93–7.
[37] Frendo JL, Vidaud M, Guibourdenche J, Luton D, Muller F, Belet D, et al. Defect of villous cytotrophoblast differentiation in syncytiotrophoblast in Down's syndrome. J Clin Endocr Metab 2000;85:3700–7.
[38] Evain-Brion D, Frendo JL, Vidaud M, Muller F. Failure of differentiation of the trophoblast in trisomy 21. Bull Acad Natl Med 2000;184:1033–45.
[39] Birken S, Krichevsky A, O'Connor J, Schlatterer J, Cole LA, Kardana A, et al. Development and characterization of antibodies to a nicked and hyperglycosylated form of hCG from a choriocarcinoma patient: generation of antibodies that differentiate between pregnancy hCG and choriocarcinoma hCG. Endocrine 1999;10:137–44.
[40] O'Connor JF, Ellish N, Kakuma T, Schlatterer J, Kovalevskaya G. Differential urinary gonadotrophin profiles in early pregnancy and early pregnancy loss. Prenat Diagn 1998;18:1232–40.
[41] Pandian R, Lu J, Ossolinska-Plewnia J. A fully automated chemiluminometric assay for hyperglycosylated hCG (invasive trophoblast antigen ITA). Clin Chem 2003;49:808–10.
[42] Shahabi S, Rinne, Oz UA, Bahado-Singh RO, Mahoney MJ, Omrani A, et al. Serum hyperglycosylated hCK a potential screening test for fetal Down syndrome. Prenat Diagn 1999;19: 488–9.
[43] Vendely PM, Lu J, Plenia J, et al. Measurement of serum invasive trophoblast antigen (ITA) for Down Syndrome (DS) screening. Clin Chem 2003;49:A132.
[44] Weinans JN, Butler SA, Mantingh A, Cole LA. Urinary hyperglycosylated hCG in first-trimester screening for chromosomal abnormalities. Prenat Diagn 2000;20:976–8.
[45] Strom CM, Palomaki GE, Knight GJ, et al. Maternal urine Invasive Trophoblast Antigen (ITA) is a useful marker for Down syndrome in the 1st trimester. Presented at the 51st Annual Meeting of the American Society of Hum Gen. San Diego, 2001.
[46] Sutton JM, Cole LA. Sialic acid-deficient invasive trophoblast antigen (sd-ITA): a new urinary variant for gestational down syndrome screening. Prenat Diagn 2004;24:194–7.
[47] Imamura S, Armstrong GA, Birken S, Cole LA, Canfield RE. Detection of desialylated forms of human chorionic gonadotropin. Clin Chim Acta 1987;163:339–49.
[48] Cole LA, Kardana A, Park SY, Braunstein GD. The deactivation of hCG by nicking and dissociation. J Clin Endocrinol Metab 1993;76:704–10.
[49] Cuckle HS, Wald NJ, Barkai G, et al. First trimester biochemical screening for Down's syndrome. Lancet 1988;8615:851–2.
[50] Wald NJ, Kennard A, Hackshaw AK. First trimester serum screening for Down's syndrome. Prenat Diagn 1995;15:1227–40.
[51] Cuckle HS, van Lith JM. Appropriate biochemical parameters in the first trimester screening for Down syndrome. Prenat Diagn 1999;19:505–12.
[52] Wald NJ, Kennard A, Hackshaw A, McGuire A. Antenatal screening for Down's syndrome. Health Technol Assess 1998;2:49–61.
[53] Wald NJ, et al. first and second trimester antenatal screening for Down's syndrome: the results of the serum, urine and ultrasound screening study (SURUSS). Health Technol Assess 2003; 7:11–34.
[54] Wald NJ, Rodeck E, Hakenshaw AK, et al. First and second trimester antenatal screening for Down's Syndrome: the results of the serum, urine and ultrasound screening study (SURUSS). J Med Screen 2003;10:56–104.
[55] Canick JA, Kellner LH. First trimester screening for aneuploidy: serum biochemical markers. Semin Perinatol 1999;5:359–68.

[56] Brambati B, Lanzania A, Tului L. Ultrasound and biochemical assessment of first trimester pregnancy. In: Chapman M, Gridzinskas JG, Chard T, editors. The embryo: normal and abnormal development and growth. London: Springer-Verlag; 1991. p. 181–94.
[57] Wald NJ, Stone R, Cuckle HS, Grudzinskas JG, et al. First trimester concentrations of pregnancy associated plasma protein A and placental protein 14 in Down's Syndrome. BMJ 1993;305:28.
[58] Brambati B, Macintosh MCM, Teisner B, Maguiness S, Shrimanker K, Lanzani A, et al. Low maternal serum levels of pregnancy placental protein A (PAPP-A) in the first trimester in association with abnormal fetal karyotype. Br J Obstet Gynecol 1993;100:324–6.
[59] Wald NJ, Kennard A, Hackshaw AK. First trimester serum screening for Down's syndrome. Prenat Diagn 1995;15:1227–40.
[60] Cuckle H. Integrating antenatal Down's syndrome screening. Curr Opin Obstet Gynecol 2001; 13:175–81.
[61] Wapner R, Thom E, Simpson JL, Pergament E, Silver R, Filkin SK, et al. First-trimester screening for trisomies 21 and 18. N Engl J Med 2003;349:1405–13.
[62] Wald NJ, Watt HC, Hackshaw AK. Integrated screening forDown's Syndrome based on tests performed during the first and second trimesters. N Engl J Med 1999;341:461–7.
[63] Copel JA, Bahado-Singh RO. Prenatal screening for Down syndrome—A search for family's values. New Engl J Med 1999;341:521–2.
[64] Staples AJ, Robertson EF, Ranieri E, Ryall RG, Haan EA. A maternal serum screen for Trisomy 18: an extension of maternal serum screening for Down syndrome. Am J Hum Genet 1991; 49:1025–33.
[65] Palomaki GE, Haddow JE, Knight GH, Wald NJ, Kennard A, Canick JA, et al. Risk-based prenatal screening for trisomy is using alpha-fetoprotein, unconjugated oeestriol and human chronic gonadotropin. Prenat Diagn 1995;15:713–23.
[66] Benn PA, Leo MV, Rodis JF, Beazoiglou T, Collins R, Horne D. Maternal serum screening for fetal trisomy 18: a comparison of fixed cutoff and patient-specific protocols. Obstet Gynecol 1999;93:707–11.
[67] Sancken V, Bartels I, Louwen F, Eiben B. A retrospective evaluation of second-trimester serum screening for fetal trisomy 18: Experience of two laboratories. Prenat Diagn 1999;19: 947–54.
[68] Benn PA, Ying J, Beasoglou T, Egan JFX. Estimates for the sensitivity and false-positive rates for second trimester serum screening for Down Syndrome and trisomy 18 with adjustment for cross-identification and double-positive results. Prenat Diagn 2001;21:46–51.
[69] Muller F. Prenatal biochemical screening for neural tube defects. Childs Nerve Syst 2003; 19:433–5.
[70] Jorgensen FS, Valentin L, Salvesen KA, Jensen FR, Bang K, et al. Multiscan—A Scandiniavian multicenter second trimester obstetric ultrasound and serum screening study. Acta Obstet Gynecol Scand 1999;78:501–10.
[71] Milunsky A, Jick SS, Bruell CL, et al. Predictive values, relative risks and overall benefits of high and low maternal serum α-feto protein screening in singleton pregnancies: new epidemiologic data. Am J Obstet Gynecol 1989;161:291.

ELSEVIER
SAUNDERS

Obstet Gynecol Clin N Am
31 (2004) 873–892

OBSTETRICS AND
GYNECOLOGY
CLINICS
OF NORTH AMERICA

Pituitary gland and pregnancy

Nastaran Foyouzi, MD[a], Yr Frisbæk, BA[b],
Errol R. Norwitz, MD, PhD[a,*]

[a]*Division of Maternal-Fetal Medicine, Department of Obstetrics, Gynecology & Reproductive Sciences, Yale–New Haven Hospital, 333 Cedar Street, New Haven, CT 06520, USA*
[b]*University of Iceland Medical School, Laeknagarôur Vatnsmýrarvegur 16, IS-101, Reykjavik, Iceland*

The hypothalamic–pituitary–adrenal axis is central to mammalian reproductive function, including conception, pregnancy maintenance, parturition, and breastfeeding. Pregnancy is associated with substantial physiologic changes within this endocrine axis to meet the demands of pregnancy, which include support of the fetus (volume support, nutritional and oxygen supply, clearance of fetal waste), protection of the fetus (from starvation, drugs, toxins), preparation of the uterus for labor, and protection of the mother from potential cardiovascular injury at delivery. This article reviews the anatomy, embryology, and physiology of the pituitary. The effect of pregnancy on pituitary structure and function, in health and disease, also is discussed.

Anatomy, embryology, and physiology

The hypophysis (pituitary) is a reddish-gray organ with a diameter of approximately 1-cm that is attached to the undersurface of the brain by a stalk or infundibulum. The adult pituitary consists primarily of an anterior and posterior lobe, with a rudimentary intermediate lobe. The anterior lobe is large and kidney-shaped. It is highly vascular with epithelial cells arranged in cord-like trabeculae. The intermediate lobe is a thin structure with few blood vessels and granular cells that are scattered among colloid material. Although of neural origin, the posterior

* Corresponding author.
E-mail address: errol.norwitz@yale.edu (E.R. Norwitz).

0889-8545/04/$ – see front matter
doi:10.1016/j.ogc.2004.08.003 obgyn.theclinics.com

lobe of the pituitary consists primarily of neuroglia cells and fibers and has few nerve cell bodies [1].

Embryologically, the pituitary gland arises from the rostral neural plate. Rathke's pouch—a primitive ectodermal invagination that is located anterior to the roof of the oral cavity—is formed by the third week of gestation. This pouch continues to invaginate and establishes itself as distinct from the oral cavity and nasopharynx; however, it remains connected to the hypothalamus through a narrow stalk. During the fourth and fifth weeks of gestation, Rathke's pouch proliferates toward the third ventricle where it fuses with an outpouching of neural ectoderm that is associated with third-ventricle development and is known as the diverticulum [1]. Rathke's pouch ultimately forms the anterior lobe of the pituitary (adenohypophysis) and the rudimentary intermediate lobe, whereas the diverticulum gives rise to the posterior lobe (neurohypophysis). The hypophyseal-portal circulation is established by 20 weeks' gestation. Remnants of pituitary tissue may persist in the midline and may give rise to functional (hormone-secreting) ectopic tumors in the nasopharynx, although this is rare.

On the basis of the hormones that they produce, the adenohypophysis contains five major distinct cell types: (1) corticotropes (that secrete primarily pro-opiomelanocortin [POMC] and its derivates, including β-lipotropin and corticotropin); (2) lactotropes (prolactin); (3) somatotropes (growth hormone [GH]); (4) gonadotropes (luteinizing hormone [LH] and follicle-stimulating hormone [FSH]); and (5) thyrotropes (thyrotropin [TSH]). The posterior pituitary produces oxytocin and vasopressin, whereas the intermediate lobe of the pituitary produces melanocyte-stimulating hormone. Within the anterior pituitary gland, the individual hormone-secreting cell types emerge in a sequential order. The ontogeny of these hormones is summarized in Fig. 1. The anterior pituitary undergoes major cellular differentiation during the first 12 weeks of gestation, by which time four of the five major secretory cell types can be identified. Corticotrope cells can be identified morphologically by 6 weeks' gestation and immunoreactive corticotropin is detectable in the region of the anterior pituitary by 7 weeks' gestation. By 8 weeks' gestation, somatotrope cells are identifiable and glycoprotein hormone–secreting cells are expressing α-subunit genes. Differentiated thyrotrope and gonadotrope cells begin to express β-subunit genes for TSH and the gonadotrope hormones (FSH and LH), respectively, at approximately 12 weeks' gestation. The exception to this pattern of expression are the lactotrope cells, which only begin to express prolactin after 24 weeks of gestation [1,2].

Although the factors that are responsible for the ontogeny of gene expression within the pituitary gland are not well-understood, it appears that direct contact of the ventral diencephalon with the developing Rathke's pouch provides critical signaling cues for proliferation and determination of the pituitary cell types [3]. Moreover, these processes seem to be regulated, at least in part, by LIM-homeobox genes. Although most of the data in support of these developmental pathways have been acquired from studies in laboratory animals, histologic and pathogenic observations suggest that similar mechanisms are likely to be critical

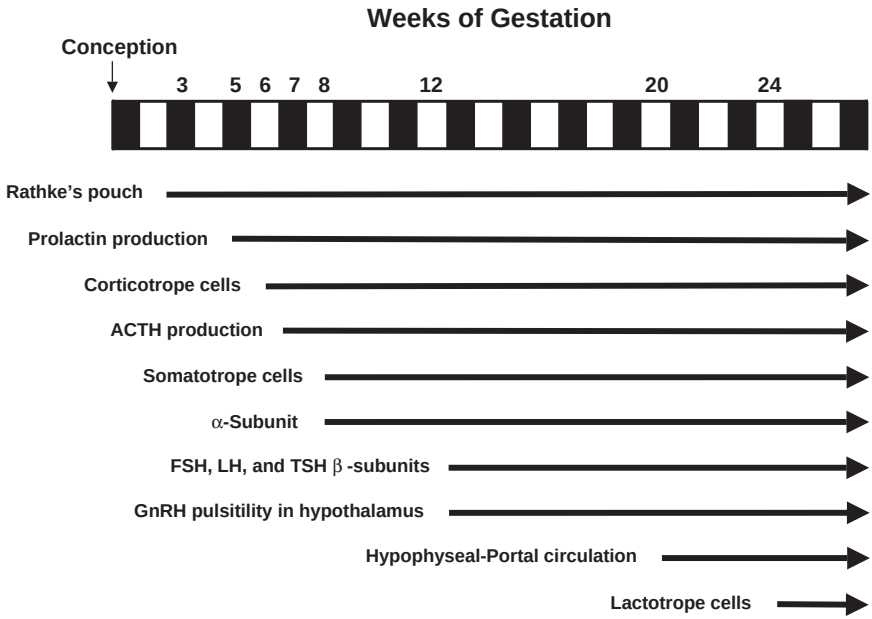


Fig. 1. Ontogeny of hormone production and cell differentiation in the anterior pituitary.

in humans as well. Multiple members of the LIM homeodomain family of transcription factors are expressed at various stages within the developing Rathke's pouch, including *Lhx2*, *Lhx3*, *Lhx4*, *and Isl-1* [4]. For example, *Lhx3* and *Lhx4* are necessary for progression from a rudimentary to a definitive Rathke's pouch [5], whereas *Isl-1* seems to be important for subsequent development. *Lhx3* and *Lhx4* also seem to be necessary for the differentiation of gonadotrope, thyrotrope, somatotrope, and lactotrope—but not corticotrope—cells [5]. Further cellular differentiation requires one or more additional specific transcription factors (eg, Pit-1 for somatotrope, lactotrope and thyrotrope cell lineages; steroidogenic factor–1 and early growth response gene-1 (Egr-1) for gonadotrope cell lines). In addition to those mentioned above, several growth factors and signaling molecules seem to be necessary at various stages of pituitary gland morphogenesis, although the precise role of many of these factors has yet to be clarified [3]. Some factors seem to be important in early pouch development, such as fibroblast growth factor (FGF), sonic hedgehog, and bone morphogenic protein (BMP)-4. Other factors may be involved in determining the expression of terminal differentiation markers of pituitary cell lineages. For example, BMP-6 and -7 stimulate the expression of FSH-β, whereas FGF activates prolactin gene expression [3].

The physiologic action of hormones that are produced by different cell types of the pituitary are summarized in Table 1 [2,6,7].

Table 1
Schematic view of physiology of pituitary

Cell type	%	Hormone	Action	Comments
Adenohypophysis (anterior pituitary)				
Lactotropes	15–25%	Prolactin	Milk production, reproduction	Pulsatile secretion (levels highest during sleep, lowest from 10 AM to noon)
Corticotropes	10%	POMC; also ACTH, β-Lipotropin (LPH), β-endorphin	Maintenance of adrenal function, promotion of steroidogenesis	Pulsatile secretion (levels highest before awakening and lowest in the evening)
Somatotropes	50%	Growth hormone	Effect on growth as well as fat, carbohydrate, and protein metabolism	Pulsatile secretion (levels highest during sleep); levels are highest during adolescence and decrease with age
Thyrotropes	5%	TSH	Synthesis and secretion of thyroid hormones	Low-amplitude pulsatile secretion (levels are highest at midnight and decline during the day)
Gonadotropes	20%	FSH, LH	Induces gonadal steroid production and gametogenesis	Pulsatile secretion (LH levels after puberty highest during the night)
Neurohypophysis (posterior pituitary)				
Vasopressinergic cells	Supraoptic nuclei	Vasopressin antidiuretic hormone (ADH)	Water homeostasis	Phasic bursts of 5–15 Hz
Oxytocinergic cells	Paraventricular nuclei	Oxytocin	Uterine contractions, milk production, and excretion	Pulsatile secretion

Data from ref. [6].

Effect of pregnancy on pituitary structure and function

The structure and function of the pituitary gland are altered significantly in pregnancy [8]. In the nonpregnant state, the pituitary gland weighs approximately 0.5 to 1.0 g. In pregnancy, the weight of the pituitary gland increases by 30% and the volume increases twofold [9,10]. The pituitary gland develops a convex, dome-shaped superior surface [11], which may impinge on the optic chiasm and account for the bitemporal hemianopia that is observed in some apparently healthy pregnant women [12,13]. Pregnancy is not associated with an increased incidence of pituitary adenoma.

The cellular composition of the adenohypophysis changes throughout pregnancy. This is especially true of the lactotrope cell population. Immunohistochemical studies showed that, in nulligravid women, approximately 20% of cells in the adenohypophysis are lactotropes [14,15]. This number increases throughout pregnancy so that by the third trimester, approximately 60% are lactotropes. By 1 month postpartum, the number of lactotropes in the anterior pituitary of nonlactating women is decreased; however, postpartum resolution of lactotrope hyperplasia is incomplete and nonpregnant multiparas have, on average, more lactotrope cells than do nulligravid women. In contrast to lactotropes, the numbers of somatotropes, gonadotropes, and α-subunit–secreting cells in the adenohypophysis decrease in pregnancy, whereas the number of thyrotropes do not change. These changes in cellular composition are associated with changes in circulating hormone levels.

Prolactin

Levels of prolactin in the maternal circulation increase throughout pregnancy and reach concentrations of approximately 140 ng/mL at term [16]. Prolactin in the maternal circulation originates primarily from the maternal pituitary, with small contributions from the maternal decidua and fetal pituitary. The maternal decidua is a major site of prolactin production and leads to elevated levels in the amniotic fluid, which peaks at approximately 6000 ng/mL at the end of the second trimester [14]. Little decidual prolactin enters the maternal circulation [17,18].

The hyperprolactinemia of pregnancy is likely due to increased circulating levels of estradiol-17β. Aside from this increase in basal levels, prolactin secretion by the maternal pituitary is stimulated by thyrotropin-releasing hormone (TRH) [19], arginine [20], meals [20], and sleep [21] in a manner that is similar to that seen in nonpregnant women. After delivery, maternal prolactin concentrations in nonlactating women decrease to prepregnancy levels within 3 months [22]. In lactating women, basal circulating prolactin levels decrease slowly to nonpregnant levels over a period of several months with intermittent episodes of ''hyperprolactinemia'' in conjunction with nursing.

Pregnancy also is associated with a shift in prolactin isoforms. In the nonpregnant state, the *N*-linked glycosylated isoform of prolactin (G-PRL) predominates in the circulation. Increasing amounts of nonglycosylated prolactin

appear in the circulation as pregnancy progresses [23]. In the third trimester, the concentration of circulating nonglycosylated prolactin exceeds that of G-PRL. Nonglycosylated prolactin seems to be more biologically active than G-PRL [24]. The precise function of the elevated circulating levels of prolactin in pregnancy is not clear, but it seems to be important in preparing breast tissue for lactation [23,25]. The role of prolactin in amniotic fluid is not known.

Pregnancy produces changes in prolactin secretion that persist long after delivery. Musey and colleagues [26] reported that the basal serum prolactin level, as well as the prolactin response to perphenazine stimulation, was lower after pregnancy than before pregnancy. They also found that the serum prolactin concentration was significantly lower in the parous women (mean, 4.8 ng/mL) than in the nulliparous women (mean, 8.9 ng/mL). These and other studies suggest that pregnancy permanently suppresses the secretion of prolactin by the maternal pituitary [27,28].

Corticotropin

Corticotropin levels in the maternal circulation increase from approximately 10 pg/mL in the nonpregnant state to 50 pg/mL at term, and increase further to approximately 300 pg/mL in labor (Fig. 2) [29]. Although the placenta produces corticotropin [30], most of the circulating corticotropin comes from the maternal pituitary [31]. Placental production of corticotropin-releasing hormone (CRH) may be a major cause of the elevated levels of corticotropin in the maternal circulation [32–34]. In nonpregnant women, maternal serum CRH levels range from approximately 10 pg/mL to 100 pg/mL. In the third trimester of pregnancy, these concentrations increase to 500 pg/mL to 3000 pg/mL; levels decrease precipitously after delivery [34]. In addition to increasing pituitary corticotropin secretion, chronically elevated levels of CRH in the maternal circulation reduce the ability of exogenous glucocorticoids to suppress the maternal corticotropin–cortisol axis [35–37], enhance the ability of vasopressin to induce an corticotropin response, and diminish the effect of exogenous CRH [32,33]. CRH binding protein (CRH-BP) inactivates CRH, thereby preventing its action on the maternal or fetal pituitary. In the last few weeks of pregnancy, CRH-BP levels in the maternal circulation decrease which results in an increase in free (biologically active) CRH [38].

Although the maternal adrenal glands do not change in size, pregnancy is associated with significant changes in the circulating concentrations of adrenal hormones. For example, serum cortisol levels increase significantly in pregnancy. Most circulating cortisol is bound to cortisol-binding globulin (CBG), which is produced by the liver. Circulating levels of CBG increase during pregnancy in response to elevated levels of estrogen; CBG may retard the clearance of these hormones. It is not surprising, therefore, that levels of total cortisol increase in pregnancy; however, levels of free cortisol in the circulation [35,36], saliva [39], and urine [35,36,40] are also increased; this likely is due to the increased levels of corticotropin in the maternal circulation. Maternal hypercortisolemia also is

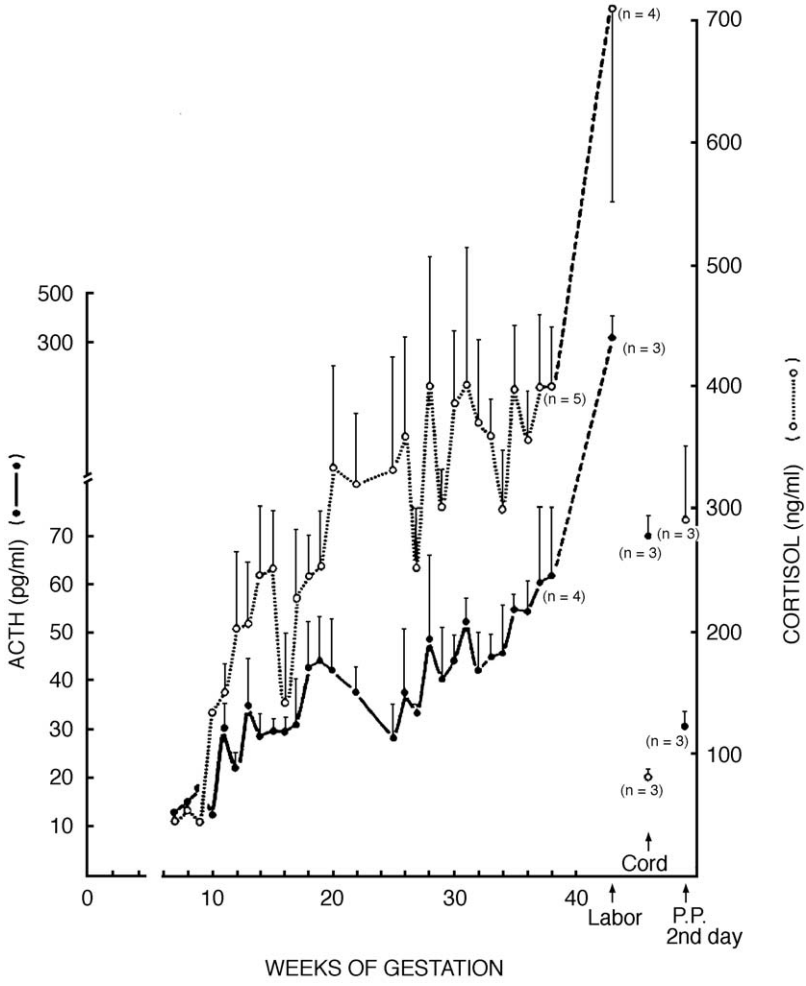


Fig. 2. ACTH and total cortisol concentration in the maternal circulation throughout gestation. P.P., postpartum. (*From* Carr BR, Parker CR, Madden JD, et al. Maternal plasma adrenocorticotropin and cortisol relationships throughout human pregnancy. Am J Obstet Gynecol 1981;139:416; with permission.)

observed in complete molar pregnancy, suggesting that the increased cortisol is not derived from a fetal source.

Growth hormone

Maternal serum GH levels begin to increase at approximately 10 weeks' gestation, plateau at approximately 28 weeks' gestation, and can remain elevated for several months postpartum [41]. Most of this GH is derived from the placenta (GH-V isoform), with a marked reduction in basal somatotropin (GH-N)

production by the maternal pituitary [42]. Moreover, the release of GH-N in response to insulin-induced hypoglycemia [43] or arginine stimulation [44] is attenuated markedly which suggests that the maternal pituitary GH secretory reserve is diminished in pregnancy.

Thyroid-stimulating hormone

Follicular cells of the thyroid produce thyroid hormone in the form of a pre-(thyroxine [T_4]) and biologically-active hormone (triiodothyronine [T_3]). Most of the biologically-active hormone is made by peripheral conversion of T_4 to T_3. Follicular cell activity is under the direct control of the hypothalamic–pituitary–thyroid axis. The hypothalamus produces TRH, a tripeptide that enters the portal circulation of the infundibular stalk and travels to the anterior lobe of the pituitary where it stimulates specific cells (thyrotropes) to produce TSH. TSH secretion varies diurnally; a peak secretion occurs between 11 PM and 4 AM. TSH enters the systemic circulation and interacts with specific, heptahelical, G-protein coupled receptors on the surface of thyroid follicular cells; this triggers a series of signal transduction cascades that culminate in the synthesis and release of thyroid hormones. Through a classic endocrine-negative feedback loop, decreased circulating levels of thyroid hormones lead to a decrease in TRH and TSH secretion, which result, in turn, in increased thyroid growth and activity.

The negative-feedback control system of the hypothalamic–pituitary–thyroid axis functions normally in pregnant women [45]. Similarly, the TSH response to

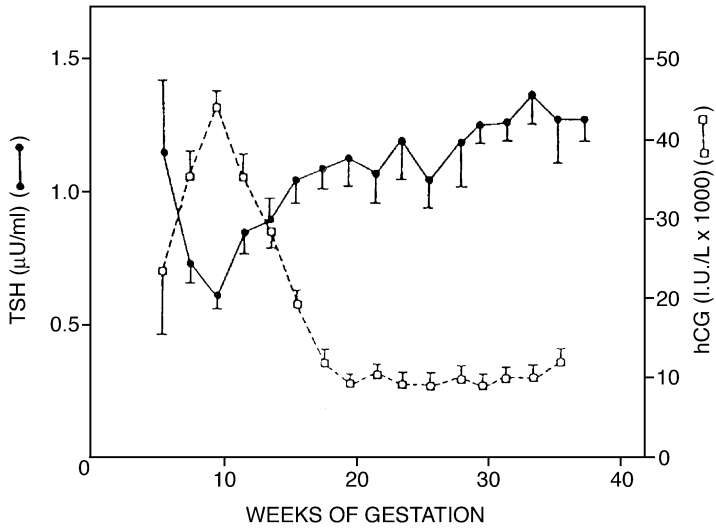


Fig. 3. Maternal concentration of serum TSH and hCG as a function of gestational age. The decrease in serum TSH at approximately 10 weeks' gestation may be due to thyrotropic effects of hCG. (*From* Glinoer D, de Nayer P, Bourdoux P, et al. Regulation of maternal thyroid during pregnancy. J Clin Endocrinol Metab 1990;71:276; with permission.)

exogenous TRH stimulation remains normal throughout pregnancy [46,47]. At 9 to 13 weeks of gestation, there is a modest decline in circulating TSH levels [48,49]. This coincides with the peak placental production of human chorionic gonadotropin (hCG); some investigators suggested that the decrease in TSH may be due to the weak thyrotropic properties of hCG (Fig. 3) [50–52]. An alternative hypothesis is that the placenta may secrete a hormone with TRH- or TSH-like properties; however, this hypothesis is not supported by most data [53]. Further details about thyroid function in pregnancy are summarized elsewhere in this issue.

Gonadotropins

Maternal serum LH and FSH levels are decreased by 6 to 7 weeks of pregnancy and are less than the limits of detection of many radioimmunoassays by the second trimester [54–56]. The marked decrease in gonadotropin immunoreac-

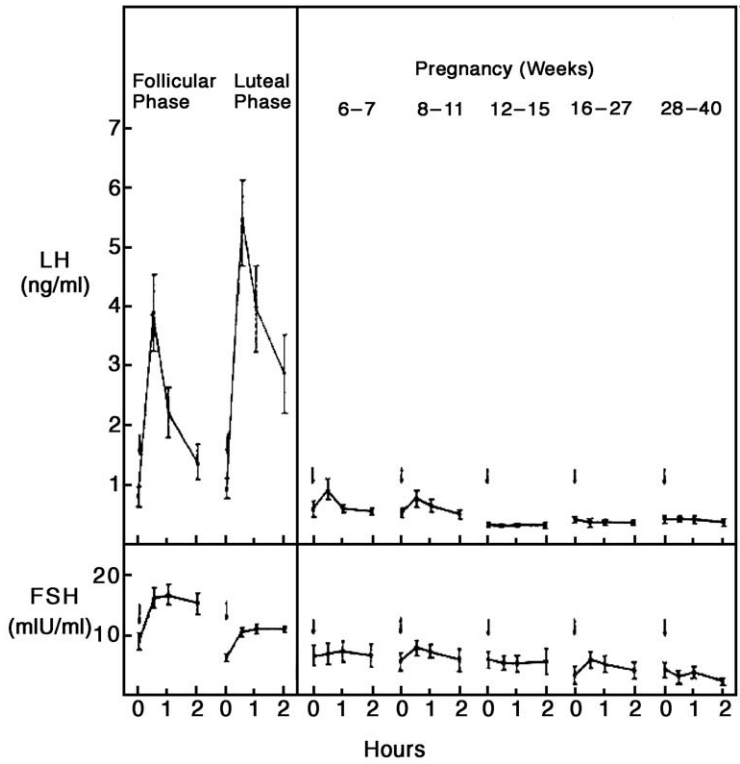


Fig. 4. Effect of pregnancy on GnRH stimulation test. Serum levels of LH and FSH before and after a 100-μg bolus of GnRH in pregnant women and normal menstruating women. During pregnancy, LH and FSH levels are markedly suppressed. (*From* Miyake A, Tanizawa O, Aono T, Kurachi K. Pituitary responses in LH secretion to LHRH during pregnancy. Obstet Gynecol 1977;49:549–51; (p. 550) with permission.)

tivity in the pituitary glands of pregnant women [14,57], coupled with the blunted LH and FSH response to exogenous GnRH stimulation (Fig. 4) [54–56], suggests that this effect is localized primarily to the pituitary. The suppression of pituitary LH and FSH synthesis and secretion likely results from elevated circulating levels of sex steroids (estradiol-17β, progesterone) and regulatory peptides (eg, inhibin) during pregnancy.

Pituitary tumors in pregnancy

Mutations are the primary cause of pituitary tumors. Most pituitary tumors are monoclonal; this indicates that a somatic mutation in a single progenitor cell was the cause of the tumor [8,58]. Other endocrine factors (eg, the levels of estradiol-17β, progesterone, dopamine) can influence tumor phenotype; changes in these hormones during pregnancy may affect tumor growth. In general, pituitary tumors are benign and slow growing.

Pituitary tumors commonly are classified according to size as microadenomas (<10 mm in diameter) or macroadenomas (>10 mm). The clinical behavior of microadenomas and macroadenomas vary considerably during pregnancy. Macroadenomas may be associated with extracellular extension, local invasion, or compression of the optic chiasm with resultant bitemporal hemianopia; such conditions may become exacerbated in pregnancy. For example, in one series of 60 pregnant women who had macroadenomas, 20% showed evidence of worsening visual field defects, significant enlargement on serial imaging studies, or developed neurologic signs [8]. Urgent neurosurgical decompression may be required during pregnancy if the tumor enlarges markedly or causes neurologic sequelae. In contrast, microadenomas tend to behave in a benign manner in pregnancy, with no evidence of functional pituitary deficiency and a low risk of neurologic complications. In a longitudinal observational study of 215 pregnant women who had microadenomas, approximately 5% of women developed headaches and less than 1% demonstrated worsening of visual field defects or developed neurologic signs [8].

Functional pituitary tumors in pregnancy

Although some pituitary tumors are nonfunctional, most produce one or more endocrine hormones. Functional pituitary tumors are classified according to the hormone that they produce.

Prolactinoma

Prolactinoma refers to a tumor of prolactin-secreting lactotrope cells and typically is associated with elevated levels of prolactin in the maternal circulation. In the initial evaluation of a suspected prolactinoma, it also is important to

measure circulating levels of T_4, TSH, and Insulin-Like Growth Factor-1 (IGF-I). This evaluation will exclude secondary causes of hyperprolactinemia, specifically hypothyroidism (T_4, TSH) and acromegaly (IGF-I). An imaging study of the hypothalamus and pituitary also is indicated and computerized evaluation of the visual fields is recommended if compression of the optic chiasm is suspected.

Women who have significant hyperprolactinemia usually are anovulatory and, as such, infertile. If a patient does not desire pregnancy, treatment with combination estrogen-progestin therapy will reduce the risk of osteoporosis and regulate the menstrual cycle. This approach seems to be safe and is associated with few tumor-related complications, including minimal risk of tumor growth [59]. For infertile women who have significant hyperprolactinemia and wish to conceive, treatment usually is required to induce ovulation. Controversy continues as to whether surgery or dopamine agonist treatment represents the best first-line therapy for such women. Some investigators recommend surgical treatment before conception to reduce the need for dopamine agonist treatment and the incidence of neurologic complications during pregnancy [60]; however, microsurgical resection of a prolactinoma can result in death (in 0.3% of cases) or serious morbidity (eg, cerebrospinal fluid leak [0.4%]) [61]. Moreover, a long-term cure can be expected in only approximately 60% of women who are treated surgically. For these reasons, medical treatment should be regarded as the best first-line therapy for infertile women who have significant hyperprolactinemia [60,62].

Having confirmed the diagnosis of a pituitary microprolactinoma, the goals of treatment are to suppress prolactin production and induce ovulation, decrease tumor size, preserve pituitary reserve, and prevent tumor recurrence. Treatment with a dopamine agonist can normalize circulating prolactin levels, establish regular ovulation, decrease tumor size, and preserve pituitary reserve [63,64]. A disadvantage of dopamine agonist treatment is that it is not effective in preventing tumor recurrence after treatment is discontinued. Four dopamine agonists are effective in the treatment of hyperprolactinemia—bromocriptine, pergolide, quinagolide, and cabergoline. Cabergoline is administered once weekly and may be more effective than bromocriptine in the treatment of microadenomas [65]; however, little information is available concerning the effects of pergolide, quinagolide, and cabergoline on pregnancy. In contrast, there is substantial experience that bromocriptine is safe in pregnancy, with no significant increase in the overall rate of fetal congenital abnormalities [66]. The most common side effects that are associated with bromocriptine therapy are nausea, vomiting, and postural hypotension. Starting with low-dosage therapy (0.625 mg/d) and increasing the dosage slowly over a period of a few weeks can minimize these side effects. In some patients, dosages that are small as 2.5 mg/d may be effective. Prolactin levels should be checked every month for 3 months and every 3 months thereafter until the levels have returned to normal.

In women who have microprolactinomas, bromocriptine can be discontinued after pregnancy is established. Most women will have no further complications during pregnancy. For women who do develop neurologic sequelae (eg, headache,

cranial nerve dysfunction), bromocriptine therapy can be reinstituted immediately. Marked enlargement of a microprolactinoma or persistence of neurologic sequelae, despite medical treatment, may be an indication for urgent neurosurgical intervention; however, such complications are rare. In contrast, pituitary insufficiency and neurosurgical complications are far more common in women who have macroadenomas. Therefore, such women should be evaluated for panhypopituitarism before dopamine agonist treatment is initiated. Women who have macroprolactinomas also are more likely to develop complications in pregnancy [8]. One approach to the management of such women is to discontinue bromocriptine treatment after pregnancy is established and to reinstitute therapy if symptoms or signs of increasing tumor volume develop [67]. An alternative plan, which is equally appropriate, is to continue bromocriptine treatment throughout pregnancy [68,69]. Lactation does not seem to worsen the clinical course of women who have prolactinomas and they should be encouraged to breastfeed [70].

