Chapter 13

Maintenance of Pregnancy and Parturition

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Key Clinical Changes

- Human pregnancy exhibits functional progesterone withdrawal and estrogen activation.
- The gene expression of contraction-associated proteins is favored at the time of human labor.
- Inflammation in pregnancy is associated with infection from microorganisms and sterile inflammation related to cellular senescence and damage.
- Novel initiators of labor, including cell-free fetal DNA and exosomes released from the placenta and fetal membranes, may provide new insight into the mechanisms of labor and be useful biomarkers for preterm labor.

13.1 ESTABLISHMENT OF PREGNANCY

Successful establishment of pregnancy begins with implantation of a viable blastocyst into a progesterone- and human chorionic gonadotropin (hCG)-primed endometrium. This process requires a two-way interaction between the maternal uterus and the semiallograft blastocyst. Implantation occurs only during a narrow window of time when the endometrium is most receptive to a blastocyst. In humans, this is during the midsecretory phase (6–10 days following ovulation on day 14), corresponding to days 20–24 of a 28-day menstrual cycle. Understanding the mechanics of embryo implantation has clinical implications, as implantation failure can lead to recurrent pregnancy loss and infertility. The natural success rate of implantation and establishment of pregnancy in humans is 25%–30% per cycle. Furthermore, only half of human embryos transferred during in vitro fertilization (IVF) will successfully implant.[1]

A typical menstrual cycle lasts 28 days and begins with menses (day 0). The proliferative phase (follicular phase) following menses is characterized by the pulsatile release of gonadotropin releasing hormone (GnRH) from the hypothalamus, acting on the anterior pituitary gland to release follicle-stimulating hormone (FSH) and luteinizing hormone (LH). FSH causes folliculogenesis in the ovaries, with subsequent selection of a dominant follicle. FSH also acts on ovarian granulosa cells to convert androgens (released from thecal cells in response to LH) into estrogen (estradiol). There is a surge of FSH and LH on day 14, leading to ovulation from the dominant follicle. The secretory phase (luteal phase) is characterized by endometrial thickening and formation of the corpus luteum from the ruptured dominant follicle after ovulation has occurred. The corpus luteum supplies progesterone and estrogen, which steadily increases into the midluteal phase, where the window of receptivity opens. In the absence of blastocyst implantation during this window, luteolysis occurs along with subsequent hormone withdrawal, and menstruation ensues. It is thought that hCG released by the blastocyst aids in making the endometrium receptive to implantation. In baboon studies, hCG administration directly modulates the function of endometrial stromal and epithelial cells.[2] Successful implantation of a blastocyst during this time allows the embedded blastocyst to continue to produce hCG, which maintains the corpus luteum. This ensures an ongoing supply of progesterone and estrogen to support the establishment of pregnancy until the placenta develops. Furthermore, hCG also prevents uterine contractions through stimulating the production of cyclic adenosine monophosphate (cAMP) within the myocytes, which prevents displacement of the conceptus in early gestation.[3] In turn, cAMP also stimulates hCG production within the trophoblasts of the blastocyst.[4]

Estrogen and progesterone mediate changes to the endometrium and cause the activation of the blastocyst required for implantation. Most of the data on hormonal regulation of endometrial receptivity and pregnancy establishment has been obtained from animal studies. Studies in mice show that progesterone receptor A (encoded by the *PGR* gene) and a preimplantation estrogen rise through estrogen receptor (ER) binding (encoded by the *ESR1* gene) is vital for blastocyst activation and successful implantation.[5] Progesterone and cAMP decidualize the endometrium, allowing trophoblast invasion

Maternal-Fetal and Neonatal Endocrinology. https://doi.org/10.1016/B978-0-12-814823-5.00013-1
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and development of the placenta. Estrogen appears to be the dominant mediator of stromal changes to the endometrium that are required for implantation.[6] Even animals that seemingly only require progesterone for successful implantation have evidence of aromatase production from blastocysts, suggesting that endogenous production of estrogen from androgens circumvents the need for maternal estrogen supply.[7,8] Following successful implantation, progesterone and cAMP maintain myometrial quiescence, while estrogen stimulates growth of the uterus by myometrial cell proliferation and hypertrophy to accommodate the growing fetus.

13.2 THE CERVIX AND ITS CHANGES IN PREGNANCY

The cervix is the opening of the uterus, composed of fibrous connective tissue (80%–85%)[9] and contractile smooth muscle (10%).[10] Its tensile strength is related to the organization of collagen within the extracellular matrix (ECM), which allows it to resist pressure from the growing fetus and remain closed throughout pregnancy. Other important components of ECM include proteoglycans, hyaluronan, and elastin. The cervix also protects the pregnancy against microbial invasion by secreting thick mucus from endocervical cells, which make up 50% of the tissue mass during pregnancy. These endocervical cells also secrete defensins and are able to activate inflammation and local immune responses to bacteria and their toxins.[11] Due to the difficulties in obtaining cervical biopsy during human pregnancy, our understanding of cervical remodeling in pregnancy is based on studies in rats.[11]

Structural remodeling of the ECM begins in early pregnancy and is key to allowing the cervix to withstand the increasing load from pregnancy. This initial process of remodeling is called *cervical softening* (see Fig. 13.1); this is a misleading term, as *softening* refers to the ECM rather than the softening of the cervix itself (which, in fact, remains firm throughout most of pregnancy). There is a decrease in collagen concentration, leading to increased tissue compliance while still maintaining tissue integrity.[11] Cervical competency, which is key to a successful pregnancy, relies on the slow occurrence of the process of cervical softening. Studies in rodents have demonstrated that relaxin, an insulinlike factor, promotes cervical softening[12]; however, the role of relaxin in cervical remodeling in human pregnancy is not fully characterized.[13]

In rat pregnancy, cervical softening ultimately leads to *cervical ripening* (see Fig. 13.1), which refers to a range of dynamic changes to the cervix itself rather than the ECM (although changes within the ECM also occur). The cervical changes include *effacement* (shortening), softening, and dilation. These changes are present in human cervix in the days (and sometimes weeks) leading up to human parturition.[11,14] The ECM remodeling that occurs in rat cervical ripening includes increases in glycosaminoglycans (e.g., hyaluronan) and aquaporin channels, resulting in increased tissue hydration that causes collagen dispersion in the cervix.[11,15] Hyaluronan, a glycosaminoglycan, also weakens the binding of collagen to scaffolding glycoproteins in the ECM (e.g., fibronectin).[16] Dispersed collagen fibers are subsequently more susceptible to degradation by proteases such as matrix metalloproteinases (MMPs).