Cushing's disease

Cushing's syndrome refers to the clinical syndrome that results from exposure to excessive circulating levels of cortisol. The most common clinical features of Cushing's syndrome include proximal muscle weakness, centripetal obesity ("potato stick" person with thick trunk and thin limbs), facial plethora, supraclavicular and dorsal ("buffalo hump") fat pads, violaceous striae, hirsutism, personality changes, and hypokalemia. In nonpregnant women, significant and persistent elevation in urinary free cortisol excretion (>200 μg/d) confirms the diagnosis. The diagnosis can be difficult to make during pregnancy because normal pregnancy is associated with physiologic hypercortisolism and increased urinary free cortisol excretion [71]. More recent studies that used high-performance liquid chromatography showed that this approach had greater sensitivity and specificity in the diagnosis [72].

After the diagnosis has been confirmed, every effort should be made to identify the cause. The excess source of cortisol can be corticotropin-dependent (eg, corticotropin-secreting pituitary adenoma [Cushing's disease], corticotropin- or CRH-secreting tumors) or corticotropin-independent (exogenous glucocorticoids, adrenal adenoma or carcinoma). In nonpregnant women, Cushing's disease (pituitary adenoma) is three times more common than adrenal adenomas. In pregnancy, however, adrenal adenomas are the most common cause of Cushing's syndrome [73]. Most cases of Cushing's disease are due to pituitary microadenomas. Functional (CRH stimulation followed by petrosal sinus sampling for corticotropin) or head imaging studies may be required to identify an corticotropin-secreting pituitary microadenoma.

Cushing's syndrome is associated with an increased risk of maternal pregnancy-related complications, including hypertension (65%), gestational diabetes (32%), preeclampsia (10%), congestive heart failure, and maternal death. The metabolic derangements that are associated with Cushing's disease also lead to an

increase in adverse perinatal outcome, including premature labor (65%), intrauterine growth restriction (26%), and perinatal death (16%).

Typically, Cushing's disease is associated with depressed gonadotropin secretion. As such, spontaneous pregnancy is rare in women with untreated Cushing's disease. Because Cushing's disease is a rare condition in pregnancy, it has not been possible to examine and compare systematically the treatment strategies for this condition. Given the high maternal and perinatal morbidity that are associated with this condition, aggressive antepartum and intrapartum management is indicated. Medical treatment options include antiglucocorticoids and inhibitors of adrenal steroidogenesis. Metyrapone, aminoglutethimide, and ketoconazole have been used to treat Cushing's syndrome in pregnancy [74]; however, the efficacy or safety of these agents during pregnancy has yet to be established. If necessary, transsphenoidal resection can be performed during pregnancy [75].

Acromegaly

Acromegaly refers to the clinical syndrome that is associated with elevated circulating levels of GH. Acromegaly often is associated with anovulation, but spontaneous pregnancy can occur [76,77]. Except for complications that are associated with pituitary enlargement, acromegaly does not seem to effect pregnancy outcome adversely. In most women, definitive treatment for acromegaly can be deferred until after delivery. Bromocriptine and transsphenoidal surgery have been used to treat acromegaly successfully during pregnancy.

Pituitary insufficiency

Sheehan's syndrome

The increase in pituitary size during pregnancy, coupled with the low-flow, low-pressure nature of the portal circulation, seems to make the pituitary and parts of the hypothalamus particularly susceptible to ischemia. Sheehan's syndrome (pituitary apoplexy) refers to the onset of acute hypothalamic-pituitary dysfunction, typically following severe obstetric hemorrhage and resultant maternal hypotension at delivery. It is the most common cause of hypopituitarism worldwide. Most cases of Sheehan's syndrome occur in developing countries where deliveries are not performed by skilled obstetric care providers in well-equipped health care facilities; this likely increases the risk of complications from obstetric hemorrhage.

The hallmark of this syndrome is a loss of anterior pituitary hormone reserve, which may be complete or partial. Prolactin and GH deficiency are the most common abnormalities that are observed in Sheehan's syndrome; however, every imaginable pattern of pituitary hormone deficiency has been described. In a study of 10 African women who had Sheehan's syndrome, Jialal and coworkers [78]

described the pituitary hormone response to a combined intravenous insulin (0.1 unit/kg), TRH (200 μg), and GnRH (100 mg) challenge test. The pattern of pituitary hormone response revealed the following loss of secretory reserve: 100% of these women had prolactin and GH deficiency, 90% had cortisol deficiency, 80% had TSH deficiency, 70% had LH deficiency, and 40% had FSH deficiency.

The initial clinical manifestations of Sheehan's syndrome include failure of lactation, failure of hair growth over areas that were shaved for delivery, poor wound healing after cesarean delivery, or generalized weakness. The best single test to confirm the diagnosis of Sheehan's syndrome is to administer intravenous TRH (100 μg) and to measure serum prolactin levels at 0 and 30 minutes. The ratio of prolactin that is measured at 30 minutes to that before TRH treatment (time 0) should be greater than 3.0 [79]. If the ratio is abnormal, a complete evaluation for panhypopituitarism should be initiated.

In addition to the loss of anterior pituitary hormone reserve, mild hypothalamic and posterior pituitary dysfunction are seen frequently in women who have Sheehan's syndrome. Detailed neuropathologic reports of autopsy specimens by Sheehan and Whitehead [80] showed that 90% of women who had postpartum hypopituitarism had evidence of atrophy and scarring of the neurohypophysis. Subsequent studies demonstrated atrophy of the supraoptic and paraventricular nuclei in such patients [81]. Several clinical studies demonstrated that most women who have Sheehan's syndrome have mild functional defects in vasopressin secretion and maximal urinary concentrating capability [82,83].

Diabetes insipidus

Arginine vasopressin–antidiuretic hormone (AVP-ADH) is a cyclic nonapeptide that is secreted by the axonal terminals of the neurohypophysis that emanate from neurosecretory neurons that are located in the supraoptic and paraventricular nuclei of the hypothalamus. Blood osmolality is monitored carefully by sensitive osmoreceptors in the anterior hypothalamus. AVP-ADH is released in response to increasing osmotic pressures or decreasing hydrostatic pressures and act on the kidney to increase water retention. This system is designed to adjust blood osmolality over a narrow range (± 1.8%) with a mean of 285 mOsm/kg in nonpregnant women [84]. Pregnancy is associated with a decrease in plasma osmolality of approximately 9 to 10 mOsm/kg; this is evident early in the first trimester and persists throughout gestation. Circulating AVP-ADH levels do not change in pregnancy [85]. These data suggest that pregnancy is associated with a modest resetting of the osmostat that leads to a 9 to 10 mOsm/kg decrease in the osmotic threshold for AVP-ADH release.

Diabetes insipidus (DI) involves the inappropriate loss of water that results from failure of adequate tubular reabsorption by the kidney. It is characterized by polyuria (defined as >3 L of urine in 24 hours), polydipsia, and plasma hyperosmolarity. The causes of DI can be divided into two groups. Central

(hypothalamic) DI refers to lesions of the hypothalamus or posterior pituitary that lead to inadequate production of AVP-ADH. The differential diagnosis of central DI includes pituitary surgery, trauma, infection, and infiltration of the neurohypophysis by tumors or inflammatory cells. Typically, central DI presents with acute onset massive polyuria of 4 to 15 L per day. Peripheral (nephrogenic) DI refers to peripheral resistance to AVP-ADH action. Measurement of plasma AVP-ADH levels may be able to distinguish these two groups (levels are decreased in central DI and elevated in nephrogenic DI).

Transient nephrogenic DI can occur in pregnancy, usually in association with preeclampsia, hemolysis, elevated liver functions, low platelets (HELLP) syndrome, or acute fatty liver of pregnancy [86]. High levels of placental vasopressinase may contribute to pregnancy-associated DI by degrading endogenous AVP-ADH. This increase in vasopressinase activity also may cause women who have partial hypothalamic AVP-ADH deficiency to develop overt DI in pregnancy. D-arginine vasopressin (DDAVP) is resistant to degradation by placental vasopressinase. As such, DDAVP may be more effective than native AVP-ADH in the treatment of women who have DI. In most cases, DI improves after delivery [87].

DI is a rare disease in pregnancy. If suspected, the diagnosis of DI should be confirmed by performing a water deprivation test. After an overnight fast, the patient is denied water until 3% of body weight is lost or urine osmolarity shows no increase in three successive hourly specimens. In women who have DI, urine osmolarity remains decreased, whereas plasma osmolarity increases significantly. Because of the risks that are associated with dehydration, this test is best performed by an endocrinologist. To help identify the cause, 10 μg of DDAVP can be administered immediately after the completion of the water deprivation test. In women who have central DI, there will be a decrease in urine output and an increase in urine osmolarity. Conversely, in women who have nephrogenic DI, there will be a minimal change in urine output and osmolarity [88].

Adrenal insufficiency

Adrenal insufficiency may be primary or secondary. Primary adrenal insufficiency (Addison's disease) results from destruction of both adrenal cortices. Secondary adrenal insufficiency results from corticotropin deficiency and resultant adrenocortical atrophy. In secondary adrenal insufficiency, the zona glomerulosa of the adrenal gland (and thus, mineralocorticoid production) are preserved because they are under the control of the renin-angiotensin system [89].

The most common symptoms of adrenal insufficiency are generalized weakness, fatigue, nausea, anorexia, diarrhea, and weight loss. Laboratory features include hyponatremia, hyperkalemia, and an increase in plasma blood urea nitrogen. The diagnosis can be confirmed using the corticotropin stimulation test in which a serum cortisol level is measured 60 minutes after an intravenous bolus of 0.25 mg synthetic corticotropin (cosyntropin). A normal corticotropin stimulation test is associated with a serum cortisol measurement of greater

than 18 μg/dL [90]. The absence of an adequate cortisol response is highly suggestive of primary adrenal insufficiency. Secondary adrenal insufficiency should be suspected if there is a suboptimal cortisol response to the corticotropin stimulation test, but a normal serum aldosterone concentration [91].

Treatment of Addison's disease should include physiologic replacement of cortisol. Typically, endogenous cortisol production rates are in the range of 20 mg/d to 30 mg/d, but may be as high as 300 mg/d. Typically, hydrocortisone (cortisol), 20 mg/d to 30 mg/d (two thirds in the morning and one third in the late afternoon or early evening), is prescribed as replacement for pregnant and nonpregnant women. Mineralocorticoid replacement is necessary for primary, but not secondary, adrenal insufficiency.

References

[1] Gray H. Anatomy of the human body. 30th American edition. Philadelphia: Lea & Febiger; 1985.
[2] Williams RH, Reed Larsen P, Kronenberg HM, et al. Williams' textbook of endocrinology. 8th edition. Philadelphia: Saunders; 1992.
[3] Burgess R, Lunyak V, Rosenfeld M. Signaling and transcriptional control of pituitary development. Curr Opin Genet Dev 2002;12(5):534–9.
[4] Scully KM, Rosenfeld MG. Pituitary development: regulatory codes in mammalian organogenesis. Science 2002;295(5563):2231–5.
[5] Sheng HZ, Westphal H. Early steps in pituitary organogenesis. Trends Genet 1999;15(6):236–40.
[6] Ganong WF. Review of medical physiology. 21st edition. New York: McGraw Hill; 2003.
[7] Speroff L. Clinical gynecologic endocrinology and infertility. 6th edition. Philadelphia: Lippincott Williams & Wilkins; 1999.
[8] Barbieri R. The maternal adenohypophysis. In: Tulchinsky D, Little B, editors. Maternal fetal endocrinology. Philadelphia: W.B. Saunders; 1994. p. 119–31.
[9] Erdheim J, Stumme E. Uber die schwangerschaftsveranderung der hypophyse. [Pregnancy and the hypophysis.] Beitr Pathol Anat 1909;46:1–132 [in German].
[10] Gonzalez JG, Elizondo G, Saldivar D, Nanez H, Todd LE, Villarreal JZ. Pituitary gland growth during normal pregnancy: an in vivo study using magnetic resonance imaging. Am J Med 1988; 85(2):217–20.
[11] Hinshaw Jr DB, Hasso AN, Thompson JR, Davidson BJ. High resolution computed tomography of the post partum pituitary gland. Neuroradiology 1984;26(4):299–301.
[12] Carvill M. Bitemporal contractions of the visual fields during pregnancy. Am J Ophthalmol 1923;6:885.
[13] Finlay C. Visual field defects in pregnancy. Arch Ophthalmol 1934;12:207.
[14] Scheithauer BW, Sano T, Kovacs KT, Young Jr WF, Ryan N, Randall RV. The pituitary gland in pregnancy: a clinicopathologic and immunohistochemical study of 69 cases. Mayo Clin Proc 1990;65(4):461–74.
[15] Asa SL, Penz G, Kovacs K, Ezrin C. Prolactin cells in the human pituitary. A quantitative immunocytochemical analysis. Arch Pathol Lab Med 1982;106(7):360–3.
[16] Rigg LA, Lein A, Yen SS. Pattern of increase in circulating prolactin levels during human gestation. Am J Obstet Gynecol 1977;129(4):454–6.
[17] Kauppila A, Chatelain P, Kirkinen P, Kivinen S, Ruokonen A. Isolated prolactin deficiency in a woman with puerperal alactogenesis. J Clin Endocrinol Metab 1987;64(2):309–12.
[18] Riddick DH, Luciano AA, Kusmik WF, Maslar IA. Evidence for a nonpituitary source of amniotic fluid prolactin. Fertil Steril 1979;31(1):35–9.
[19] Hershman JM, Burrow GN. Lack of release of human chorionic gonadotropin by thyrotropin-releasing hormone. J Clin Endocrinol Metab 1976;42(5):970–2.

[20] Quigley ME, Ishizuka B, Ropert JF, Yen SS. The food-entrained prolactin and cortisol release in late pregnancy and prolactinoma patients. J Clin Endocrinol Metab 1982;54(6):1109–12.
[21] Boyar RM, Finkelstein JW, Kapen S, Hellman L. Twenty-four hour prolactin (PRL) secretory patterns during pregnancy. J Clin Endocrinol Metab 1975;40(6):1117–20.
[22] Bonnar J, Franklin M, Nott PN, McNeilly AS. Effect of breast-feeding on pituitary-ovarian function after childbirth. BMJ 1975;4(5988):82–4.
[23] Markoff E, Lee DW, Hollingsworth DR. Glycosylated and nonglycosylated prolactin in serum during pregnancy. J Clin Endocrinol Metab 1988;67(3):519–23.
[24] Pellegrini I, Gunz G, Ronin C, et al. Polymorphism of prolactin secreted by human prolactinoma cells: immunological, receptor binding, and biological properties of the glycosylated and nonglycosylated forms. Endocrinology 1988;122(6):2667–74.
[25] Andersen JR. Prolactin in amniotic fluid and maternal serum during uncomplicated human pregnancy. A review. Dan Med Bull 1982;29(6):266–74.
[26] Musey VC, Collins DC, Musey PI, Martino-Saltzman D, Preedy JR. Long-term effect of a first pregnancy on the secretion of prolactin. N Engl J Med 1987;316(5):229–34.
[27] Yu MC, Gerkins VR, Henderson BE, Brown JB, Pike MC. Elevated levels of prolactin in nulliparous women. Br J Cancer 1981;43(6):826–31.
[28] Kwa HG, Cleton F, Bulbrook RD, Wang DY, Hayward JL. Plasma prolactin levels and breast cancer: relation to parity, weight and height, and age at first birth. Int J Cancer 1981;28(1):31–4.
[29] Carr BR, Parker Jr CR, Madden JD, MacDonald PC, Porter JC. Maternal plasma adrenocorticotropin and cortisol relationships throughout human pregnancy. Am J Obstet Gynecol 1981;139(4):416–22.
[30] Genazzani AR, Fraioli F, Hurlimann J, Fioretti P, Felber JP. Immunoreactive ACTH and cortisol plasma levels during pregnancy. Detection and partial purification of corticotrophin-like placental hormone: the human chorionic corticotrophin (HCC). Clin Endocrinol (Oxf) 1975;4(1):1–14.
[31] Genazzani AR, Felber JP, Fioretti P. Immunoreactive ACTH, immunoreactive human chorionic somatomammotrophin (HCS) and 11-OH steroids plasma levels in normal and pathological pregnancies. Acta Endocrinol (Copenh) 1976;83(4):800–10.
[32] Goland RS, Stark RI, Wardlaw SL. Response to corticotropin-releasing hormone during pregnancy in the baboon. J Clin Endocrinol Metab 1990;70(4):925–9.
[33] Goland RS, Wardlaw SL, MacCarter G, Warren WB, Stark RI. Adrenocorticotropin and cortisol responses to vasopressin during pregnancy. J Clin Endocrinol Metab 1991;73(2):257–61.
[34] Goland RS, Wardlaw SL, Stark RI, Brown Jr LS, Frantz AG. High levels of corticotropin-releasing hormone immunoactivity in maternal and fetal plasma during pregnancy. J Clin Endocrinol Metab 1986;63(5):1199–203.
[35] Nolten WE, Lindheimer MD, Rueckert PA, Oparil S, Ehrlich EN. Diurnal patterns and regulation of cortisol secretion in pregnancy. J Clin Endocrinol Metab 1980;51(3):466–72.
[36] Nolten WE, Rueckert PA. Elevated free cortisol index in pregnancy: possible regulatory mechanisms. Am J Obstet Gynecol 1981;139(4):492–8.
[37] Rees LH, Burke CW, Chard T, Evans SW, Letchworth AT. Possible placental origin of ACTH in normal human pregnancy. Nature 1975;254(5501):620–2.
[38] Hobel CJ, Arora CP, Korst LM. Corticotrophin-releasing hormone and CRH-binding protein. Differences between patients at risk for preterm birth and hypertension. Ann N Y Acad Sci 1999;897:54–65.
[39] Schulte HM, Weisner D, Allolio B. The corticotrophin releasing hormone test in late pregnancy: lack of adrenocorticotrophin and cortisol response. Clin Endocrinol (Oxf) 1990;33(1):99–106.
[40] Cousins L, Rigg L, Hollingsworth D, et al. Qualitative and quantitative assessment of the circadian rhythm of cortisol in pregnancy. Am J Obstet Gynecol 1983;145(4):411–6.
[41] Kletzky OA, Rossman F, Bertolli SI, Platt LD, Mishell Jr DR. Dynamics of human chorionic gonadotropin, prolactin, and growth hormone in serum and amniotic fluid throughout normal human pregnancy. Am J Obstet Gynecol 1985;151(7):878–84.
[42] Daughaday WH, Trivedi B, Winn HN, Yan H. Hypersomatotropism in pregnant women, as measured by a human liver radioreceptor assay. J Clin Endocrinol Metab 1990;70(1):215–21.

[43] Yen SS, Vela P, Tsai CC. Impairment of growth hormone secretion in response to hypoglycemia during early and late pregnancy. J Clin Endocrinol Metab 1970;31(1):29–32.
[44] Samaan NA, Goplerud CP, Bradbury JT. Effect of arginine infusion on plasma levels of growth hormone, insulin, and glucose during pregnancy and the puerperium. Am J Obstet Gynecol 1970;107(7):1002–7.
[45] Burrow GN, Fisher DA, Larsen PR. Maternal and fetal thyroid function. N Engl J Med 1994; 331(16):1072–8.
[46] Hershman JM, Kojima A, Friesen HG. Effect of thyrotropin-releasing hormone on human pituitary thyrotropin, prolactin, placental lactogen, and chorionic thyrotropin. J Clin Endocrinol Metab 1973;36(3):497–501.
[47] Kannan V, Sinha MK, Devi PK, Rastogi GK. Plasma thyrotropin and its response to thyrotropin releasing hormone in normal pregnancy. Obstet Gynecol 1973;42(4):547–9.
[48] Glinoer D, de Nayer P, Bourdoux P, et al. Regulation of maternal thyroid during pregnancy. J Clin Endocrinol Metab 1990;71(2):276–87.
[49] Harada A, Hershman JM, Reed AW, et al. Comparison of thyroid stimulators and thyroid hormone concentrations in the sera of pregnant women. J Clin Endocrinol Metab 1979;48(5): 793–7.
[50] Nisula BC, Ketelslegers JM. Thyroid-stimulating activity and chorionic gonadotropin. J Clin Invest 1974;54(2):494–9.
[51] Silverberg J, O'Donnell J, Sugenoya A, Row VV, Volpe R. Effect of human chorionic gonadotropin on human thyroid tissue in vitro. J Clin Endocrinol Metab 1978;46(3):420–4.
[52] Ballabio M, Poshychinda M, Ekins RP. Pregnancy-induced changes in thyroid function: role of human chorionic gonadotropin as putative regulator of maternal thyroid. J Clin Endocrinol Metab 1991;73(4):824–31.
[53] Harada A, Hershman JM. Extraction of human chorionic thyrotropin (hCT) from term placentas: failure to recover thyrotropic activity. J Clin Endocrinol Metab 1978;47(3):681–5.
[54] Miyake A, Tanizawa O, Aono T, Kurachi K. Pituitary responses in LH secretion to LHRH during pregnancy. Obstet Gynecol 1977;49(5):549–51.
[55] Reyes FI, Winter JS, Faiman C. Pituitary gonadotropin function during human pregnancy: serum FSH and LH levels before and after LHRH administration. J Clin Endocrinol Metab 1976; 42(3):590–2.
[56] Rubinstein LM, Parlow AF, Derzko C, Hershman J. Pituitary gonadotropin response to LHRH in human pregnancy. Obstet Gynecol 1978;52(2):172–5.
[57] De la Lastra M, Llados C. Luteinizing hormone content of the pituitary gland in pregnant and non-pregnant women. J Clin Endocrinol Metab 1977;44(5):921–3.
[58] Herman V, Fagin J, Gonsky R, Kovacs K, Melmed S. Clonal origin of pituitary adenomas. J Clin Endocrinol Metab 1990;71(6):1427–33.
[59] Corenblum B, Donovan L. The safety of physiological estrogen plus progestin replacement therapy and with oral contraceptive therapy in women with pathological hyperprolactinemia. Fertil Steril 1993;59(3):671–3.
[60] Molitch ME, Thorner MO, Wilson C. Management of prolactinomas. J Clin Endocrinol Metab 1997;82(4):996–1000.
[61] Molitch ME. Prolactinoma. In: Melmed S, editor. The pituitary. Boston: Blackwell Science Publications; 1995. p. 443–77.
[62] Barbieri RL, Ryan KJ. Bromocriptine: endocrine pharmacology and therapeutic applications. Fertil Steril 1983;39(6):727–41.
[63] McGregor AM, Scanlon MF, Hall R, Hall K. Effects of bromocriptine on pituitary tumour size. BMJ 1979;2(6192):700–3.
[64] Molitch ME, Elton RL, Blackwell RE, et al. Bromocriptine as primary therapy for prolactin-secreting macroadenomas: results of a prospective multicenter study. J Clin Endocrinol Metab 1985;60(4):698–705.
[65] Webster J, Piscitelli G, Polli A, Ferrari CI, Ismail I, Scanlon MF. A comparison of cabergoline and bromocriptine in the treatment of hyperprolactinemic amenorrhea. Cabergoline Comparative Study Group. N Engl J Med 1994;331(14):904–9.

[66] Raymond JP, Goldstein E, Konopka P, Leleu MF, Merceron RE, Loria Y. Follow-up of children born of bromocriptine-treated mothers. Horm Res 1985;22(3):239–46.
[67] Bergh T, Nillius SJ, Wide L. Clinical course and outcome of pregnancies in amenorrhoeic women with hyperprolactinaemia and pituitary tumors. BMJ 1978;1(6117):875–80.
[68] Konopka P, Raymond JP, Merceron RE, Seneze J. Continuous administration of bromocriptine in the prevention of neurological complications in pregnant women with prolactinomas. Am J Obstet Gynecol 1983;146(8):935–8.
[69] Holmgren U, Bergstrand G, Hagenfeldt K, Werner S. Women with prolactinoma—effect of pregnancy and lactation on serum prolactin and on tumour growth. Acta Endocrinol (Copenh) 1986;111(4):452–9.
[70] Molitch ME. Pregnancy and the hyperprolactinemic woman. N Engl J Med 1985;312(21): 1364–70.
[71] Lindholm J, Schultz-Moller N. Plasma and urinary cortisol in pregnancy and during estrogen-gestagen treatment. Scand J Clin Lab Invest 1973;31(1):119–22.
[72] Lin CL, Wu TJ, Machacek DA, Jiang NS, Kao PC. Urinary free cortisol and cortisone determined by high performance liquid chromatography in the diagnosis of Cushing's syndrome. J Clin Endocrinol Metab 1997;82(1):151–5.
[73] Buescher MA, McClamrock HD, Adashi EY. Cushing syndrome in pregnancy. Obstet Gynecol 1992;79(1):130–7.
[74] Aron DC, Schnall AM, Sheeler LR. Cushing's syndrome and pregnancy. Am J Obstet Gynecol 1990;162(1):244–52.
[75] Casson IF, Davis JC, Jeffreys RV, Silas JH, Williams J, Belchetz PE. Successful management of Cushing's disease during pregnancy by transsphenoidal adenectomy. Clin Endocrinol (Oxf) 1987;27(4):423–8.
[76] Abelove WA, Rupp JJ, Paschkis KE. Acromegaly and pregnancy. J Clin Endocrinol Metab 1954;14(1):32–44.
[77] Finkler RS. Acromegaly and pregnancy; case report. J Clin Endocrinol Metab 1954;14(10): 1245–6.
[78] Jialal I, Naidoo C, Norman RJ, Rajput MC, Omar MA, Joubert SM. Pituitary function in Sheehan's syndrome. Obstet Gynecol 1984;63(1):15–9.
[79] Barbieri RL, Cooper DS, Daniels GH, Nathan D, Klibanski A, Ridgway EC. Prolactin response to thyrotropin-releasing hormone (TRH) in patients with hypothalamic-pituitary disease. Fertil Steril 1985;43(1):66–73.
[80] Sheehan HL, Whitehead R. The neurohypophysis in post-partum hypopituitarism. J Pathol Bacteriol 1963;85:145–69.
[81] Whitehead R. The hypothalamus in post-partum hypopituitarism. J Pathol Bacteriol 1963;86: 55–67.
[82] Bakiri F, Benmiloud M, Vallotton MB. Arginine-vasopressin in postpartum panhypopituitarism: urinary excretion and kidney response to osmolar load. J Clin Endocrinol Metab 1984;58(3): 511–5.
[83] Bakiri F, Benmiloud M. Antidiuretic function in Sheehan's syndrome. Br Med J (Clin Res Ed) 1984;289(6445):579–80.
[84] Miller M, Moses A. Clinical status due to alteration of ADH release and action. In: Moses A, Share L, editors. Neurohypophysis. Basel (Switzerland): Karger; 1977. p. 153.
[85] Barron WM, Lindheimer MD. Renal sodium and water handling in pregnancy. Obstet Gynecol Annu 1984;13:35–69.
[86] Durr JA. Diabetes insipidus in pregnancy. Am J Kidney Dis 1987;9(4):276–83.
[87] Iwasaki Y, Oiso Y, Yamauchi K, et al. Neurohypophyseal function in postpartum hypopituitarism: impaired plasma vasopressin response to osmotic stimuli. J Clin Endocrinol Metab 1989; 68(3):560–5.
[88] Robertson G. Posterior pituitary. In: Fleg P, Baxter J, Broadus A, et al, editors. Endocrinology and Metabolism. New York: Mc Graw-Hill; 1995. p. 338–77.
[89] Swartz SL, Williams GH, Hollenberg NK, Dluhy RG, Moore TJ. Primacy of the renin-

angiotensin system in mediating the aldosterone response to sodium restriction. J Clin Endocrinol Metab 1980;50(6):1071–4.

[90] Speckart PF, Nicoloff JT, Bethune JE. Screening for adrenocortical insufficiency with cosyntropin (synthetic ACTH). Arch Intern Med 1971;128(5):761–3.

[91] Dluhy RG, Himathongkam T, Greenfield M. Rapid ACTH test with plasma aldosterone levels. Improved diagnostic discrimination. Ann Intern Med 1974;80(6):693–6.

ELSEVIER
SAUNDERS

Obstet Gynecol Clin N Am
31 (2004) 893–905

OBSTETRICS AND
GYNECOLOGY
CLINICS
OF NORTH AMERICA

Thyroid disease in pregnancy

Donna Neale, MD[a,*], Gerard Burrow, MD[b]

[a]*Department of Obstetrics and Gynecology, Division of Maternal-Fetal Medicine, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520-8063, USA*
[b]*Mystic Aquarium & Institute for Exploration, 55 Coogan Boulevard, Mystic, CT 06355, USA*

Thirteen million Americans are affected with thyroid disease. Thyroid disease often manifests itself during the reproductive period of a woman's life and is the second most common endocrinopathy that affects women of childbearing age. The physiologic changes of pregnancy can mimic thyroid disease or cause a true remission or exacerbation of underlying disease. In addition, thyroid hormones are key players in fetal brain development.

Maternal, fetal and neonatal thyroid are discussed here. Moreover, this article serves as a review of the more common thyroid diseases that are encountered during pregnancy and the postnatal period, their treatments, and their potential effects on pregnancy.

Maternal physiology

Normal thyroid physiology

The thyroid gland is composed of two lobes that are connected by the isthmus. Each lobe is divided into lobules; each lobule contains 20 to 40 follicles. Each follicle consists of colloid, a glycoprotein-rich material, which is surrounded by follicular cells that produce thyroid hormone.

The hypothalamic pituitary axis is responsible for the maintenance of thyroid hormone production. Thyrotropin-releasing hormone (TRH) is produced in a

* Corresponding author.
E-mail address: donna.neale@yale.edu (D. Neale).

0889-8545/04/$ – see front matter
doi:10.1016/j.ogc.2004.09.001 obgyn.theclinics.com

tonic fashion in the paraventricular nucleus of the hypothalamus. It reaches the pituitary gland by way of the pituitary stalk and stimulates the production and release of thyrotropin (TSH). TSH has an α and a β subunit; the β subunit confers specificity. In addition to the direct stimulatory effect that TRH has on TSH secretion, TSH secretion is regulated by negative feedback from circulating thyroid hormone, dopamine, and somatostatin. TSH then stimulates the thyroid gland to produce, as well as secrete, thyroxine (T_4) and triiodothyronine (T_3).

The rate-limiting step in thyroid hormone production is iodide trapping, which is mediated by TSH. In the nonpregnant state, 80 μg/d to 100 μg/d of iodine are taken up by the thyroid gland. Dietary iodine is reduced to iodide and 20% of the intake is cleared by the thyroid gland, whereas the remainder is cleared renally. In the thyroid gland, the iodide is converted back to iodine and is organified by binding to tyrosyl residues, which are a part of the glycoprotein, thyroglobulin. The binding of the iodine to the tyrosyl residues is dependent on the enzyme, thyroid peroxidase. The binding of one iodine molecule to the tyrosyl residue leads to the formulation of monoiodotyrosine (MIT), whereas the binding of two iodine molecules leads to diiodotyrosine (DIT). Coupling of two DITs yields T_4 and a DIT and a MIT forms T_3. After iodination occurs, the thyroglobulin is transported out of the cell into the colloid. TSH is involved in the secretion of thyroid hormone from the follicle. The thyroglobulin is digested and then T_4 and T_3 are released into the circulation. The daily secretion rates of T_4 and T_3 are 90 μg and 30 μg, respectively. Most of the circulating T_4 and T_3 is bound to thyroid-binding globulin (TBG). Other binding proteins that are involved in thyroid hormone transport are thyroxine-binding prealbumin and albumin. Less than 1% of circulating thyroid hormone is in the free form and it is the free form of hormone that enters cells. Once inside of the target cell, thyroid hormone has an affinity for nuclear receptors to initiate its cellular responses [1]. The cellular responses that are modulated by thyroid hormone include growth, development, and metabolism, as well as transcription and translation of new proteins.

Several differences between T_4 and T_3 are worth mentioning. First, T_3 has a 10-fold greater affinity for the nuclear receptors; this explains its greater biologic activity. Secondly, whereas T_4 is synthesized completely in the thyroid gland, only 20% of T_3 comes from the thyroid gland. Most (80%) circulating T_3 is derived from peripheral conversion of T_4. T_4 gets metabolized in extrathyroidal tissues (eg, liver and kidneys) to T_3 and reverse T_3 by deiodination. Removal of an iodine by 5′ monodeiodination from the outer ring of T_4 results in T_3. When iodine is removed from the inner ring, reverse T_3—which is metabolically inactive—is formed. Approximately 35% of T_4 gets converted to T_3, whereas 40% is converted to reverse T_3. In catabolic states (eg, severe illness, starvation) even more of the T_4 is converted to metabolic inactive reverse T_3 [2]. Monodeiondinase types I and II catalyze the formation of T_3, whereas monodeiodinase type III is responsible for the deiodination that yields reverse T_3. Monodeiodinase types II and III are found abundantly in the placenta [3]. A third difference between the two thyroid hormones is their half-lives. The half-life of T_4 is 1 week, whereas the half-life of T_3 is 1 day.

Physiologic adaptation during pregnancy

Starting in early pregnancy, there is an increase in thyroid-binding globulin secondary to an estrogenic stimulation of TBG synthesis and reduced hepatic clearance of TBG [3]. Basal levels increase two to threefold. Therefore, levels of bound proteins, total thyroxine, and total triiodothyronine are increased and resin triiodothyronine uptake (RT_3U) is decreased. Also in the first trimester, as human chorionic gonadotropin (hCG) levels peak, there is partial inhibition of the pituitary gland (by cross-reactivity of the α subunit) that yields a transient decrease in TSH between Weeks 8 and 14 of gestation [3]. In about 20% of normal women, TSH levels decrease to less than the lower limit of normal. Women who have the highest hCG concentrations have the greatest reduction in TSH. A decrease in basal TSH of 0.1 mU/L was observed for every 10,000 IU/L increment in hCG [4]. In most pregnant women, this change has minimal clinical consequences.

The second physiologic adaptation is a reduction in plasma iodide. Plasma iodine levels decrease during pregnancy because of fetal use of iodide (monodeiodinase types II and III are found in the placenta) as well as increased maternal renal clearance of iodide [5]. For this reason, the World Health Organization recommends 200 micrograms/day of iodine for pregnant women. The normal daily-recommended dosage for adults is 150 micrograms.

This decrease in plasma iodide levels is associated with a noticeable increase in thyroid gland size in approximately 15% of women [3]. Ultrasound measurement of thyroid glands in more than 600 women who did not have thyroid disease confirmed a mean increase in size of 18%; the gland returns to normal in the postpartum period. Despite the change in size of the gland, none of the women had abnormal thyroid function tests [6].

Fetal thyroid physiology

The fetal thyroid gland forms as a midline outpouching of the anterior pharyngeal floor. It migrates caudally and reaches its final position by 7 weeks of gestation. The bilateral shape is apparent by 9 weeks of gestation. Active trapping of iodine is apparent by 12 weeks of gestation; there is evidence of T_4 products by the 14th week of gestation. Fetal thyroid function is under the pituitary TSH control by midgestation [7]; however, hypothalamic TRH is detectable as early as 9 weeks of gestation and the pituitary portal circulation is functioning by the end of the first trimester. At 20 weeks' gestation, concentrations of fetal TSH, T_4, TBG, and free T_4 start to increase and reach adults levels by 36 weeks of gestation [8]. Levels of fetal T_3 and free T_3 do not increase to the adult levels because the placental type III deiodinase rapidly converts most fetal T_4 to reverse T_3. Tissues that depend on T_3 for development (eg, brain) have abundant levels of type II deiodinase that convert T_4 to T_3.

Maternal T_4 and T_3 cross the placenta, whereas maternal TSH does not. It is believed that enough maternal T_4 crosses the placenta to sustain fetal thyroid function because it was shown that umbilical cord T_4 levels in neonates who have congenital hypothyroidism are up to 50% of the normal [9]. There also is transplacental transfer of TRH, iodine, thyroid stimulatory immunoglobulin (TSI), propylthiouracil (PTU), methimazole, and β- blockers. Amniotic fluid measurements of TSH, T_4, and T_3 levels reflect fetal serum levels.

Neonatal thyroid function

At birth, there is a surge of TRH and TSH that lead to an increase in T_3 and T_4 [3]. By the first week of life, the TSH levels return to normal adult levels and by 6 weeks of life, T_4 and T_3 levels return to normal adult levels. In premature infants, the surging pattern of postnatal thyroid hormone is not seen. Instead, free T_4 is decreased and TSH is in the normal adult range.

Hyperthyroidism and pregnancy

Hyperthyroidism occurs in 0.2% of pregnancies. Symptoms of hyperthyroidism include tachycardia, nervousness, tremors, heat intolerance, weight loss, goiter, frequent stools, excessive sweating, palpitations, and hypertension. Although some of these signs and symptoms mimic normal physiologic changes of pregnancy, a thyroid function test will differentiate thyroid disease from normal pregnancy. In normal pregnancy, TSH, free T_4, and free thyroxine index (fTI) should not change. In hyperthyroidism, TSH is depressed and fT_4 and fTI are increased. The RT_3U that normally is decreased in pregnancy is increased in hyperthyroidism.

Graves' disease is the most common cause of hyperthyroidism during pregnancy and accounts for 95% of the cases [10]. Other causes of hyperthyroidism during pregnancy include gestational trophoblastic disease, nodular goiter or solitary toxic adenoma, viral thyroiditis (de Quervain), and tumors of the pituitary gland or ovary (struma ovarii). Women also can present with transient hyperthyroidism as seen in cases of hyperemesis gravidarum and gestational transient thyrotoxicity (GET).

Preferably, thyroid disease should be controlled before conception. The importance of diagnosing and treating hyperthyroidism during pregnancy is its association with adverse perinatal outcomes. Severe maternal hyperthyroidism is associated with increased risk of stillbirth, preterm delivery, intrauterine growth restriction, preeclampsia, and heart failure [11]. Thyrotoxicosis at conception also increases the risk for spontaneous abortion [12] although the mechanism for this finding is not known.

Transient hyperthyroidism during pregnancy

In most cases of hyperemesis gravidarum, elevated T_4 and suppressed TSH levels are seen. This change is believed to be related to hCG stimulation of the thyroid gland [13]. Normalization of T_4 levels usually occurs by midgestation. Treatment is supportive care. In cases where antithyroid medication is needed, PTU and methimazole can be used, if tolerated. Methimazole has the advantage in this setting because it is available in suppository form.

GET occurs in the first trimester in women who do not have a personal or family history of autoimmune disease. It also is believed to be related to hCG stimulation of the thyroid gland [14]. The patient will present with symptoms of hyperthyroidism and elevated free T_4 levels. The thyroid gland usually is not enlarged. The resolution of symptoms parallels the decline in hCG levels. Patients rarely need treatment but β-blockers can be used for symptomatic relief.

Graves' disease

Graves' disease accounts for 95% of cases of thyrotoxicosis during pregnancy. This is an organ-specific autoimmune disease that is mediated by thyroid stimulatory immunoglobins. These autoantibodies mimic TSH and its ability to stimulate thyroid function. They are responsible for thyroid hyperfunction and thyroid gland hypertrophy. There does not seem to be any clinical correlation between levels of antibody activity and disease severity. These antibodies, however, can cross the placenta and cause neonatal Graves' disease. In addition to the traditional signs and symptoms of hyperthyroidism that were outlined above, proptosis and external ocular muscle palsies along with localized or pretibial myxedema are distinctive symptoms of Graves' disease.

The goal of treatment of hyperthyroidism during pregnancy is to keep the patient euthyroid with the free T_4 in the upper limit of normal range so as not to cause fetal or neonatal hypothyroidism. In the United States, PTU is the drug of choice, whereas in Canada and Europe, methimazole or carbimazole (metabolized to methimazole) often are used. Both are thionamides, which inhibit iodination of thyroglobulin and thyroglobulin synthesis by competing with iodine for the enzyme peroxidase [15]. PTU also blocks the conversion of T_4 and T_3.

PTU is started at dosages of 100 mg to 150 mg every 8 hours (total daily dose should be 300 mg–450 mg, depending on severity of disease). It may take 6 to 8 weeks to see a clinical change. Free T_4 levels should be monitored monthly. After the mother is euthyroid, the dosage of PTU should be tapered. The side effects of PTU include rash (5%), pruritus, hepatitis, lupus-like syndrome, drug fever, and bronchospasm. The risks of uncontrolled thyroid disease outweigh these risks, so daily dosages of up to 600 mg can be used. An alternative thionamide also can be tried, but there is a 50% cross sensitivity. Agranulocytosis is a rare, but serious, side effect that occurs in 0.1% of patients; it is seen particularly in older

patients and in those who receive high dosages [16]. Agranulocytosis is a contraindication to further thionamide therapy. The white blood count should improve on its own over days to weeks after the medication is discontinued.

Methimazole is not used often in the United States because of its possible association with a rare scalp deformity, cutis aplasia, and its possible increased placental passage. Further review of the use of this agent during pregnancy has not shown such a strong association [17,18]. There also have been reports of a methimazole embryopathy that is manifested by tracheoesophageal fistula and facial abnormalities [19]. A study that compared the use of PTU with methimazole during pregnancy found the incidence of major malformations were similar—3% with PTU and 2.7% with methimazole [20].

Neonatal thyrotoxicosis can occur in approximately 1% of infants who are born to mothers who have Graves' disease secondary to transplacental passage of maternal TSIs [12]. This process even can occur in instances in which the mother remains euthyroid throughout the pregnancy or has had surgical or radioactive ^{131}I treatments before pregnancy. Because the women in the latter group do not require any therapy, the risk is even higher for neonatal disease because of the lack of suppressive effect of thionamides (which cross the placenta) [21]. For these reasons, maternal TSI levels should be checked at the onset of pregnancy as well as during the third trimester. In addition, fetal ultrasound should be performed to exclude evidence of fetal thyrotoxicosis (eg, an anterior fetal neck mass) or fetal tachycardia. Women who have Graves' disease also have thyroid-stimulating hormone-binding inhibitory immunoglobulin (TBII), which mediates thyroid inhibition. Like the TSIs, these antibodies can cross the placenta and cause transient neonatal hypothyroidism [22]. Ultrasound can be used to assess for signs of fetal hypothyroidism, including fetal bradycardia, goiter, and growth restriction. If there is concern for fetal thyroid dysfunction, amniocentesis or cordocentesis can be performed to assess fetal thyroid function. Amniotic fluid measurements of TSH, T_4, and T_3 levels reflect fetal serum levels.

PTU and methimazole are considered to be compatible with breastfeeding. These medications should be taken just after breastfeeding; a 3- to 4-hour interval should lapse before the mother lactates again to minimize their concentration in breast milk. It is imperative to continue medications throughout the postpartum period because exacerbation of Graves' disease is common during this time.

If PTU or methimazole cannot be given, β-blockers can be used to control the adrenergic symptoms that are seen with thyrotoxicosis, particularly tachycardia. In addition, the β-blockers block the peripheral conversion of T4 to T3. Propranolol is the agent that is used most commonly, starting at 20 mg to 40 mg, by mouth, two or three times a day. In acute settings, intravenous (IV) esmolol can be used. This is an ultrashort-acting cardioselective agent. The use of β-blockers for a prolonged period during pregnancy has been associated with intrauterine growth restriction, fetal brachycardia, and neonatal hypoglycemia.

Surgery is reserved for the most severe cases. As with other surgeries during pregnancy, if thyroidectomy is necessary it should be performed in the second trimester. Preoperatively, the woman should receive 2 weeks of iodine therapy.

The surgical risks include hypoparathyroidism and paralysis of the recurrent laryngeal nerve as well as anesthetic risks.

^{131}I is contraindicated in pregnancy because the fetal thyroid can take it up after 10 weeks of gestation. If a pregnant woman inadvertently receives ^{131}I then she should receive potassium iodide (SSKI) and PTU within 1 week of exposure [23]. This combination acts to block organification and reduce radiation exposure to the fetal thyroid by a factor of 100. It also reduces the radiation exposure to the whole body of the fetus by a factor of 10.

Thyroid storm

Thyroid storm during pregnancy is an obstetric emergency that is marked by an extreme metabolic state. It occurs in only 10% of pregnant women who have hyperthyroidism, but is associated with a high risk of maternal cardiac failure.

This diagnosis is suspected when patients present with a combination of fever, change in mental status, seizures, nausea, diarrhea, and cardiac arrhythmias. An inciting event (eg, infection, surgery, labor/delivery) can be identified and a source of infection always should be excluded; however, after the diagnosis is suspected, treatment should begin immediately, even if the results of serum free T_4, free T_3, and TSH levels are not known. The consequences of untreated thyroid storm can be shock, stupor, and coma.

Adequate treatment of thyroid storm is achieved using a combination of pharmacologic agents with maternal supportive measures (eg, oxygen, IV fluids, electrolyte replacement, antipyretics, cooling blankets) and fetal assessment. These patients should be cared for in labor and delivery or in an ICU.

Each pharmacologic agent that is used has a synergistic role in the suppression of thyroid function. PTU or methimazole blocks additional synthesis of thyroid hormone by inhibiting the iodination of thyroglobulin. In addition, PTU blocks the peripheral conversion of T_4 to T_3. If the patient is not able to take the medication by mouth, methimazole rectal suppositories can be used. SSKI and sodium iodide block the release of thyroid hormone from the thyroid gland. Dexamethasone decreases thyroid hormone release and blocks the peripheral conversion of T_4 to T_3. β-blockers are used to treat the adrenergic effects of the excessive thyroid hormone. If the patient has a contraindication to β-blockade (ie, history of severe bronchospasm) then Reserpine or Diltiazem can be used instead. Phenobarbital has been used to reduce extreme agitation and it may increase the catabolism of thyroid hormone [24].

Assessment of intravascular volume status is crucial. Invasive central monitoring and continuous maternal cardiac monitoring may be indicated. The perceived underlying cause of the thyroid storm should be treated. Fetal status should be assessed with ultrasound, biophysical profile, or nonstress test, depending on the gestational age of the fetus. Delivery should be reserved for fetal indications that outweigh the risks to the woman.

Hypothyroidism in pregnancy

Hypothyroidism was reported to occur in 0.05% of pregnancies [25]; however, population-screening studies have suggested a higher incidence. In a U.S. study, 49 of 2000 women who had their serum TSH levels checked between 15 and 18 weeks of gestation had levels that were elevated to 6 mU/L or greater. Of these 49 women, 58% had positive thyroid antibodies compared with an 11% rate in the group that was euthyroid [26]. In a more recent study that examined midtrimester TSH levels in pregnant women, only 75 of the 25,215 women (0.30%) had elevated TSH levels [27]. The urge to determine the true incidence of hypothyroidism in pregnancy is driven by the knowledge that these women have increased rates of miscarriage, preeclampsia, placental abruption, growth restriction, prematurity and stillbirths and their fetuses are at risk for impaired neurologic development.

Symptoms of hypothyroidism often can be masked by the hypermetabolic state of pregnancy. Mild symptoms include modest weight gain, lethargy, decrease in exercise capacity, and intolerance to cold. In more symptomatic women, constipation, hoarseness, hair loss, brittle nails, dry skin, goiter, or delay in the relaxation phase of the deep tendon reflexes may been appreciated. Patients who undergo thyroidectomy for Graves' disease may become hypothyroid if not supplemented with thyroid hormone postoperatively. Therefore, in patients who have a thyroidectomy scar together with the above symptoms, the diagnosis of hypothyroidism should be entertained. In normal pregnancy, TSH and free T_4 levels should not change. In hypothyroidism, TSH can be elevated with or without suppressed levels of free T_4.

Frequently, antithyroid autoantibodies (eg, antithyroglobulin, antithyroid peroxidase) are present in cases of hypothyroidism. Other laboratory abnormalities include elevated creatine phosphokinase, cholesterol, and liver function tests. Because having an autoimmune disease increases the likelihood of developing another, some patients present with type I diabetes mellitus. There is a 5% to 8% prevalence of hypothyroidism in type I diabetes mellitus and women who have type I diabetes have a 25% risk of developing postpartum thyroid dysfunction [28].

Causes of hypothyroidism

Worldwide, the most common cause of hypothyroidism is iodine deficiency. One to 1.5 billion people are at risk; 500 million live in areas of overt iodine deficiency [29]. Because the transplacental passage of maternal T_4 is necessary for fetal brain development early in the first trimester before the development of the fetal thyroid gland, lack of iodine during this time may lead to impaired neurologic development. Moreover, even when the fetal thyroid gland has developed, if there is no iodine substrate for the gland to use, then the fetus is unable to synthesize its own thyroid hormones. The result of severe iodine deficiency (intake of 20–25 μg/dg) is endemic cretinism. These infants are characterized by severe mental retardation, deafness, muteness, and pyramidal or extrapyramidal syndromes; the most common cause of mental retardation worldwide is iodine deficiency.

In the United States, the most common cause of hypothyroidism is a primary thyroid abnormality. Hashimoto's thyroiditis, also known as chronic lymphocytic thyroiditis, occurs in 8% to 10% of women of reproductive age; it accounts for most cases of hypothyroidism during pregnancy. This is an autoimmune disorder; therefore, titers of antithyroglobulin are elevated in 50% to 70% of patients and almost all of the patients have antiperoxidase antibodies [30]. These women most often present with a firm, painless goiter. Histologically, plasma cells and lymphocytes infiltrate the gland. Despite the goiter, some women can be euthyroid and subsequently become hypothyroid. Sometimes the thyroid gland will become atrophic and the antithyroid antibodies are absent. This presentation is termed idiopathic hypothyroidism.

Other causes of hypothyroidism are ^{131}I treatment for Graves' disease and thyroidectomy. Ten to 20% of women who receive ^{131}I are hypothyroid within 6 months and 2% to 4% become hypothyroid each year thereafter [31]. Subacute viral thyroiditis and suppurative thyroiditis also can result in hypothyroidism. Drugs that are used commonly during pregnancy can interfere with the metabolism of thyroid hormones, and thus, can lead to depressed thyroid function. Ferrous sulfate and sucralfate interfere with the intestinal absorption of thyroxine. Carbamazepine, phenytoin, and rifampin can increase the clearance of T_4. Hypothyroidism that is secondary to hypothalamic or pituitary disease is rare but it can be seen in conditions (eg, Sheehan's syndrome, lymphocytic hypophysitis) that occur disproportionately in relation to pregnancy.

Treatment of hypothyroidism

Treatment should be initiated as soon as the diagnosis of hypothyroidism is made. The starting dosage of thyroxine is 0.1 mg/d to 0.15 mg/d. The dosage is adjusted every 4 weeks to keep the TSH at the lower end of normal. Women who are euthyroid or on thyroxine at the beginning of pregnancy should have their TSH and free T_4 levels checked every 8 weeks. T_4 requirements most likely will increase as the pregnancy progresses. This increase in requirement can be secondary to the increased demand for T_4 during pregnancy as well as its inadequate intestinal absorption that is caused by ferrous sulfate. Therefore, during pregnancy, ferrous sulfate and thyroxine dosages should be spaced at least 4 hours apart.

Postpartum thyroid disease

Postpartum thyroiditis

The diagnosis of postpartum thyroiditis is made by documenting abnormal TSH (elevated or suppressed) levels during the first year postpartum in the absence of positive TSI or a toxic nodule [32]. This disease occurs in 6% to 9% of women who do not have a history of the thyroid disease.

Women can present with hypo- or hyperthyroidism. The classic presentation is a transient hyperthyroid phase that occurs 6 weeks to 6 months postpartum which is followed by a hypothyroid phase that lasts for up to 1 year postpartum. Studies have shown, however, that only approximately a quarter of women have this classic presentation, whereas more than a third present with solely hypothyroidism or hyperthyroidism.

This is an autoimmune disorder with a self-limited hyperthyroid phase; therefore, antithyroid drugs have no real role. The adrenergic symptoms can be intolerable so β-blockers may be useful. In contrast, the hypothyroid phase may require treatment and some women never may recover their thyroid function. Tacchi et al [33] reported that 77% of women recovered during the first postpartum year and remained euthyroid; permanent hypothyroidism developed in the remaining 23%. A lower rate (11%) of residual hypothyroidism was reported by other investigators. Furthermore, it seems that women who have the highest levels of TSH and antithyroid peroxidase antibodies have the greatest risk for developing permanent hypothyroidism [34]. Because of this significant rate of residual hypothyroidism, some investigators recommend that women maintain their thyroxine treatment—if they have a predominant hypothyroid presentation—until childbearing is complete, with an attempt to wean off medication 1 year after the last delivery. Using this plan, all at-risk mother–child pairs should have adequate thyroid replacement coverage to prevent adverse perinatal and neonatal outcomes.

The early signs of postpartum thyroiditis may present around the time of the traditional "postpartum visit." Therefore, patients should be assessed for signs/symptoms of thyroid dysfunction at this visit. Clearly, many of these symptoms mimic normal postpartum changes so if there is any concern, TSH, free T_4, and antithyroid antibodies levels should be assessed. In addition, depression is a common postpartum event; the question has been raised as to whether postpartum depression and postpartum thyroiditis are related. Four studies attempted to evaluate this question. Two studies demonstrated an association between postpartum thyroiditis and depression [35,36], one study demonstrated an association between antithyroid antibodies and depression [37], and one study showed a relationship between antithyroid antibodies and postpartum thyroiditis [38].

Postpartum Graves' disease

Graves' disease may present in the postpartum period; 60% of patients who have Graves' disease in the reproductive years have a history of postpartum onset [39]. In euthyroid patients with Graves' disease, patients who had TSI at the end of pregnancy had an increased risk of developing recurrent Graves' disease if antithyroid medication was withheld. The shift from a TH2 (anti-inflammatory) environment that predominates during pregnancy to a pro-TH1 (proinflammatory) environment may explain this postpartum autoimmune response. Other explanations include a reduced number of fetal cells that leads to loss of maternal tolerance to remaining microchimeric cells and loss of placental major histocompatibility complex peptides, which were inducing T-cell anergy during

pregnancy [40]. The presence of TSIs differentiate postpartum Graves' disease from postpartum thyroiditis with a hyperthyroid component.

Thyroid cancer

Thyroid tumors are the most common endocrine neoplasms. Most of the nodules are benign hyperplastic (or colloid) nodules. The actual rate of malignancy is low; thyroid cancer accounts for 1% of all cancers. Three out of four tumors occur in women; half present during the reproductive years. Therefore, whenever a solitary or dominant nodule is found within the thyroid gland, biopsy is recommended. Serum TSH and free T_4 levels should be obtained. Radionucleotide scanning is contraindicated during pregnancy; however, ultrasonography can be used to characterize the lesion. Fine needle aspiration can be performed safely during pregnancy. A benign lesion can be followed conservatively and reaspirated if it enlarges again. If a lesion is frankly malignant or suspicious for papillary cancer, surgery should ensue at the earliest safe period. There is no evidence that pregnancy causes a reactivation of thyroid cancer or that exposure to radioactive iodine poses a risk to future pregnancies [41]. Patients should be maintained on thyroid replacement therapy with monitoring of TSH and free T_4 levels every 8 weeks.

To screen or not to screen?

It appears that the first phase of neuronal multiplication and organization corresponds to the phase during which the supply of thyroid hormones to the fetus is almost exclusively of maternal origin [7]. The second phase of maximum fetal brain growth occurs during the third trimester and extends to 2 to 3 years of age. During this time, the supply of thyroid hormones is of fetal and neonatal origin. Thus, it seems that if maternal concentrations of T_4 are decreased early in pregnancy, irreversible neurologic changes may result in the offspring. As more knowledge about the regulatory role of thyroid hormone in fetal brain development has been gained, more attention has been focused on the subject of maternal subclinical hypothyroidism.

In an observational study in which 25,216 serum samples—previously collected from second trimester Alpha fetoprotein (AFP) screening—were screened for TSH levels. Seventy-five of the samples (0.03%) had TSH levels that were greater than the 99.7th percentile [27]. Neuropsychologic testing was performed at 8 years of age on 62 children of the hypothyroid women found in this cohort and 124 children of matched women who had normal thyroid function. No difference in IQ was found; however, there was a significant difference in mean IQ scores between children of untreated women who had hypothyroidism compared with controls. Nineteen percent of the children of the

untreated women had an IQ score that was up to 85 compared with only 5% of children of women who had normal thyroid function. From these observations, the American Association of Clinical Endocrinologists recommended in 2002 that routine TSH levels be performed preconceptionally or during the first trimester in all pregnant women [42]. The American College of Obstetricians and Gynecologists responded by stating that although the data are consistent with the possibility that maternal hypothyroidism is associated with a decrement in some neuropsychologic testing, no intervention trials have demonstrated the efficacy of screening and treatment to improve neuropsychologic performance in the offspring of hypothyroid women. For this reason, they do not support routine screening of asymptomatic pregnant women. Instead, the College recommends that thyroid function tests be performed on gravidas who have a personal history of thyroid disease or symptoms of thyroid disease [43].

References

[1] Brent GA. The molecular basis of thyroid hormone action. N Engl J Med 1994;331:847–53.
[2] Brennan MD, Bahn RS. Thyroid hormone and illness. Endocr Pract 1998;4:396.
[3] Burrow GN, Fischer DA, Larsen PR. Maternal and fetal thyroid function. N Engl J Med 1994;331:1072–8.
[4] Grun JP, Meuris S, DeNayer P, et al. The thyrotrophic role of human chorionic gonadotropin (hCG) in the early stage of twin (versus single) pregnancy. Clin Endocrinol 1997;46:719–25.
[5] Ferris TF. Renal disease. In: Burrow GN, Ferris TF, editors. Medical complications during pregnancy. Philadelphia: WB Saunders; 1988.
[6] Rasmussen NG, Hornnes PJ, Hegedus L. Ultrasonographically determined thyroid size in pregnancy and post partum: the goitrogenic effect of pregnancy. Am J Obstet Gynecol 1989; 160:1216–20.
[7] Glinoer D, Derange F. The potential repercussions of maternal, fetal and neonatal hypothyroxinemia on the progeny. Thyroid 2000;10:871–87.
[8] Thorpe-Beeston JG, Nicolaides KH, Felton CV, Butler J, et al. Maturation of the secretion of thyroid hormone and thyroid stimulating hormone in the fetus. N Engl J Med 1991;324:532–6.
[9] Vulsma T, Gons MH, deVijlder JJ. Maternal-fetal transfer of thyroxine in congenital hypothyroidism due to total organification defect or thyroid agenesis. N Engl J Med 1989;321:13.
[10] Eeker JL, Musci TJ. Thyroid function and disease in pregnancy. Curr Probl Obstet Gynecol Fertil 2000;23:109–22.
[11] Davis LE, Lucas MJ, Hawkins GD, et al. Thyrotoxicosis complicating pregnancy. Am J Obstet Gynecol 1989;160:63–70.
[12] Weetman AP. Graves disease. N Engl J Med 2000;343:1236–48.
[13] Goodwin TM, Montoro M, Mestman JH. Transient hyperthyroidism and hyperemesis gravidarum: Clinical aspects. Am J Obstet Gynecol 1992;167:648–52.
[14] Glinoer D, DeNayer P, Robyn C, et al. Serum levels of intact human chorionic gonadotropin (hCG) and its free α and β subunits, in relation to maternal thyroid stimulation during normal pregnancy. J Endocrinol Invest 1993;16:881–8.
[15] Mandel SJ, Cooper DS. The use of antithyroid drugs in pregnancy and lactation. J Clin Endocrinol Metab 2001;86:2354–9.
[16] Cooper DS, Goldminz D, Levin AD, et al. Agranulocytosis associated with antithyroid drugs. Ann Intern Med 1983;98:26–9.
[17] Van Dijke UP, Heydendael RJ, De Kleine MJ, et al. Methisazole, carbimazole and congenital skin defects. Ann Intern Med 1987;106:60–1.

[18] Mortimer RH, Cannell GR, Addison RS. Methisazole and propylthiouracil equally cover the perfused human term placental lobule. J Clin Endocrinol Metab 1997;82:3099–102.
[19] DiGianantonio E, Schaefer C, Mastrioacovo PP, et al. Adverse effects of premature methisazone exposure. Teratology 2001;64:262.
[20] Wing DA, Millar LK, Koonings PP, et al. A comparison of propylthiouracil versus methimazole in the treatment of hyperthyroidism in pregnancy. Am J Obstet Gynecol 1994;170:90–5.
[21] Laurberg P, Nygaard B, Glinoer D, et al. Guidelines for TSH-receptor antibody measurements in pregnancy: results of an evidence-based symposium organized by the European Thyroid Association. Eur J Endocrinol 1998;139:584–6.
[22] Matsuura N, Harada S, Ohyama Y, et al. The mechanisms of transient hypothyroidism in infants born to mothers with Graves' disease. Pediatr Res 1997;42:214–8.
[23] Gorman CA. Radio iodine and pregnancy. Thyroid 1999;9:721–6.
[24] Molitch ME. Endocrine emergencies in pregnancy. Baillieres Clin Endocrinol Metab 1992; 6:167–91.
[25] Montoro MN. Management of hypothyroidism during pregnancy. Clin Obstet Gynecol 1997; 40:65.
[26] Klein RZ, Haddow JE, Faix JD. Prevalence of thyroid deficiency in pregnant women. Clin Endocrinol 1991;35:41–8.
[27] Haddow JE, Palomaki GE, Allan WC, et al. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. N Engl J Med 1999;341:549–55.
[28] Alvarez-Marfany M, Roman SH, Drexler AJ, et al. Long term prospective study of postpartum thyroid dysfunction in women with insulin dependent diabetes mellitus. J Clin Endocrinol Metab 1994;79:10–6.
[29] Glinoer D. Pregnancy and iodine. Thyroid 2001;11:471–81.
[30] Weetman AP, McGregor AM. Autoimmune thyroid disease: further development is an understanding. Endocrinol Rev 1994;15:788–830.
[31] Werner S. Modification of the classification of eye changes of Graves' disease: recommendations of the Ad Hoc Committee of the American Thyroid Association. J Clin Endocrinol Metab 1977;44:203–4.
[32] Stagnaro-Green AS. Recognizing, understanding and treating postpartum thyroiditis. Endocrinol Metab Clinics NA 2000;29:417–30.
[33] Tachi J, Amino N, Tamaski M, et al. Long term follow-up and HLA association in patients with postpartum hypothyroidism. J Clin Endocrinol Metab 1988;66:480–4.
[34] Lucas A, Pizarro E, Granada ML, et al. Postpartum thyroiditis: epidemiology and clinical evolution in a non selected population. Thyroid 2000;10:71–7.
[35] Pop VSM, de Rooy HAM, Vader HL, et al. Postpartum thyroid dysfunction and depression in an unselected population. N Engl J Med 1991;324:1815–6.
[36] Harris B, Fong M, Johns S, et al. Transient postpartum thyroid dysfunction and postnatal depression. J Affect Disord 1989;17:243.
[37] Harris B, Othman S, Davies JA, et al. Association between postpartum thyroid dysfunction and thyroid antibodies and depression. BMJ 1992;305:152–6.
[38] Pop VSM, deVries E, van Baar AL, et al. Microsomal antibodies during gestation in relation to postpartum thyroid dysfunction and depression. Acta Endocrinol 1993;129:26–30.
[39] Janssen R, Dahlberg PA, Winsa B, et al. The postpartum period constitutes an Important risk for development of clinical Graves' disease in young women. Acta Endocrinol 1987;116:321–5.
[40] Davies TF. The thyroid immunology of the postpartum period. Thyroid 1999;9:675–84.
[41] Balan KK, Critchley M. Outcome of pregnancy following treatment of well differentiated thyroid cancer with 131 Iodine. Br J Obstet Gynec 1992;99:1021–4.
[42] AACE Thyroid Task Force. American Association of Clinical Endocrinologists. Medical guidelines for clinical practice for the evaluation and treatment of hyperthyroidism and hypothyroidism. Endocr Pract 2002;8:457–69.
[43] American College of Obstetricians and Gynecologists. Thyroid disease in pregnancy. Clinical management guidelines for obstetrician-gynecologists. ACOG Practice Bulletin No. 37. Obstet Gynecol 2002;100:387–96.

ELSEVIER
SAUNDERS

Obstet Gynecol Clin N Am
31 (2004) 907–933

OBSTETRICS AND
GYNECOLOGY
CLINICS
OF NORTH AMERICA

Diabetes mellitus in pregnancy

France Galerneau, MD[a], Silvio E. Inzucchi, MD[b,*]

[a]*Yale University School of Medicine, Department of Obstetrics and Gynecology, 333 Cedar Street, P.O. Box 208063, New Haven, CT 06520-8063, USA*
[b]*Yale University School of Medicine, Section of Endocrinology, 333 Cedar Street, New Haven, CT 06520-8020, USA*

Nearly 4% of pregnant women in the United States are diabetic. Of those, 88% have gestational diabetes and 12% have pre-existing diabetes [1]. Because the outcome of pregnancy in diabetic women and the long-term course of diabetes have improved dramatically over the past few decades, more women who have diabetes are choosing to have babies. Moreover, there has been a significant increase in the prevalence of type 2 and gestational diabetes in the American population, particularly in women of African, Hispanic, and Asian descent [2]. A large proportion of these women are within reproductive age. This article reviews normal and abnormal carbohydrate metabolism in pregnancy, with an emphasis on the challenges that are faced by those who care for the pregnant woman who has hyperglycemia. The growing problem of type 2 diabetes in pregnancy, the controversial use of oral antihyperglycemic agents for the treatment of gestational diabetes, and the long-term issue of diabetes prevention in those whose hyperglycemia resolves postpartum also are addressed.

Carbohydrate metabolism

Normal carbohydrate metabolism

The body's energy requirements depend on a continuous supply of glucose from the circulation. The regulation of blood glucose concentrations is of critical importance and is regulated by the hormone, insulin. After the ingestion of

* Corresponding author.
E-mail address: silvio.inzucchi@yale.edu (S.E. Inzucchi).

0889-8545/04/$ – see front matter
doi:10.1016/j.ogc.2004.09.002 obgyn.theclinics.com

carbohydrates, plasma glucose increases quickly; this change is detected by the pancreatic β-cells, which, in turn, produce insulin. After binding to its receptor, insulin promotes glucose uptake by skeletal muscle, stimulates glycogen synthesis and suppresses glucose production by the liver, and suppresses lipolysis by adipocytes. Several hours after a meal, as glucose concentrations return to baseline, insulin production is decreased concomitantly. The liver now converts from an organ of net glucose uptake to one of net glucose production and supports circulating glucose concentrations until the next meal. After prolonged fasting, insulin levels decline further and allow for muscle breakdown and lipolysis; these provide substrates for gluconeogenesis and ketone body production by the liver, which can be used as an alternative fuel. In the fed state, insulin serves as an anabolic and as an anticatabolic factor, thereby enhancing the supply of energy to cells. In contrast, during fasting, the relative absence of insulin allows for the maintenance of energy homeostasis, by way of endogenous glucose production and muscle and fat catabolism.

Carbohydrate metabolism during pregnancy

During pregnancy, several important changes in normal glucose metabolism are observed. The major change is the development of insulin resistance—chiefly manifested in skeletal muscle—where there is an approximate 50% reduction in insulin sensitivity by the third trimester [3]. This change is attributed to several humoral factors of maternal and placental sources (eg, human placental lactogen, placental growth hormone variant) [4]. A significant increase in the maternal circulating concentrations of cortisol, another counterregulatory hormone, also is observed [5]. Elevated concentrations of estrogen, progesterone, and prolactin also may affect insulin sensitivity during pregnancy. Other contributing factors include increased body weight and increased caloric intake. It was proposed that the insulin resistance of pregnancy serves to shunt nutrients preferentially to the growing fetus, while simultaneously allowing for the accumulation of calorie storage in maternal adipose tissue [6]. Because of insulin resistance, pregnancy is characterized by elevated circulating insulin concentrations as the maternal pancreas compensates for increased peripheral demands. Therefore, in the normal situation, maternal glucose levels during gestation remain normal or near normal [7]. If adequate compensation does not occur, gestational diabetes results (see later discussion).

In the fasted state, two other important changes occur in maternal intermediary metabolism—a general decrease in plasma glucose concentrations and increased fat catabolism. The former may result from less gluconeogenic precursors provided to the liver or further nutrient shunting to the fetoplacental unit [8]. This results in larger glucose fluctuations from the postprandial to the fasting state. The latter likely reflects the lipolytic effects of placental hormones [9] and results in increased free fatty acid levels, which serve as substrate to the liver for ketone body production.

In summary, in the pregnant woman, carbohydrate metabolism is something of a paradox. After meals, there is a tendency for increased glucose and insulin levels and fat storage is promoted. During fasting, however, glucose levels decrease and lipolysis is stimulated. These changes may have developed to ensure adequate nutrient supply to the mother and fetus.

The growth of the fetus during the 9 months of gestation is dependent on the transport of a large amount and assortment of nutrients from mother to fetus; this demand creates a significant metabolic stress for the mother. The placenta serves as the conduit for most of these important factors. Glucose molecules pass the placental circulation through the process of facilitated transport [10]. Fetal glucose concentrations are 20 mg/dL to 40 mg/dL less than the simultaneous maternal levels. Access of the hormones that control maternal glucose concentrations, including insulin, however, are blocked by the placenta. Instead, metabolism in the fetus is regulated by insulin that is produced by the fetal pancreas in response to glucose. This distinction between maternal glucose and maternal insulin supply is an important consideration in the setting of poorly-controlled diabetes. Here, increased maternal glucose concentrations have ready access to the fetal circulation and stimulate more fetal insulin secretion. Macrosomia is the result; it is analogous to the consequences of overeating in the postnatal state.

Diabetes overview

Definition

Diabetes mellitus is a chronic metabolic condition that is marked by increased circulating concentrations of glucose, which is associated with the development of long-term vascular complications. There are two predominant forms—type 1 and type 2.

Diagnostic criteria

In the nonpregnant individual, the diagnosis of diabetes can be made in several ways. In the fasting state, a plasma glucose of 126 mg/dL or more establishes the diagnosis. Alternatively, the diagnosis can be made when any ‘‘casual’’ (ie, without respect to meals) plasma glucose reaches or exceeds 200 mg/dL, as long as classic symptoms of diabetes (blurred vision, polyuria, polydipsia) also are present. If a 75-g oral glucose tolerance test (OGTT) is performed, a 2-hour glucose value of 200 mg/dL or more also would indicate the disease [11].

Two categories of milder abnormalities of glucose metabolism are also defined. ‘‘Impaired glucose tolerance’’ occurs when the glucose level reaches 140 mg/dL to 199 mg/dL at the 2-hour mark during an OGTT. ‘‘Impaired fasting glucose’’ is the term that is used for patients who have fasting plasma glucose levels that are between 100 mg/dL and 125 mg/dL. These individuals are at

higher risk for the future development of frank diabetes; therefore, these categories are grouped together as ''prediabetes.''

Classification

Type 1 diabetes mellitus

Type 1 diabetes mellitus (T1DM), formerly referred to as ''juvenile-onset diabetes mellitus'' or ''insulin-dependent diabetes mellitus,'' mainly afflicts lean children, teens, and young adults. It is characterized by absolute insulin deficiency that results from autoimmune destruction of pancreatic islet cells. Usually, anti–islet cell antibodies are detectable. Clinically, the onset usually is abrupt and severe, with marked hyperglycemia that develops over several days to weeks that is associated with weight loss, fatigue, polyuria, polydipsia, blurring of vision, and evidence of volume contraction. The hyperglycemic emergency, diabetic ketoacidosis (DKA), indicates absolute insulin deficiency and leads to profound hyperglycemia, dehydration, unrestrained lipolysis, and ketoacid production.

Type 2 diabetes mellitus

Type 2 diabetes mellitus (T2DM), previously referred to as ''adult-onset diabetes mellitus'' or ''noninsulin-dependent diabetes mellitus'' is a different disease and has a slower onset. It is responsible for 90% to 95% of diabetes worldwide. Patients who have T2DM usually are older than 40 years and overweight or obese. Recently, however, because of the increasing obesity rates in the young, it is not uncommon for teenagers or even older children to present with T2DM [12].

T2DM is a disease of dual defects—insulin resistance and relative insulin deficiency [13]. Typically, autoimmune markers of T1DM are absent. It develops from an initial period of insulin resistance and relatively preserved insulin secretion, as the pancreas attempts to maintain euglycemia. Pancreatic β-cell function ultimately falters and no longer is able to meet peripheral demands. Insulin levels decline and hyperglycemia ensues. Because insulin secretion persists to some extent in virtually all patients, ketoacidosis is rare. In patients who have T2DM, other clinical and biochemical features often are present, including central adiposity, hypertension, and dyslipidemia; these place patients at an increased risk of cardiovascular disease. This constellation of findings often is referred to as the ''metabolic syndrome'' or the ''insulin resistance syndrome'' [14]. In women of childbearing age, polycystic ovary syndrome is a common manifestation of insulin resistance.

Other forms

In addition to gestational diabetes, other forms include so-called ''secondary'' diabetes mellitus—when hyperglycemia results from a separate disease process. These include diseases of the endocrine pancreas (pancreatitis, cystic fibrosis, hemochromatosis), hormonal abnormalities (hyperthyroidism, Cushing's syndrome, acromegaly, pheochromocytoma), and several genetic syndromes (Klinefelter's, Prader-Willi). A fascinating group of hyperglycemic diseases—''maturity

onset diabetes of the young'' (''MODY'')—develops early in life with a dominant inheritance pattern. Although patients who have MODY may present with severe hyperglycemia, their diabetes usually is mild. Several subtypes of MODY have been described that are associated with specific genetic defects in enzymes or transcription factors that affect the pancreatic islet cell's ability to sense ambient glucose concentrations [15].

Complications

Acute

The major acute complications of diabetes and its management include hyperglycemic and hypoglycemic emergencies. The former are DKA in T1DM and hyperglycemic hyperosmolar syndrome in T2DM [16]. If untreated, these conditions can result in severe complications and require urgent medical attention, including aggressive rehydration, insulin administration, and cautious monitoring of metabolic and hemodynamic status. Initially, hypoglycemia (plasma glucose <50–60 mg/dL) leads to hyperadrenergic signs and symptoms—diaphoresis, pallor, tachycardia, and tremor. If untreated, neuroglycopenic manifestations may follow, including personality change, cognitive impairment, loss of consciousness, and seizures.

Chronic

The major chronic complications of diabetes are those related to vascular disease. These are distinguished by the size of vessel affected.