MMPs are a group of proteases that have various target substrates: MMP-1 and MMP-8 are collagenases, MMP-2 and MMP-9 are gelatinases, and MMP-3 is a stromelysin.[17] The transcripts of these MMPs are increased in cervical tissue from

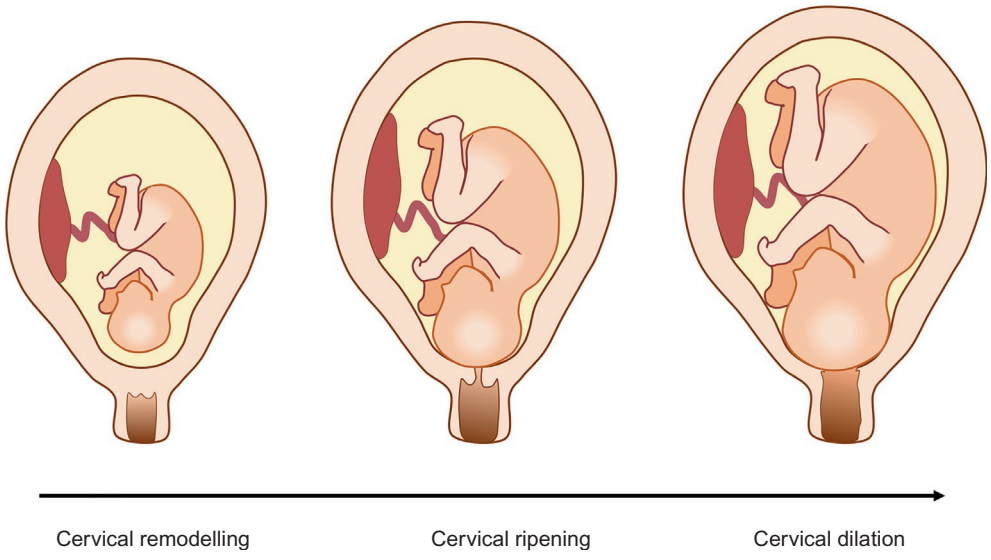

FIG. 13.1 Cervical remodeling, ripening, and dilation.

pregnant women at term, implicating that they play a role in the remodeling of the cervix prior to the onset of labor.[18,19] These MMPs appear to be regulated by leukocytes and inflammatory cytokines, such as IL-8, IL-1β, IL-6, and tumor necrosis factor-alpha (TNF-α),[20] which are also upregulated at the time of labor.[21] The role of inflammation in labor is discussed later in this chapter.

What is known about the regulation of cervical remodelling in human pregnancy is largely based on animal studies. A body of evidence from mice experiments suggests that hormonal regulation (particularly progesterone withdrawal) is key to initiating cervical ripening and dilation.[22] In pregnant mice, local metabolism of progesterone is important for labor, as the majority of mice deficient in 5a reductase type I do not labor secondary to failure of cervical ripening.[23] There is evidence of local hormone metabolism in the human cervix.[24] Further, 17β-hydroxysteroid dehydrogenase (17βHSD) type 2 is found in the pregnant human cervix and converts 20α-hydroxyprogesterone to bioactive progesterone, and estradiol to the less biologically active estrone. 17βHSD levels drop during labor and is thought to play a role in localized progesterone withdrawal that facilitates cervical remodeling in humans.[24] Following labor, the cervix undergoes a reparative process that restores the cervix to its prepregnancy state.

13.3 MAINTAINING QUIESCENCE IN THE PREGNANT UTERUS

13.3.1 Human Chorionic Gonadotropin

As the fetus grows with gestation, so does the uterus. Key hormones in the establishment of pregnancy continue to increase with advancing gestation, including estrogen (both estradiol and estriol), progesterone, and hCG.[25,26] A successful pregnancy relies on quiescence in the myometrium, so that uterine contraction is delayed until the fetus has completed growth and maturation at term.

A number of factors are important in maintaining uterine quiescence. First, circulating hCG levels continue to gradually rise in the second and third trimesters of pregnancy,[25] where it acts on hCG receptors in the myometrium to maintain myometrium relaxation by inhibiting the production of gap junctions required for labor.[27] Evidence implicating its direct role in myometrial relaxation comes from in vitro studies demonstrating hCG administration to human myometrium tissue strips in pregnancy led to reduced oxytocin-induced contractility.[28] hCG also indirectly maintains quiescence by stimulating cAMP, whose role in maintenance of pregnancy is discussed later in this chapter.[3] Furthermore, there is a decrease in hCG levels 2 weeks prior to the onset of labor,[25] as well as a reduced number of myometrial hCG receptors in labor compared to nonlabor states.[29] This temporal relationship suggests that hCG contributes to pregnancy quiescence, and its declining activity may play a role in parturition in humans.

13.3.2 Progesterone

Progesterone is a steroid hormone important to establishing and maintaining pregnancy. During the menstrual cycle, progesterone inhibits contractions and thickens the lining of the endometrium, allowing blastocyst implantation. Progesterone is initially suppled by the corpus luteum in early pregnancy and subsequently by the developing placenta from approximately 7 weeks gestation. Following the establishment of pregnancy, progesterone acts to prolong gestation and maintain uterine quiescence in many species, including humans.

George Corner, and later Arpad Csapo, first described the concept of a "progesterone block" in the 1940s and 1950s, when they observed that progesterone prevented stimulation of contractions with oxytocin in animal models. That is, the presence of progesterone created a block against drivers of uterine contractions and labor. This progesterone-dependent delay in uterine contraction allows fetal growth and development. Metaphorically, progesterone acts as a biological brake to the onset of labor, which, once lifted, enables the uterus to undergo transformation from a noncontractile phenotype into a contractile phenotype.

Progesterone prolongs pregnancy predominantly through anti-inflammatory effects.[30] In vitro studies have demonstrated that progesterone blocks various inflammatory pathways and works to supress inflammation-induced *PTGS2* expression (and hence inhibits prostaglandin production).[31,32] Progesterone also reduces the activation of activator protein 1 (AP-1), a transcription factor expressed in response to human myometrial stretch and thought to be important in labor initiation.[33,34] Progesterone also increases the expression of *ZEB1* and *ZEB2*, which have shown repressive activity over both the *OXTR* and *GJA1* genes, which encode the oxytocin receptor (OTR) and connexin-43 proteins, respectively.[35]

A great body of evidence from animal models shows that decreased progesterone production from the ovary or placenta leads to a reduction in circulating progesterone levels, and hence a reduction in its blocking effects on myometrial contractility. Removing the progesterone block lifts the brake on parturition toward the end of gestation. In rats, mice, rabbits,

goats, and pigs, progesterone supply is primarily dependent on the corpus luteum; and luteolysis due to the action of $PGF_{2\alpha}$ results in a measurable drop in systemic progesterone, which coincides with labor. In sheep, active conversion of progesterone to estrogen via 17α-hydroxylase within the placenta appears to be the main driver of labor. There is no measurable drop in progesterone levels that precedes labor in human pregnancy. In fact, serum progesterone levels increase with gestation and during labor. Despite this observation, mifepristone, a prostaglandin analog with antiprogestin effects administered at any point throughout human gestation, initiates cervical ripening and subsequent uterine contractions, leading to expulsion of the conceptus. As such, a functional progesterone withdrawal has been hypothesized as an alternative means to remove the progesterone block in human pregnancy. How this is achieved and the mechanisms that regulate this process in normal and pathological labor remain unknown. A number of hypotheses have been put forward: a loss of progesterone receptors (PRs), increase in progesterone metabolism, local production of antiprogestogens, sequestration of progesterone into cellular lipoproteins, unidirectional binding to proteins, and localization to the fetal membranes.[36,37] The most widely reported hypothesis on functional progesterone withdrawal in human pregnancy revolves around the interplay among the various PR isoforms.[38]