Microangiopathy. Diabetic microvascular disease predominately affects the kidneys, eyes, and nerves. Although still controversial, most of the evidence points to elevated glucose concentrations as the cause; microvascular injury occurs through a complex series of intracellular derangements that are the sequelae of oxidative stress. Duration of diabetes and inadequacy of glucose control are the best predictors of these complications.

Diabetes remains one of the most common causes of renal failure in the world. Diabetic nephropathy begins with a period of glomerular hyperfiltration and intraglomerular hypertension. After years, glomerular injury develops with the eventual loss of filtration rate. Frequently, coexisting hypertension hastens this decline. Eventually, azotemia may develop and dialytic therapy is required. Abnormal albumin excretion is the first sign of glomerular disease and may progress slowly from microalbuminuria (20–300 mg/d) to macroalbuminuria (>300 mg/d) to frank nephrotic syndrome. Aggressive blood pressure control, particularly with angiotensin-converting enzyme (ACE) inhibitors or angiotensin-II receptor blockers (ARBs), delays this progression.

The retinal microcirculation also is affected in diabetes. Background diabetic retinopathy does not result in visual impairment. It is marked by early fundoscopic changes that include lipid deposits (hard exudates), microaneurysms, and minor hemorrhages. This may progress to preproliferative retinopathy that is

characterized by areas of retinal infarction (“cotton-wool spots”). The ischemia provides an angiogenic stimulus that leads to the growth of new and more fragile blood vessels. As the disease progresses further, proliferative retinopathy develops; retinal and vitreous hemorrhage result directly from those abnormal vessels, and, eventually there is loss of vision. A separate, though related, form of diabetic retinopathy is macular edema, in which central vision may be affected severely. Regular and careful screening by eye care specialists is necessary to detect the early changes of diabetic retinopathy. When progressive disease is documented, laser photocoagulation can prevent visual loss.

Also troubling to many patients is diabetic neuropathy. Injury—which stems from ischemic and metabolic insult—occurs to sensory, motor, and autonomic nerves; therefore, this condition has various manifestations. Loss of sensation in a “stocking-glove” distribution that may be associated with paresthesias or painful dysesthesias is the most common presentation. Loss of sensation in the lower extremities also is a major factor in the development of foot ulcerations that can lead to limb loss. Less commonly, acute mononeuropathies may involve cranial or peripheral nerves, and sometimes, spinal nerve roots. Autonomic neuropathy can present as erectile dysfunction in men and orthostatic hypotension, gastroparesis, diabetic diarrhea, and atonic bladder. Other than improving glucose control, there is no direct treatment for diabetic neuropathy.

Macroangiopathy. Atherosclerosis is the major cause of death in patients who have diabetes, predominately as a result of coronary artery and cerebrovascular disease that leads to myocardial infarction, congestive heart failure, and stroke. Peripheral vascular disease with limb ischemia contributes to foot ulcerations, infections, and gangrene. Diabetic patients have a twofold to fourfold increased risk of dying from a macrovascular event [17]. In patients who have T2DM, the injurious effect of hyperglycemia on the vasculature is compounded by the associated features of the insulin resistance syndrome [18]. These include hypertension, dyslipidemia, hypercoagulability, and vascular inflammation. The proper treatment of any patient who has diabetes must include comprehensive multi-factorial risk reduction strategies, including aggressive control of blood pressure and lipids and prophylactic antiplatelet therapy [19].

Other morbidities

Chronic hyperglycemia also delays wound healing and impairs normal function of the immune system. As a result, patients who have diabetes are prone to infections and complications that are related to surgical and nonsurgical wounds.

Therapy

For patients who have T1DM, the only treatment is insulin replacement that is administered by subcutaneous injection. Many insulin formulations are available, including the rapid-acting insulin analogs that have an onset that is measured in minutes and once daily long-acting and peakless products. Table 1 shows a

Table 1
Human insulins and their pharmacokinetics

Insulin Type	Onset	Peak	Duration
Rapid-acting			
Insulin lispro	10–15 min	1–2 h	3–5 h
Insulin aspart	10–15 min	1–2 h	3–5 h
Short-acting			
Regular insulin	0.5–1 h	2–4 h	4–8 h
Intermediate-acting			
NPH	1–3 h	4–10 h	10–18 h
Lente	2–4 h	4–12 h	12–20 h
Long-acting			
Ultralente	6–8 h	–	16–24 h
Insulin glargine[a]	2–3 h	–	24 h

[a] Not approved for use during pregnancy.

complete list of insulins and their pharmacokinetic profiles. Although most patients who have T1DM can prevent ketoacidosis with one injection of intermediate or long-acting insulin per day, dosing at least twice daily is required for any reasonable degree of glucose control. Preferably, more intensive regimens of three to four daily injections that involve combinations of long- and short-acting insulins are recommended [20]. Patients do even better with an insulin pump, which provides a continuous subcutaneous infusion of insulin, with calibrated basal rates and measured "boluses" before meals. Any comprehensive management program also must include adequate self-management education, a well-proportioned meal plan, and regular physical exercise.

Typically, patients who have T2DM are treated initially with a calorie-restricted diet, weight loss (if necessary), and exercise. Most patients are not able to achieve adequate glucose control with these interventions alone and pharmacotherapy is required [21]. A variety of oral agents is available; each targets a distinct aspect of the pathophysiology of this disease [22]. A complete list of agents and their mechanisms of action is shown in Table 2; however, none of these is approved for use during pregnancy. Therapy is started with a single agent; if control is not achieved, drugs from different classes are added in combination. Among the more common combinations include metformin with a secretagogue or metformin plus a thiazolidinedione. T2DM is a progressive disease that is marked by continued β-cell dysfunction [23]. Ultimately, a significant proportion of patients develop enough insulin deficiency to require exogenous insulin injections [24]. Frequently, such patients may achieve good control with less intensive insulin regimens than in T1DM.

Glucose control

It is now well-accepted that glycemic control that approaches the normal range is desirable for all patients who have diabetes. This has been demonstrated in T1DM and T2DM by prospective, randomized clinical trials [25–27]. Microvascular complications are reduced and there is a trend toward improved macro-

Table 2
Oral pharmacological agents for type 2 diabetes

Agent	Efficacy (Δ HbA1c)	Mechanism of antihyperglycemic action
Sulfonylureas Glyburide Glipizide Glimepiride	−1 to 2%	Binds to sulfonylurea receptor on pancreatic β-cells, stimulating insulin release
Meglitinides Repaglinide Nateglinide	−1 to 2% (Nateglinide may be less potent)	Binds to sulfonylurea receptor on pancreatic β-cells, stimulating insulin release (more rapid onset and disappearance of action that sulfonylureas)
Biguanides Metformin	−1 to 2%	Decreases hepatic glucose production
α-Glucosidase inhibitors Acarbose Miglitol	−0.5 to 1%	Slows intestinal carbohydrate absorption
Thiazolidinediones Rosiglitazone Pioglitazone	−1 to 2%	Increases glucose uptake by skeletal muscle and fat cells (reduces insulin resistance)

vascular outcomes. In light of these results, professional organizations, including the American Diabetes Association (ADA), promote strict control as the core of any treatment program. The most reliable assessment of overall glycemic status is the periodic measurement of the hemoglobin A1c (HbA1c). With this single test, the practitioner can determine the average degree of glycemia over the previous 2 to 3 months. Frequent home capillary glucose monitoring is a helpful and a more immediate adjunct to the HbA1c measurement. It also provides information to the patient on intensive regimens that can be used to determine insulin dosing. Current ADA guidelines are to maintain HbA1c at less than 7% (normal range 4%–6%) [28]. Plasma glucose should be kept between 90 mg/dL and 120 mg/dL before meals and less than 180 mg/dL 2 hours after meals. During pregnancy, stricter targets are advised (see later discussion).

Pre-existing diabetes in pregnancy (pregestational diabetes)

Over the past 4 decades, the importance of maternal glucose control before and during pregnancy has become clear. Generally, glycemic control during the preconception period and in the early phases of gestation determine the risk of fetal congenital malformations. In contrast, maternal outcomes and the risk of fetal macrosomia (and related obstetric sequelae) are predicted best by glucose control during the second and third trimesters. With better care, perinatal mortality has improved greatly during this time as the intensive approach toward the diabetic pregnancy has become routine [29]. Until recently, most pregnant women who had pre-existing diabetes had T1DM. Because of two societal

Table 3
Original White classification of diabetes in pregnancy

Class	Characteristics
A	Abnormal glucose tolerance test (asymptomatic; normal glucose levels achieved with diet alone)
B	Adult-onset diabetes (≥ age 20) and limited disease duration (<10 years)
C	Youth-onset diabetes (age 1–19) or relatively long disease duration (10–19 years)
D	Childhood onset (< age 10), very long disease duration (≥ 20 years), or evidence of background retinopathy
E	Any diabetes with evidence of vascular disease observed in the pelvis by plain radiograph
F	Any diabetes with the presence of renal disease
R	Any diabetes with the presence of proliferative retinopathy
RF	Any diabetes with both renal disease and proliferative retinopathy
G	Any diabetes with a previous history of multiple failures during pregnancy
H	Any diabetes with heart disease
T	Any diabetes following renal transplantation

Adapted from Hare JW. Gestational diabetes and the White classification. Diabetes Care 1980;3:394; with permission.

phenomena—increasing maternal age at first pregnancy and increasing rates of obesity (with resultant insulin resistance)—pre-existing T2DM is becoming a more frequent phenomenon [30].

Classification

The use of the traditional classification schema for diabetic pregnancy by White [31] (Table 3) is no longer widespread. Classifications that are similar to that in Box 1 have become more popular and puts the pregnancy in the context of the specific diabetes type and its vascular complications [32].

Impact on the mother

When advanced microvascular complications are present in a woman who has pregestational diabetes, pregnancy may exacerbate the condition.

Retinopathy

Generally, background retinopathy does not progress to proliferative retinopathy during pregnancy [33]. In contrast, however, established proliferative retinopathy may advance during gestation, particularly if severe, as demonstrated in the Diabetes in Early Pregnancy Study. In this observational investigation, more than 50% of women who had moderate to severe proliferative changes during the preconception period progressed during pregnancy [34]. Predictors of

Box 1. Classification of diabetes in pregnancy

Pregestational diabetes

1. Type 1 diabetes
   a. Uncomplicated (no hypertension, retinopathy, nephropathy, neuropathy, or cardiovascular disease)
   b. Complicated (presence of at least one of the above)
2. Type 2 diabetes
   a. Uncomplicated (no hypertension, retinopathy, nephropathy, neuropathy, or cardiovascular disease)
   b. Complicated (presence of at least one of the above)

Gestational diabetes mellitus

A-1. Fasting plasma glucose less than 105 mg/dL
A-2. Fasting plasma glucose greater than 105 mg/dL

retinopathy deterioration include the quality of glycemic control as well as the coexistence of hypertension. It was recommend that laser photocoagulation therapy be administered to women who have proliferative retinal changes before conception. Evaluation and follow-up by an ophthalmologist is recommended in pregnant women who have had diabetes for at least 5 years. In women who have established proliferative retinopathy, evaluation by an ophthalmologist—at least each trimester—is mandatory.

Nephropathy

Similarly, early diabetic nephropathy tends not to progress during pregnancy, whereas more advanced stages may deteriorate, especially when hypertension coexists [35]. An uncomplicated pregnancy in a diabetic woman who has normal renal function does not increase her risk of subsequent nephropathy. In a woman who has mild pre-existing nephropathy, the expected progression of her renal function should not be altered [36]; however, the glomerular filtration rate may decline more rapidly in those who have established chronic renal failure or advanced proteinuria [37]. Therefore, close follow-up of blood pressure, serum creatinine, and urine albumin excretion rate are important during the diabetic pregnancy. Also, the frequently used ACE inhibitors and ARBs should be discontinued after pregnancy is confirmed because of possible teratogenic effects [38].

Neuropathy

There is little evidence that neuropathy worsens during pregnancy, although women who have pre-existing carpal tunnel syndrome are at risk for worsening

symptoms during gestation. In women who have advanced autonomic neuropathy and severe gastroparesis, pregnancy and its accompanying gastrointestinal manifestations most assuredly will worsen during pregnancy and may pose management challenges. In general, pregnancy is poorly tolerated in women who have severe autonomic dysfunction.

Cardiovascular disease

Active coronary artery disease is a strong contraindication to pregnancy in women who have diabetes. Any woman who has pre-existing macrovascular disease should undergo a comprehensive evaluation by a cardiologist, including exercise tolerance testing with perfusion imaging or echocardiography before conception. Revascularization should be considered if significant myocardial ischemia is detected.

Obstetric complications

Diabetic pregnancies are at increased risk for obstetric and medical complications, such as hypertension, preterm labor, urinary tract and other infections, periodontal disease, cesarean section, and obstetric trauma.

The prevalence of preeclampsia is reported 10% to 20% compared with 5% to 8% in nondiabetics [39,40]. The rate of preeclampsia increases with the severity of the diabetes (White class B, 11%; class C, 22%; class D, 21%; class R or higher, 36%) [40], and with the presence of proteinuria at the onset of pregnancy [40,41]. Even in diabetics who do not have pre-existing hypertension or renal disease, the rate of preeclampsia is increased at 8% to 9% [39,42].

Iatrogenic and spontaneous preterm delivery is increased in diabetic pregnancies (22% versus 3% and 16% versus 11%, respectively) [43]. The frequency of preterm delivery before 35 weeks' gestation also increases with the severity of the diabetes and with the presence of proteinuria at the onset of the pregnancy [40]. One third of all preterm deliveries in diabetic women are the result of hypertensive complications [44]. The reasons for the increased rate of spontaneous premature labor are unclear but may be related to poor glycemic control, polyhydramnios, or infection. One study found a 37% increased risk for preterm delivery with each 1% increase in HbA1C before delivery [45].

Impact on the pregnancy and the neonate

The abnormal metabolic environment that is created by hyperglycemia has a significant impact on the pregnancy and the fetus. Increased rates of spontaneous miscarriages are reported in women who have pre-existing diabetes (eg, Relative Risk (RR) = 3 with HbA1 >14.4%) [46]. Numerous studies have linked the increased rates of miscarriages and fetal anomalies to poor glycemic control. HbA1c values that are greater than 8% are particularly concerning with a risk for malformations that is three to six times greater than when the HbA1c is maintained at less than this cut-off point [47]. Overall, the risk for major malformations is up

to eight times greater than in nondiabetics [48]. In particular, the relative risk for central nervous system and cardiovascular anomalies is as high as 15.5 and 18, respectively [48]. Caudal regression syndrome, although extremely rare, is seen almost exclusively in diabetic pregnancies [49]. Fetal malformations are responsible for about 50% of the perinatal deaths in this population.

Although the perinatal mortality rate has declined dramatically over the past 3 decades (from 250 per 1000 to 20 per 1000), the fetus of a diabetic mother remains at increased risk for perinatal asphyxia [50]. One study of 162 infants of diabetic mothers reported a 27% prevalence rate for perinatal asphyxia as described by fetal distress in labor (repetitive late decelerations or persistent bradycardia), 1-minute Apgar of 6 or less, or intrauterine fetal death [51]. Perinatal asphyxia correlated with new-onset nephropathy during the pregnancy, maternal hyperglycemia before delivery, and prematurity.

Fetuses who are exposed to chronic maternal hyperglycemia respond by increasing their pancreatic production of insulin. This results in increased fetal growth, and hence, a rate of macrosomia (birth weight >4 kg) that is as high as 45% compared with 8% to 9% in controls [52]. A tighter relationship between maternal postprandial glucose and fetal macrosomia has been observed, as compared with fasting glucose [53]. Frequently, the pattern of macrosomia in diabetics is asymmetric with an increased ponderal index. Babies who have this disproportionate growth pattern are at increased risk for hypoglycemia, hyperbilirubinemia, and acidosis [52]. Fetal macrosomia is related directly to an increased risk of shoulder dystocia [54]. The highest risk for shoulder dystocia is seen in macrosomic infants of diabetic mothers who are delivered by vacuum or forceps [54]. Shoulder dystocia increases the risk for perinatal asphyxia and birth trauma, particularly Erb's palsy [54]. Ultrasound has not proven to be superior to clinical examination in predicting macrosomia [55]; however, ultrasound measurements may allow the clinician to identify an asymmetric pattern of growth with a decreased head circumference over abdominal circumference ratio. Diabetic vasculopathy and excessively tight control have been linked to intrauterine growth restriction (IUGR) [52].

Polyhydramnios is seen often in diabetic pregnancies. The exact mechanism by which the amniotic fluid level increases is not well-understood. Fetal polyuria that results from maternal and fetal hyperglycemia has been suggested as a possible explanation. Unlike polyhydramnios that results from malformations or twin-to-twin transfusion syndrome, hydramnios that is associated with diabetes does not seem to increase the risk for adverse outcomes.

Babies of diabetic mothers are at increased risk of neonatal metabolic complications, such as hypoglycemia, hyperbilirubinemia, hypocalcemia, and hypomagnesemia. Hypoglycemia (plasma glucose <30–40 mg/dL) results from the persistence of hyperinsulinemia in the neonatal period, after the maternal supply of glucose by way of the placental circulation is no longer available. Typically, this occurs within a few hours of birth. Macrosomic fetuses are at increased risk. Growth restricted and premature fetuses also are predisposed because their glycogen reserves are reduced [56]. Neonatal hypoglycemia is more

frequent in babies of mothers who have more advanced diabetes (class C and above) [57]. One study showed a correlation between neonatal hypoglycemia and maternal hyperglycemia in labor, but not with HbA1c during pregnancy [58]. Hypoglycemia can be treated with enteral feedings alone in 50% of cases and is less frequent among breastfed infants [57].

Neonatal hypocalcemia and hypomagnesemia often are associated, usually are asymptomatic, and frequently resolve without treatment [59]. Hyperbilirubinemia is associated with poor maternal glycemic control, fetal macrosomia, polycythemia (increased hemolysis), and prematurity [60].

Respiratory distress syndrome (RDS) is more common in babies of diabetic mothers, compared with normal infants at the same gestational age before 38.5 weeks [61]. The postulated mechanism is delayed biochemical (surfactant) maturation as a result of the antagonism of insulin on glucocorticoids [62,63]. Insulin inhibits the normal stimulatory effect of cortisol on lecithin synthesis [53]. Other possible causes of respiratory distress include pneumonia, hypertrophic cardiomyopathy, and transient tachypnea of the newborn. The use of fetal lung maturity tests has reduced the risk of neonatal death from RDS. The presence of phosphatidylglycerol in the amniotic fluid is the best predictor of lung maturity in the diabetic pregnancy [64]. A lecithin:sphingomyelin ratio that is greater than 2.0 is usually predictive of fetal lung maturity, although some centers use a level that is greater than 3.5 in diabetic pregnancies [64,65].

Neurodevelopmental outcome of babies of diabetic mothers can be expected to be normal with good glycemic control. There is a negative correlation between smaller head circumference at age 3 and intellectual development and HbA1c levels during pregnancy [66]. Children of diabetic mothers who had early IUGR are at increased risk of abnormal psychomotor development at age 4 [67].

Children of diabetic parents are at increased risk of developing the same type of diabetes (5%–6% for T1DM and 10%–15% for T2DM) [68]; however, the risk for developing T1DM is higher if the father is the parent who has T1DM (6% versus 1% if the mother is the parent who has T1DM) [69].

Type 2 diabetes in pregnancy

The prevalence of T2DM has increased, particularly among women of reproductive age who are of African, Asian, or Hispanic background [70,71]; more pregnancies may now be complicated by T2DM than T1DM [70]. Despite this, the literature on pregnancy and T2DM is still limited. Such pregnancies seem to be at least at the same risk as those of women who have T1DM [70–73]. Women who have T2DM tend to be older, more obese, and have higher rates of unplanned pregnancies in comparison with those who have T1DM [71]. Studies showed increased rates of obstetric complications (miscarriages, gestational hypertension, preterm delivery, cesarean section), fetopathy (congenital anomalies, macrosomia, polyhydramnios, stillbirth), and neonatal mortality and morbidity (metabolic complications, RDS, obstetric trauma, perinatal asphyxia)

Table 4
Complications of pregnancy by type of diabetes

	Normal	GDM	Type 1	Type 2
Unplanned pregnancies	–	–	10	55
Miscarriages	5–15	–	9–17	8–15
HbA1c >8% in 1st trimester	–	–	26.4	46.6
Congenital anomalies	1–2.2	1–2.2	10	10–15
Preeclampsia	4	–	10–20	8–20
Pre-term delivery	4–11	4–11	22–25	26–46
Infections (at least one episode during pregnancy)	25	–	–	80
Cesarean section	15–25	–	32–45	40–50
Postpartum hemorrhage	6	–	–	34
Macrosomia (>4 kg)	8–10	17–29	9–28	9–14
Polyhydramnios	1–5	–	–	9
Stillbirth	0.5	0.5	2.5	1–1.5
Perinatal mortality	0.7	0.7	3	5
Admission to NICU	–	29	–	37–40
Neonatal hypoglycemia	–	1	5–25	51
RDS (requiring support)	–	–	2–6	40

Blanks left where references used did not provide information or where not applicable. The wide range in some sections reflects the different populations studied in the different sources. All numbers are expressed in %.
Abbreviations: GDM, gestational diabetes mellitus; NICU, neonatal intensive care unit.
Data from Refs. [70–74].

at rates that are similar to those of women who have T1DM (Table 4) [70–74]. Therefore, metabolic goals for the management of T2DM in pregnancy should be the same as for T1DM (see later discussion).

Management

Prepregnancy counseling

Prepregnancy counseling should be provided to all diabetic women of reproductive age. Because there is a direct relationship between preconception glycemic control and the development of congenital malformations and miscarriages, the importance of planning pregnancies should be emphasized repeatedly. A Cochrane review of one case-matched controlled study and six observational studies found that preconception care of diabetic women is associated with improved glycemic control in early pregnancy [75]. Preconception care also resulted in less maternal hospitalizations, less use of neonatal intensive care, a reduction in major congenital anomalies (1.2%–5% versus 10.9%–14%), and a reduction in fetal and neonatal deaths (6.5% versus 21.1%) [75]. The same results were found in a meta-analysis that examined the importance of preconceptual glycemic control [76]. The goal should be to bring HbA1c as close to the normal range as possible [77], certainly within 1% of the

upper normal range. Successful preconception programs used a premeal capillary plasma glucose target of 80 mg/dL to 110 mg/dL and 2-hour postprandial target of less than 155 mg/dL [78]. Hypoglycemia has not been linked with embryopathy, although profoundly low blood glucose levels place the mother at increased risk of injury. Also, tight glycemic control that is achieved rapidly was linked with a worsening of pre-existing retinopathy. Therefore, care should be taken to improve glycemic control progressively and with close monitoring of any retinal disease [34,79,80]. Patients should be instructed to use a reliable method of contraception until they have achieved the desired goal of glycemic control. Glycemic control in the first trimester was shown to lower the incidence of macrosomia in some studies.

Folic acid supplement in early pregnancy reduces the risk for neural tube defects and other congenital anomalies [81]. Because diabetics are in a high-risk category for these malformations, they should be placed on a high daily dosage (4–5 mg) during the preconception period and throughout the first trimester [82].

A thorough assessment of the patient should be done before conception, including an ophthalmologic examination, renal function tests, EKG, and thyroid function tests. Patients should be counseled about the potential risks in pregnancy based on their specific condition. Antihypertensive medications should be stopped if the patient's blood pressure is less than 130/80 mm Hg and restarted as needed with the goal of maintaining blood pressure at less than 140/90 mm Hg. Drugs that modulate the renin-angiotensin-aldosterone axis are contraindicated in pregnancy and should be discontinued before conception.

Glycemic control during gestation

The progressive insulin resistance of pregnancy leads to increased insulin requirements, which can be more than double the preconception dose. Most of the increase occurs in the second and third trimesters, when placental hormones are circulating in increased concentrations. In addition, the propensity for blood glucose excursions to be accentuated after meals and for relative hypoglycemia in the fasting state can present additional management challenges. There also is a tendency for increased ketosis during pregnancy. Careful monitoring of blood glucose levels and clinical status is of utmost importance during the diabetic pregnancy.

The metabolic goal during pregnancy is to maintain blood glucose as close to the normal range as possible, while avoiding severe hypoglycemia. This approach improves clinical outcomes for mother and fetus [83]. Most authorities recommend that fasting plasma glucose levels be maintained at 70 mg/dL to 105 mg/dL (whole blood, 60–95 mg/dL) before meals and no higher than 130 mg/dL (whole blood, <120 mg/dL) at 2 hours postprandial [83,84]. The HbA1c concentration should be measured every 4 to 6 weeks, with the goal being a normal value (<6%). These targets are substantially tighter than what would be considered optimal control for nonpregnant individuals. To achieve these targets, office/clinic visits must occur on a regular basis and telephone or

e-mail contact should be frequent—sometimes daily. Occasionally, a poorly controlled patient will benefit from hospitalization to ensure rapid attainment of the euglycemic state. A multi-disciplinary team that includes the obstetrician, internist or endocrinologist, and diabetes educator works best. Alternatively, if available, specialized care at high-risk centers with interest and expertise in diabetes during pregnancy should be considered [85]. Self-management training is a key component of diabetes management at any stage, with particular attention to diet, exercise, and frequent (four to six times per day) blood glucose monitoring.

Adequate understanding of the tenets of dietary therapy by the patient is imperative, especially the relationship between certain types of carbohydrates and glucose excursions [86]. Foods of lower glycemic index, for example, seem to be suited best for women who have diabetes during pregnancy. A formal nutritional consultation should be arranged. In general, the diet should consist of 30 kcal/kg/d to 35 kcal/kg/d—in the form of three main meals and one to two snacks—that consist of 50% to 60% carbohydrates, less than 30% fat, and adequate amounts of dietary fiber. Further restriction of carbohydrates to 40% of total calories may assist in the management of uncontrolled postprandial glucose readings. Regular physical exercise, as tolerated, also should be encouraged.

There are numerous insulin options for women who have diabetes during pregnancy. If the preconception regimen is working well in those who have pregestational T1DM, this should be continued. Commonly, however, the patient requires intensification of her regimen to achieve the stricter targets. The actual type of insulin that is used in an individual patient is not as important as the quality of glycemic control that is achieved. At times, creative regimens may need to be devised for individual patients, depending on their meal preferences and work schedules. The best treatment regimens typically combine ‘‘basal’’ insulin—in the form of intermediate or long-acting formulations (ie, NPH, Lente, Ultralente) that are administered once or twice daily—and mealtime short-acting insulin analogs (ie, insulin lispro, insulin aspart) [87,88]. The latter have pharmacokinetics that are ideally suited to address the postprandial excursions that characterize the diabetic pregnancy. These largely have replaced regular insulin, whose onset and duration of action may result in inadequate glucose control in the immediate postprandial time period and a greater risk of late postprandial hypoglycemia. Optimally, prandial insulins should be dosed while taking into account the anticipated carbohydrate content of the meal (‘‘carbohydrate counting’’), the preprandial blood glucose reading, and any anticipated activity level after the meal. In early pregnancy, 1 unit of insulin for every 15 g of carbohydrates consumed may suffice. As insulin resistance worsens during pregnancy, the carbohydrate: insulin ratio may decrease to 10:1 or less (ie, dosage increases). To compensate for preprandial hyperglycemia, an additional ‘‘correction bolus’’ of insulin (ie, 1–2 units for every 25–50 mg/dL more than 120 mg/dL) should be considered.

Alternatively, an insulin pump can be used, although this typically requires ongoing management by a diabetologist who is trained in this form of therapy [89].

All insulin types, except for insulin glargine [90], are approved for use during pregnancy. Glargine should be avoided because the safety of this basal insulin analog has not been confirmed in this setting [91]. In the near future, other insulin delivery systems will be available, including inhaled insulin which uses the vast pulmonary alveolar surface area for hormone absorption [92]. The precise role of these devices for diabetes management, especially for pregnant women, remains unclear. Not surprisingly, they are perceived by many patients to be preferable to injections.

In the woman who has pregestational T2DM, it is recommended that any oral agent regimen be converted to insulin during the preconception phase. There has been increasing interest in the use of sulfonylureas, and even metformin, during gestation (see later discussion), but these drugs are not approved for use in pregnancy. As in the nonpregnant patient, gravidas who have T2DM may be able to achieve good control with less intensive insulin programs, although an individualized approach is necessary.

Through frequent contact with the patient and careful analysis of home glucose monitoring logs, insulin dosages should be adjusted aggressively to achieve targets. For instance, elevated fasting blood glucoses should lead to an increase of approximately 10% in the dosage of the long- or intermediate-acting insulin that is injected at night. Suboptimal control before dinner is addressed by increasing the long- or intermediate-acting insulin dosage that is given in the morning. If postprandial hyperglycemia is noted, the preprandial rapid-acting insulin dosage should be adjusted similarly. In general, insulin dosage changes should not be made more frequently than every 2 to 3 days and should be influenced by glycemic trends, not individual glucose readings.

Because of enhanced lipolysis during pregnancy, in T1DM, maternal ketoacidosis may occur more frequently and at decreased plasma glucose concentrations. There are known deleterious fetal effects of ketoacidosis; this hyperglycemic decompensation should be avoided at all costs. In addition, frequent and severe episodes of hypoglycemia also should be avoided [93], although there is no proof that mild maternal hypoglycemia is dangerous for the fetus [94].

Fetal assessment

Screening for fetal anomalies should be done with first and second trimester ultrasound and a fetal echocardiogram between 20 and 22 weeks' gestation. As for all other pregnant woman, aneuploidy screening with first or second trimester maternal serum markers should be offered. The maternal serum α-fetoprotein (MSAFP), unconjugated estriol, and inhibin-A are reduced in diabetic pregnancies, a pattern that otherwise is suggestive of Down's syndrome. Therefore, laboratories must adjust the multiples of the median values for these markers in diabetic pregnant women. If the patient declines aneuploidy screening, MSAFP measurement between 15 and 20 weeks' gestation should be offered as an adjunct to ultrasound in screening for neural tube defects.

Usually, follow-up ultrasounds are performed at least every trimester to identify fetal macrosomia and polyhydramnios. Most, if not all, centers use pro-

tocols for routine fetal surveillance (nonstress test, biophysical profile, or Doppler) at or near term, as recommended by the American College of Obstetricians and Gynecologists (ACOG). There are no data from randomized trials to make specific evidence-based recommendations on the type of test or the initiation and frequency of testing. Therefore, fetal surveillance protocols are based upon institutional expert opinion.

Timing and mode of delivery

In the absence of complications, diabetic expectant mothers who have good glycemic control may be allowed to reach term. If elective delivery is considered before 38.5 weeks' gestation, amniocentesis should be done to confirm fetal lung maturity. Elective cesarean section for delivery of the macrosomic fetus should be considered. Its potential benefits in decreasing the risk for shoulder dystocia and birth trauma must be weighed against its potential risks to the mother. The ACOG considers an elective cesarean delivery to be an acceptable means to prevent brachial plexus injury for diabetic pregnancies with an estimated fetal weight of more than 4500 g.

Glycemic management during labor

Typically, insulin requirements decrease during labor, partly as a result of energy use and partly as a result of the mother's fasting status. Nonetheless, it is advisable to give all women who have insulin-treated pregestational diabetes an insulin infusion during labor and delivery, especially those who have T1DM. This allows for the tight regulation of plasma glucose within the physiologic range of 70 mg/dL to 100 mg/dL. An initial dosage of 1 to 2 units per hour will suffice in most women. A more individualized regimen could be estimated from the woman's total daily insulin intake. Using this method, 50% of the total daily dosage is divided by 24 hours to calculate an initial hourly rate. Concurrently, a dextrose infusion (D5W at 75–125 mL/h) should be provided to prevent catabolism. Blood glucose must be checked hourly during intravenous insulin infusions.

Postpartum care and lactation

The insulin resistance of pregnancy resolves within hours after delivery. As a result, the insulin requirements of a woman who has pregestational diabetes may decrease dramatically. Insulin dosing during the first 24 to 48 hours postpartum will need to be reassessed carefully to avoid hypoglycemia. Patients who have T1DM will remain insulin dependent. A resumption of the prepregnancy regimen may suffice; however, if the insulin strategy has been intensified successfully during gestation, it may be in the patient's best long-term interest to maintain this greater degree of regimentation. Admittedly, however, the patient's motivation for compulsive glucose control after childbirth may not be as high as during pregnancy. Those who have T2DM may be able to be switched

back to oral agents soon, although insulin should be continued if breastfeeding is planned.

Breastfeeding can be associated with more difficulty in maintaining good glucose control, with a tendency for hypoglycemia [95]. As a result, frequent monitoring and cautious follow-up is necessary during this time period.

The prevalence of postpartum thyroiditis is high (up to 25%) in women who have T1DM [96]. Therefore, signs and symptoms of thyrotoxicosis or hypothyroidism should be evaluated promptly by measuring a thyroid hormone panel. Clearly, given the physical and sometimes emotional upheaval during this period, subtle signs of thyroid dysfunction may be overlooked.

Contraception

All contraceptive methods, including oral contraceptives (OCs), are available to diabetic women. The ACOG recommends limiting the use of combined OCs in diabetic patients who do not smoke, are less than 35 years old, and who do not have hypertension or vasculopathy. For these women, a progestin-only pill may be an acceptable alternative.

Gestational diabetes mellitus

Gestational diabetes mellitus (GDM) indicates carbohydrate intolerance that is diagnosed during pregnancy [97]. It occurs when the pregnant woman's pancreas is unable to respond to the insulin resistance that is created by the normal hormonal changes of pregnancy. Depending on the population and diagnostic tests that are used, the prevalence of gestational diabetes ranges from 1% to 14% [98]. Overall, about 7% of all pregnancies are complicated by GDM. A proportion of these women likely have overt diabetes rather than purely GDM. One study of 1190 diabetic women found that when diabetes was diagnosed before 24 weeks' gestation, pregnancy outcomes were similar to those of White class B and higher [99]. Other studies corroborated these findings [100]. Women who have GDM have a 50% likelihood of developing overt diabetes within a decade after delivery [101]. Fasting hyperglycemia during pregnancy and the need for insulin therapy, particularly before 24 weeks' gestation, are associated strongly with postpartum persistence of diabetes [102,103]. Women who have other cardiovascular risk factors (eg, obesity, elevated triglycerides, hypertension) are at greater risk for persistence of diabetes postpartum [104].

There are no universally agreed upon criteria for the diagnosis of GDM. In Europe, the 75-g 2-hour OGTT that is recommended by the World Health Organization is used. In North America, the 100-g 3 hour OGTT that is performed after an overnight fast is the standard [105].

GDM has been linked with an increased risk for fetal macrosomia and its related maternal and perinatal complications. Treatment produces a small reduction in fetal size. No well-designed studies have demonstrated any additional benefit in treating GDM [106]. Children of women who have GDM

are at increased risk for obesity, glucose intolerance, and diabetes as adults [98]. This suggests a fundamental "reprogramming" effect of the glucose-insulin milieu during fetal life on future adipocyte biology, insulin sensitivity, or pancreatic endocrine function.

Another controversy is whether screening for GDM should be universal or reserved for women who have risk factors; at present, there is a lack of evidence to support universal screening. In populations who are at risk, however, universal screening may be more practical. Women who are at low risk for GDM are white, younger than 25 years of age, have a normal prepregnancy weight, and do not have a history of diabetes in first degree relatives, abnormal glucose tolerance, or poor obstetric outcome. One study that compared the institutional effects of changing screening from selective to universal demonstrated that although the number of women tested was doubled, the number of women who was diagnosed with GDM remained the same [107]. Moreover, universal screening did not change perinatal outcomes.

At present, the ADA makes the following recommendations for the diagnosis of diabetes in pregnancy [98]:

(1) Established diagnosis of diabetes: no need for glucose challenge
(2) Screening between 24 and 28 weeks' gestation for all women who are at risk with 3-hour 100-g OGTT or 1-hour 50-g oral glucose challenge test (GCT), followed by 3-hour OGTT if:
    • GCT is > 140 mg/dL (for 80% detection)
    • GCT is > 130 mg/dL (for 90% detection)
(3) Diagnostic criteria for gestational diabetes (by O'Sullivan and Mahan, as modified by Carpenter and Coustan) [108]: at least two abnormal values (venous plasma) on 3-hour OGTT with 100-g glucose load:
    • Fasting: 95 mg/dL or 5.3 mmol/L
    • 1 hour 180 mg/dL 10.0 mmol/L
    • 2 hour 155 mg/dL 8.6 mmol/L
    • 3 hour 140 mg/dL 7.8 mmol/L

Current management of gestational diabetes starts with nutritional counseling. Insulin is added when diet alone is insufficient to maintain glycemia at the following levels: fasting plasma glucose up to 105 mg/dL, or 1 hour after meals up to 155 mg/dL, or 2 hour after meals up to 130 mg/dL.

Traditionally, oral hypoglycemic agents have not been recommended during pregnancy, although they may be more acceptable to patients and result in better compliance. There have been several investigations into their use in GDM, with encouraging results. In one randomized trial that compared glyburide with conventional insulin therapy in 400 women who had GDM, there was no difference in glycemic control, the incidence of macrosomia, or the risk of fetal anomalies or other neonatal complications [109]. Metformin also was studied in this setting and seems to be safe [110]; however, despite these reports, oral agents are not indicated for use during gestation.

Pregnancies that are complicated by GDM are followed closely with weekly fetal surveillance near term .The ACOG recommends fetal surveillance in pregnancies that have GDM if control is poor, insulin is required, or other complications develop [105]. Delivery before 40 weeks' gestation is not indicated if the diabetes is well-controlled. An elective cesarean section may be offered for the macrosomic fetus, for the same reason as in pregnancies in which there is pre-existing diabetes. In labor, the goal is to maintain normoglycemia. Usually, women who have GDM do not require insulin during labor. If needed, insulin may be administered, preferably as an intravenous infusion.

Postpartum care

It is recognized that a history of GDM is a major risk factor for the future development of T2DM. Several studies estimated this risk to be approximately 50% within 10 years of pregnancy [111]. The risk seems to be highest in the most overweight women and in those whose greater fasting glucose levels are within the normal range postpartum. As a result, it is now recommended that all women who have a history of GDM undergo formal re-evaluation with OGTT at 6 to 8 weeks postpartum or after breastfeeding has been discontinued. In addition, long-term monitoring for the development of diabetes is advisable—at least annually with fasting blood glucose and, perhaps, HbA1c determinations. An argument can be made for continuing to use the OGTT as a more sensitive screening tool; however, this more cumbersome technique is not widely recommended for routine use after the first postpartum evaluation.

Because of the frequent development of T2DM in women who had GDM, these patients are ideal candidates for diabetes prevention strategies. In patients who have prediabetes, it was demonstrated convincingly that lifestyle modification with diet, exercise, and weight loss dramatically reduced the risk of T2DM [112,113]. Metformin therapy also seems to mitigate this risk, although not as effectively as lifestyle change. The TRoglitazone In Prevention Of Diabetes (TRIPOD) study assessed the effectiveness of the original thiazolidinedione, troglitazone, in a group of predominately Hispanic woman who had a recent history of GDM [114]. The investigators proposed that by improving insulin sensitivity, β-cell function could be preserved and diabetes could be prevented. In TRIPOD, 266 women were randomized to placebo or troglitazone and were followed for approximately 3 years. Subjects were assessed by fasting glucose levels and OGTTs. The mean annual prevalence of diabetes was 12.1% and 5.4% in the groups who were given placebo or troglitazone ($P<.01$), respectively. This translated to an impressive 55% relative risk reduction with active therapy. Troglitazone is no longer on the market because of hepatotoxicity. Another thiazolidinedione, pioglitazone is being tested by the same group of investigators in an open label study design (PIoglitazone In Prevention Of Diabetes study) [115]. No pharmacologic agent is approved by the U.S. Food and Drug Administration for the prevention of diabetes in at-risk individuals.

Summary

Because of several societal trends, the presence of diabetes during pregnancy is an increasingly common phenomenon. Rigid glycemic control improves fetal and maternal outcomes and should be encouraged in all patients. Modern monitoring techniques and newer insulin types and delivery systems have made optimizing glucose control easier, which translates into a greater chance of a successful pregnancy in all women who have diabetes.

References

[1] Engelgau MM, Herman WH, Smith PJ, German RR, Aubert RE. The epidemiology of diabetes and pregnancy in the US. Diabetes Care 1995;18:1029–33.
[2] Mazze RS. Measuring and managing hyperglycemia in pregnancy: from glycosuria to continuous blood glucose monitoring. Sem Perinatol 2002;26(3):171–80.
[3] Buchanan T, Metzger B, Freinkel N, Bergman R. Insulin sensitivity and B-cell responsiveness to glucose during late pregnancy in lean and moderately obese women with normal glucose tolerance of mild gestational diabetes. Am J Obstet Gynecol 1990;162:1008–14.
[4] Ryan EA. Hormones and insulin resistance during pregnancy. Lancet 2003;362(9398):1777–8.
[5] Demey-Ponsart E, Foidart J, Sulon J, Sodoyez J. Serum CBG, free and total cortisol and circadian patterns of adrenal function in normal pregnancy. J Steroid Biochem 1982;16:165–9.
[6] Freinkel N. The Banting Lecture 1980. Of pregnancy and progency. Diabetes 1980;29: 1023–35.
[7] Lind T, Billewicz W, Brown G. A serial study of the changes occurring in the oral glucose tolerance test during pregnancy. J Obstet Gynecol Br Commonw 1973;80:1033–4.
[8] Chiasson J, el Achkar G, Ducros F, Bourque J, Maheux P. Glucose turnover and gluconeogenesis during pregnancy in women with and without insulin-dependent diabetes mellitus. Clin Invest Med 1997;20:140–51.
[9] Turtel J, Kipnis D. The lipolytic action of human placental lactogen in isolated fat cells. Biochim Biophys Acta 1967;144:583–8.
[10] Thorens B, Charron M, Lodish H. Molecular physiology of glucose transporters. Diabetes Care 1990;13:209–18.
[11] Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diab Care 2004; 27(Suppl 1):S5–20.
[12] Fagot-Campagna A, Pettitt DJ, Engelgau MM, et al. Type 2 diabetes among North American children and adolescents: an epidemiologic review and a public health perspective. J Pediatr 2000;136:664–72.
[13] Ferrannini E. Insulin resistance versus insulin deficiency in non-insulin-dependent diabetes mellitus: problems and prospects. Endocr Rev 1998;19:477–90.
[14] Reaven G. The metabolic syndrome or the insulin resistance syndrome? Different names, different concepts, and different goals. Endocrinol Metab Clin North Am 2004;33:283–303.
[15] Fajans SS, Bell GI, Polonsky KS. Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. N Engl J Med 2001;345:971–80.
[16] Kitabchi AE, Umpierrez GE, Murphy MB, et al. Management of hyperglycemic crises in patients with diabetes. Diabetes Care 2001;24:131–53.
[17] Inzucchi SE. Noninvasive assessment of the diabetic patient for coronary artery disease. Diabetes Care 2001;24:1519–21.
[18] Ginsberg HN. Insulin resistance and cardiovascular disease. J Clin Invest 2000;106:453–8.
[19] Gaede P, Vedel P, Larsen N, Jensen GV, Parving HH, Pedersen O. Multifactorial interven-

tion and cardiovascular disease in patients with type 2 diabetes. N Engl J Med 2003;348: 383–93.
[20] Atkinson MA, Eisenbarth GS. Type 1 diabetes: new perspectives on disease pathogenesis and treatment. Lancet 2001;358:221–9.
[21] Mayerson AB, Inzucchi SE. Type 2 diabetes therapy. A pathophysiologically based approach. Postgrad Med 2002;111(3):83–95.
[22] Inzucchi SE. Oral antihyperglycemic therapy for type 2 diabetes: scientific review. JAMA 2002;287:360–72.
[23] Kahn SE. Clinical review 135: the importance of beta-cell failure in the development and progression of type 2 diabetes. J Clin Endocrinol Metab 2001;86:4047–58.
[24] Turner RC, Cull CA, Frighi V, Holman RR. Glycemic control with diet, sulfonylurea, metformin, or insulin in patients with type 2 diabetes mellitus: progressive requirement for multiple therapies (UKPDS 49). JAMA 1999;281:2005–12.
[25] The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329:977–86.
[26] Prospective Diabetes Study UK. (UKPDS) Group. Intensive blood-glucose control with sulphoylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 1998;352:837–53.
[27] Prospective Diabetes Study UK. (UKPDS) Group. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). Lancet 1998;352:854–65.
[28] American Diabetes Association. Standards of medical care in diabetes. Diabetes Care 2004; 27(Suppl 1):S15–35.
[29] Gregory R, Scott A, Mohajer M, Tattersall R. Diabetic pregnancy 1977–1990: have we reached a plateau? J R Coll Physicians Lond 1992;26:162–6.
[30] Green A, Christian Hirsch N, Pramming SK. The changing world demography of type 2 diabetes. Diabet Metab Res Rev 2003;19:3–7.
[31] White P. Pregnancy complicating diabetes. Am J Med 1949;7(5):609–16.
[32] Metzger BE, Phelps RL, Dooley SL. The mother in pregnancies complicated by diabetes mellitus. In: Porte D, Sherwin RS, Baron A, editors. Ellenberg and Rifkin's diabetes mellitus. New York: McGraw-Hill; 2002. p. 626.
[33] Klein B, Moss S, Klein R. Effect of pregnancy on progression of diabetic retinopathy. Diabetes Care 1990;13:34.
[34] Chew E, Mills J, Metzger B, Remaley N, Jovanovic-Peterson L, Knopp R, et al. Metabolic control and progression of retinopathy. National Institute of Child Health and Human Development Diabetes in Early Pregnancy Study. Diabetes Care 1995;18(5):631–7.
[35] Rossing K, Jacobsen P, Hommel E, Mathiesen E, Svenningsen A, Rossing P, et al. Pregnancy and progression of diabetic nephropathy. Diabetologia 2002;45(1):36–41.
[36] Miodovnik M, Rosenn B, Khoury J, Grigsby J, Siddiqi T. Does pregnancy increase the risk for development and progression of diabetic nephropathy. Am J Obstet Gynecol 1996;174:1180–9 [discussion 1189–91].
[37] Biesenbach G, Stoger H, Zazgornik J. Influence of pregnancy on progression of diabetic nephropathy and subsequent requirement of renal replacement therapy in female type 1 diabetic patients with impaired renal function. Nephrol Dial Trans 1992;7:105–9.
[38] Rosenthal T, Oparil S. The effect of antihypertensive drugs on the fetus. J Hum Hyperten 2002; 16:293–8.
[39] Garner PR, D'Alton ME, Dudley DK, Huard P, Hardie M. Pre-eclampsia in diabetic pregnancies. Am J Obstet Gynecol 1990;163(2):505–8.
[40] Sibai BM, Caritis S, Hauth J, et al. Risks of pre-eclampsia and adverse neonatal outcomes among women with pre-gestational diabetes mellitus. National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units. Am J Obstet Gynecol 2000; 182(2):364–9.
[41] Ekbom P, Damm P, Feldt-Rasmussen B, Feldt-Rasmussen V, Molvig J, Mathieson ER.

Pregnancy outcome in type I diabetic women with micro albuminuria. Diabetes Care 2001; 24(10):1739–44.
[42] Acler PB, Barss VA. Obstetrical complications. In: Brown FM, Hare JW, editors. Diabetes complications in pregnancy. 2nd edition. New York: Wiley-Liss; 1995. p. 153.
[43] Sibai BM, Caritis SN, Hauth JC, et al. Preterm delivery in women with pre-gestational diabetes mellitus or chronic hypertension relative to women with uncomplicated pregnancies. The National Institute of Child Health and Human Development. Maternal-Fetal Medicine Units Network. Am J Obstet Gynecol 2000;183:1520–4.
[44] Greene MF, Hare JW. Prematurity among insulin-requiring diabetic gravid women. Am J Obstet Gynecol 1989;161(1):106–11.
[45] Kovilam O, Khoury J, Miodovnik M, Chames M, Spinnato J, Sibai B. Spontaneous preterm delivery in the type I diabetic pregnancy: the role of glycemic control. J Matern Fetal Neonatal Med 2002;11(4):245–8.
[46] Greene MF, Hare JW, Cloherty JP, Benacerraf BR, Soeldner JS. First trimester hemoglobin A1 and risk for major malformation and spontaneous abortion in diabetic pregnancy. Teratology 1989;39(3):225–31.
[47] Ylinen K, Aula P, Steinman UH, et al. Risk of minor and major fetal malformations in diabetics with high haemoglobin Alc values in early pregnancy. Br Med J (Clin Res Ed) 1984;289(6441): 345–6.
[48] Becerra JE, Khoury MJ, Cordero JF, Erickson JD. Diabetes mellitus during pregnancy and the risks for specific birth defects: a population-based case-control study. Pediatrics 1990; 85(1):1–9.
[49] Khoury MJ, Becerra JE, Cordero JF, Erickson JD. Clinical-epidemiologic assessment of pattern of birth defects associated with human teratogens: application to diabetic embryopathy. Pediatrics 1989;84(4):658–65.
[50] Weintrob N, Karp M, Hod M. Short and long-range complications in offspring of diabetic mothers. J Diab Complic 1996;10(5):294–301.
[51] Mimouni F, Miodovnik M, Siddiqi TA, Khoury J, Tsang RC. Perinatal asphyxia in infants of insulin-dependent diabetic mothers. J Pediatr 1988;113(2):345–53.
[52] Ballard JL, Rosenn B, Khoury JC, Miodovnik M. Diabetic fetal macrosomia: significance of disproportionate growth. J Pediatr 1993;122(1):115–9.
[53] Combs CA, Gunderson E, Kitzmiller JD, Gavin LA, Main EK. Relationship of fetal macrosomia to maternal postprandial glucose control during pregnancy. Diabetes Care 1992; 15(10):1251–7.
[54] Nesbitt TS, Gilbert WM, Herrchem B. Shoulder dystocia and associated risk factors with macrosomic infants born in California. Am J Obstet Gynecol 1998;179(2):476–80.
[55] Johnstone FD, Prescott RJ, Steel JM, Mao JH, Chambers S, Muir N. Clinical and ultrasound prediction of macrosomia in diabetic pregnancy. Br J Obstet Gynaecol 1996;103(8):747–54.
[56] Kalhan SC, Savin SM, Adam PA. Attenuated glucose production rate in newborn infants of insulin-dependent diabetic mothers. N Engl J Med 1977;296:375–6.
[57] Cordero L, Treuer SH, Landon MB, Gabbe SG. Management of infants of diabetic mothers. Arch Pediatr Adolesc Med 1998;152(3):249–54.
[58] Taylor R, Lee C, Kyne-Grzebalski D, Marshall SM, Davison JM. Clinical outcomes of pregnancy in women with type 1 diabetes. Obset Gynecol 2002;99(4):537–41.
[59] Mimouni F, Tsang RC, Hertzberg VS, Miodovnik M. Polycythemia, hypomagnesemia, and hypocalcemia in infants of diabetic mothers. Am J Dis Child 1986;140(8):798–800.
[60] Rosenn B, Miodovnik M, Tsang R. Common clinical manifestations of maternal diabetes in newborn infants: implications for the practicing pediatrician. Pediatr Ann 1996;25(4):212–22.
[61] Robert MF, Neff RK, Hubbell JP, et al. Association between maternal diabetes and the respiratory distress syndrome in the newborn. N Engl J Med 1976;294(7):357–60.
[62] Bourbon JR, Farrell PM. Fetal lung development in the diabetic pregnancy. Pediatr Res 1985;19(3):253–67.
[63] Smith BT, Giroud CJ, Robert M, Avery ME. Insulin antagonism of cortisol action on lecithin synthesis by cultured fetal lung cells. J Pediatr 1975;87(6 Pt 1):953–5.

[64] Tyden O, Eriksson UJ, Berne C. Fetal lung maturation in diabetic pregnancy. Acta Endocrinol Suppl (Copenh) 1986;277:101–6.
[65] Kjos SL, Walther FJ, Montoro M, Paul RH, Diaz F, Stabler M. Prevalence and etiology of respiratory distress in infants of diabetic mothers: predictive value of fetal lung maturation tests. Am J Obstet Gynecol 1990;163(3):898–903.
[66] Sells CJ, Robinson NM, Brown Z, Knopp RH. Long-term developmental follow-up of infants of diabetic mothers. J Pediatr 1994;125(1):9–17.
[67] Petersen MB, Pedersen SA, Greisen G, Pedersen JF, Molsted-Pedersen L. Early growth delay in diabetic pregnancy: relation to psychomotor development at age 4. Br Med J (Clin Res Ed) 1988;296(6622):598–600.
[68] Atkinson MA, Maclaren NK. The pathogenesis of insulin-dependent diabetes mellitus. N Engl J Med 1994;331(21):1428–36.
[69] Warram JH, Krolewski AS, Gottlieb MS, Kahn CR. Differences in risk of insulin-dependent diabetes in offspring of diabetic mothers and diabetic fathers. N Engl J Med 1984;311(3): 149–52.
[70] Homko CJ, Reece EA. Development of early-onset type 2 diabetes in the young: implications for child bearing. Curr Diab Rep 2003;3(4):313–8.
[71] Hieronimum S, Cupelli C, Durand-Reville M, Bongain A, Fenichel P. Pregnancy and type 2 diabetes: which fetal prognosis? Gynecol Obstet Fertil 2004;32(1):23–7.
[72] Dunne F, Brydon P, Smith K, Gee H. Pregnancy in women with type 2 diabetes: 12 years outcome data 1990–2002. Diabet Med 2003;20(9):734–8.
[73] Watson D, Rowan J, Neale L, Battin MR. Admissions to neonatal intensive care unit following pregnancies complicated by gestational or type 2 diabetes. Australian & New Zealand Journal of Obstetrics and Gynecology 2003;43(6):429–32.
[74] Casson IF, Clarke CA, Howard CV, et al. Outcomes of pregnancy in insulin dependent diabetic women: results of a five year population cohort study. BMJ 1997;315:275–9.
[75] Korenbrot CC, Steinberg A, Bender C, Newberry S. Preconception care: a systematic review. Matern Child Health J 2002;6(2):75–88.
[76] Ray JG, O'Brien TE, Chan WS. Preconception care and the risk of congenital anomalies in the offspring of women with diabetes mellitus: a meta-analysis. QJM 2001;94(8):435–44.
[77] Kitzmiller JL, Buchanan TA, Kjos S, Combs CA, Ratner RE. Preconception care of diabetes, congenital malformations, and spontaneous abortions. Diabetes Care 1996;19(5):514–41.
[78] American Diabetes Association. Preconception care of women with diabetes. Diabetes Care 2004;27(Suppl 1):S76–8.
[79] Wang PH, Lau J, Chalmers TC. Meta-analysis of effects of intensive blood-glucose control on late complications of type I diabetes. Lancet 1993;341(8856):1306–9.
[80] Blood glucose control and the evolution of diabetic retinopathy and albuminuria. A preliminary multicenter trial. The Kroc Collaborative Study Group. N Engl J Med 1984;311(6):365–72.
[81] Milunsky A, Jick H. Multivitamin/folic acid supplementation in early pregnancy versus the prevalence of neural tube defects. JAMA 1989;262:2847–52.
[82] Wilson RD, Davies G, Desilets V, Reid GJ, Summers A, Wyuatt P, et al. Genetics Committee and Executive and Council of the Society of Obstetricians and Gynaecologists of Canada. J Obstet Gynaecol Can 2003;25(11):959–73.
[83] Hagay Z, Reece EA. Diabetes mellitus in pregnancy. In: Reece EA, Hobbins JC, editors. Medicine of the fetus and mother. 2nd edition. Philadelphia: Lippincott-Raven; 1999. p. 1068–9.
[84] Gabbe SG, Graves CR. Management of diabetes mellitus complicating pregnancy. Obstet Gynecol 2003;102(4):857–68.
[85] Bailey B, Caldwell M. A team approach to managing preexisting diabetes complicated by pregnancy. Diabetes Educ 1996;22:111–7.
[86] Jovanovic-Peterson L, Peterson C. Pregnancy in the diabetic woman. Guidelines for a successful outcome. Endocrinol Metab Clin North Am 1992;21:433–56.
[87] Rosenstock J. Insulin therapy: optimizing control in type 1 and type 2 diabetes. Clin Cornerstone 2001;4(2):50–64.

[88] Simmons D. The utility and efficacy of the new insulins in the management of diabetes and pregnancy. Curr Diab Rep 2002;2:331–6.
[89] Bode BW, Tamborlane WV, Davidson PC. Insulin pump therapy in the 21st century. Strategies for successful use in adults, adolescents, and children with diabetes. Postgrad Med 2002; 111:69–77.
[90] Dunn CJ, Plosker GL, Keating GM, McKeage K, Scott LJ. Insulin glargine: an updated review of its use in the management of diabetes mellitus. Drugs 2003;63:1743–78.
[91] Hofmann T, Horstmann G, Stammberger I. Evaluation of the reproductive toxicity and embryotoxicity of insulin glargine (LANTUS) in rats and rabbits. Int J Toxicol 2002;21:181–9.
[92] Owens DR, Zinman B, Bolli G. Alternative routes of insulin delivery. Diab Med 2003;20: 886–98.
[93] Rizzo A, Dooley S, Metzger B, Cho N, Ogata E, Silverman B. Prenatal and perinatal influences on long-term psychomotor development in offspring of diabetic mothers. Am J Obstet Gynecol 1994;173:1753–7.
[94] ter Braak EW, Evers IM, Willem Erkelens D, Visser GH. Maternal hypoglycemia during pregnancy in type 1 diabetes: maternal and fetal consequences. Diab Metab Res Rev 2003; 18(2):96–105.
[95] Gagne M, Leff E, Jefferis S. The breast-feeding experience of women with type 1 diabetes. Health Care Wom Int 1992;13:249–53.
[96] Alvarez-Marfany M, Roman S, Drexler A, Robertson C, Stagnaro-Green A. Long-term prospective study of postpartum thyroid dysfunction in women with insulin dependent diabetes mellitus. J Clin Endocrinol Metab 1994;79:10–16.
[97] Metzger BE, Coustan DR. Proceedings of the 4th. Diabetes Care 1998;21(Suppl 2):B161–7.
[98] ADA Position Statement: gestational diabetes mellitus. Diabetes Care 2004;27(Suppl 1): S88–90.
[99] Sheffield JS, Casey BM, Lucas MJ, Rezai K, McIntire DD, Leveno KJ. Gestational diabetes: effects of the degree of hyperglycemia and the gestational age at diagnosis. Soc Gyn Inv 1999; 6:6A–8A.
[100] Bartha JL, Martinez-Del-Fresno P, Comino-Delgado R. Gestational diabetes mellitus diagnosed during early pregnancy. Am J Obstet Gynecol 2000;182:346–50.
[101] O'Sullivan JB. Body weight and subsequent diabetes mellitus. JAMA 1982;248:949–52.
[102] Metzger BE, Bybee DE, Freinkel N, Phelps RL, Radvany RM, Vaisrub N. Gestational diabetes mellitus: correlations between the phenotypic and genotypic characteristics of the mother and abnormal glucose tolerance during the first year postpartum. Diabetes 1985;34(Suppl 2):111–5.
[103] Greenberg LR, Moore TR, Murphy H. Gestational diabetes mellitus: antenatal variables as predictors of postpartum glucose intolerance. Obstet Gynecol 1995;86:97–101.
[104] Pallardo F, Herranz L, Garcia-Ingelmo T, Grande C, Martin-Vaquero P, Janez M, et al. Early postpartum metabolic assessment in women with prior gestational diabetes. Diabetes Care 1999;22:1053–8.
[105] ACOG. Pract Bull 2001.
[106] Tuffnal DJ, West J, Walinshaw SA. Treatments for gestational diabetes and impaired glucose tolerance in pregnancy(Cochrane Review). The Cochrane Library. Chichester (UK): John Wiley & Sons, LTD; 2004 [issue 3].
[107] Casey BM, Lucas MJ, McIntire DD, Leveno KJ. Population impact of universal screening for gestational diabetes. Am J Obstet Gyn 1999;180:536–9.
[108] Carpenter MW, Coustan DR. Criteria for screening tests for gestational diabetes. Am J Obstet Gynecol 1982;144:768–73.
[109] Langer O, Conway DL, Berkus MD, Xenakis EM, Gonzales O. A comparison of glyburide and insulin in women with gestational diabetes mellitus. N Engl J Med 2000;343(16):1134–8.
[110] Hundal RS, Inzucchi SE. Metformin: new understandings, new uses. Drugs 2003;63:1879–94.
[111] Kim C, et al. Gestational diabetes and the incidence of type 2 diabetes: a systematic review. Diabetes Care 2002;25:1862–8.
[112] Tuomilehto J, Lindstrom J, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P, et al.

Finnish Diabetes Prevention Study Group. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med 2001;344:1343–50.

[113] Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, et al. Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med 2002;346:393–403.

[114] Buchanan TA, Xiang AH, Peters RK, Kjos SL, Marroquin A, Goico J, et al. Preservation of pancreatic beta cell function and prevention of type 2 diabetes by pharmacological treatment of insulin resistance in high risk Hispanic women. Diabetes 2002;51:2796–803.

[115] Xiang A, Peters R, Siri K, Maroquin A, Ochoa C, Tan S, et al. Continued protection from diabetes during treatment of the TRIPOD cohort with pioglitazone. Diabetes 2003; 52(Suppl 1):A75.

ELSEVIER
SAUNDERS

Obstet Gynecol Clin N Am
31 (2004) 935–947

OBSTETRICS AND
GYNECOLOGY
CLINICS
OF NORTH AMERICA

The initiation of parturition at term

Charles J. Lockwood, MD

Department of Obstetrics, Gynecology and Reproductive Sciences,
Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520, USA

Although the precise mechanisms that underlie the initiation of parturition in humans remain to be elucidated, a series of natural experiments and clinical observations provide valuable insights. Conditions that disrupt the fetal hypothalamic–pituitary–adrenal (HPA) axis (eg, anencephaly in the absence of polyhydramnios) or the synthesis of estrogen by the placenta (eg, placental sulfatase deficiency) lead to prolonged gestation. That prostaglandins play a crucial role is suggested by the finding that prostaglandin synthase inhibitors delay parturition, whereas administration of prostaglandins initiate parturition. Thus, theories of the initiation of parturition in humans must reconcile the need for an intact fetal HPA axis, increasing placental estrogen synthesis, and enhanced reproductive tract prostaglandin activity.

Human parturition is similar yet different from that of other species

The initiation of parturition in humans shares many features with that of other nonprimate and primate species. For example, in the sheep, pig, horse, human, and many other mammalian species, fetal plasma cortisol levels increase rapidly in the last 14 to 17 days before labor begins [1,2]. In most mammalian species, including humans, estrogen levels increase in the amniotic fluid and plasma before the onset of term parturition [3,4]. Finally, in virtually all mammalian species, prostaglandins play a pivotal role in the onset of parturition.

E-mail address: chairobgyn@yale.edu

0889-8545/04/$ – see front matter
doi:10.1016/j.ogc.2004.08.012 obgyn.theclinics.com

In humans, concentrations of prostaglandins in maternal plasma are increased just before and during parturition [5–9]. Amniotic fluid prostaglandin concentrations also increase in labor and levels seem to increase before the onset of contractions [10]. In addition, in humans, myometrial prostaglandin receptors seem to be down-regulated during most of pregnancy [11]; this coincides with uterine quiescence, but increase with the onset of labor [12]. Although longitudinal studies of myometrial prostaglandin receptor expression are not feasible in humans for ethical reasons, in baboons, labor is associated with increased expression of the uterotonic prostaglandin E2 receptors, 1 and 3 (EP-1 and -3) in the uterine fundus [13]. These receptors, as well as that of prostaglandin F2α (PgF2α) (FP) are coupled to various G proteins (ie, Gaq and Gai) that lead to increased intracellular calcium concentrations [14]. Labor in the baboon also is associated with increased expression of the prostaglandin E2 (PgE2) receptors 2 and 4 (EP-2 and 4) in the lower uterine segment [13]. These latter receptors are coupled to an alternative G protein (Gas) that enhances adenyl cyclase activity to inhibit uterine contractions [14]. It is believed that in humans, such a topographic distribution of myometrial prostanoid receptor subtypes would facilitate effective contractions in the fundus and corpus of the uterus with relaxation of the lower uterine segment to facilitate expulsion of the fetus [15].

In most species, prostaglandins mediate the onset of labor by stimulating uterine contractions through direct enhancement of myometrial intracellular calcium concentrations by increasing sarcoplasmic and transmembrane calcium fluxes as well as through indirect effects on myometrial contractile protein expression. The latter include increasing steady-state myometrial mRNA and protein levels of oxytocin receptor, connexin 43 (gap junctions), EP-1–4, FP, and the crucial mediator of prostaglandin synthesis, prostaglandin endoperoxide H synthase (PGHS-II), which also is known as cyclooxygenase-2 (Cox-2) [14,16,17]. Prostaglandins also promote parturition by enhancing the synthesis of matrix metalloproteinases (MMPs) in the fetal membranes and cervix and by increasing cervical expression of interleukin (IL)-8, which recruits and activates neutrophils and releases additional MMPs [14,18,19]. These actions directly promote cervical change with or without fetal membrane rupture.

The initiation of parturition in humans and higher-order primates also has one major difference when compared with other species; it is not associated with obvious reductions in circulating progesterone levels [20]. Administration of the antiprogestin, RU486, although it enhances cervical ripening, it does not induce parturition in humans [21]. In most mammals, maturation of the fetal HPA axis and development of the transient ‘‘fetal inner zone’’ of the fetal adrenal gland cause an abrupt increase in circulating cortisol levels that activate the placental 17 α-hydroxylase-17,20-lyase enzyme to shunt steroid precursors away from the progesterone to the estradiol synthetic pathway. Parturition in humans and higher primates cannot result from such direct progesterone withdrawal, however, because of the absence of the glucocorticoid-inducible form of this enzyme in the placenta. However, activation of the fetal HPA axis does seem to play a crucial role in the onset of human parturition.

Fetal control of human parturition: the role of the fetal hypothalamic–pituitary–adrenal axis

That the fetus should exercise control over the initiation of parturition holds great teleologic appeal. Through such a mechanism, our species can ensure that the labor will be delayed until vital fetal organs (eg, lungs) are biochemically and anatomically mature enough to sustain extrauterine survival. Many lines of evidence suggest that ontogeny and maturation of the fetal HPA axis are the primary regulators of the onset of parturition.

Corticotropin-releasing hormone

Corticotropin-releasing hormone (CRH) is a 41–amino acid peptide that is produced in different parts of the brain as well as the reproductive tract and sites of inflammation [22]. Its production and release into the portal circulation from the hypothalamus mediates pituitary corticotropin secretion. The latter enhances adrenal cortisol secretion which inhibits hypothalamic CRH release. CRH also is expressed by trophoblasts in placenta and chorion, as well as by amnion and decidual cells [22–25]. Plasma CRH levels increase dramatically during the second half of pregnancy, peak during labor, and rapidly decline in the postpartum period, whereas levels of its inactivating binding protein decrease in the third trimester [22,23]. McLean et al [26] conducted a prospective study of 485 pregnant women. They reported an exponential increase in placental-derived maternal plasma CRH concentrations with advancing pregnancy that was associated with a concomitant decrease in concentrations of its binding protein in late pregnancy. This combination results in a rapid increase in circulating levels of bioavailable CRH that coincides with the onset of parturition. Although CRH levels increase sharply at term, labor also is associated with increased expression of the CRH receptor-2 in the chorion and myometrium and of the type-1 receptor in the amnion, chorion, and myometrium [27,28].

Although glucocorticoids inhibit the hypothalamic release of CRH, they increase expression of CRH by cultured trophoblasts, amnion, chorion, and decidual cells [23,29,30]. Maternal cortisol levels also increase steadily during the second half of gestation; this suggests that placental CRH is driving a "relative" hypercortisolism in the mother [22,31]. Using data that were obtained from cordocenteses, we showed that fetal CRH and cortisol also increase during the second half of gestation and that fetal cortisol levels correlate most strongly with placental CRH secretion [31]. This suggests that placental CRH expression drives fetal HPA activation. One explanation for this paradoxic cortisol stimulation of placental CRH expression may rest with the decreased levels of progesterone receptor expression in trophoblasts and progesterone's weak antagonist effects on the glucocorticoid receptor (GR). Thus, before term, the increased relative amounts of progesterone compete with the decreased amounts of cortisol for binding to trophoblast GR binding sites [32]. Increasing maternal and fetal

cortisol levels at term progressively overcome progesterone's tonic inhibition of cortisol/GR-mediated CRH expression, however. The resulting increase in placental-derived CRH "inappropriately" stimulates maternal and fetal pituitary corticotropin production, which normally would be suppressed significantly by the increasing cortisol levels. That this feed-forward loop is more pronounced in the fetus than in the mother is suggested by our observation that maternal corticotropin concentrations decrease modestly ($r = -0.21$; $P = .04$), whereas fetal corticotropin levels increase ($r = 0.35$; $P < .003$) with increasing gestational age, despite increasing cortisol levels in both compartments [31]. Thus, further maternal and, especially fetal, adrenal cortisol production is stimulated paradoxically [31] to establish an endocrine or systemic feed-forward loop (Fig. 1).

Because prostaglandins act as direct and indirect uterotonins and enhance fetal membrane and cervical protease production to promote cervical change and fetal membrane rupture, the observation that CRH enhances prostanoid production by isolated amnion, chorion, and decidual cells directly implicates it in the onset of parturition [33,34]. In addition, incubation of an amnion cell line with CRH results in a dose-dependent increase in EP-1 receptor protein and mRNA levels further implicating CRH in the onset of parturition [35]. Finally, prostaglandins seem to stimulate CRH production in the placenta and amnion which may create a paracrine feed-forward loop (see Fig. 1) [36].

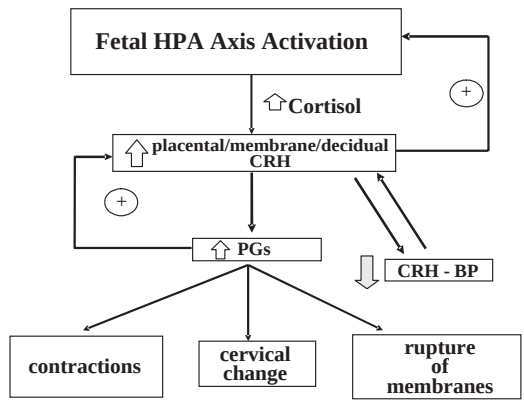


Fig. 1. The feed-forward loops that lead to fetal HPA axis activation and parturition. The role of CRH in the activation of the fetal HPA axis involves the paradoxic induction of placental, and to a lesser extent, decidual and fetal membrane CRH expression by cortisol. Increasing fetal CRH levels drive "inappropriate" fetal pituitary corticotropin to further enhance fetal adrenal cortisol production; this causes further increases in placental CRH production. In addition, a local or paracrine feed-forward loop is established by the induction of placental, decidual, chorion, and amnion prostaglandin (PG) and PG receptor production by CRH because these prostaglandins can enhance placental and amnion CRH expression. Finally, the system is activated further by a rapid reduction in CRH–binding protein (CRH-BP) near term caused by the excess production of CRH with increase clearance of the CRH/CRH-BP complexes.

Cortisol

Increasing fetal and amniotic fluid cortisol levels that result from progressive activation of the fetal HPA axis also may exert a direct effect on fetal membrane prostaglandin production. There is evidence that cortisol and other corticosteroids increase the production of PgE2 and the activity of PGHS-II/Cox-2 in cultured amnion fibroblast cells, although corticosteroids are considered to be potent anti-inflammatants and inhibit prostanoid synthesis in many other cell types [37–39]. In addition, corticosteroids reduce prostaglandin degradation in the chorion by inhibiting NAD^+-dependent 15–hydroxy-prostaglandin dehydrogenase (PGDH), the key enzyme that controls the metabolism of PgE2, PgF2α, and other prostanoids. The PGDH enzyme is localized to syncytiotrophoblasts in placenta and to the extravillous cytotrophoblasts of the chorion [40]. Labor is associated with decreased PGDH activity in placenta or chorion and cortisol exhibits a dose-dependent inhibitory effect on PGDH enzymatic activity and mRNA expression in placental and chorion trophoblasts cells in vitro; this effect is inhibited by progestins [40]. Thus, cortisol can enhance amnion PgE2 production and decrease its metabolism and decrease degradation of decidual-derived PgF2α.

There is evidence that prostaglandins augment local concentrations of cortisol in the fetal membranes by enhancing expression of chorionic 11 β-hydroxysteroid oxidase type 1 (11 β-HSD1) to convert biologically-inactive cortisone to cortisol [41]. Thus, decidual cell-derived PgF2α rapidly increases 11 β-HSD1 reductase activity to increase local concentrations of amniochorion cortisol. This cortisol

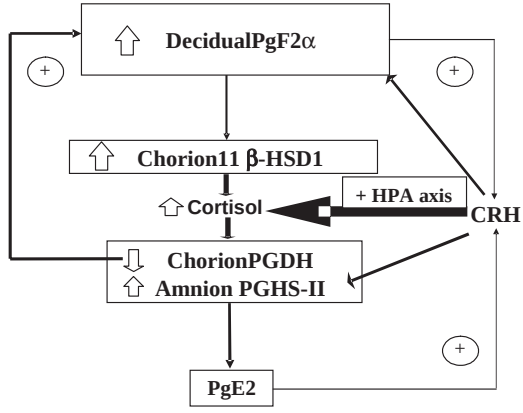


Fig. 2. The local feed-forward loop for cortisol-mediated fetal membrane prostaglandin production. Cortisol induces amnion PGHS-II/Cox-2 to increase synthesis of PgE2. Cortisol also inhibits chorionic PGDH and leads to reduced metabolism of decidual PgF2α and amnionic PgE2. Decidual PgF2α increases chorionic 11 β-HSD1 which increases local conversion of inactive cortisone to the active cortisol. The latter inhibits chorionic PGDH and increases amnionic PGHS-II to drive further PgE2 production in the amnion and to decrease the metabolism of decidual PgF2α. These prostaglandins, in turn, increase CRH production to trigger further prostaglandin production locally and to activate further the fetal HPA axis to generate more cortisol.

enhances amnionic PGHS-II/Cox-2 activity and decreases chorionic PGDH expression to cause a net increase in amnion-derived PgE2 and decidual-derived PgF2α concentrations [41,42]. Cortisol and, potentially, these prostaglandins also should drive additional decidual chorion or amnion CRH production which further amplifies amnion and decidual prostaglandin synthesis and activates the fetal HPA axis. Thus, progressive increases in decidual and fetal membrane prostanoid production may establish a third, combined paracrine–endocrine, feed-forward loop to promote parturition (Fig. 2).