Classically, the PR functions as a transcription factor when it is liganded by progesterone. Once liganded, the PR translocates into the nucleus, where it binds to progesterone-specific promoter sequences on target genes to activate or inhibit their transcription, which appears to occur in a tissue-dependent manner. However, recent studies suggest that unliganded PR is also able to function as a transcription factor.[39] In humans, PR transcripts exist as two major isoforms that are encoded by the same gene but driven by different promotors.[40] The PR-B protein is a strong activator of progesterone-dependent genes and appears to drive the transcription of genes that aid in uterine quiescence. The PR-A protein is a truncated form of PR-B, lacking the first 164 amino acids at the N-terminal where the activator element 3 (AF3) is located.[41] Progesterone receptor A (PR-A) has a predominantly inhibitory function to gene transcription, and when coexpressed with progesterone receptor B (PR-B), it represses PR-B activity.[36] Progesterone receptor C (PR-C) is a third isoform, which is a truncated form of PR-B that lacks a DNA-binding domain and is found only in the cytosolic fraction.[42] Due to its missing DNA-binding site, it lacks transactivation ability but is able to bind to PR-B transcripts, rendering it biologically inactive. In vitro studies using an immortalized pregnant human cell line showed that overexpression of *PR-C* represses activity of PR-B.[42] PR-C was also upregulated in myometrium from women in labor.[42]

As repression of PR-B action appears to be the predominant mechanism by which progesterone withdrawal occurs in human and primate parturition,[38,43] understanding the regulation of PR-A throughout gestation is important. Prostaglandins (PGE_2 more so than $PG_{2\alpha}$), released in response to inflammation, increase both PR-A and PR-B messenger ribonucleic acid (mRNA) abundance in myometrium.[44] There appears to be higher PR-A expression relative to PR-B, leading to an increased PR-A-to-PR-B ratio, and thus an overall progesterone-reducing effect.[44] An important observation during human labor is that despite the fact that there is increased circulating progesterone, there is a decrease in progesterone levels in the nucleus and the majority of PR is unliganded during labor.[39] There is a corresponding increase in the progesterone-metabolizing enzyme, 20a hydroxysteroid dehydrogenase (20aHSD), which leads to localized progesterone withdrawal that encourages uncoupling of PR to its ligand.[39] In studies using immortalized human myometrial cells in pregnancy, liganded PR-B localizes to the nucleus and liganded PR-A is found in the cytoplasm. In their unliganded state, PR-A is found predominantly in the nucleus and PR-B was found only in the cytoplasm.[39] Taken together, liganded PR-B predominates within the nucleus and encourages myometrial quiescence throughout pregnancy. However, at the time of labor when PR are unliganded, PR-A is the predominant isoform found within the nucleus, where it favors the transformation of the myometrium into a contractile phenotype (see Fig. 13.2). For instance, unliganded PR-A functions as a transcription factor to activate GJA1, a known contraction-associated protein (discussed later in this chapter).[39]

A number of epigenetic changes, including histone modification at PR-A promotor regions and cytokine-mediated stabilization of PR-A stability, also work to increase the transrepressive activity of PR-A.[45–48]

13.3.3 Cyclic Adenosine Monophosphate

cAMP is a diffusible second messenger generated by the conversion of adenosine triphosphate (ATP) by adenylate cyclase (AC) enzymes. AC activation occurs in response to ligand binding to nuclear G-protein-coupled receptors (GPCRs). cAMP signaling is involved in a number of cellular pathways and is tightly regulated by AC activation, but also through phosphodiesterase (PDE) enzymes, which degrade free cAMP. Classically, cAMP binds to protein kinase A (PKA), which releases a catalytic subunit that is free to phosphorylate various targets, including the transcription factor cAMP response element-binding protein (CREB).

In the myometrium, cAMP is thought to induce relaxation through the inhibition of calcium signaling by PKA-led phosphorylation of PLC and the inactivation of myosin light chain kinase (MLCK), which prevent the phosphorylation of

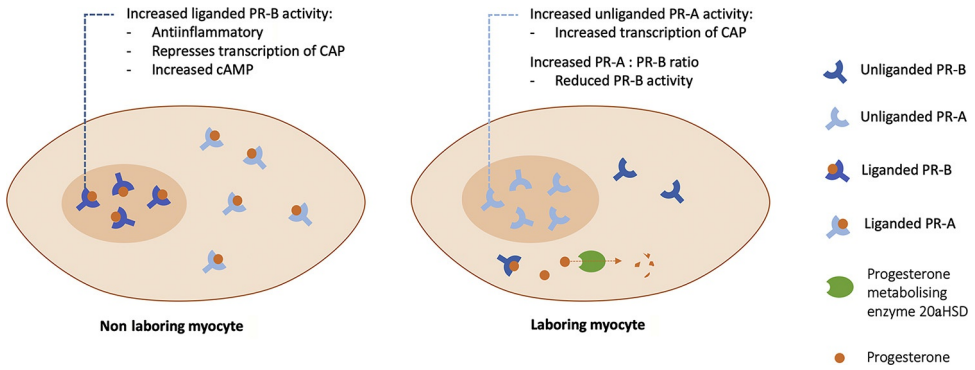

FIG. 13.2 Localization and unliganding of PR-A and PR-B during labor. PR-A increased relative to PR-B during labor.

myosin, which is required for smooth muscle contraction.[49] PKA-activated transcription factors also has been shown to repress inflammatory pathways,[50] and reduce the expression of *OXTR*.[51] Furthermore, cAMP enhances the binding of PR-B to the progesterone response element (PRE) of progesterone-dependent genes, increasing their expression, and subsequently the progesterone block effect as well.[52]

13.4 REQUIREMENTS FOR LABOR CONTRACTIONS IN THE UTERUS

13.4.1 Corticotropin-Releasing Hormone

The term *placental clock* refers to placenta-derived corticotropin-releasing hormone (CRH) and its role in determining the length of human pregnancy.[53] In the nonpregnant state, CRH is a stress peptide hormone released from the hypothalamus as part of the hypothalamic-pituitary-adrenal (HPA) axis. CRH acts on the anterior pituitary to release adrenocorticotropic hormone (ACTH), which in turn acts on the adrenal glands to stimulate the release of cortisol, a glucocorticoid stress hormone (see Fig. 13.3).

Placental CRH is secreted by trophoblasts during human pregnancy and is first detectable by radioimmunoassay (RIA) early in the second trimester.[53] CRH levels rise with gestation in an exponential manner, peaking at the onset of labor.[26,53,54] This sudden increase in CRH level coincides with a reduction in CRH-binding hormone (CRH-BP), an endogenous inhibitor that renders CRH biologically inactive.[55–58] Reductions in CRH-BP levels occur approximately 30 days

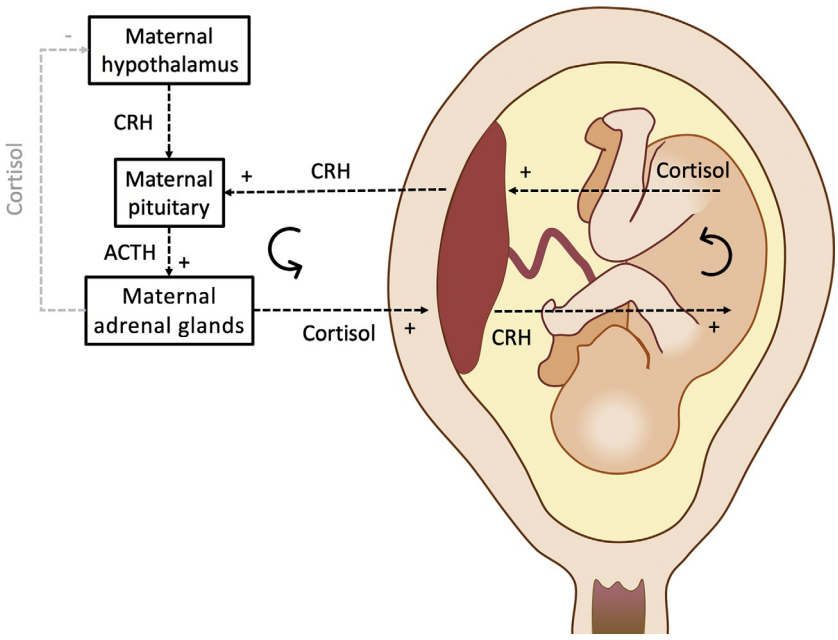

FIG. 13.3 A CRH feed-forward loop.

prior to the onset of labor, which contributes to increases in overall bioactive CRH.[53,55] Multiple studies have demonstrated that an increase in maternal serum CRH levels in the early third trimester is associated with preterm birth.[59,60] Furthermore, serial measurements of maternal CRH levels with advancing gestation have found the following:

- Women who delivered preterm had higher levels of CRH at any given gestation and a faster rate of increase with gestation compared to women who delivered at term.[53]
- Women who delivered postterm had lower levels of CRH at any given gestation compared to women that labored at term.[53]
- CRH levels do not increase in women who deliver preterm in the context of chorioamnionitis, suggesting the pathogenesis of PTB in these women differs to those that labor spontaneously in the absence of infection.[61]

While these results would suggest early processes in the placenta could play a role in determining the length of human pregnancy, heterogeneity between women that deliver preterm limits the use of CRH as a predictive test. Some have investigated the usefulness of a single CRH level in the third trimester,[60] while others have assessed the predictor of serial measurements[62]; however, overall CRH has moderate sensitivity (63%) and modest specificity (52%) when applied as a screening tool.[63]

While cortisol downregulates CRH production in the hypothalamus in a negative feedback loop within the HPA axis, it upregulates CRH production in the placenta in a feed-forward system during pregnancy. Placental CRH is also released into the fetal circulation,[64] where it stimulates the fetal adrenal glands to produce cortisol, from the outer definitive zone,[65] and dehydroepiandrosterone sulfate (DHEAS), from the inner fetal zone.[66] Fetal contributions of cortisol add to the feed-forward loop stimulating further CRH production, eventually resulting in the aforementioned CRH surge. DHEAS, on the other hand, is the precursor for the placental production of estradiol and the substrate for liver 16-hydroxylase. Here, 16-hydroxylated DHEAS is the precursor for placental production of estriol. Furthermore, increasing cortisol production by the fetal adrenal glands is necessary for fetal lung development and maturation. In mice, maturation of the fetal lungs also produces proinflammatory phospholipids and surfactant protein A, which have been found to permeate the amniotic fluid, where they are able to attract immune cells that initiate inflammatory pathways seen at the time of labor.[67]

While there is no doubt that CRH is important in regulating human gestational length, its exact role in the uterus is not fully characterized. Its production is primarily driven by binding of CREB to the CRH gene, which is stimulated by mediators of uterine quiescence (cAMP and PR-B), as discussed previously, but also by mediators of uterine contractions (PR-A and estrogen).[68,69] Studies of human myometrial tissue strips showed that at lower physiological concentrations, CRH caused reduced contractions in human myometrium strips from preterm and term women.[70] This relaxation effect was enhanced 3.5-fold by progesterone supplementation.[70] This tocolytic effect appears to be related to cAMP production, which increases upon activation of the CRH receptor in myometrium through increased adenylyl cyclase (AC) (see Section 13.3.3). At higher concentrations of CRH, its receptor becomes desensitized, leading to reduced CRH receptors coupling to $G\alpha_s$ protein, subsequently reduced AC activation, and thus impaired cAMP production.[71] Furthermore, CRH may have synergistic roles with oxytocin[72] and prostaglandin $F_{2\alpha}$[73] in myometrial contractility; however, high doses of CRH were not able to induce or enhance contractions.[70,74] CRH, therefore, plays a paradoxical role in maintaining uterine quiescence throughout gestation, but it also may aid the transition of myometrium into a contractile phenotype during labor.[75]

13.4.2 Estrogen

Three types of estrogen are produced in humans—estrone (E1), estradiol (E2), and estriol (E3)—which have varying degrees of potency in their interaction with ERs. Estradiol and estriol are the predominant estrogens produced during pregnancy, with estradiol considered to have higher estrogenic activity than estriol. In early pregnancy, the corpus luteum and the maternal ovaries supply estradiol and from the eighth week of gestation, production is taken over by the placenta. However, the placenta is an incomplete steroid-generating organ, in that it lacks the ability to produce DHEAS due to an absence of the enzyme 17α-hydroxylase (P450c17). Because DHEAS and its hydroxylated form, 16-hydroxy-DHEAS, are important precursors required for estrogen synthesis, the placenta relies on maternal and fetal contributions of these substrates. DHEAS produced predominantly by the maternal adrenal is converted to estradiol in the placenta.[76] On the other hand, DHEAS produced by the fetal adrenals undergoes hydroxylation in the fetal liver by 16α-hydroxylase to form 16-OH-DHEAS, which is converted to estriol in the placenta. Production of DHEAS increases with gestation and is regulated by CRH, which is discussed elsewhere in this chapter.

Throughout gestation, estradiol and estriol both steadily increase in equimolar concentrations.[26] At equimolar concentrations, estradiol and estriol block one another's actions through the formation of heterodimers. However, when the ratio of

estriol to estradiol exceeds 10:1, there is an increase of estrogenic activity, as estriol is able to form homodimers.[77] In a longitudinal study measuring hormones in pregnancy, there is a rise in the ratio of estriol to estradiol at the time of parturition (driven by rising placenta CRH production, which acts directly on the fetal adrenal to stimulate DHEAS synthesis), demonstrating a functional activation of estrogen toward labor.[26] Interestingly, in the case of fetal death, levels of estriol fall as estradiol levels remain elevated, resulting in a change in ratio that favors estradiol, which also results in the activation of labor.[4,26] This redundant mechanism may be an evolutionary mechanism to preserve maternal life even with fetal death. Switching to an estrogenic environment upregulates a number of contraction-associated proteins, including gap junction alpha-1 protein (encoded by the *GJA1* gene and also known as *connexin 43*),[78,79] OTR (encoded by the *OXTR* gene)[80] and prostaglandin-synthesizing enzymes (see Section 13.4.4).[38] These changes occur upon ligand binding to ESR1 receptors, which displays upregulation at the time of labor.[38]

In vitro studies have also examined estrogen action through the seven-transmembrane receptor GPR30, encoded by *GPER1*. *GPER1* transcripts are found in pregnant human myometrium, and the GPR30 protein is localized to the cell membrane of myometrium.[81] Treatment of pregnant human myometrial strips with the GPR30 agonist, G-1, and estradiol enhanced the phosphorylation of heat shock protein 27 (HSP27) and oxytocin-induced contractility.[81,82] These changes facilitate myometrial contractility by stabilizing actin filaments and increasing calcium signaling, respectively.