Estrogen

Activation of the fetal HPA axis by increasing circulating levels of placental-derived CRH enhances fetal corticotropin production, and thus, fetal adrenal cortisol synthesis [31]. This process is accompanied by enhanced fetal adrenal dehydroepiandrosterone sulfate (DHEAS) synthesis. DHEAS molecule is 16-hydroxylated in the fetal liver to 16-hydroxy DHEAS. In addition to these corticotropin effects, CRH can augment fetal adrenal androgen production directly [43,44]. In the placenta, sulfatases cleave the sulfate conjugates of DHEA and 16-hydroxy DHEA and allow their conversion to estrogens. The former acts as a substrate for estradiol (E2) and estrone (E1), whereas the latter is converted to estriol (E3). Before 20 weeks of gestation, placental estrogen production uses maternal androgen precursors; thereafter, fetal adrenal androgens are the primary substrate. Beginning at 36 weeks' gestation, there is an accelerated rate of estrogen production that corresponds with the rapid development of the developmentally transient adrenal inner "fetal zone" [45,46].

A possible trigger for the escalation in fetal adrenal DHEA–placental estrogen production and fetal HPA axis activation is estrogen's stimulation of placental 11 β-HSD1 activity. In the baboon, the first half of gestation is marked by transplacental activation of maternal cortisone to cortisol, whereas the second half is associated with progressive oxidation of cortisol to the inactive cortisone. Estradiol seems to regulate the latter process [47]. A parallel phenomenon seems to occur in humans because placental 11 β-HSD1 mRNA abundance increases significantly with spontaneous labor [48]. These changes would reduce the maternal contribution to circulating fetal cortisol and drive increased fetal HPA axis activity, pituitary ACTH release, and thus, fetal adrenal DHEAS generation [48].

Estrogens facilitate parturition by enhancing transcription of a variety of uterine activation protein genes. For example, in human myometrial cells, estradiol and estriol increase the expression of connexin 43 (Cx43) gap junction proteins as demonstrated by immunocytochemistry and Western blotting [49]. Estradiol seems to augment connexin 43 gene transcription by increasing levels of the estrogen-responsive transcription factor, c-jun [50]. Increases in gap junctions facilitate cell–cell communication in the myometrium and efficient transmission of contractions.

Similar estradiol effects have been observed for myometrial oxytocin receptor expression. Levels of oxytocin receptor protein increase in human myometrium

across gestation with further increases at term and in labor [51]; these are commensurate with increasing estradiol, estrone, and estriol levels. In nonpregnant human uteri, estrogen enhances oxytocin receptor gene expression with maximal expression in the fundus [52]. Wu et al [53] examined topologic, gestational age, and labor-related changes in oxytocin receptor expression in the pregnant baboon myometrium. They observed increases in mRNA levels with increasing gestational age ($R^2 = 0.81$, $P < .05$) and greater expression in the fundus than in the lower uterine segment during spontaneous labor. They suggested that this may assist fetal expulsion by uterine contractions during labor.

Animal studies suggest that estrogen induces increased myometrial expression of other uterine activation proteins, including L-type calcium channels (rat) [54]; myosin light chain kinase (MLCK), and calmodulin (rabbit) [55]; as well as PGHS-II/Cox-2 (sheep) [56]. Although studies of estradiol induction of these other contractile-associated genes are limited in humans, it is clear that increasing estradiol, estrone, and estriol levels are implicated in myometrial activation.

Changes in circulating estrogen levels seem to be accompanied by dramatic changes in myometrial cell expression of sex steroid receptor isoforms. In general, the "A" form of the progesterone receptor (PR) exerts antagonist (antiprogesterone) effects on many progestin-sensitive end points, whereas the α form of the estrogen receptor (ER) can exert agonist (proestrogen) effects. There is now evidence of increases in PR-A, PR-A:PR-B ratio, and ERα mRNA in laboring myometrium, whereas ERβ mRNA levels are decreased and remain unchanged [57]. These data indicate that in humans, labor is associated with functional progesterone withdrawal and functional estrogen activation in the myometrium. It remains to be seen whether increasing circulating levels of cortisol, CRH, estrogen, prostaglandins, or other potential mediators of parturition mediate to cause these steroid receptor isoform changes.

Recently, Condon et al [58] proposed that this parturition-initiating functional progesterone withdrawal also may occur at a subreceptor molecular level. Using semiquantitative and real-time reverse transcriptase polymerase chain reaction (RT-PCR), immunohistochemistry, and immunoblotting, they observed decreased expression of the PR coactivators cyclic adenosine monophosphate (cAMP)-response element-binding (CREB) protein and steroid receptor coactivators 2 and 3 in fundal uterine tissue of women who were in labor. Some coactivators act through their histone acetyltransferase activity to catalyze the acetylation of histones and cause chromatin remodeling and increased access of transcription factors and polymerase to gene promoters. Therefore, they next examined human and murine uterine tissue and found that decreased levels of acetylated histone H3 were associated with labor. Moreover, administration of a potent histone deacetylase inhibitor to pregnant mice late in gestation increased histone acetylation and delayed the initiation of parturition by 24 to 48 hours [58]. Their findings suggest that the declines in PR coactivator expression and histone acetylation in the uterus near term causes progesterone withdrawal at a molecular level that results in decreased expression of PR-responsive genes. Again, it is unclear whether cortisol, CRH, estrogens, prostanoids, or other factors mediate

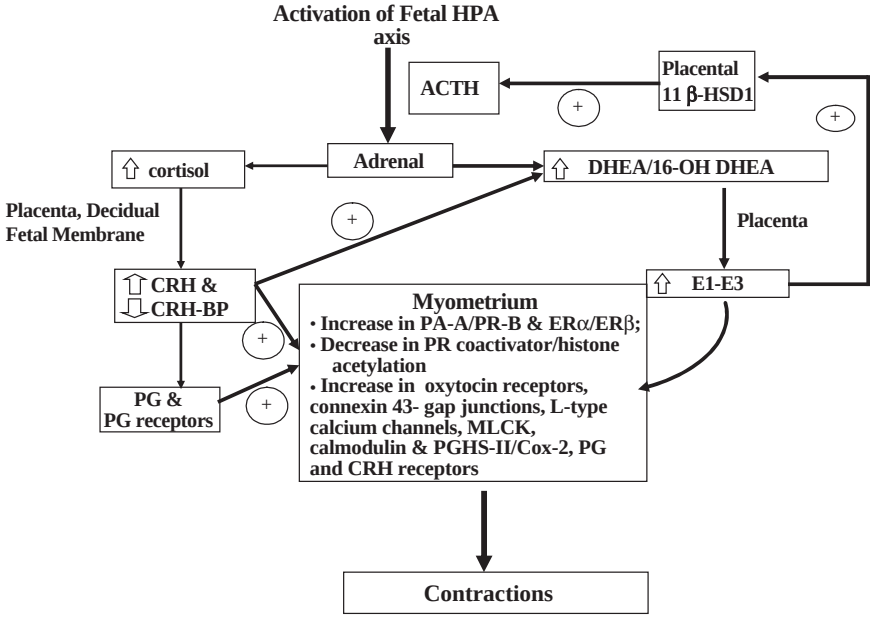


Fig. 3. The role of estrogen in mediating the onset of parturition. Fetal adrenal DHEAS production increases after 36 weeks' gestation commensurate with the development of the inner zone of the fetal adrenal as well as increasing levels of CRH which indirectly stimulate adrenal DHEAS synthesis by increasing pituitary corticotropin release and directly augment fetal adrenal cell DHEAS synthesis. Another key regulator of this increase in fetal adrenal DHEAS production is estradiol stimulation of placental 11 β-HSD1activity. This decreases transplacental maternal to fetal cortisol passage and further drives fetal pituitary corticotropin production. These androgens are desulfated and converted in the placenta to estrogens, which together with increasing reproductive tract prostaglandin production, activate the myometrium by increasing expression of gap junction connexin 43, oxytocin receptor, L-type calcium channels, PGHS-II/Cox-2, MLCK, and calmodulin genes. Induction of these uterine activation proteins converts ineffective contractures to contractions. Commensurate with the increasing estradiol, estrone, and estriol levels is a change in myometrial PR-B to PR-A and ER-β to ER-α isoforms and a reduction in PR receptor coactivator levels. The mediators of these changes in myometrial steroid receptor isoforms and steroid receptor coactivator expression are unknown but they may augment estrogen effects greatly.

the declines in PR coactivator expression and histone acetylation. Fig. 3 summarizes the role of estrogens in the genesis of labor in humans.

Other putative fetal-initiators of parturition

It is well-established that a variety of pathologic mechanisms can trigger preterm parturition, including ascending genital tract infections. The leading theory behind infection-associated prematurity is that activation of the maternal and fetal immune system leads to increased decidual and fetal membrane

macrophage activation. The latter causes the release of tumor necrosis factor–α and IL-1β which activates the NF-κB transcription factor in decidual, amnion, chorion, myometrial, and cervical cells to promote the release of decidual and amnion prostaglandins, the inhibition of chorionic PGDH, and increases in MMP and IL-8 release in the cervix, decidua, and fetal membranes (for reviews see [59–61]). Although such infectious mechanisms also may lead to term parturition, there is now intriguing evidence that the fetus may initiate parturition by a similar, albeit noninfectious, mechanism.

Condon et al [62] reported that maturation of the murine fetal lung may be linked to the onset of parturition. They demonstrated that the lung surfactant protein A (SP-A) stimulated IL-1 and NF-κB expression in cultured amnionic cavity macrophages. Studies that used the Lac-Z mouse strain, which allows investigators to discriminate between fetal and maternal-derived cells, demonstrated that SP-A–activated amnionic cavity macrophages migrated to the uterus where they released IL-1 to promote myometrial activation by way of the transcription factor, NF-κB. They next demonstrated that intra-amniotic injection of SP-A caused the preterm delivery of fetuses within 6 to 24 hours, whereas injection of SP-A antibodies or NF-κB inhibitors delayed labor by more than 24 hours. If confirmed in humans, such a mechanism would couple fetal lung maturation tightly to the onset of parturition. Moreover, because increasing cortisol levels are crucial to the induction of fetal lung maturation, this observation would further link maturation of the fetal HPA axis to the onset of parturition.

Summary

The initiation of human parturition requires orchestration of the sequential onset of a series of tightly coupled endocrine and paracrine feed-forward loops that are designed to ensure that parturition will occur when the fetus is capable of extended extrauterine survival. The leitmotif of this process is maturation of the fetal HPA axis (Fig. 4). It also depends upon the gradual acceleration of feed-forward hormonal signals between the placenta (CRH and estrogen), fetal pituitary (corticotropin), fetal adrenal gland (cortisol and DHEA), and possibly, the fetal lung (SP-A). A third theme is the absolute requirement for induction of hormone receptors (ie, steroid, prostaglandin, CRH, and oxytocin) which act in a permissive fashion to facilitate the establishment of the requisite feed-forward loops. A fourth theme is the induction of requisite families of transcription factors that ultimately mediate the transcription of parturition-associated genes (NF-κB, c-jun, and the PR coactivators, CREB protein, and steroid receptor coactivators 2 and 3). The final theme of human parturition is the requirement for induction of the ultimate arbiters of myometrial contractions (eg, PGHS-II/Cox-2, gap junctions, MLCK, and calmodulin) and cervical change (eg, MMPs, IL-8). The success of this rich tapestry of biochemical threads can be seen in the Earth's

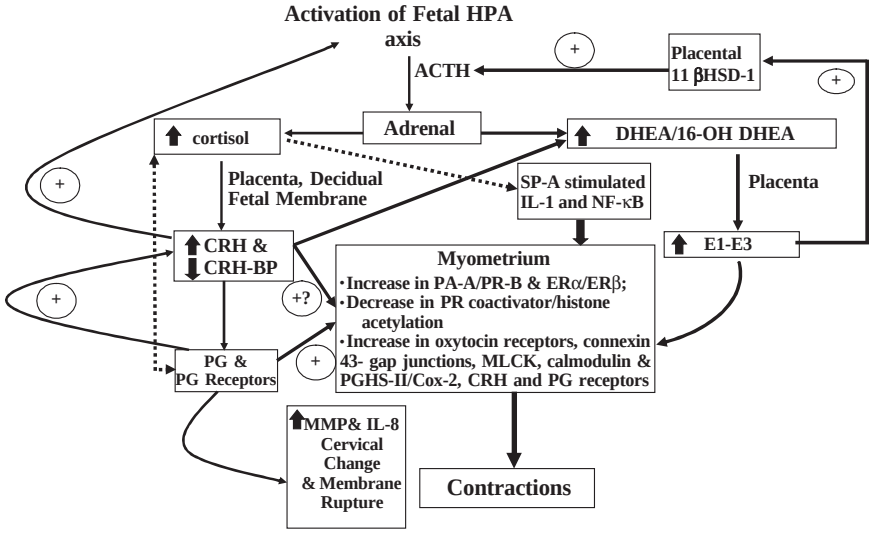


Fig. 4. An integrative theory of human parturition at term. Parturition is the net result of the initiation of an integrated series of endocrine and paracrine feed-forward loops. The crucial step is activation of the fetal HPA axis. The latter requires development of the "fetal inner zone" of the adrenal gland accompanied by progressive increases in fetal pituitary-derived corticotropin; it is facilitated by the induction of placental 11 β-HSD1 by estradiol which inactivates maternal-derived cortisol and decreases transplacental cortisol transfer to drive fetal corticotropin production. The ultimate driver of fetal HPA axis activation is the unique and paradoxic enhancing effect of cortisol on placental CRH production. Thus, increasing fetal cortisol levels stimulate further placental CRH release to drive "excess" fetal pituitary corticotropin and cause further cortisol production to establish a systemic or endocrine feed-forward loop.

dense population, its failure, in our persistently high rates of both prematurity and postmaturity.

References

[1] Magyar DM, Fridshal D, Elsner CW, Glatz T, Eliot J, Klein AH, et al. Time-trend analysis of plasma cortisol concentrations in the fetal sheep in relation to parturition. Endocrinology 1980; 107:155–9.

[2] McNellis D, Challis JRG, MacDonald PC, Nathanielsz PW, Roberts JM, editors. The onset of labour: cellular and integrative mechanisms. Ithaca (NY): Perinatology Press; 1988.

[3] Chaim W, Mazor M. The relationship between hormones and human parturition. Arch Gynecol Obstet 1998;262:43–51.

[4] Challis JRG, Matthews SG, Gibb W, Lye SJ. Endocrine and paracrine regulation of birth at term and preterm. Endocr Rev 2000;21:514–50.

[5] Husslein P, Sinzinger H. Concentration of 13,14-dihydro-15-keto-prostaglandin E2 in the maternal peripheral plasma during labour of spontaneous onset. Br J Obstet Gynaecol 1984;91: 228–31.

[6] Sellers SM, Hodgson HT, Mitchell MD, Anderson AB, Turnbull AC. Raised prostaglandin levels in the third stage of labor. Am J Obstet Gynecol 1982;144:209–12.

[7] Keirse MJNC. Endogenous prostaglandins in human parturition. In: Keirse MJNC, Anderson ABM, Bennebroek-Gravenhorstm J, editors. Human parturition. Leiden (The Netherlands): Leiden University Press; 1979. p. 101.
[8] Keirse MJNC, Turnbull AC. Prostaglandins in amniotic fluid during late pregnancy and labour. J Obstet Gynaecol Br Commonw 1973;80:970–3.
[9] Casey ML, MacDonald PC. Biomolecular processes in the initiation of parturition: decidual activation. Clin Obstet Gynecol 1988;31:533–52.
[10] Romero R, Munoz H, Gomez R, Parra M, Polanco M, Valverde V, et al. Increase in prostaglandin bioavailability precedes the onset of human parturition. Prostaglandins Leukot Essent Fatty Acids 1996;54:187–91.
[11] Matsumoto T, Sagawa N, Yoshida M, Mori T, Tanaka I, Mukoyama M, et al. The prostaglandin E2 and F2 alpha receptor genes are expressed in human myometrium and are down-regulated during pregnancy. Biochem Biophys Res Commun 1997;238:838–41.
[12] Brodt-Eppley J, Myatt L. Prostaglandin receptors in lower uterine segment myometrium during gestation and labor. Obstet Gynecol 1999;93:89–93.
[13] Smith GC, Wu WX, Nathanielsz PW. Effects of gestational age and labor on expression of prostanoid receptor genes in baboon uterus. Biol Reprod 2001;64:1131–7.
[14] Olson DM. The role of prostaglandins in the initiation of parturition. Best Pract Res Clin Obstet Gynaecol 2003;17:717–30.
[15] Myatt L, Lye SJ. Expression, localization and function of prostaglandin receptors in myometrium. Prostaglandins Leukot Essent Fatty Acids 2004;70:137–48.
[16] Lye SJ, Challis JRG. Paracrine and endocrine controlof myometrial activity. In: Gluckman PD, Johnson BM, Nathanielsz PW, editors. Advances in fetal physiology: reviews in honour of G.C. Liggins. Advances in Perinatal Medicine (VII). Ithaca (NY): Perinatology Press; 1989. p. 361–75.
[17] Cook JL, Zaragoza DB, Sung DH, Olson DM. Expression of myometrial activation and stimulation genes in a mouse model of preterm labor: myometrial activation, stimulation, and preterm labor. Endocrinology 2000;141:1718–28.
[18] Yoshida M, Sagawa N, Itoh H, Yura S, Takemura M, Wada Y, et al. Prostaglandin F(2alpha), cytokines and cyclic mechanical stretch augment matrix metalloproteinase-1 secretion from cultured human uterine cervical fibroblast cells. Mol Hum Reprod 2002;8:681–7.
[19] Denison FC, Calder AA, Kelly RW. The action of prostaglandin E2 on the human cervix: stimulation of interleukin 8 and inhibition of secretory leukocyte protease inhibitor. Am J Obstet Gynecol 1999;180:614–20.
[20] Turnbull AC. The endocrine control of labour. In: Turnbull AC, Chamberlain G, editors. Obstetrics. London: Churchill Livingston; 1989. p. 189.
[21] Frydman R, Lelaidier C, Baton-Saint-Mleux C, Fernandez H, Vial M, Bourget P. Labor induction in women at term with mifepristone (RU 486): a double-blind, randomized, placebo-controlled study. Obstet Gynecol 1992;80:972–5.
[22] Mastorakos G, Ilias I. Maternal and fetal hypothalamic-pituitary-adrenal axes during pregnancy and postpartum. Ann N Y Acad Sci 2003;997:136–49.
[23] Fadalti M, Pezzani I, Cobellis L, Springolo F, Petrovec MM, Ambrosini G, et al. Placental corticotropin-releasing factor. An update. Ann N Y Acad Sci 2000;900:89–94.
[24] Perkins AV, Linton EA. Identification and isolation of corticotrophin-releasing hormone-positive cells from the human placenta. Placenta 1995;16:233–43.
[25] Warren WB, Silverman AJ. Cellular localization of corticotrophin releasing hormone in the human placenta, fetal membranes and decidua. Placenta 1995;16:147–56.
[26] McLean M, Bisits A, Davies J, Woods R, Lowry P, Smith R. A placental clock controlling the length of human pregnancy. Nat Med 1995;1:460–3.
[27] Jirecek S, Tringler B, Knofler M, Bauer S, Topcuoglu A, Egarter C. Redistribution of corticotropin-releasing hormone receptor R2 using fluorescence immunohistochemistry in fetal membranes of women delivering preterm or at term. Wien Klin Wochenschr 2002;114:1008–12.

[28] Stevens MY, Challis JR, Lye SJ. Corticotropin-releasing hormone receptor subtype 1 is significantly up-regulated at the time of labor in the human myometrium. J Clin Endocrinol Metab 1998;83:4107–15.
[29] Majzoub JA, McGregor JA, Lockwood CJ, Smith R, Taggart MS, Schulkin J. A central theory of preterm and term labor: putative role for corticotropin-releasing hormone. Am J Obstet Gynecol 1999;180:S232–41.
[30] Jones SA, Brooks AN, Challis JR. Steroids modulate corticotropin-releasing hormone production in human fetal membranes and placenta. J Clin Endocrinol Metab 1989;68:825–30.
[31] Lockwood CJ, Radunovic N, Nastic D, Petkovic S, Aigner S, Berkowitz GS. Corticotropin-releasing hormone and related pituitary-adrenal axis hormones in fetal and maternal blood during the second half of pregnancy. J Perinat Med 1996;24:243–51.
[32] Karalis K, Goodwin G, Majzoub JA. Cortisol blockade of progesterone: a possible molecular mechanism involved in the initiation of human labor. Nat Med 1996;2:556–60.
[33] Jones SA, Challis JR. Local stimulation of prostaglandin production by corticotropin-releasing hormone in human fetal membranes and placenta. Biochem Biophys Res Commun 1989;159:192–9.
[34] Jones SA, Challis JR. Effects of corticotropin-releasing hormone and adrenocorticotropin on prostaglandin output by human placenta and fetal membranes. Gynecol Obstet Invest 1990;29:165–8.
[35] Spaziani EP, O'Brien WF, Benoit RR, Gould SF. Corticotropin-releasing hormone increases the expression of the prostaglandin E(2) receptor subtype EP1 in amnion WISH cells. Biol Reprod 2000;62:23–6.
[36] Jones SA, Challis JR. Steroid, corticotrophin-releasing hormone, ACTH and prostaglandin interactions in the amnion and placenta of early pregnancy in man. J Endocrinol 1990;125:153–9.
[37] Zakar T, Hirst JJ, Mijovic JE, Olson DM. Glucocorticoids stimulate the expression of prostaglandin endoperoxide H synthase-2 in amnion cells. Endocrinology 1995;136:1610–9.
[38] Economopoulos P, Sun M, Purgina B, Gibb W. Glucocorticoids stimulate prostaglandin H synthase type-2 (PGHS-2) in the fibroblast cells in human amnion cultures. Mol Cell Endocrinol 1996;117:141–7.
[39] Sun K, Ma R, Cui X, Campos B, Webster R, Brockman D, et al. Glucocorticoids induce cytosolic phospholipase A2 and prostaglandin H synthase type 2 but not microsomal prostaglandin E synthase (PGES) and cytosolic PGES expression in cultured primary human amnion cells. J Clin Endocrinol Metab 2003;88:5564–71.
[40] Patel FA, Clifton VL, Chwalisz K, Challis JR. Steroid regulation of prostaglandin dehydrogenase activity and expression in human term placenta and chorio-decidua in relation to labor. J Clin Endocrinol Metab 1999;84:291–9.
[41] Alfaidy N, Xiong ZG, Myatt L, Lye SJ, MacDonald JF, Challis JR. Prostaglandin F2alpha potentiates cortisol production by stimulating 11beta-hydroxysteroid dehydrogenase 1: a novel feedback loop that may contribute to human labor. J Clin Endocrinol Metab 2001;86:5585–92.
[42] Patel FA, Sun K, Challis JR. Local modulation by 11beta-hydroxysteroid dehydrogenase of glucocorticoid effects on the activity of 15-hydroxyprostaglandin dehydrogenase in human chorion and placental trophoblast cells. J Clin Endocrinol Metab 1999;84:395–400.
[43] Parker Jr CR, Stankovic AM, Goland RS. Corticotropin-releasing hormone stimulates steroidogenesis in cultured human adrenal cells. Mol Cell Endocrinol 1999;155:19–25.
[44] Chakravorty A, Mesiano S, Jaffe RB. Corticotropin-releasing hormone stimulates P450 17alpha-hydroxylase/17,20-lyase in human fetal adrenal cells via protein kinase C. J Clin Endocrinol Metab 1999;84:3732–8.
[45] Speroff L, Glass RH, Kase NG. Clinical gynecologic endocrinology and infertility. 4th edition. Baltimore (MD): William & Wilkins; 1989.
[46] Tulchinsky D, Little AB, editors. Maternal-fetal endocrinology. 2nd edition. Philadelphia: WB Saunders; 1994.
[47] Baggia S, Albrecht ED, Pepe GJ. Regulation of 11 beta-hydroxysteroid dehydrogenase activity in the baboon placenta by estrogen. Endocrinology 1990;126:2742–8.

[48] Murphy VE, Clifton VL. Alterations in human placental 11beta-hydroxysteroid dehydrogenase type 1 and 2 with gestational age and labour. Placenta 2003;24:739–44.
[49] Di WL, Lachelin GC, McGarrigle HH, Thomas NS, Becker DL. Oestriol and oestradiol increase cell to cell communication and connexin43 protein expression in human myometrium. Mol Hum Reprod 2001;7:671–9.
[50] Geimonen E, Boylston E, Royek A, Andersen J. Elevated connexin-43 expression in term human myometrium correlates with elevated c-Jun expression and is independent of myometrial estrogen receptors. J Clin Endocrinol Metab 1998;83:1177–85.
[51] Kimura T, Takemura M, Nomura S, Nobunaga T, Kubota Y, Inoue T, et al. Expression of oxytocin receptor in human pregnant myometrium. Endocrinology 1996;137:780–5.
[52] Richter ON, Kubler K, Schmolling J, Kupka M, Reinsberg J, Ulrich U, et al. Oxytocin receptor gene expression of estrogen-stimulated human myometrium in extracorporeally perfused non-pregnant uteri. Mol Hum Reprod 2004;10:339–46.
[53] Wu WX, Ma XH, Zhang Q, Nathanielsz PW. Characterization of topology-, gestation- and labor-related changes of a cassette of myometrial contraction-associated protein mRNA in the pregnant baboon myometrium. J Endocrinol 2001;171:445–53.
[54] Helguera G, Olcese R, Song M, Toro L, Stefani E. Tissue-specific regulation of Ca(2+) channel protein expression by sex hormones. Biochim Biophys Acta 2002;1569:59–66.
[55] Matsui K, Higashi K, Fukunaga K, Miyazaki K, Maeyama M, Miyamoto E. Hormone treatments and pregnancy alter myosin light chain kinase and calmodulin levels in rabbit myometrium. J Endocrinol 1983;97:11–9.
[56] Wu WX, Ma XH, Zhang Q, Buchwalder L, Nathanielsz PW. Regulation of prostaglandin endoperoxide H synthase 1 and 2 by estradiol and progesterone in nonpregnant ovine myometrium and endometrium in vivo. Endocrinology 1997;138:4005–12.
[57] Mesiano S, Chan EC, Fitter JT, Kwek K, Yeo G, Smith R. Progesterone withdrawal and estrogen activation in human parturition are coordinated by progesterone receptor A expression in the myometrium. J Clin Endocrinol Metab 2002;87:2924–30.
[58] Condon JC, Jeyasuria P, Faust JM, Wilson JW, Mendelson CR. A decline in the levels of progesterone receptor coactivators in the pregnant uterus at term may antagonize progesterone receptor function and contribute to the initiation of parturition. Proc Natl Acad Sci USA 2003; 100:9518–23.
[59] Wickelgren I. Premature labor. Resetting pregnancy's clock. Science 2004;304:666–8.
[60] Lockwood CJ. Predicting premature delivery—no easy task. N Engl J Med 2002;346:282–4.
[61] Lockwood CJ. Biochemical predictors of prematurity. Front Horm Res 2001;27:258–68.
[62] Condon JC, Jeyasuria P, Faust JM, Mendelson CR. Surfactant protein secreted by the maturing mouse fetal lung acts as a hormone that signals the initiation of parturition. Proc Natl Acad Sci USA 2004;101:4978–83.

ELSEVIER
SAUNDERS

Obstet Gynecol Clin N Am
31 (2004) 949–961

OBSTETRICS AND
GYNECOLOGY
CLINICS
OF NORTH AMERICA

Effects of hormones on fetal lung development

Matthew J. Bizzarro, MD, Ian Gross, MD*

Division of Perinatal Medicine, Department of Pediatrics, Yale University School of Medicine, Children's Hospital WP 493, 333 Cedar Street, P.O. Box 208064, New Haven, CT 06520-8064, USA

Fetal lung development

Lung development in the human fetus comprises five overlapping stages that begin at approximately 3 weeks' postconception and extend into the second year of postnatal life. The embryonic stage is the initial period of lung development that spans from 3 to 7 weeks' postconception. During this time endodermally-derived lung buds are formed from the ventral outpouching of the anterior foregut into the adjacent mesoderm. The pseudoglandular stage begins at 5 weeks' gestation and is characterized by the development and branching of the bronchial tree and the pulmonary arterial system. This stage is completed at approximately 17 weeks' postconceptual age—a week after the onset of the canalicular stage. The canalicular stage spans 10 weeks' of gestation and has the formation of the capillary network of the lung and the differentiation of alveolar type I and II cells as its hallmark. The period of time from 24 to 38 weeks' postconception is known as the saccular stage of fetal lung development. During this period of time, primitive alveoli (ie, saccules) are formed and the type II alveolar cells begin to secrete surfactant. Finally, the alveolar stage begins at 36 weeks' gestation and extends into the second year of postnatal life. This phase is characterized by the septation of saccules to form alveoli and the thinning of the squamous epithelial lining to facilitate gas exchange with a well-developed capillary network [1].

During this developmental period, several hormones, transcription factors, and other agents play a part in the initiation and regulation of fetal lung development. The major focus of clinical studies has been on the role of endogenous hormones;

Dr. Bizzarro was supported by NICHD Training Grant T32 HD 07094.
* Corresponding author.
E-mail address: ian.gross@yale.edu (I. Gross).

0889-8545/04/$ – see front matter
doi:10.1016/j.ogc.2004.08.001 obgyn.theclinics.com

although several hormones may play a role in this process, glucocorticoids and thyroid hormones have received the most attention. [2].

Glucocorticoids and their role in fetal lung development

In 1969, Liggins [3] observed that antenatal infusion of fetal lambs with glucocorticoids, before preterm delivery, resulted in improved respiratory status and survival in the newborn period. Since that discovery, many studies have explored the biochemical and physiologic roles of glucocorticoids in the developing lung. Intracellular glucocorticoid receptors facilitate the binding and transport of the hormone into the cell nucleus. Once in the nucleus, the glucocorticoid-receptor complex binds to specific sites on DNA which results in the transcription of mRNA molecules. These mRNA molecules then direct translation of specific proteins, many of which (eg, surfactant proteins B and C) play a major role in lung function and development [4]. In addition, glucocorticoids seem to stimulate the production and activation of several pulmonary-specific enzymes that are responsible for the development and protection of the lung [5].

Endogenous glucocorticoids modulate production of many components of surfactant, particularly surfactant proteins B and C that—together with surfactant phospholipids—are essential for decreasing the surface tension at the air–alveolar interface [2,6,7]. In addition to surfactant protein production, animal studies demonstrated that glucocorticoids increase the activity of antioxidant enzymes (eg, superoxide dismutase) that are responsible for protecting the lung against damaging oxygen-free radicals [8]. Glucocorticoids also may influence and enhance the structural development of the lung through increased production of collagen and elastin and thinning of alveolar septae to facilitate gas exchange [9]. These hormones also play an active role in the production of sodium and potassium adenosine triphosphatase to facilitate the clearance of fetal lung fluid [10,11].

The biochemical influence of glucocorticoids on the developing lung is reflected in physiologic changes. In particular, increased lung compliance and volume occur as a result of increased surfactant production and structural remodeling of the lung. Also, decreased vascular permeability and enhanced clearance of fetal lung fluid, which result from the action of glucocorticoids on specific sodium channels, improve gas exchange and ease the fetal to neonatal transition [2]. These findings provided a rationale for clinical trials on the use of antenatal exogenous glucocorticoids in an attempt to accelerate fetal lung maturation and improve respiratory function and, ultimately, survival in preterm neonates.

Thyroid hormones and their role in fetal lung development

Thyroid hormones, like glucocorticoids, are believed to play an important part in the development of the fetal lung, although their role is less well-defined.

Triiodothyronine (T_3) and thyroxine (T_4) bind to specific receptors in the cells of the lung [12]. Although specific target proteins for the thyroid hormones have not been identified, the hormones play a significant role in the production of surfactant phospholipids, particularly phosphatidylcholine [13–15]. Thyroid hormone, however, seems to have no effect on the production of surfactant proteins and may, in fact, decrease production of surfactant protein A and B mRNA [16–19]. In addition, thyroid hormone may decrease the activity of protective antioxidant enzymes in the developing lung [19]. When exogenous T_3 was administered to early embryonic mouse lung tissue, epithelial and mesenchymal cell differentiation was accelerated, whereas there was a significant reduction in branching morphogenesis and lung growth [20]. Thus, the overall role of thyroid hormone in the developing lung remains unclear.

There is evidence that an additive effect of thyroid hormone and glucocorticoids on fetal lung development exists. T_3 and T_4 do not cross the placenta readily from mother to fetus, whereas thyrotropin-releasing hormone (TRH) does. Research that used TRH and glucocorticoids demonstrated a synergistic effect with respect to the production of phosphatidylcholine [14,15]. In animal models, there was an improvement in lung function—particularly compliance and maximal volume capacity—when TRH, betamethasone, and surfactant were administered together as compared with the administration of betamethasone and surfactant alone [21,22]. These encouraging findings resulted in clinical trials to test the effects of this combined hormonal therapy on premature neonates. The results are discussed below.

Clinical trials of antenatal glucocorticoids

Premature delivery and care of the premature neonate remain significant problems in perinatal medicine [23]. In particular, the immaturity of the lungs presents a difficult challenge to the neonatologist; the respiratory distress syndrome (RDS) and its associated complications are a contributing or direct cause of much of the mortality and long-term morbidity that are seen in premature infants [24]. As a result of this growing population and the encouraging investigative work on glucocorticoids and their role in fetal lung development, a series of clinical trials were undertaken from 1972 to 1995 in an attempt to define a therapeutic role for antenatal glucocorticoids in the treatment of RDS.

A systematic review was performed by Patricia Crowley [25] in 1995 to assess the effects of antenatal glucocorticoid administration on fetal lung maturity. Eighteen randomized and quasi-randomized controlled trials were identified from 1972 to 1995; data on more than 3700 neonates were collected and analyzed. These trials included the use of betamethasone (24 mg), dexamethasone (24 mg), and hydrocortisone (2 g) in pregnant women who were expected to deliver prematurely. These therapies were compared with the use of placebo or no treatment. The major outcomes that were assessed included the overall incidence

of RDS, neonatal or infant death, and long-term developmental or neurologic disabilities [25].

Antenatal glucocorticoid administration and respiratory distress syndrome

The systematic review indicated that maternal treatment with antenatal glucocorticoids resulted in a substantial reduction (approximately 50%)—regardless of race or gender—in the overall incidence of RDS in the preterm neonate; the most significant effect was observed in neonates who were delivered before 32 weeks' gestational age. In the subgroup of newborns who was delivered at less than 28 weeks' gestation, a trend toward a reduction in the incidence of RDS was seen in the treatment group as compared with the control group; however, this trend was not statistically significant. This analysis included only 48 subjects (17 treatment and 31 control) [25].

The meta-analysis also showed that the most significant reduction in the incidence of RDS was in the group of neonates who were delivered at more than 48 hours but less than 7 days after the initiation of maternal glucocorticoid administration. There was a trend toward a reduction in RDS in the treatment group when therapy was administered at less than 48 hours or greater than 7 days before delivery; however, this trend was not statistically significant [25]. In addition to the timing of administration, glucocorticoid selection also was investigated. In the review, betamethasone and dexamethasone seemed to be associated with a significant reduction in RDS; however, the data that were collected from the few trials that used hydrocortisone showed no significant effect [25].

Antenatal glucocorticoid administration and neonatal morbidity and mortality

In the meta-analysis, antenatal administration of glucocorticoids also resulted in a significant reduction in neonatal death. This effect was most apparent in trials that were performed before 1980 when the incidence of death from RDS was more substantial. A trend toward a reduction in neonatal mortality in the subgroup that was treated after 1980 was observed [25].

The incidences of intraventricular hemorrhage (IVH), necrotizing enterocolitis (NEC), infection, and chronic lung disease (CLD) also were compared in each group. There was a statistically significant reduction in IVH in the treatment group, but no difference in CLD or NEC between groups. In addition, no significant difference between the treatment and control groups was observed with respect to fetal or neonatal infection [25]. Review of a large database suggested that use of antenatal glucocorticoids might be associated with a reduction in the incidence of NEC [10].

The meta-analysis also evaluated the possible effects of antenatal glucocorticoid administration on long-term growth and development. Three trials—the U.S. Collaborative Antenatal Steroid Therapy Trial, the Auckland Trial, and the Amsterdam Trial—published data on long-term developmental outcome at 3, 6, and 10 to 12 years, respectively [26–29]. The trials used betamethasone and dexamethasone and found that neither therapy, when administered in the antenatal period, had any adverse effect on physical growth or development [25]. Development at 12 years was assessed in 90 children through formal testing in the areas of intelligence and reasoning, memory, visual perception, motor development, scholastic achievement, and social and emotional functioning. No statistically significant difference was observed between treated and control groups [29].