Estradiol is also responsible for the rapid expansion and growth of the uterus throughout gestation,[83] which allows accommodation of the growing fetus. The effect of the growing fetus on the uterine wall during human pregnancy is not well understood. Studies in pregnant mice provide insights into myometrial growth patterns in pregnancy, changing from hyperplasia to hypertrophy at midgestation.[84] This is about the same time that the fetus is most spherical and thought to be exerting the greatest tension on the uterine wall.[84,85] As the uterus slows its growth, there is an increase in intrauterine tension. This leads to a combination of stretch, hypoxia, and reduced blood flow that activates caspase enzymes, particularly caspase 3.[85] Caspase 3 (encoded by the *CASP3* gene) contributes to uterine quiescence in mouse pregnancy by cleaving smooth muscle actin in a progesterone-dependent manner.[86] *CASP3* transcripts reduce at the end of mouse gestation, which coincide with reduced progesterone levels and restoration of full-length actin.[84]

Myometrial stretch is also an important initiator of human labor, as women with multiple gestations[87] or polyhydramnios (where there is uterine overdistention) are at higher risk of PTB.[87,88] Evidence suggests that myometrial stretch activates mitogen-activated protein kinases (MAPKs), which are a group of enzymes that phosphorylate serine and threonine amino acids in order to modulate extracellular signals that direct gene expression of contraction-associated genes that favor a contractile phenotype in the uterus.[33] While there is increasing stretch-activated expression of prolabor genes with advancing gestation, most pregnancies do not deliver until term. Furthermore, estrogen levels throughout early gestation to midgestation in human pregnancy are already significantly higher than humans in nonpregnant states. Despite this, the uterus does not switch to a contractile phenotype until the end of pregnancy. This is likely due to progesterone blocking. Upon withdrawal of progesterone's effects, the uterus is able to respond to a multitude of stimuli (including estrogen and myometrial stretch) that enable it to transform from a quiescent to a contractile state. Premature withdrawal of progesterone, therefore, may contribute to premature labor. Evidence of this is observed in studies in which abrupt progesterone withdrawal by ovariectomy in mice leads to PTB, which is associated with an increase in oxytocin and labor.[89]

13.4.3 Oxytocin and the OTR

Sir Henry Dale first discovered oxytocin when one of his studies elicited uterine contractions following the administration of posterior pituitary extracts in pregnant cats.[90] The modern-day use of synthetic oxytocin to cause uterine contractions to induce labor comes from a body of research that is still predominantly from animal studies. There is an increase in oxytocin levels at the time of labor in animal studies (involving rodents, rhesus monkeys, and sheep) that is not consistently reproducible in human labor.[91–96] In women, oxytocin is released from the posterior pituitary in a pulsatile manner and has a relatively short half-life and high clearance rate[97] by placental oxytocinase.[98] Oxytocin binds to the OTR, a protein that increases in expression in the myometrium with advancing gestation.[99,100] While it would seem logical that OTR expression would increase at the time of labor, there is no consensus in the published literature, with some studies showing decreased myometrial expression,[101,102] and others showing increased expression with the onset of labor.[103–105] Interestingly, the action of oxytocin and OTR is not a requirement for labor onset in mice,[106] nor in humans, as reports exist of women with panhypopituitarism who labored spontaneously.[107] The mechanisms of how oxytocin affects smooth muscle contractility is covered in the following discussion.

Besides the myometrium, oxytocin and OTR are found in chorion-amnion and decidua,[108] where it stimulates the production of prostaglandins. In vitro studies demonstrate oxytocin treatment of human amnion cells stimulated the synthesis of prostaglandin E_2 (PGE_2),[109] while the treatment of human decidua cells resulted in increased prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)

production.[110] These prostaglandins are lipid molecules that play vital roles in parturition by encouraging smooth muscle contractions. The mechanism by which oxytocin increases prostaglandin production appears to be the activation of cytoplasmic phospholipase A_2 that causes release of a 20-carbon unsaturated fatty acid called *arachidonic acid (AA)*.[111] Prostaglandin G/H synthase (PTGS), or cyclooxygenase, is the group of enzymes that then convert AA into prostaglandin G_2 (PGG_2), which is then immediately converted to prostaglandin H_2 (PGH_2), the substrate for production of various prostaglandins, including PGE_2, $PGF_{2\alpha}$, and PGD_2, as well as prostacyclin (PGI_2) and thromboxane (TxA2).[110–113] There is increased AA metabolism at the time of labor,[114] leading to increased prostaglandin action that promotes myometrial contractility.

13.4.4 Prostaglandins and Prostaglandin Receptors

In human pregnancy, the majority of prostaglandin is produced in amnion cells by *PTGS2* (and not its other isoforms).[115,116] Evidence indicating the role of PTGS2 in these processes include increased concentrations of prostaglandin in amniotic fluid of pregnant women[117,118] and increased transcripts of *PTGS2* in human myometrium,[119] chorion-amnion,[114,116,120] and decidua at the time of labor.[121] Furthermore, myometrial stretch and proinflammatory mediators, such as lipopolysaccharide (LPS), IL-1β, and TNF-α, stimulate increased PTGS2 production in vitro and in animal studies, suggesting that inflammation at the onset of labor coincides with an increase in prostaglandin activity.

Prostaglandins are also used clinically to ripen the cervix for induction of labor, and its analog, misoprostol, is commonly used for termination of pregnancy or induction of labor following midgestation fetal demise. PTGS2 inhibitors like celecoxib delay labor in animal models[122]; however, in human pregnancy, the use of a PTGS inhibitor called *indomethacin* causes premature closure of the ductus arteriosus in the fetal heart, reducing its use as a treatment for preterm labor.[123]

A prostaglandin inactivator, prostaglandin dehydrogenase (PGDH), is present in human chorion and trophoblastic cells. It causes irreversible conversion of PGE_2 and $PGF_{2\alpha}$ into their biologically inactive forms. Prostaglandins are primarily produced by the amnion, so the fact that PGDH are produced in high quantities in adjacent tissue suggests that it plays a role in the regulation of prostaglandins during pregnancy. Studies have demonstrated that PGDH may be stimulated by progesterone, while CRH, estrogen, and other inflammatory mediators stimulate *PTGS2*. The balance of the opposing actions of PGDH and PTGS2 (and their respective regulators), therefore, may determine whether a contractile or quiescent state is achieved in the uterus. The balance favors contractility at the time of labor.

An understanding of prostaglandin effect, therefore, is important in uncovering the pathway to parturition. Prostaglandins exhibit various effects upon binding to specific GPCRs in target tissue. There are four PGE_2 receptors: EP1, EP2, EP3, and EP4, and two receptors for PGF2α, FP (types a and β). EP1 and FP promote contraction by increasing intracellular Ca^{2+} levels, whereas EP4 has a relaxing effect through stimulation of cAMP. EP2 and EP3 have dual actions that promote contraction and relaxation—in particular, EP2 receptor working via $G_{\alpha s}$ to promote cAMP signaling maintains myometrial quiescence, while EP2 working via $G_{\alpha q/11}$ promotes Ca^{2+} signaling and myometrial contractility. Differences in expression of the four PGE_2 receptors are observed in the myometrium during labor,[124] and thus regulation of these receptors is likely to determine the balance between uterine quiescence and contractility.