Antenatal glucocorticoid administration and complicated pregnancies

Crowley's [25] systematic review analyzed the potential effects of antenatal glucocorticoid administration on pregnancies that were complicated by factors other than premature labor. In particular, subgroup analysis was attempted on women who had premature rupture of the membranes (PROM), gestational or pregestational diabetes, and hypertension. The meta-analysis revealed that antenatal glucocorticoid administration did not result in an increased risk of maternal, fetal, or neonatal infection in the subgroup of women who had PROM. Insufficient data (N = 35) were available to make a recommendation for the administration or withholding of glucocorticoids in women with diabetes during pregnancy, although glucocorticoid therapy may potentiate insulin resistance and problems with diabetic control [24].

Some concern was raised with respect to the administration of antenatal glucocorticoids to pregnant women who had hypertensive crisis when the Auckland Trial, in 1972, reported a significantly higher incidence of stillbirths (26% versus 7%) in the group that was treated with antenatal glucocorticoids [30]. Three subsequent trials included data on this subgroup and observed no reported stillbirths in the treatment or control groups (a total of 149 subjects) [26,31,32]; however, no definitive statement has been made to support or reject the use of antenatal glucocorticoids in this population.

The 1994 National Institutes of Health Consensus Conference

Despite a significant amount of data of high methodologic quality to support the use of antenatal glucocorticoids in pregnant women who are at risk of preterm delivery, initially, this therapeutic intervention was not adopted widely. In the 1987 UK Ten Centre study and the 1991 U.S. Exosurf trial, which investigated the use of surfactant therapy on premature newborns, only 12% and 20% of the neonates that were enrolled, respectively, had received antenatal glucocorticoids

[33,34]. In the United States in 1993, it was estimated that only 12% to 18% of neonates who were delivered at less than 1500 g had been born to mothers who had received antenatal glucocorticoid therapy [35]. After thoroughly reviewing the available clinical evidence, the Royal College of Obstetricians and Gynecologists in 1993, and the National Institutes of Health (NIH) in 1994, published recommendations for the use of antenatal glucocorticoid therapy in women who are at risk of delivering prematurely [35,36].

The recommendations of the NIH on the use of antenatal glucocorticoids are summarized in Box 1. The committee also addressed other pertinent topics that were not covered in the summary consensus statement. The NIH consensus committee believed that insufficient evidence existed to make definitive recommendations with respect to the use of glucocorticoids in certain maternal and fetal high-risk conditions. For pregnancies that are complicated by maternal

Box 1. Recommendations of the 1994 NIH consensus development conference on the effect of corticosteroids for fetal maturation on perinatal outcomes

All fetuses between 24 and 34 weeks' gestation who are at risk of preterm delivery, regardless of race or gender, are candidates for antenatal treatment with glucocorticoids.

Patients who are eligible for therapy with tocolytics also should be eligible for treatment with antenatal glucocorticoids.

Treatment consists of two dosages of betamethasone,12 mg, given intramuscularly 24 hours apart or four dosages of dexamethasone, 6 mg, given intramuscularly 12 hours apart.

Antenatal glucocorticoids should be given unless immediate delivery is anticipated because treatment with corticosteroids for less than 24 hours is associated with significant reductions in neonatal mortality, RDS, and IVH.

In the absence of clinical chorioamnionitis, antenatal glucocorticoid therapy is recommended in premature prolonged rupture of membranes (PPROM) at less than 30 to 32 weeks' gestation.

In complicated pregnancies where delivery before 34 weeks' gestation is likely, antenatal glucocorticoid use is recommended unless there is evidence that such therapy will have an adverse effect on the mother or unless delivery is imminent.

Adapted from: Gilstrap LC, Christensen R, Clewell WH, et al. NIH Consensus Development Panel on the Effect of Corticosteroids for Fetal Maturation of Perinatal Outcomes. Effect of corticosteroids for fetal maturation on perinatal outcomes. JAMA 1995;273(5):413–8.

hypertension, maternal diabetes, multiple gestation pregnancies, fetal intrauterine growth restriction, and hydrops fetalis, the NIH stated that "it may be reasonable to treat" but offered no conclusive recommendations [35].

Although the 1994 NIH consensus development conference statement offered long overdue support for antenatal glucocorticoid therapy, several related issues arose over the next decade. In particular, the issues of repeat dosing of antenatal steroids, combined therapy with TRH, the safety and efficacy of betamethasone versus dexamethasone therapy, and the potential toxicities and long-term consequences of antenatal glucocorticoids became topics of great interest and debate.

Repeat courses of antenatal glucocorticoids

The systematic review by Crowley [25] revealed that the most significant effect of glucocorticoids on RDS was observed when therapy was initiated 48 hours to 7 days before delivery and that no statistically significant effect was observed in newborns who were delivered 7 days after treatment. As a result, some obstetricians advocated the use of repeated courses of antenatal glucocorticoids in pregnancies that were threatened by preterm delivery. In 2000, an NIH Consensus Development Conference recommended that "because of insufficient data from randomized clinical trials regarding efficacy and safety, repeat courses of corticosteroids should not be used routinely" [37]. The American College of Obstetricians and Gynecologists (ACOG) published a statement in 2002 that supported this recommendation [38]. Both organizations called for further randomized controlled trials to address the question of the safety and efficacy of repeat courses of antenatal glucocorticoids. Several trials were underway at the time that these statements were made [39–41] and their data eventually were compiled, analyzed, and summarized in a 2003 systematic review [42].

This meta-analysis reviewed three randomized clinical trials that assessed the efficacy and safety of repeat versus single courses of antenatal glucocorticoids. A total of 551 pregnant women who already had received a single course of betamethasone or dexamethasone were enrolled at between 24 and 30 weeks' gestation and were randomized to receive weekly courses of glucocorticoids or placebo until 33 to 34 weeks' gestation or delivery [42]. No statistically significant difference was observed between groups with respect to incidence of RDS; chronic lung disease; periventricular hemorrhage (PVH); periventricular leukomalacia (PVL); and fetal, neonatal, or infant death [42]. One trial (N = 500), however, reported a significant reduction in the severity of lung disease in the group that received repeated doses of glucocorticoids [40]; two of the trials indicated that fewer neonates in the group that received repeat treatment received surfactant as compared with those in the placebo group [42].

The systematic review seemed to show some benefit to repeat courses of antenatal glucocorticoids; however, one important issue—safety—still needed to be addressed. The concern over the use of repetitive courses of antenatal glu-

cocorticoids stems from animal and human studies that reported potential adverse long-term outcomes with respect to growth restriction [43–45]; suppression of brain growth [46], neuronal myelination [47], and adrenal function [48]; and increased risk of mortality [48]. In addition, a cohort study that was published in 2004 showed that repeat courses of glucocorticoids may be associated with an increased risk of aggressive/destructive and hyperactive behavior in children [49]. Although most of these studies were not conducted in a randomized, controlled fashion, enough concern was raised to support the recommendation of the NIH Consensus Development Conference and ACOG that repeat courses of antenatal glucocorticoids should not be administered routinely to mothers who are at risk for preterm delivery. The randomized controlled trials that were included in the meta-analysis did not provide sufficient evidence to challenge this recommendation [42].

Betamethasone versus dexamethasone

Some controversy exists as to the use of betamethasone or dexamethasone as the antenatal glucocorticoid of choice. The NIH and ACOG recommend either therapy in the antenatal period; however, there is accumulating evidence that betamethasone is the preferred agent.

Dexamethasone and betamethasone are structurally similar glucocorticoids with equal ability to cross the placenta and stimulate similar biologic function; they differ only by the configuration of a single methyl group [50,51]. There may be differences in the benefits and in the comparative risks of the two therapies. Studies in fetal mice revealed that betamethasone may be a more effective therapy for accelerating fetal lung maturity than dexamethasone [52]. In addition, the antenatal administration of betamethasone to mice resulted in an improvement in some neurobehavioral development tasks, including memory enhancement, as compared with dexamethasone [53]. This, together with data from Crowley's systematic review which demonstrated a significant reduction in neonatal mortality in the subgroup that was treated with betamethasone, seems to point toward a potential therapeutic benefit to betamethasone [25,54].

In addition to the therapeutic benefits of betamethasone, significant concern has been raised as to the potential toxicities that are associated with the use of antenatal dexamethasone. In 1999, Baud et al [50] published retrospective data on 883 neonates who were born between 24 and 31 weeks' gestational age. These newborns had been exposed to antenatal betamethasone or dexamethasone or no glucocorticoid treatment. After adjusting for differences in gestational age, number of courses of glucocorticoids, and several other possible confounding variables, the researchers demonstrated no significant difference between glucocorticoids with respect to incidence of RDS, bronchopulmonary dysplasia, IVH, or NEC [50]. There was a significant reduction in the risk of cystic PVL in the group that was treated with betamethasone (adjusted odds ratio of 0.5). In

addition, the neonates who had received antenatal dexamethasone demonstrated an increased risk of cystic PVL compared with newborns whose mothers had not received treatment (adjusted odds ratio of 1.5) [50].

There are two theories as to why dexamethasone may be more toxic than betamethasone. Sulfite preservatives are used specifically in dexamethasone preparations. In vitro and in vivo exposure to pure betamethasone and dexamethasone, compared with commercial preparations of these glucocorticoids and with isolated sulfites that are identical to those that are used in the dexamethasone preparation, was investigated in mice [55]. There was an increase in in vivo and in vitro neuronal death after treatment with prepared dexamethasone or its isolated sulfites [55]; however, the dosing of glucocorticoids in this study was approximately 10 times that recommended for antenatal administration. It also was pointed out that a small amount of sulfite would cross from mother to fetus after administration of dexamethasone; other drugs that contain sulfites do not seem to be toxic to neonates. An alternative, and more plausible, explanation relates to the fact that rapidly absorbed dexamethasone reaches higher peak blood levels than does the slow release form of betamethasone. It is possible that increased circulating levels of dexamethasone may potentiate the observed neuronal damage [56]. Whatever the etiology, the potential toxicity of dexamethasone has raised concerns as to the safety and efficacy of its administration; however, ACOG and the NIH have not revised their recommendations that support its use. In addition, periodic shortages in the availability of betamethasone have necessitated the use of antenatal dexamethasone.

Thyrotropin-releasing hormone and glucocorticoids

Animal studies indicated that there were synergistic, beneficial effects of antenatal glucocorticoid plus TRH administration on the developing lung [14,15,21,22]. As a result, several clinical trials were undertaken in an attempt to evaluate the clinical efficacy of this combined therapeutic approach. In 2004, a systematic review evaluated data from 13 randomized, clinical trials in which more than 4600 women were recruited and treated antenatally with TRH plus glucocorticoids or glucocorticoids alone [57]. The data revealed that there was no statistically significant difference with respect to death before hospital discharge, death or need for oxygen at 28 days, the risk of RDS or severe RDS, gestational age at birth, severe PVH, NEC, or the use of surfactant between the two groups [57].

In contrast, there was a higher risk of decreased 5-minute Apgar scores and the need for respiratory support in the group that was treated with TRH. In addition, maternal side effects, such as nausea, vomiting, light-headedness, facial flushing, and an increase in systolic and diastolic blood pressure also were seen in this group [57]. Two of the trials evaluated long-term outcome in children at 12 months [58,59], whereas one trial evaluated long-term outcome at 24 months [59]. The data at 12 months revealed an increase risk of motor delay, motor

impairment, sensory impairment, and social delay in the group that was given TRH. Follow-up at 24 months also demonstrated a higher risk of developmental delay in this group. These findings remained consistent when subgroup analysis was performed on trials of highest methodologic quality, timing of delivery, and TRH dosage [57]. As a result, the administration of TRH in conjunction with glucocorticoids in the antenatal period is not recommended. A recent review indicated that the combination of TRH and glucocorticoids did have a beneficial effect in terms of lung disease in infants who were delivered from 1 to 10 days after the first dosage of hormones and when postnatal surfactant therapy was not used [60]. Antenatal TRH seems to provide no further benefit to that of antenatal glucocorticoid and postnatal surfactant and is associated with adverse developmental effects.

Summary

The use of antenatal glucocorticoids has been an issue of intense research and debate since Liggins [3] first published his report on the subject in 1969. Since that time, significant progress has been made with respect to identifying the efficacy of this therapy in reducing the incidence of RDS, IVH, and neonatal mortality in preterm newborns. Questions remain as to the choice of glucocorticoid preparation, optimal dosage, and scheduling of antenatal glucocorticoid administration. Although experimental and clinical data seem to favor a single course of betamethasone, more information is needed with respect to long-term growth and neurodevelopmental outcome. In addition, further trials are required to address the issue of optimal dosing regimens. At present, the evidence favors the use of betamethasone under the conditions that were described by the NIH Consensus Conference.

References

[1] Weri SM. Normal and abnormal structural development of the lung. In: Polin RA, Fox WW, Abman SH, editors. Fetal and neonatal physiology. 3rd edition, volume 1. Philadelphia: W.B. Saunders; 2004. p. 783–94.
[2] Ballard PL, Ballard RA. Scientific basis and therapeutic regimens for use of antenatal glucocorticoids. Am J Obstet Gynecol 1995;173(1):254–62.
[3] Liggins GC. Premature delivery of foetal lambs infused with glucocorticoids. Endocrinology 1969;45(4):515–23.
[4] Kresch MJ, Gross I. The biochemistry of fetal lung development. Clin Perinatol 1987;14(3): 481–507.
[5] Gross I. Regulation of fetal lung maturation. Am J Physiol 1990;259(6 part 1):L337–44.
[6] Rooney SA, Smart DA, Weinhold PA, Feldman DA. Dexamethasone increases the activity but not the amount of choline-phosphate cytidylyltransferase in fetal rat lung. Biochim Biophys Acta 1990;1044(3):385–9.
[7] Beneke S, Rooney SA. Glucocorticoids regulate expression of the fatty acid synthase gene in fetal rat type II cells. Biochim Biophys Acta 2001;1534(1):56–63.

[8] Connelly IH, Hammond GL, Harding PG, Possmayer F. Levels of surfactant-associated protein messenger ribonucleic acids in rabbit lung during prenatal development and after hormonal treatment. Endocrinology 1991;129(5):2583–91.
[9] Kikkawa Y, Kaibara M, Motoyama EK, Orzalesi MM, Cook CD. Morphologic development of fetal rabbit lung and its acceleration with cortisol. Am J Pathol 1971;64(2):423–42.
[10] NIH Consensus Development Panel on the Effect of Corticosteroids for Fetal Maturation on Perinatal Outcomes. Effect of corticosteroids for fetal maturation on perinatal outcomes. JAMA 1995;273(5):413–8.
[11] Creuwels LA, van Golde LM, Haagsman HP. The pulmonary surfactant system: biochemical and clinical aspects. Lung 1997;175(1):1–39.
[12] Ballard PL, Hovey ML, Gonzales LK. Thyroid hormone stimulation of phosphatidylcholine synthesis in cultured fetal rabbit lung. J Clin Invest 1984;74(3):898–905.
[13] Ballard PL, Benson BJ, Brehier A, Carter JP, Kriz BM, Jorgensen EC. Transplacental stimulation of lung development in the fetal rabbit by 3,5-dimethyl-3′-isopropyl-L-thyronine. J Clin Invest 1980;65(6):1407–17.
[14] Gonzales LW, Ballard PL, Ertsey R, Williams MC. Glucocorticoids and thyroid hormone stimulate biochemical and morphological differentiation of human fetal lung in organ culture. J Clin Endocrinol Metab 1986;62(4):678–91.
[15] Gross I, Wilson CM. Fetal lung in organ culture. IV. Supra-additive hormone interactions. J Appl Physiol 1982;52(6):1420–5.
[16] Liley HG, White R, Benson BJ, Hagwood S, Ballard PL. Regulation of messenger RNAs for the hydrophobic surfactant proteins in human lung. J Clin Invest 1989;83(4):1191–7.
[17] Nichols KV, Dynia DW, Floros J, Veletza SV, Lequillo R, Gross I. Hormonal regulation of fetal rat surfactant proteins A and B mRNA. A comparison [abstract]. Pediatr Res 1989;57A:25.
[18] Nichols KV, Floros J, Dynia DW, Veletza SV, Wilson CM, Gross I. Regulation of surfactant protein A mRNA by hormones and butyrate in cultured fetal lung. Am J Physiol 1990; 259:L488–95.
[19] Ramadurai SM, Nielsen HC. Differential effects in vivo of thyroid hormone on the expression of surfactant phospholipids, surfactant protein mRNA and antioxidant enzyme mRNA in fetal rat lung. Exp Lung Res 1998;24(5):641–57.
[20] Archavachotikul K, Ciccone TJ, Chinoy MR, Nielsen HC, Volpe MV. Pre- and postnatal lung development, maturation, and plasticity. Thyroid hormone affects embryonic mouse lung branching morphogenesis and cellular differentiation. Am J Physiol Lung Cell Mol Physiol 2002;282(3):L359–69.
[21] Ikegami M, Jobe AH, Pettenazo A, Seidner SR, Berry DD, Ruffini L. Effects of maternal treatment with corticosteroids, T_3, thyrotropin-releasing hormone and their combinations on lung function of ventilated preterm rabbits with and without surfactant treatments. Am Rev Respir Dis 1987;136(4):892–8.
[22] Ikegami M, Polk D, Tabor B, Lewis J, Yamada T, Jobe AH. Corticosteroid and thyrotropin-releasing hormone effects on preterm sheep lung function. J Appl Physiol 1991;70(5):2268–78.
[23] Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. N Engl J Med 2000;342(20):1500–7.
[24] Crowley P. Antenatal corticosteroid therapy: a meta-analysis of the randomized trials, 1972–1994. Am J Obstet Gynecol 1995;173(1):322–34.
[25] Crowley P. Prophylactic corticosteroids for preterm birth (Cochrane Review). The Cochrane Library, Issue 2. Chichester (UK): John Wiley & Sons, Ltd.; 2004.
[26] Collaborative Group on Antenatal Steroid Therapy. Effects of antenatal dexamethasone administration in the infant: long-term follow-up. J Pediatr 1984;104(2):259–67.
[27] MacArthur BA, Howie RN, Dezoete JA, Elkins J. School progress and cognitive development of 6-year-old children whose mothers were treated antenatally with betamethasone. Pediatrics 1982;70(1):99–105.
[28] Smolders-de Haas H, Neuvel J, Schmand B, Treffers PE, Koppe JG, Hoeks J. Physical development and medical history of children who were treated antenatally with corticosteroids to prevent respiratory distress syndrome: a 10- to 12-year follow-up. Pediatrics 1990;86(1):65–70.

[29] Schmand B, Neuvel J, Smolders-de Haas H, Hoeks J, Treffers PE, Koppe JG. Psychological development of children who were treated antenatally with corticosteroids to prevent respiratory distress syndrome. Pediatrics 1990;86(1):58–64.
[30] Howie RN, Liggins GC. Clinical trial of antepartum betamethasone therapy for prevention of respiratory distress in preterm infants. In: Anderson ABM, Beard RW, Brudenell JM, Dunn PM, editors. Preterm labor: proceeding of the Fifth Study Group of the Royal College of Obstetricians and Gynecologists. London: Royal College of Obstetricians and Gynecologists; 1977. p. 281–9.
[31] Gamsu HR, Mullinger BM, Donnai P, Dash CH. Antenatal administration of betamethasone to prevent respiratory distress syndrome in preterm infants: report of a UK multicentre trial. Br J Obstet Gynaecol 1989;96(4):401–10.
[32] Kari MA, Hallman M, Eronen M, Teramo K, Vitanen M, Koivisto M, et al. Prenatal dexamethasone in conjunction with rescue therapy of human surfactant-a randomized placebo controlled multicenter study. Pediatrics 1994;93(5):730–6.
[33] Ten Centre Study Group. Ten centre trial of artificial surfactant (artificial lung expanding compound) in very premature babies. BMJ 1987;294(6578):991–6.
[34] Corbet A, Bucciarelli R, Goldman S, Mammel M, Wold D, Long W, US Exosurf Pediatric Group I. Decreased mortality rate among small premature infants treated at birth with a single dose of synthetic surfactant: a multicenter controlled trial. J Pediatr 1991;118(2):277–84.
[35] Gilstrap LC, Christensen R, Clewell WH, et al. NIH Consensus Development Panel on the Effect of Corticosteroids for Fetal Maturation on Perinatal Outcomes. Effect of corticosteroids for fetal maturation on perinatal outcomes. JAMA 1995;273(5):413–8.
[36] Scientific Advisory Committee. Royal College of Obstetricians and Gynecologists. Antenatal corticosteroid administration reduces the incidence of neonatal respiratory distress syndrome. London: Royal College of Obstetricians and Gynecologists; 1992.
[37] Gilstrap LC, Clewell WH, D'Alton ME, et al. Antenatal corticosteroids revisited: repeat courses - National Institutes of Health Consensus Development Conference Statement, August 17–18, 2000. Obstet Gynecol 2001;98(1):144–50.
[38] American College of Obstetricians and Gynecologists. Antenatal corticosteroid therapy for fetal maturation. Committee Opinions 2002;273:9–11.
[39] Aghajafari F, Murphy K, Ohlsson A, Amankwah K, Matthews S, Hannah M. Multiple versus single courses of antenatal corticosteroids for preterm birth: a pilot study. J Obstet Gynaecol Canada 2002;24(4):321–9.
[40] Guinn D, Atkinson M, Sullivan L, Lee M, MacGregor S, Parilla B, et al. Single versus weekly courses of antenatal corticosteroids for women at risk of preterm delivery. JAMA 2001;286(13):1581–7.
[41] McEvoy C, Bowling S, Williamson R, Lozano D, Tolaymat L, Izquierdo L, et al. The effect of a single remote course versus weekly courses of antenatal corticosteroids on functional residual capacity in preterm infants: a randomized trial. Pediatrics 2002;110(2 part 1):280–4.
[42] Crowther CA, Harding J. Repeat doses of prenatal corticosteroids for women at risk of preterm birth for preventing neonatal respiratory disease (Cochrane Review). The Cochrane Library, Issue 2. Chichester (UK): John Wiley & Sons Ltd.; 2004.
[43] Jobe AH, Wada N, Berry LM, Ikegami M, Ervin MG. Single and repetitive maternal glucocorticoid exposure reduces fetal growth in sheep. Am J Obstet Gynecol 1998;178(5):880–5.
[44] Ikegami M, Jobe AH, Newnham J, Polk DH, Willet KE, Sly P. Repetitive prenatal corticosteroids improve lung function and decrease growth in preterm lambs. Am J Respir Crit Care Med 1997;156(1):178–84.
[45] Abbasi S, Hirsch D, Davis J, Tolosa J, Stouffer N, Debbs R, et al. Effects of single versus multiple courses of antenatal corticosteroids on maternal and neonatal outcome. Am J Obstet Gynecol 2000;182(5):1243–9.
[46] Modi N, Lewis H, Al-Naqeeb N, Ajayi-Obe M, Dore CJ, Rutherford M. The effects of repeated antenatal glucocorticoid therapy on the developing brain. Pediatr Res 2001;50(5):581–5.
[47] Dunlop SA, Archer MA, Quinlivan JA, Beazley LD, Newnham JP. Repeated prenatal corticosteroids delay myelination in the ovine central nervous system. J Matern Fet Med 1997;6(6):309–13.

[48] Banks BA, Cnaan A, Morgan MA, Parer JT, Merrill JD, Ballard PL, et al. Multiple courses of antenatal corticosteroids and outcome of premature neonates North American Thyrotopin-Releasing Hormone Study Group. Am J Obstet Gynecol 1999;181(3):709–17.
[49] French NP, Hagan R, Evans SF, Mullan A, Newnham JP. Repeated antenatal corticosteroids: effects on cerebral palsy and childhood behavior. Am J Obstet Gynecol 2004;190(3):588–95.
[50] Baud O, Foix-L'Helias L, Kaminski M, Audibert F, Jarreau P-H, Papiernik E, et al. Antenatal glucocorticoid treatment and cystic periventricular leukomalacia in very premature infants. N Engl J Med 1999;341(16):1190–6.
[51] Baud O. Antenatal corticosteroid therapy: benefits and risks. Acta Paediatr 2004;93(Suppl 444): 6–10.
[52] Christensen HD, Sienko AE, Rayburn WF, Gonzalez CL. A placebo-controlled comparison between betamethasone and dexamethasone to enhance lung maturation in the fetal mouse. J Soc Gynecol Invest 1997;4(3):130–4.
[53] Rayburn WF, Christensen HD, Gonzalez CL. A placebo-controlled comparison between beta-methasone and dexamethasone for fetal maturation: differences in neurobehavioral development in mice offspring. Am J Obstet Gynecol 1997;176(4):842–50.
[54] Jobe AH, Soll RF. Choice and dose of corticosteroid for antenatal treatments. Am J Obstet Gynecol 2004;190(4):878–81.
[55] Baud O, Laudenbach V, Laudenbach E, Laudenbach P, Gressens P. Neurotoxic effects of fluorinated glucocorticoid preparations on the developing mouse brain: role of preservatives. Pediatr Res 2001;50(6):706–11.
[56] Thebaud B, Lacaze-Moasmonteil T, Watterberg K. Postnatal glucocorticoids in very preterm infants: "the good the bad and the ugly"? Pediatrics 2001;107(2):413–5.
[57] Crowther CA, Alfirevic Z, Haslam RR. Thyrotropin-releasing hormone added to corticosteroids for women at risk of preterm birth for preventing neonatal respiratory disease (Cochrane Review). In: The Cochrane Library, Issue 2. Chichester (UK): John Wiley & Sons, Ltd.; 2004.
[58] Crowther CA, Hiller JE, Haslam RR, Robinson JS. Australian collaborative trial of antenatal thyrotropin-releasing hormone: adverse effects at 12 month follow-up. ACTOBAT Study Group. Pediatrics 1997;99(3):311–7.
[59] Briet JM, van Sonderen L, Buimer M, Boer K, Kok JH. Neurodevelopmental outcome of children treated with antenatal thyrotropin-releasing hormone. Pediatrics 2002;110(2 Part 1): 249–53.
[60] Gross I, Moya FR. Is there a role for antenatal TRH in the prevention of neonatal respiratory disorders. A meta-analysis. Sem Perinatol 2001;25:406–16.

ELSEVIER
SAUNDERS

Obstet Gynecol Clin N Am
31 (2004) 963–979

OBSTETRICS AND
GYNECOLOGY
CLINICS
OF NORTH AMERICA

Endocrinology of lactation

Catalin S. Buhimschi, MD

Yale University School of Medicine, Department of Obstetrics, Gynecology and Reproductive Sciences, 333 Cedar Street, New Haven, CT 06520, USA

Throughout human development, lactation proved to be of paramount importance to the survival, development, and growth of mammalian species. Parturition and lactation are two processes that are closely coordinated. Profound changes in several key hormones occur early in pregnancy and parturition; these prepare and assure milk production by the mammary gland after delivery. The word mammal comes from the Latin word "mamma" which means "resembling milk gland or breast." In the absence of successful lactation or appropriate human intervention, the process of reproduction is a failure because the neonate will not be able to survive. Breastfeeding has been embraced as a way to provide milk to the newborn but equally important, it creates a strong bonding experience between the mother and her newborn child [1].

The past 20 years were characterized by increased efforts to put forward a policy of breastfeeding; however, societal, cultural transformations, and world epidemics (eg, AIDS) created the context under which large groups of disadvantaged women—whose newborns would benefit most from breastfeeding—are less likely to initiate such behavior. The advantage of breastfeeding over bottle feeding continues to be a subject of debate; however, the benefits of breastfeeding are so well demonstrated that all obstetricians should continue to encourage such conduct if it is not contraindicated medically.

Mammary gland

Mammary gland: embryology

The mammary gland first begins to develop during the sixth week [2] within the ectodermal ridges that form the ventral surface of the embryo. From the

E-mail address: catalin.buhimschi@yale.edu

0889-8545/04/$ – see front matter
doi:10.1016/j.ogc.2004.08.002 obgyn.theclinics.com

multitude of ventral ectodermal buds, just two that are located in the pectoral region of the embryo continue to develop as mammary glands. The two mammary buds begin to grow and develop in utero and ultimately form 15 to 20 secondary buds that will provide the basis for the ductal system in the mature breast. Only the main ducts are formed at birth; the mammary gland remains underdeveloped until puberty [2].

Mammary gland: morphology

The breasts secrete milk and serve as accessory glands of the reproductive system. The left breast usually is a little bit larger than the right. Breasts are separated from the pectoral muscle by the pectoral fascia. Male and female humans have mammary glands, but in males they remain in a rudimentary state.

The mature mammary gland is composed of 15 to 25 lobes [3] that are separated by septa that emerge down from the fibrous tissue which covers the entire surface of the breast. Fatty tissue surrounds the surface of the breast and space between lobes; however, its abundance affects the shape and size of breasts. Lobes are distributed radially; each consists of several lobules that are made up of large numbers of sack like structures called ''alveoli.'' Alveoli join their ducts (intralobular ducts) to form a unique lobar duct, which, in turn, converges separately toward the nipple (lactiferous ducts). Before their opening into the nipple, the lactiferous ducts open in lactiferous sinuses (ampullae) that serve as small milk reservoirs during pregnancy. The nipple is surrounded by a specialized pigmented tissue—areola—that contains sweat and sebaceous glands (glands of Montgomery). The areola becomes important during puerperium; its glands secrete a characteristic fatty substance that protects and lubricates the nipple during lactation.

Histologically, the adult breast consists of glandular tissue that is surrounded by connective and adipose tissue (stroma) that is populated by blood vessels, nerves, and lymphatics. The structure of the mammary gland is simple and consistent with that of a compound tubulo-alveolar gland. The alveoli (approximately 0.2 mm in diameter) are arranged in lobuli (~10–100 alveoli per lobule). During pregnancy, the glandular tissue becomes developed under the influence of estrogens and progesterone and the intralobular ducts undergo rapid development to form buds that become alveoli. Each alveolus is surrounded by a mesh of myoepithelial tissue and a capillary network. The myoepithelial cells that surround the alveolus serve as milk ejectors during lactation.

The arterial supply of the breast consists of branches from axillary, intercostal, and thoracic vessels. Veins describe an anastomotic circle that surrounds the nipple which is called ''Haller circle.'' The lymphatics, for the most part, run along the inferior border of the pectoralis muscle toward the axillary lymphatic nodes. The lymphatics on the inner side of the breast perforate the intercostal space and drain lymph into anterior mediastinal lymphatic nodes. Nerves are derived from the anterior and lateral cutaneous nerves of the thorax. The nipple and the areola are innervated abundantly, which is in contrast with the rest of the

breast [3]. Free sensory endings are disseminated in the peripheral area of the breast and areola. Tactile corpuscles represent the main nerve supply of the areola and nipple and contribute to the great sensitivity of these areas to perceptible stimuli. The myoepithelial cells that surround the alveolus are not innervated.

The first significant postnatal change in the mammary gland alveolar system occurs at puberty. Growth of the milk-producing system is dependent on numerous hormonal stimuli; estrogens represent the major breast-growth factor at this age. Outside of pregnancy, the alveoli are small, solid, and filled with a mass of granular tissue. The final differentiation of the breast glandular tissue occurs during pregnancy [4] through the concerted effects of progesterone, estrogen, insulin, prolactin, thyroid hormones, and other multiple growth factors that impact on mammary gland growth and function. A new role for galanin, a hormone that augments mammary development during pregnancy in concert with prolactin, was reported recently [5].

Early in pregnancy, cells from the central part of the alveoli suffer fatty degeneration and are excreted in the first few days of postpartum as colostrum. Morphologic changes occur frequently as the periphery of the alveoli differentiate into secretor epithelium that consist of a single layer of alveolar cells. The glandular epithelium differs in morphology, depending on its secretor activity. Cells are columnar and tall when the alveolar lumen is empty and become flattened when the glandular lumen becomes filled with milk. During a state of activity, alveolar cells are synthesizing oil globules to be ejected into the lactiferous duct lumen as milk globules.

Mammogenesis/lactogenesis

The human mammary gland is a highly evolved skin gland, which is best described as an apocrine (ie, milk components are synthesized and secreted without destruction of the glandular cells) or merocrine (ie, the gland is repeatedly functional without cellular destruction) glandular structure. The main function of the mammary gland is milk production; it is one of a few human organs that undergoes repeated cycles of structural development, differentiation, and regression.

Two complex phenomena occur during pregnancy: mammogenesis and lactogenesis. Although before birth mammogenesis represents a structural developmental process, lactogenesis occurs most frequently following birth and represents the process of milk synthesis and secretion. Milk production is maintained until the neonate no longer needs it or until the factors that are involved in maintenance and stimulation of lactation no longer act to stimulate the gland. The mammary gland undergoes involution and the cycle can be repeated in a new reproductive cycle.

Mammogenesis is initiated early in pregnancy [3]. Starting at 6 to 8 weeks' gestation, the breasts increase in size and volume. Significant vascular changes occur in parallel as part of the preparative phenomenon. The nipples become enlarged, pigmented, and erectile. In the second trimester, multiplicative

processes dominate with a progressive increase in the size of the lobules [6]. This cellular hyperplasia is replaced by cellular hypertrophy. Halfway through pregnancy, proliferation of the alveolar epithelium ceases; this process is reflected as a marked decrease in the number of cell mitosis. Following this, the alveolar tissue begins to differentiate into a secretory epithelium. An increased amount of Golgi apparatus and rough endoplasmic reticulum also reflects an increased secretory state. Toward the end of gestation, the alveoli begin to fill up with amorphous material that consists of proteins, desquamated cells, and leukocytes. The stromal structure is replaced almost entirely by glandular tissue before the delivery of the fetus.

Lactogenesis is entirely hormonal dependent. Following delivery, the number and size of the intracellular secretor organelles increase rapidly to assure full development of the mammary gland in just a few days [7]. The alveoli distend with milk, whereas the lumen becomes flattened. The epithelium regains its shape following emptying of the breast. Vascular regulation of the milk volume and quality is essential because milk production can vary between 500 mL and 600 mL per day.

Hormonal regulation of lactogenesis

Our knowledge about mammogenic/lactogenic hormones and the minimal requirements to assure success of the reproductive process were described in human and animals [8,9]. The endocrine control of lactation is one of the most complex physiologic mechanisms of human reproduction. Mammogenesis, lactogenesis, galactopoiesis (maintenance of lactation), and galactokinesis (extrusion of milk from the mammary gland) are all essential to assure proper lactation.

Although the process starts early in life, mammogenesis continues and reaches its maximum only at puberty. The initial growth of the ductal system is dependent on estrogens, whereas growth hormones and cortisol have a synergistic effect [10]. Pregnancy continues to be a critical time because progesterone, estrogen, cortisol, placental lactogen, and insulin should prepare the gland for lactation and they should act in concert and proper sequence. The development of the tubuloalveolar system requires prolactin, estrogen, and progesterone [11]. The synthesis of milk proteins (casein and lactalbumin) is regulated principally by prolactin, but is facilitated by growth hormones, cortisol, and insulin [12].

Mammogenic/lactogenic hormones

Prolactin: a reproductive hormone

Prolactin is the key hormone of lactation [8]. Its structure resembles that of a single polypeptide that contains 198 amino acid residues and has a molecular weight of approximately 22 kd. A high degree of similarity between the structure of prolactin, human growth hormone (hGH), and placental lactogen is well-recognized [8].

Owerbach et al [13] reported that the human prolactin gene is localized on chromosome 6. Prolactin, growth hormone, and chorionic somatomammotropin form a set of hormones that is believed to have evolved from a common ancestral gene [14]. This assumption is based on several lines of evidence: (1) overlap in their biologic and immunologic properties, (2) similarities in their amino acid sequences, and (3) homologies in the nucleic acid sequences of their structural genes. As a corollary to these hypotheses, Cooke et al [14] speculated that the chromosomal segregation of human prolactin and hGH occurred about a million years ago and that growth hormone and chorionic somatomammotropin underwent an intrachromosomal recombination within the last 10 million years.

Human prolactin is synthesized and released by the adenohypophysis lactotroph cells [15]. Ultrastructural immunohistochemistry studies demonstrated that human pituitary gland lactotroph cells represent approximately 32% to 55% of the total pituitary cell population [15]. During gestation, the human adenohypophysis gland enlarges to reach volumes that are almost double that of its normal, nonpregnant size. An intense hyperplastic and hypertrophic process of the lactotroph population apparently is responsible for such a dramatic increase in volume [16]. Marked differences in the proliferative ability of the lactotroph population was demonstrated most recently in rodents. Using immunostaining techniques for prolactin and 5-bromo-2′-deoxyuridine, Yin and Arita [17] demonstrated marked variation in proliferative activity of the pituitary lactotroph cell population; maximal proliferative capacity was demonstrated between the day of parturition and Day 2 of lactation. Future studies need to confirm similar findings in humans.

Following its synthesis, prolactin is stored in cytoplasmic secretory granules until its systemic delivery. The secretor mechanism of prolactin is complex because it is under direct control of a dual regulatory system that involves inhibitory and stimulatory prolactin factors. A variety of hypothalamic prolactin-inhibitory factors (dopamine, γ-aminobutyric acid [GABA] system) act in concert with hypothalamic prolactin-releasing factors (thyrotropin, vascular intestinal peptide, angiotensin II) [8]. Other factors (eg, oxytocin, serotonin, opioids, histamine, substance P, arginine-leucine) modulate prolactin release by means of an autocrine/paracrine mechanism, whereas estrogen and progesterone hormones can act at hypothalamic and adenohypophysial levels.