13.4.5 Calcium Signaling and Myometrial (Smooth Muscle) Contractility

Despite its uncertain role in the initiation of parturition, oxytocin is a powerful uterotonic that is used in clinical practice to induce and augment labor, as well as in the prophylaxis and treatment of postpartum hemorrhage. OXTR is a rhodopsin-type GPCR. Activation of the $G_{q/11}$ protein activates phospholipase C-β, which hydrolyzes phosphatidylinositol bisphosphate (PIP_2) to form inositol triphosphate (IP_3) and diacylglycerol (DAG). IP_3 binds to receptors on the sarcoplasmic reticulum, causing release of Ca^{2+} into the cytosol. The increase in intracellular Ca^{2+} results in four Ca^{2+} ions binding to calmodulin, forming a Ca^{2+}-calmodulin complex that activates the enzyme MLCK, which phosphorylates serine-19 of the regulatory myosin light chain (MLC). Phosphorylated MLC is then able to interact with actin filaments in an ATP-dependent manner known as *cross-bridge cycling,* generating force and contraction. This process is reversed by MLC phosphatase (MLCP). DAG activates protein kinase C (PKC), which phosphorylates MLCP into its inactive form. DAG also stimulates an inhibitor of MLCP called *C-kinase-activated protein phosphatase-1 inhibitor 17 kDa (CPI-17).* Together, PKC increases the phosphorylation of MLC by reducing the action of MLCP. On the other hand, activation of the $G_{12/13}$ protein triggers activation of Rho kinase (ROCK). ROCK is able to phosphorylate myosin-binding subunit 1 (MYPT1) of MLCP, which inactivates its ability to dephosphorylate MLC. This pathway is independent of Ca^{2+} and is activated upon oxytocin binding.[125]

Transient increases in intracellular Ca^{2+}, therefore, are required for cross-bridge cycling and smooth muscle contraction. Excessive cytosolic Ca^{2+} leads to cellular damage and apoptosis. Therefore, Ca^{2+} signaling is tightly regulated through numerous transporters and ion pumps. The resting membrane potential in uterine smooth muscle lies between −35 and −80 mV and is a reflection of ionic concentrations across the cell membrane, particularly Ca^{2+}, Na^+, K^+, and Cl^-.[126] This resting membrane potential is maintained via an ATPase-dependent Na^+-K^+ channel that pumps three Na^+ ions across the cell membrane into the extracellular space in exchange for two K^+ ions. These K^+ ions then passively diffuse out of the cell via K^+ channels (which are opened by β-sympathomimetics), creating a negatively charged intracellular environment that is refractory to depolarization. Therefore, K+ channels maintain uterine quiescence and their diminished activity at parturition encourages uterine contractility.[127] Binding of oxytocin and prostaglandins to their respective receptors causes the opening of ligand-operated Ca^{2+} channels, promoting intracellular increases in Ca^{2+}. The increase in intracellular Ca^{2+} levels lowers the electronegativity of the cell, which activates voltage-gated Ca^{2+} channels, further increasing the influx of Ca^{2+} into the cell. This leads to depolarization of the cell and activation of contraction mechanisms. Other nonselective cation channels may also be involved in the regulation of uterine contractility, including transient receptor potential vanilloid 4 (TRPV4) channels. When activated by agonists in pregnant rats, these channels allow increase cytosolic calcium levels in myometrium and increase uterine contractility.[128] These channels are increased in the myometrium in pregnant women compared to nonpregnant women, but their role in parturition has yet to be defined.

13.4.6 Gap Junctions

The phenomenon discussed previously occurs in each individual myocyte cell—but how does a collective group of myocytes depolarize in unison? Unlike the smooth muscles of the gastrointestinal (which possess interstitial cells of Cajal, which are able to generate rhythmic action potentials) or the myocytes of the heart (which are controlled by the sinoatrial node), there is no equivalent pacemaker in the uterus to orchestrate myometrial contractions. However, labor is characterized by strong, regular, and rhythmic contractions, which is possible due to myometrial electrical coupling through gap junctions. At the time of parturition, myocytes become increasingly connected via the gap junction protein, connexin-43 (see Fig. 13.4). Regulation of connexin-43 in early pregnancy occurs through a progesterone-induced transcription factor called *zinc finger E-box-binding homeobox 1 (ZEB1),* which works to inhibit the formation of gap junctions.[35] However, as term approaches, the formation of gap junctions increases dramatically. Leading up to labor,

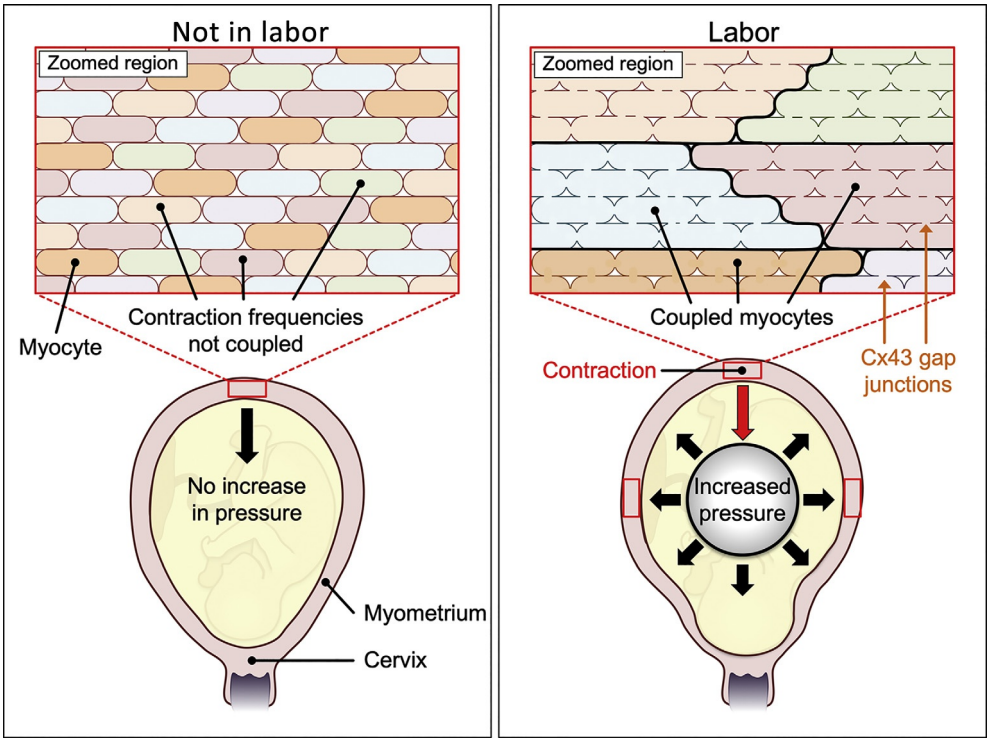

FIG. 13.4 Increased gap-junction connections between myocytes at the time of labor.

decreasing progesterone activity reduces ZEB1 activity, and increasing estrogen activity activates micro-RNA (miRNA) that also works to inhibit ZEB1.[129] When a threshold of myocyte connectivity is reached that allows a sufficiently large area of myometrium to depolarize in unison, there is a rise in intrauterine pressure that stretches the uterine walls as a whole; the stretch causes depolarization of the rest of the uterus leading to synchronous contractions.[130] Prior to reaching this threshold, nonpainful contractions experienced by women in the third trimester, called *Braxton-Hicks contractions*, may represent patches of uterine activity in the presence of insufficient gap-junction connectivity. These contractions increase in frequency toward term and are often referred to as *practice contractions*.