Although the prolactin gene is programmed genetically to respond specifically to many endocrine factors, estrogens remain the major transcriptional modulators—by way of estrogen responsive elements—in the prolactin gene [18,19]. Transcription of the prolactin gene is regulated primarily by the pituitary specific transcriptional factor, Pit-1 [20]. Pit-1 activates and regulates transcription of prolactin and the growth hormone gene. Gene activation by estrogen requires interaction with Pit-1, thyroid-releasing hormone, and other growth factors. Prolactin increases during pregnancy in primates with the increasing levels of placental progesterone; the mechanism of action through which progesterone exerts its action remains to be discovered. Progesterone receptors are not found in

pituitary lactotrophs only in gonadotroph cell population [21,22]. Studies that were conducted by Rakoff and Yen [21] and Sprangers et al [22] put forth the hypothesis that the effect of progesterone on prolactin is mediated through a reduction of hypothalamic dopamine.

Dopamine is a small, simple molecule that fulfills diverse functions within the brain. The role of hypothalamic dopaminergic neurons in modulating the secretor activity of prolactin is well-demonstrated [23]. In the cerebrum, dopamine acts as a classic neurotransmitter and reaches the pituitary gland by way of the hypophysial portal blood system that surrounds several hypothalamic nerve tracts and whose activity is regulated by prolactin, estrogens, several neuropeptides, and several neurotransmitters. Dopamine binds to type-2 dopamine receptors (the predominant pituitary dopamine receptor), which are linked functionally to membrane channels and G-proteins, which, in turn, suppress the high intrinsic secretor activity of the pituitary lactotrophs [24]. Administration of L-dopa (a dopamine antagonist) is followed rapidly by a decrease in prolactin levels [25]. This inhibitory effect is followed by a rebound in prolactin secretion; this suggests that dopamine mostly has a secretory inhibitor effect and does not alter prolactin synthesis. In addition to inhibiting prolactin release, dopamine activates several interacting intracellular signaling pathways and suppresses prolactin gene expression and lactotroph proliferation. Several lines of research confirmed that in addition to dopamine, GABA may exhibit a significant inhibitory role over prolactin secretion [8,26].

To exert its function, prolactin should act in concert with placental lactogen and placental growth hormones; they are all lactogenic hormones [27,28]. The role that is played by the growth hormones has not been established firmly because development of the mammary gland remains intact in the congenital absence of growth hormones. Recently, investigators evaluated how growth factors interact to regulate estradiol action on lactotroph cell proliferation and the regulatory role of transforming growth factor–β-related peptides and estradiol action on lactotropes [29]. These studies suggest that transforming growth factor–β and fibroblast growth factor interact to facilitate the communication between lactotropes and folliculostellate cells that are necessary for the mitogenic action of estradiol [29]. Although not part of the lactogen hormone family, thyroid hormones also are required to assure successful activity of prolactin [30,31]. Further studies remain to identify the role, if any, that is played by a newly-described brain neuropeptide, galanin. Studies in rodents found that galanin is a potent lactotroph; its immunoneutralization inhibited the mitogenic effect of the lactotroph [32]. Future studies remain to confirm galanin's role on human lactation.

Prolactin: during pregnancy

Serum prolactin begins to increase during first trimester of human pregnancy—most probably as a reflection of hypertrophy and hyperplasia of the lactotrophs [8]. During pregnancy, prolactin levels increase linearly under direct

estrogen stimulation and reach levels that are almost 10 times higher than those of nonpregnant women [33,34].

Heterogeneity of the human prolactin molecule was described and explains the marked variation between clinical findings and radioimmunoassayable serum prolactin levels [35]. Earlier studies demonstrated marked differences between immunoactivity and bioactivity that occurred, secondary to various ratios between glycosylated and nonglycosylated forms of prolactin [8,36]. During pregnancy, there is a shift between different prolactin isoforms, with predominance of the bioactive, nonglycosylated isoforms over the less biologically active, glycosylated isoforms [37]. Whether this shift has any biologic significance continues to be under scientific scrutiny. The metabolic effect of prolactin, specifically its anti-insulin role, combined with the catabolic role of cortisol, may serve as part of the complex, integrated endocrine control of metabolism during pregnancy.

Prolactin secretion in the puerperium period follows a remarkable multi-phasic pattern, which was not identified in patients who underwent an elective cesarean section without labor [38]. There is a highly significant decline in prolactin levels during active labor which reaches a nadir about 2 hours before delivery [38]. Immediately after delivery, a surge of prolactin occurs and it reaches peak levels within 2 hours postpartum. Thereafter, prolactin levels decrease and reach a second nadir approximately 9 hours postpartum; this low level is maintained for a variable period of time up to 24 hours following delivery. This multi-phasic pattern of prolactin secretion is not correlated with changes in serum concentrations of cortisol, estrogen (estradiol, estrone), or progesterone. Prolactin levels in all pregnant patients at term were unaffected by the administration of synthetic narcotic analgesic agents, anesthesia, or operative stress. Instead, the rapid decline in prolactin levels during active labor may be explained by a rapid and dramatic release of oxytocin, which was shown to inhibit prolactin release at the pituitary level [39].

Studies in vitro and in vivo established that morphogenesis and preparation of the alveolar epithelial cells require a fine interplay between prolactin and several steroid and polypeptide hormones [40]. Although the ovarian and adrenal steroids favor mammogenic processes, the combination of adrenal steroids (cortisol) and prolactin is essential for lactation.

During pregnancy, although lactation is virtually absent, the increased levels of prolactin, placental lactogen, estrogen, and progesterone favor the development of the milk secretor alveolar apparatus. This inhibitory phenomenon probably is mostly attributable to progesterone [41]. First, progesterone has an inhibitory effect over prolactin's ability to up-regulate its own receptors [41]. Second, because most of the progesterone receptors are localized within the mammary glandular epithelium, the modulator effect of progesterone over the estrogen receptor seems to be restricted to the epithelial component of the mammary gland. During pregnancy and lactation, major mammary gland developmental changes occur within the alveolar epithelium. Estrogen/progesterone receptor modulation may serve a regulatory function within the mammary gland

[42]. Furthermore, there is an inhibitory effect of progesterone against cortisol-induced stimulation of casein accumulation in the mammary cells [43].

Isolated prolactin deficiency as a clinical entity was reported by Turkington [44]. Such a clinical entity may be an autosomal recessive trait. The affected women generally are healthy but are unable to nurse following parturition and have no detectable prolactin secretion after stimulation with phenothiazine [45]. Kauppila et al [46] reported a woman who had puerperal alactogenesis who, despite undetectable immunoactive serum prolactin measurements, conceived normal pregnancies without benefit of ovulation-inducing medications.

Placental lactogen and growth hormones

The human placenta performs various and important functions for the maintenance of pregnancy, development of the fetus, and initiation and preservation of lactogenesis. Probably, its secretor diversity surpasses any of the other endocrine organs. The placenta is provided with precursors of hormones by the mother as well as by the fetus. The placenta synthesizes and secretes steroids, protein hormones, growth factors, cytokines, and coagulation factors. During pregnancy—and with special relevance for lactation—human placental lactogen or human chorionic somatomammotropin can substitute for pituitary prolactin and growth factors [3]. Prolactin, growth hormones, and placental lactogen can act as circulating hormones or as paracrine/autocrine factors to stimulate or inhibit various stages of formation and remodeling of new blood vessels, including endothelial cell proliferation, migration, protease production, and apoptosis [47].

The human chorionic somatomammotropin is a single-chain polypeptide of 191 amino acids that shares 85% amino acid homology with hGH [48]. Both hormones are related more closely to each other than to human prolactin, with which they share only 35% amino acid identity [49]. The genes for human chorionic somatomammotropin and hGH are clustered on chromosome 17 [50]. Josimovich and MacLaren [51] first identified this hormone in peripheral maternal serum and named it ‘‘placental lactogen’’ because it promoted milk production by the pseudo-pregnant rabbit mammary gland. Its lactogenic properties in humans remain to be established [8,52].

The syncytiotrophoblastic layer of the placenta secretes human chorionic somatomammotropin. Its membrane receptors have binding and molecular characteristics that are similar to lactogenic receptors from other mammalian species [53]; however, measurements of hormone concentrations in the plasma of pregnant animals show considerable differences in the pattern of secretion of lactogenic hormones among species. In humans, serum levels of human placental lactogen increase gradually as pregnancy progresses and reach peak levels during the last 4 weeks of gestation [54]. The timing of progesterone withdrawal correlates well with lactogenesis, but species differ in their mechanisms at parturition. The mean and range of plasma levels of human placental lactogen at 24 hours after delivery were within the range of levels that were found in nonlactating women [55].

The mammary gland is subjected to major morphologic and biochemical changes during the lactation cycle. An important regulatory role is played by growth factors that modulate cell survival (epidermal growth factor, amphiregulin, transforming growth factor–α, insulin like growth factor, tumor necrosis factor–α) or apoptosis (tumor necrosis factor–α, transforming growth factor–β) of the mammary cells. The importance of insulin and insulin growth factors in the support of lactogenesis and lactation was proposed previously [56]. Insulin growth factor–I and –II stimulate casein synthesis and exert a significant augmentation of prolactin-induced lactogenesis [57].

Current evidence strongly suggests that human placental lactogen and growth factor hormones may play a pivotal role during pregnancy and act to regulate and coordinate growth and metabolism of the mother and its fetus [58]. The correlation between the molecular, functional evolution, and metabolic role of prolactogen hormones is inconsistent. It was suggested that human placental lactogen is the ''growth hormone'' of pregnancy [59,60]. Grumbach [61] proposed that human chorionic somatomammotropin exerts its catabolic effect on the mother, and thus, supports the growing fetus. Because the overall metabolic role of human placental lactogen is anti-insulinic, an enhanced elevation in plasma free fatty acids and impaired glucose uptake was recognized previously [62]. Such actions ensure a steady supply of glucose toward the fetus, and thus, its growth. Metabolic hormones, whose main role is to regulate metabolic responses to nutrient intake or stress, often have direct effects on the mammary gland as well.

Galactopoiesis

Galactopoiesis is supported by prolactin and cortisol, which act directly on enzyme activities and differentiation of the alveolar cells [63]. Milk synthesis begins in the second half of pregnancy; however, the full onset of milk production takes place during the first 4 days postpartum and involves a carefully programmed set of changes in milk volume and its composition. Galactopoiesis usually is measured by the output of milk carbohydrates or the most abundant milk proteins—lactoglobulin and casein [3]. The molecular mechanisms by which prolactin regulates milk protein synthesis are the subject of numerous studies; however, the specific mechanisms through which progesterone and milk removal interact with the mammary epithelial cell at parturition have not been studied. Such studies may have been hampered by the lack of an in vitro model system that could mimic lactogenesis or because of the complexity of the changes that must be coordinated during this process [64]. The evidence suggests that the abrupt progesterone withdrawal at the time of parturition removes the inhibitory effect of progesterone on the production of lactalbumin and provides the trigger for lactogenesis in the presence of high plasma concentrations of prolactin and adequate plasma concentrations of cortisol [65].

Following delivery, estrogen and progesterone levels decrease rapidly [66]; however, this withdrawal creates the context under which the unopposed pro-

lactin autoregulatory mechanism up-regulates its own receptors [67]. Although prolactin seems to be the single most important galactopoietic hormone, prolactin levels are not elevated during lactation and there is solid evidence to suggest that approximately 2 to 3 weeks postpartum the serum prolactin levels reach the upper level of normal for nonpregnant woman [33]. By 15 weeks postpartum, most nonbreastfeeding mothers had resumed ovulation and menstruation. The mean time to first ovulation was approximately 36 weeks (range, 15 to 66 weeks) [68]. The importance of prolactin in galactopoiesis is proved because elective inhibition of prolactin by a long-acting bromocriptine inhibited lactation [69]. Prolactin can be identified in epithelial cells of the lactating gland as well as in the milk [70,71]. There is little doubt that a portion of the prolactin that is found in the milk originates in the pituitary lactotroph population. Apparently, prolactin reaches the milk by way of the mammary epithelial basal membrane [72], and ultimately, is responsible for activation of the two milk proteins genes—α-lactoglobulin and β-casein [73].

It is well-accepted that pituitary lactotrophs have spontaneous secretor activity [67]. Therefore, the pituitary is under predominant dopamine inhibitory hypothalamic effect secondary to its release into the hypophysial portal system [74]. Powerful evidence suggests that suckling and increased levels of estrogens are the most important physiologic stimuli for prolactin release [75,76]. After delivery of the fetus and placenta, the estrogen levels decline; therefore, frequent suckling is necessary to maintain the elevated prolactin levels that are critical for adequate galactopoiesis.

The prolactin response to suckling was studied during the first week of the puerperium by Howie and his collaborators [77]. Mean basal levels of prolactin showed no significant change during the first week postpartum; however, there were progressive increases in the maximum suckling-induced response, which reached peak values on the fourth day after delivery. Large variations in basal prolactin levels, peak suckling-induced response, and total prolactin response were reported between individuals. Much less variability was recorded within individuals between consecutive feeds.

Oxytocin and prolactin responses to suckling were evaluated extensively by McNeilly et al [78]; however, the control of prolactin release remains enigmatic. The finding that suckling is associated with increased prolactin levels sparked numerous ideas and controversy concerning the responsible mechanism [79]. Although oxytocin is released in a pulsatile manner during suckling, the response is not related to milk volume, parity, or intensity of prolactin response to breastfeeding. Although there is ample evidence to suggest that stimulation of the nipple that is associated with suckling is a strong impulse for prolactin release, oxytocin is not considered to be the mediator for such a reaction [75]. It is well-known that dopamine inhibits prolactin release, whereas mechanical stimulation during suckling releases this inhibitory influence [75]. The release of prolactin is induced most probably by way of a neurogenic pathway from nipple to hypothalamus and is proportionate to the length of nursing and to the intensity of the stimulus. There is strong evidence to support catecholamine/serotonin control

of prolactin release and the influence of changes in hypothalamic dopamine turnover [75,80].

Failure of early removal of colostrum from the breast is associated with high milk sodium and poor prognosis for successful lactation in many women. Some investigators hypothesized that this problem may result from accumulation of inhibitory factors in the mammary alveolus that inhibit lactogenesis, even when there are appropriate hormonal changes after parturition [81]. Lactation suppression occurs naturally when the stimulation of suckling is absent. Physiologically, the suckling-induced increment in plasma prolactin diminishes with advancing lactation, most probably secondary to a gradual decrease in the number of nursing episodes. The postpartum decrease in serum prolactin is quicker among women who breastfeed less frequently [82]. Because serum prolactin levels return to normal ranges within 6 months postpartum among women who breastfed their children one to three times a day, elevated prolactin levels could be maintained only if the baby is nursed at least six times a day.

Recent studies demonstrated that overweight/obese women had a lower prolactin response to suckling . This would be expected to compromise the ability of overweight/obese women to produce milk and, over time, could lead to premature cessation of lactation. These findings are important because just before and after lactogenesis—the time of onset of copious milk secretion—the prolactin response to suckling is more important for milk production than it is later in lactation [83].

Increased attention has been paid to drugs that can modulate prolactin release during lactation, and thus, stop, initiate, or maintain appropriate galactopoiesis. Galactogogues are medications that aid in initiating and maintaining adequate milk production. Most exert their pharmacologic effects through interactions with dopamine receptors and result in increased prolactin levels, and thereby, augment the milk supply [84]. Metoclopramide remains the galactogogue of choice because of its documented record of efficacy and safety in women and infants. Studies that were conducted by Kauppila et al [85] demonstrated that metoclopramide is useful in the treatment of deficient puerperal lactation and does not stimulate the pituitary lactotropes or thyrotrophs of the nursing infants. Similarly, thyrotropin-releasing hormone improved defective lactation [86]. Pharmacologic manipulation of serum prolactin levels is as well achieved by bromoergocriptine, L-dopa, thyrotropin-releasing hormone, or phenothiazine administration [87,88].

Galactokinetic hormones

Milk accumulation in the alveoli and lactiferous sinuses cannot flow passively into the ductus. The importance of the endocrine milieu in lactation is emphasized clearly based on the fact that the myoepithelial cells that surround the alveolus are not innervated.

Oxytocin

Oxytocin is the most powerful galactokinetic hormone [3]. Haeger and Jacobsohn [89] emphasized the important role that is played by oxytocin in the process of milk ejection. High-affinity binding sites for oxytocin were demonstrated in particulate fractions from mammary glands; this confirmed that similar to myometrial cells, alveolar myoepithelial cells contain oxytocin receptors [90]. Studies in rodents suggest that there is a gradual increase in the mammary gland oxytocin receptors that seems to be gestational dependent [91]. In the mammary glands, the increase is gradual and reaches a maximum in the first week postpartum [92].

Oxytocin is the most abundant neuropeptide in the hypothalamus. Release of oxytocin into the vicinity of the long portal vessels that connect the hypothalamus with the anterior pituitary gland and the presence of short portal vessels that connect the posterior lobe to the anterior pituitary, established the potential for the peptide to act in a neuroendocrine fashion to control the release of one or several adenohypophysial hormones. Oxytocin receptors are present in the gland; numerous trophic effects of the peptide were described, some with apparent physiologic relevance. Thus, under defined physiologic conditions, a participatory role for oxytocin in the physiologic regulation of at least two hormones—prolactin and adrenocorticotropin—has been shown [93].

A detailed secretory profile of oxytocin during suckling and parturition showed that the sensory nerve endings that mediate the afferent path of the neuroendocrine reflex lie in the peripheral area of the breast and areola [94]. Contrary to expectation. plasma oxytocin concentrations increased minutes before suckling began [67]. These results clearly indicate that the milk ejection reflex—with release of oxytocin—occurs in most women before the tactile stimulus of suckling. A second release of oxytocin follows in response to the suckling stimulus. Oxytocin seems to be released for only several minutes which is followed by a abrupt decrease approximately 20 minutes following the initiation of nipple stimulation [95].

Oxytocin release is essential for milk removal because only the mechanical forces of suckling can extract the milk, which is stored in the lactiferous sinuses under the areola. Removal of milk from the alveoli is necessary for continuation of milk secretion. The flattened alveolar cells cease their milk production. Thus, frequent suckling, coupled with adequate milk evacuation, represent the most important stimuli for milk production.

The importance of oxytocin as a galactokinetic hormone was demonstrated most recently by experiments that were conducted in knockout mice. Mice that lacked oxytocin were viable and fertile. Female mice that lacked oxytocin had no obvious deficits in fertility or reproduction, including gestation and parturition. Although oxytocin-deficient females demonstrated normal maternal behavior, all offspring died shortly after birth because of the dam's inability to nurse. Postpartum administration of oxytocin restored milk ejection and rescued the offspring [96].

Summary

The endocrine control of lactation is one of the most complex physiologic mechanisms of human parturition. Mammogenesis, lactogenesis, galactopoiesis, and galactokinesis are essential to assure proper lactation. Prolactin is the key hormone of lactation and seems to be the single most important galactopoietic hormone. Oxytocin, serotonin, opioids, histamine, substance P, and arginine-leucine modulate prolactin release by means of an autocrine/paracrine mechanisms, whereas estrogen and progesterone hormones can act at hypothalamic and adenohypophysial levels. Human placental lactogen and growth factors may play essential roles in successful lactation during pregnancy. Oxytocin is the most powerful galactokinetic hormone.

References

[1] Holman DJ, Grimes MA. Patterns for the initiation of breastfeeding in humans. Am J Hum Biol 2003;15:765–80.
[2] Moore KL. The integumentary system. The skin, the cutaneous appendages, and the teeth. In: Moore KL, editor. The developing human. Clinically oriented embryology. 3rd edition. Philadelphia: W.B. Saunders; 1982. p. 432–46.
[3] Fuchs AR. Physiology and endocrinology of lactation. In: Gabbe SG, Niebyl JR, Simpson JL, editors. Obstetrics, normal & problem pregnancies. 1st edition. New York: Churchill Livingstone; 1986. p. 549–77.
[4] Glass RH, Kase NG. The breast. In: Speroff L, Glass RH, Kase NG, editors. Clinical gynecologic endocrinology and infertility. 6th edition. Philadelphia: Lippincott Williams and Wilkins; 1999. p. 595–641.
[5] Naylor MJ, Ginsburg E, Iismaa TP, Vonderhaar BK, Wynick D, Ormandy CJ. The neuropeptide galanin augments lobuloalveolar development. J Biol Chem 2003;278:29145–52.
[6] Ferguson DJP, Anderson TJ. A morphologic study of the changes which occur during pregnancy in the human breast. Virchows Arch Pathol Anat 1983;401:163–75.
[7] Tobon H, Salazar H. Ultrastructure of the human mammary gland. Postpartum lactogenesis. J Clin Endocrinol Metabol 1975;40:834–44.
[8] Jaffe RB, Yuen BH, Keye WR, Midgley Jr AR. Physiologic and pathologic profiles of circulating hormone prolactin. Am J Obstet Gynecol 1973;117:757–73.
[9] Soares MJ, Talamantes F. Gestational effects on placental and serum androgen, progesterone and prolactin-like activity in the mouse. J Endocrinol 1982;95:29–36.
[10] Yen SS, Jaffe RB. Prolactin in human reproduction. In: Yen SC, Jaffe RB, Barbieri RL, editors. Reproductive endocrinology. Physiology, pathophysiology and clinical management. 4th edition. Philadelphia: W.B. Saunders; 1999. p. 257–83.
[11] Lyons WR. Hormonal synergism in mammary growth. Proc R Soc Lond 1958;149:303–25.
[12] Topper YJ, Freeman CS. Multiple hormone interaction in the developmental biology of the mammary gland. Physiol Rev 1980;60:1049–106.
[13] Owerbach D, Rutter WJ, Cooke NE, Martial JA, Shows TB. The prolactin gene is located on chromosome 6 in humans. Science 1981;212:815–6.
[14] Cooke NE, Coit D, Shine J, Baxter JD, Martial JA. Human prolactin. cDNA structural analysis and evolutionary comparisons. J Biol Chem 1981;256:4007–16.
[15] Lloyd RV, Anagnostou D, Cano M, Barkan AL, Chandler WF. Analysis of mammosomatotropic cells in normal and neoplastic human pituitary tissues by the reverse hemolytic plaque assay and immunocytochemistry. J Clin Endocrinol Metab 1988;66:1103–10.

[16] Goluboff LG, Ezrin C. Effect of pregnancy on the somatotroph and the prolactin cell of the human adenohypophysis. J Clin Endocrinol Metab 1969;29:1533–8.
[17] Yin P, Arita J. Differential regulation of prolactin release and lactotrope proliferation during pregnancy, lactation and the estrous cycle. Neuroendocrinology 2000;72:72–9.
[18] Pantic VR. Estrogen as modulator of genes expression involved in control of reproduction. Ital J Anat Embryol 2001;106:223–32.
[19] Anderson I, Gorski J. Estrogen receptor alpha interaction with estrogen response element half-sites from the rat prolactin gene. Biochemistry 2000;39:3842–7.
[20] Nelson C, Albert VR, Elsholtz HP, Lu LI, Rosenfeld MG. Activation of cell-specific expression of rat growth hormone and prolactin genes by a common transcription factor. Science 1988; 239:1400–5.
[21] Rakoff JS, Yen SS. Progesterone induced acute release of prolactin in estrogen primed ovariectomized women. J Clin Endocrinol Metab 1978;47:918–21.
[22] Sprangers SA, Brenner RM, Bethea CL. Estrogen and progestin receptor immunocytochemistry in lactotropes versus gonadotropes of monkey pituitary cell cultures. Endocrinology 1989;124: 1462–70.
[23] MacLeod RM, Lehmeyer JE. Studies on the mechanism of the dopamine-mediated inhibition of prolactin secretion. Endocrinology 1974;94:1077–85.
[24] Ben-Jonathan N, Hnasko R. Dopamine as a prolactin (PRL) inhibitor. Endocr Rev 2001;22: 724–63.
[25] Leblanc H, Lachelin GC, Abu-Fadil S, Yen SS. Effects of dopamine infusion on pituitary hormone secretion in humans. J Clin Endocrinol Metab 1976;43:668–74.
[26] Lux-Lantos V, Becu-Villalobos D, Bianchi M, Rey-Roldan E, Chamson-Reig A, Pignataro O, et al. GABA(B) receptors in anterior pituitary cells. Mechanism of action coupled to endocrine effects. Neuroendocrinology 2001;73:334–43.
[27] Rosen JM, Humphreys R, Krnacik S. The regulation of mammary gland development by hormones, growth factors and oncogenes. Prog Clin Biol Res 1994;387:95–111.
[28] Lee PA, Xenakis T, Winer J, Matsenbaugh S. Puberty in girls: correlation of serum levels of gonadotropins, prolactin, androgens, estrogens and progestins with physical changes. J Clin Endocrinol Metab 1976;43:775–84.
[29] Hentges S, Sarkar DK. Transforming growth factor-beta regulation of estradiol-induced prolactinomas. Front Neuroendocrinol 2001;22:340–63.
[30] Capuco AV, Kahl S, Jack LJ, Bishop JO, Wallace H. Prolactin and growth hormone stimulation of lactation in mice requires thyroid hormones. Proc Soc Exp Biol Med 1999;22:345–51.
[31] Motil KJ, Thotathuchery M, Montandon CM, Hachey DL, Boutton TW, Klein PD, et al. Insulin, cortisol and thyroid hormones modulate maternal protein status and milk production and composition in humans. J Nutr 1994;124:1248–57.
[32] Cunnningham MJ, Krasnow SM, Gevers EF, Chen P, Thompson CK, Robinson IC, et al. Regulation of galanin-like peptide gene expression by pituitary hormones and their downstream targets. J Neuroendocrinol 2004;16:10–8.
[33] Rigg LA, Lein A, Yen SS. Pattern of increase in circulating prolactin levels during human gestation. Am J Obstet Gynecol 1977;129:454–6.
[34] Andersen JR. Prolactin in amniotic fluid and maternal serum during uncomplicated human pregnancy. Dan Med Bull 1982;29:266–74.
[35] Farkouh NH, Packer MG, Frantz AG. Large molecular size prolactin with reduced receptor activity in human serum: high proportion in basal state and reduction after thyrotropin-releasing hormone. J Clin Endocrinol Metab 1979;48:1026–32.
[36] Haro LS, Lee DW, Singh RN, Bee G, Markoff E, Lewis UJ. Glycosylated human prolactin: alterations in glycosylation pattern modify affinity for lactogen receptor and values in prolactin radioimmunoassay. J Clin Endocrinol Metab 1990;71:379–83.
[37] Markoff E, Lee DW, Hollingsworth DR. Glycosylated and nonglycosylated prolactin in serum during pregnancy. J Clin Endocrinol Metab 1988;67:519–23.
[38] Rigg LA, Yen SS. Multiphasic prolactin secretion during parturition in human subjects. Am J Obstet Gynecol 1977;128:215–8.

[39] Samson WK, Lumpkin MD, McCann SM. Evidence for a physiological role for oxytocin in the control of prolactin secretion. Endocrinology 1986;119:554–60.
[40] Ganguly R, Ganguly N, Mehta NM, Banerjee MR. Absolute requirement of glucocorticoid for expression of the casein gene in the presence of prolactin. Proc Natl Acad Sci USA 1980;77: 6003–6.
[41] Djiane J, Durand P. Prolactin-progesterone antagonism in self regulation of prolactin receptors in the mammary gland. Nature 1977;266:641–3.
[42] Haslam SZ, Shyamala G. Relative distribution of estrogen and progesterone receptors among the epithelial, adipose, and connective tissue components of the normal mammary gland. Endocrinology 1981;108:825–30.
[43] Ganguly R, Majumder PK, Ganguly N, Banerjee MR. The mechanism of progesterone-glucocorticoid interaction in regulation of casein gene expression. J Biol Chem 1982;257: 2182–7.
[44] Turkington RW. Phenothiazine stimulation test for prolactin reserve: the syndrome of isolated prolactin deficiency. J Clin Endocr 1972;34:247–9.
[45] Falk RJ. Isolated prolactin deficiency: a case report. Fertil Steril 1992;58:1060–2.
[46] Kauppila A, Chatelain P, Kirkinen P, Kivinen S, Ruokonen A. Isolated prolactin deficiency in a woman with puerperal alactogenesis. J Clin Endocr Metab 1987;64:309–12.
[47] Corbacho AM, Martinez De La Escalera G, Clapp C. Roles of prolactin and related members of the prolactin/growth hormone/placental lactogen family in angiogenesis. J Endocrinol 2002;173: 219–38.
[48] Li CH, Dixon JS, Chung D. Primary structure of the human chorionic somatomammotropin (HCS) molecule. Science 1971;173:56–8.
[49] Niall HD, Hogan ML, Sauer R, Rosenblum IY, Greenwood FC. Sequences of pituitary and placental lactogenic and growth hormones: evolution from a primordial peptide by gene reduplication. Proc Natl Acad Sci USA 1971;68:866–70.
[50] Owerbach D, Rutter WJ, Martial JA, Baxter JD, Shows TB. Genes for growth hormone, chorionic somatommammotropin, and growth hormones-like gene on chromosome 17 in humans. Science 1980;209:289–92.
[51] Josimovich JB, MacLaren JA. Presence in human placenta and term serum of highly lactogenic substance immunologically related to pituitary growth hormone. Endocrinology 1962;71: 209–20.
[52] West CP, McNeilly AS. Hormonal profiles in lactating and non-lactating women immediately after delivery and their relationship to breast engorgement. Br J Obstet Gynaecol 1979;86: 501–6.
[53] Ormandy CJ, Clarke CL, Sutherland RL. Solubilization and characterization of a lactogenic receptor from human placental chorion membranes. J Cell Biochem 1990;43:1–15.
[54] Kaplan SL, Gurpide E, Sciarra JJ, Grumbach MM. Metabolic clearance rate and production rate of chorionic growth hormone-prolactin in late pregnancy. J Clin Endocrinol Metab 1968;28: 1450–60.
[55] West CP, McNeilly AS. Hormonal profiles in lactating and non-lactating women immediately after delivery and their relationship to breast engorgement. Br J Obstet Gynaecol 1979;86: 501–6.
[56] Lamote I, Meyer E, Massart-Leen AM, Burvenich C. Sex steroids and growth factors in the regulation of mammary gland proliferation, differentiation, and involution. Steroids 2004;69: 145–59.
[57] Duclos M, Houdebine LM, Djiane J. Comparison of insulin-like growth factor 1 and insulin effects on prolactin-induced lactogenesis in the rabbit mammary gland in vitro. Mol Cell Endocrinol 1989;65:129–34.
[58] Handwerger S. Clinical counterpoint: the physiology of placental lactogen in human pregnancy. Endocr Rev 1991;12:329–36
[59] Grumbach MM, Kaplan SL, Sciarra JJ, Burr IM. Chorionic growth hormone-prolactin (CGP): secretion, disposition, biologic activity in man, and postulated function as the ''growth hormone'' of the 2d half of pregnancy. Ann N Y Acad Sci 1968;5(148):501–31.

[60] Josimovich JB, Archer DF. The role of lactogenic hormones in the pregnant woman and the fetus. Am J Obstet Gynecol 1977;129:777–80.
[61] Grumbach MM. Growth hormone and growth. Pediatrics 1966;37:245–8.
[62] Barbour LA, Shao J, Qiao L, Pulawa LK, Jensen DR, Bartke A, et al. Human placental growth hormone causes severe insulin resistance in transgenic mice. Am J Obstet Gynecol 2002;186: 512–7.
[63] Bellmann O. Hormonal regulation of lactation. Klin Padiatr 1976;188:385–95.
[64] Neville MC, Morton J, Umemura S. Lactogenesis. The transition from pregnancy to lactation. Pediatr Clin North Am 2001;48:35–52.
[65] Kuhn NJ. Specificity of progesterone inhibition of lactogenesis. J Endocrinol 1969;45:615–6.
[66] Said S, Johansson ED, Gemzell C. Serum oestrogens and progesterone after normal delivery. J Obstet Gynaecol Br Commonw 1973;80:542–5.
[67] Freeman ME, Kanyicska B, Lerant A, Nagy G. Prolactin: structure, function, and regulation of secretion. Physiol Rev 2000;80:1523–631.
[68] Howie PW, McNeilly AS. Breast-feeding and postpartum ovulation. IPPF Med Bull 1982;16: 1–3.
[69] Peters F, Del Pozo E, Conti A, Breckwoldt M. Inhibition of lactation by a long-acting bromocriptine. Obstet Gynecol 1986;67:82–5.
[70] Nolin JM, Witorsch RJ. Detection of endogenous immunoreactive prolactin in rat mammary epithelial cells during lactation. Endocrinology 1976;99:949–58.
[71] Buts JP. Bioactive factors in milk. Arch Pediatr 1998;5:298–306.
[72] Ollivier-Bousquet M, Kann G, Durand G. Prolactin transit through mammary epithelial cells and appearance in milk. Endocr Regul 1993;27:115–24.
[73] Lochnan HA, Buteau H, Richards S, Edery M, Kelly PA. Functional activity of the human prolactin receptor and its ligands. Mol Cell Endocrinol 1995;114:91–9.
[74] Frawley LS, Neill JD. Brief decreases in dopamine result in surges of prolactin secretion in monkeys. Am J Physiol 1984;247:E778–80.
[75] de Greef WJ, Plotsky PM, Neill JD. Dopamine levels in hypophysial stalk plasma and prolactin levels in peripheral plasma of the lactating rat: effects of a simulated suckling stimulus. Neuroendocrinology 1981;32:229–33.
[76] Neill JD, Freeman ME, Tillson SA. Control of the proestrus surge of prolactin and luteinizing hormone secretion by estrogens in the rat. Endocrinology 1971;89:1448–53.
[77] Howie PW, McNeilly AS, McArdle T, Smart L, Houston M. The relationship between suckling-induced prolactin response and lactogenesis. J Clin Endocrinol Metab 1980;50:670–3.
[78] McNeilly AS, Robinson IC, Houston MJ, Howie PW. Release of oxytocin and prolactin in response to suckling. Br Med J (Clin Res Ed) 1983;286:257–9.
[79] Tyson JE, Hwang P, Guyda H, Friesen HG. Studies of prolactin secretion in human pregnancy. Am J Obstet Gynecol 1972;113:14–20.
[80] Tyson JE. Mechanisms of puerperal lactation. Med Clin North Am 1977;61:153–63.
[81] Neville MC, Morton J. Physiology and endocrine changes underlying human lactogenesis II. J Nutr 2001;131:3005S–8S.
[82] Delvoye P, Demaegd M, Delogne-Desnoeck J. The influence of the frequency of nursing and of previous lactation experience on serum prolactin in lactating mothers. J Biosoc Sci 1977; 9:447–51.
[83] Rasmussen KM, Kjolhede CL. Prepregnant overweight and obesity diminish the prolactin response to suckling in the first week postpartum. Pediatrics 2004;113:e465–71.
[84] Gabay MP. Galactogogues: medications that induce lactation. J Hum Lact 2002;18:274–9.
[85] Kauppila A, Anunti P, Kivinen S, Koivisto M, Ruokonen A. Metoclopramide and breast feeding: efficacy and anterior pituitary responses of the mother and the child. Eur J Obstet Gynecol Reprod Biol 1985;19:19–22.
[86] Peters F, Schulze-Tollert J, Schuth W. Thyrotrophin-releasing hormone—a lactation-promoting agent? Br J Obstet Gynaecol 1991;98:880–5.
[87] Ibrahim II, Abdalla MI, Badraoui MH, Abdelaal AE. Thyroid function in lactating women using the Progestasert system. Contracept Deliv Syst 1981;2:139–44.

[88] Mornex R, Orgiazzi J, Hugues B, Gagnaire JC, Claustrat B. Normal pregnancies after treatment of hyperprolactinemia with bromoergocryptine, despite suspected pituitary tumors. J Clin Endocrinol Metab 1978;47:290–5.
[89] Haeger K, Jacobsohn D. A contribution to the study of milk ejection in women. Acta Physiol Scand Suppl 1953;111:152–60.
[90] Soloff MS, Alexandrova M, Fernstrom MJ. Oxytocin receptors: triggers for parturition and lactation? Science 1979;204:1313–5.
[91] Soloff MS, Schroeder BT, Chakraborty J, Pearlmutter AF. Characterization of oxytocin receptors in the uterus and mammary gland. Fed Proc 1977;36:1861–6.
[92] Sala NL, Althabe O. The milk ejecting effect induced by oxytocin during human lactation. Acta Physiol Lat Am 1968;18:88–95.
[93] Samson WK, Schell DA. Oxytocin and the anterior pituitary gland. Adv Exp Med Biol 1995; 395:355–64.
[94] Jaspars JJ, Posma AN, van Immerseel AA, Gittenberger-de Groot AC. The cutaneous innervation of the female breast and nipple-areola complex: implications for surgery. Br J Plast Surg 1997;50:249–59.
[95] Dawood MY, Khan-Dawood FS, Wahi RS, Fuchs F. Oxytocin release and plasma anterior pituitary and gonadal hormones in women during lactation. J Clin Endocrinol Metab 1981; 52:678–83.
[96] Nishimori K, Young LJ, Guo Q, Wang Z, Insel TR, Matzuk MM. Oxytocin is required for nursing but is not essential for parturition or reproductive behavior. Proc Natl Acad Sci USA 1996;93:11699–704.