13.4.7 Micro-RNA

A family of miRNAs, miR-200, has also been shown to regulate parturition processes.[35] Here, miRNA is noncoding, single-stranded RNA that is 19–25 nucleotides in length[50] and regulates at least a third of the human genome. It binds to the 3′ untranslated region of mRNA to regulate gene repression, predominantly at the posttranscriptional level. Further, miR-200 downregulates ZEB1 and ZEB2 transcription factors, which repress the expression of contraction-associated proteins such as *GJA1* and *OTXR*.[35] Furthermore, miR-200a (a member of the miR-200 family) also increases during labor, where it inhibits STAT5B,[131] a transcriptional factor that represses 20aHSD and its progesterone-metabolizing activity.[132] Deletion of the *STAT5B* gene in mice leads to sustained high levels of circulating progesterone and causes a delay in labor.[132]

Also, miRNA expression profiles are altered through exogenous oxytocin administration in pregnant women,[133] and various miRNA changes at midgestation have been associated with spontaneous preterm birth.[134] Taken together, it appears that miRNAs are important in both normal and preterm labor.

13.5 INFLAMMATION IN PREGNANCY

13.5.1 Sterile and Nonsterile (Microbial) Inflammation and the Innate Immune System

Inflammation is a result of activation of the innate immune system—the first line of defense against harm.[135] It leads to leucocyte infiltration and production of cytokines and chemokines that alter gene expression to affect protective mechanisms of the host in response to perceived harm. This protective response is mediated by antigen-presenting immune cells that contain pattern-recognition receptors (PRRs), such as toll-like-receptors (TLRs).[136] These PRRs recognize potential pathogenic components, such as bacterial nucleic acids, lipoproteins, glycoproteins, and components of bacterial membranes, including peptidoglycans, lipoteichoic acid, lipopolysaccharide, and glycosylphosphatidylinositol. These components are collectively known as *pathogen-associated molecular patterns (PAMPs)*. The innate immune system can also be activated by cellular damage in the absence of microorganisms, which can be caused by trauma and ischemia. These components are called *damage-associated molecular patterns (DAMPs)*[137] and include protein components derived from the nucleus, cytoplasm, exosomes, or extracellular matrix, or within plasma, as well as nonprotein DAMPs, such as nucleic acids, ATP, and uric acid. Activation of PRRs by either PAMPs or DAMPs initiates cellular pathways and alters gene expression to produce proinflammatory chemokines, cytokines, and other molecules required to mount an immune response.

13.5.2 Inflammation in Parturition

The fetus is a semiallograft and relies on tight regulation of maternal cytokines at the maternal-fetal interface as a means of allowing pregnancy to continue without fetal cells being attacked by the maternal immune system. Progesterone appears to exert anti-inflammatory effects that allow maternal tolerance of the fetus, which begins in early gestation through the action of the leukemia inhibitory factor (LIF),[138] while throughout gestation, the production of the anti-inflammatory cytokine, IL-10, at the maternal-fetal interface suppresses proinflammatory cytokines.[139,140] This tolerance, however, appears to be lost or withdrawn at the time of labor, which coincides with an excess production of inflammatory markers. This has been demonstrated by studies showing an increase in neutrophils and macrophages in the cervix and myometrium during labor,[141] as well as an increase in the proinflammatory chemokines that promote the migration of these leucocytes into the chorion-amnion, decidua, amniotic fluid, placenta, cervix, and myometrium.[142,143]

Inflammation can also be a response to infection.[144] Microorganisms gain access to the otherwise-sterile amniotic cavity via ascending the genitourinary tract[145] or via hematogenous spread, as in the case of periodontal infection.[146,147] In fact, microbial-associated intra-amniotic inflammation has been causally linked to PTB, as a number of bacterial components are recognized to initiate inflammation at the onset of labor.[148] Inflammation stimulates the release of numerous

proinflammation cytokines and chemokines that contribute to various aspects of parturition, reflected in their contributions to (1) remodeling of the cervix to allow dilation, (2) weakening of fetal membranes leading to membrane rupture, and (3) initiating rhythmic contractions.

In the cervix, infiltration of leucocytes leads to the production of IL-8, IL-1β, IL-6, and TNF-α.[149,150] MMP-1, MMP-2, MMP-3, MMP-8, and MMP-9 transcripts are increased in cervical biopsies from pregnant women at term.[18,19] IL-1β and TNF-α are able to increase the production of MMPs (in particular, MMP-1, MMP-3, and MMP-9) and proteases (cathepsin), which work to break down the extracellular matrix of collagen and elastin within the cervix.[20] IL-1β also represses the production of an inhibitor of MMP-2, called the *tissue inhibitor of metalloproteinase 2 (TIMP-2).*[20] IL-8 is a chemotactic to neutrophils and also acts to increase the production of MMP-8.[151] Together, these processes lead to softening, shortening, and dilation of the cervix.

In the fetal membranes, similar degradation of the extracellular matrix leads to reduced membrane integrity, increasing the likelihood of spontaneous rupture. This is facilitated by IL-1β- and TNF-α-induced increases in MMP-9 and reduction in TIMP-2.[152,153] Furthermore, in vitro treatment of amnion and chorion cells with IL-1β and TNF-α leads to increased PGE_2 in a PTGS2-dependent manner.[154,155] PGE_2 produced by the amnion further increases MMP-9 levels to perpetuate the effects described previously.[156] IL-1β and TNF-α also reduce the production of PGDH, the inactivator of PGS, within the chorion.[157]

Perhaps unsurprisingly, transcripts of IL-1β and TNF-α are also increased in the myometrium at the time of labor.[149] In the myometrium, these cytokines cause phospholipid metabolism and the production of arachidonic acid,[158] which are the substrates required for PTGS to produce prostaglandins.[159] IL-1β also appears to stimulate expression of *PTGS2* through inflammatory pathways.[160] Increased IL-6 levels in the myometrium work to increase expression of *OXTR,*[161] and alongside IL-1β, they also increase the secretion of oxytocin from myometrial cells in vitro.[162] IL-8, which is consistently increased in the myometrium at the time of both term and preterm labor, appears to attract more leucocytes to propagate the effects of IL-1β.[151]

With advancing gestation, the likelihood of infection-associated PTB decreases. Work by Romero and colleagues found that prior to 25 weeks, microbial-associated intra-amniotic inflammation was more common, while sterile intra-amniotic inflammation was more common from 25 to 33 weeks of gestation.[144] DAMPs, such as high-mobility group box 1 (HMGB1), are elevated in patients with sterile intra-amniotic inflammation with intact membranes,[163] but mechanisms behind sterile intra-amniotic inflammation are not well defined. Certainly, a number of other mechanisms have been postulated to contribute to sterile inflammation, including oxidative stress (leading to cellular necrosis, apoptosis, or senescence), cell-free DNA, mechanical stretch, bleeding (e.g., antepartum hemorrhage or placental abruption), and release of fetal surfactant protein A into amniotic fluid.

13.6 OTHER MECHANISMS INVOLVED IN THE ACTIVATION OF PARTURITION

13.6.1 Oxidative Stress, Aging, and Senescence

Aging is the increasing probability of frailty, malfunction, and death that occurs because of accumulation of damage to molecules, cells, and tissues over a lifetime. Cells that undergo a terminal state of arrest but are still viable and functioning are in a state of senescence, which is distinct from cellular apoptosis. Cellular senescence is triggered by numerous stimuli, including oxidative stress, telomere shortening, and mitochondrial dysfunction—factors that often coexist. Senescence of fetal membrane cells as a result of cumulative oxidative stress has been postulated to release inflammatory mediators that may contribute to labor.[164] Menon et al. have demonstrated numerous markers of oxidative stress and cellular senescence in fetal membranes at term, including structural changes to mitochondria, endoplasmic reticulum, and the nucleus associated with senescence; increased expression of p38 mitogen-activated protein kinase stress-responsive kinases; and increased proportion of cells that are positive for senescence-associated beta-galactosidase.[165]

Cellular senescence is also associated with the release of inflammatory markers called *senescence-associated secretory proteins (SASPs),*[166,167] which include cytokines, chemokines, growth factors, enzymes that degrade the matrix, and cell adhesion molecules and inhibitors.[166] Due to the degree of overlap between SASPs and inflammatory mediators seen during parturition (including IL-1β, IL-6, IL-8, TNF-α, and MMPs),[166,168,169] there is biological plausibility that SASPs released in response to cellular senescence may be a contributing factor to the inflammatory response seen at the onset of labor. Furthermore, SASPs such as MMPs could contribute to the weakening and spontaneous rupture of membranes that are seen in women at term. Interestingly, the concept of premature senescence of fetal membranes may be involved in the pathophysiology of premature rupture of membranes (PPROM) and subsequent preterm birth.[170]

While the evidence of fetal membrane senescence contributing to human parturition may seem plausible, local inflammation confined to the fetal membrane is unlikely to be sufficient to initiate contractions in the myometrium. SASP and DAMP signals released from fetal membranes are thought to be able to effect changes in adjacent tissue, such as the myometrium, decidua, or cervix, by either direct chemical diffusion or by encapsulation and transportation in exosomes.[171] As some SASPs and DAMPs are readily inactivated by acetylation and oxidation,[172] diffusion is unlikely to enable safe trafficking of these molecules to other tissues. On the other hand, exosome transportation enables protected transportation across the fetal-maternal interface. Exosomes are cell-derived vesicles that are created in the process of exocytosis. Placental-derived exosomes increase in maternal blood with gestation and are able to carry a number of molecules, including SASP and DAMP, in a highly stable manner. Whether they are transported from one compartment to another through blood circulation or directly through tissue layers is still under investigation.[171] Tracking of exosomes in human pregnancy has not been well studied, but the hypothesis that SASP/DAMP created from fetal membranes can be delivered to myometrium and cervix tissue where they activate inflammatory pathways is being investigated as an initiator of parturition.

13.6.2 Cell-Free Fetal Dna

Cell-free fetal DNA (cffDNA) is extracellular DNA of fetal origin that is found in the maternal circulation in a fraction ranging between 3.4% and 6.2% of total cell-free DNA that increases with gestation.[173] Its use has predominantly been in noninvasive prenatal screening for aneuploidy, but cffDNA fractions were noted to be higher in women who delivered preterm.[174,175] Higher cffDNA measured in the second trimester also increased the odds of delivery before 34 weeks (OR 22.0 95% CI 5.02–96.9).[176] The link between cffDNA and inflammation was hypothesized to be through activation of TLR-9, a PRR that recognized hypomethylated DNA, usually from bacterial DNA.[177] Also, cffDNA is hypomethylated and released from senescent placental and fetal membranes. Hence, recognition by TLR-9 provided a framework to suggest increasing cffDNA with gestation-activated sterile inflammation seen at the time of labor. This hypothesis was underpinned by animal data demonstrating that cffDNA injected into pregnant mice led to labor—effects that were mitigated in TLR9-deficient mice.[178] It has since been demonstrated that women in labor actually have a higher proportion of methylated cffDNA compared to nonlaboring women.[179] This seemingly refutes the hypothesis of TLR-9 activation; however, hypomethylation increases from 28 to 36 weeks, and the total amount of cffDNA in laboring women is higher than in nonlaboring women. Hence, it remains possible that there is a relative increase in TLR-9 activation at the time of labor.[179]

13.7 A SUMMARY OF HUMAN PARTURITION

In early pregnancy, progesterone, estrogen, and hCG are key hormones that enable endometrium priming, blastocyst activation, implantation, and placental decidualization. The action of progesterone is a brake on the uterus, which halts contractions until the fetus has completed development and maturation. It functions to inhibit the expression of contraction-associated proteins (OXTR, GJA1, and PTGS2), which are required in order for the myometrium of the uterus to contract during labor.

Mechanistically, progesterone inhibits inflammatory pathways, decreases stretch-activated transcription factors, and increases ZEB1/ZEB2, which work to repress contraction-associated proteins. Secondary messenger cAMP works to inactivate MLCK, a key kinase that phosphorylates myosin to enable interaction with actin. It also inhibits PLC, a key molecule in calcium signaling required for smooth muscle contraction. Further, cAMP enhances the progesterone block effect by increasing the binding of liganded PR-B to PRE-promotors on progesterone-dependent genes. In addition, cAMP is stimulated by hCG and CRH, both of which increase with gestational age and decline just prior to the onset of labor.

As increasing levels of CRH contributes to myometrial quiescence by increasing levels of cAMP, it also stimulates the production of fetal DHEAS, which is hydroxylated in the fetal liver before being converted to estriol by the placenta. Similarly, the placenta also produces estradiol from maternally derived DHEAS in equimolar concentrations to estriol, which causes the formation of biologically inactive heterodimers. Prior to the onset of labor, biologically active estriol homodimers are formed as production of estriol outstrips that of estradiol, leading to a functional activation of estrogen. There is also an upregulation of ER-a receptors in the myometrium.

Through the ER-α receptor, estrogen increases the expression of contraction-associated proteins. Oxytocin and prostaglandins increase intracellular Ca^{2+}, which combines with calmodulin to activate MLCK, which in turn is able to phosphorylate myosin. Phosphorylated myosin is able to interact with actin filaments in an ATP-dependent manner to cause smooth muscle contraction. The increase in myocyte connectivity via gap-junction proteins (e.g., connexin 43) allows a cluster of myocytes to depolarize in unison and function as coupled oscillators. As myocytes are increasingly connected

toward labor, patches of myometrium are able to contract in unison to generate sufficient force to cause increases in intrauterine pressure, which provides a global stimulus for all myocytes within the uterus to contract in unison.

The expression of contraction-associated proteins is further increased by inflammatory markers that are released in response to a number of stimuli, including microbial infection, uterine stretch, haemorrhage, and trauma in the setting of placental abruption, cellular senescence in fetal membranes, or inflammatory mediators such as surfactant proteins from the developing fetal lungs. Importantly, all of these stimuli are more likely to occur toward term, when the majority of women go into labor. Their presence attracts leukocytes and other immune cells, which release further inflammatory mediators, making parturition a highly inflammatory process. Inflammation also increases the production of MMPs and decreases their inhibitors in order to accelerate the cervical-ripening process, leading to cervical ripening and eventually dilation to allow passage of the fetus at birth.

At the same time as the observed inflammatory process, human parturition is characterized by a functional withdrawal of progesterone in the setting of increasing circulating progesterone levels. PR-A is able to inhibit the activity of PR-B (which is the predominant isoform that maintains pregnancy quiescence), and its expression is increased relative to PR-B at the time of labor. There is also increased hydroxysteroid dehydrogenase (HSD) enzyme activity, causing cellular metabolism of progesterone and leading to PR to become unliganded from progesterone during labor. Unliganded PR-A is able to act as a transcription factor in order to increase the expression of contraction-associated proteins in the myometrium.

The exact pathway leading to progesterone withdrawal is unclear; however, attractive hypotheses include functional estrogen activation and inflammation-led activation. It is also unclear whether hormonal processes (such as functional progesterone withdrawal and estrogen activation) initiate the process of labor, and therefore, inflammation is a consequence of labor; or whether inflammation is the initiating process that drives the hormonal changes that lead to labor.

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