Molecular Mechanisms of Preterm Delivery

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Abstract

Preterm delivery occurs in approximately 10% of all pregnancies and it is a leading cause of infant morbidity and mortality, risking lifelong health problems in those who survive. Spontaneous preterm delivery and preterm premature rupture of membrane (pPROM) result from multiple causes, such as infection or inflammation, intrauterine bleeding, maternal stress and nutrition, and uterine overdistension. Infection is a leading cause of preterm delivery. Bacteria are recognized by pattern recognition receptors—such as toll-like receptors, which induces the release of inflammatory chemokines and cytokines. Chemokines and cytokines also result in decline of progesterone receptor (PR) function and initiate myometrial contraction, and part of PR function is regulated by microRNAs. Maternal stresses increase hypothalamic corticotropin-releasing hormone (CRH) and plasma glucocorticoid, which in turn stimulate the release of placental CRH as "placental clock," enhancing prostaglandin (PG) synthesis. Fetal fibronectin or thrombin increases matrix metalloproteinases and PGE2 synthesis in amnion mesenchymal cells, which lead to membrane rupture, cervical ripening, and myometrial contraction. Here, the current understanding of the molecular mechanisms of preterm delivery is summarized.

Keywords

Preterm delivery • Preterm premature rupture of membrane • Infection • Progesterone • Corticotropin-releasing hormone • Thrombin • Fetal fibronectin • Matrix metalloproteinase • Prostaglandin

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15.1 Introduction

Preterm delivery occurs in approximately 5% of all births in Japan and in 10% of births in the United States [1]. The preterm delivery rate in developed countries has recently been increasing due to advanced maternal age and multiple pregnancies by assisted reproductive techniques. There are several risk factors of preterm delivery: infection, low socioeconomic status, low body mass index, previous preterm delivery, obesity, cigarette smoking, maternal poor nutrition, young or advanced age, periodontal disease, poverty, and genital bleeding in the first and second trimester [1]. Adenomyosis is also considered as a risk factor of preterm delivery due to increased synthesis of prostaglandins [2]. Recent advances of molecular biology have revealed the new mechanism of preterm delivery, but vast majority still remains unknown. This is evident from the fact that there are no newly developed drugs that efficiently prevent preterm delivery for decades, so the continuing pursuit of basic research is necessary.

15.2 Stress-Associated Preterm Delivery

Maternal stresses such as depression, anxiety, and chronic stress are associated with preterm delivery [3]. Moreover, the risk of preterm delivery is increased in women who work long hours [1]. In the hypothalamus, glucocorticoids inhibit release of corticotropin-releasing hormone (CRH). This decreases expression of adrenal glucocorticoid to establish classic negative feedback. During pregnancy, in contrast, glucocorticoids stimulate release of CRH from placenta and fetal membrane, and increased CRH enhances production of prostaglandins from fetal membrane [4, 5], which play pivotal roles in human parturition by stimulating cervical ripening, myometrial contraction, and fetal membrane rupture [6] (Fig. 15.1). Increase of fetal CRH also upregulates secretion ACTH from fetal pituitary, which enhances production of cortisol and dehydroepiandrosterone (DHEA) sulfate by the fetal adrenal. DHEA sulfate is subsequently metabolized to DHEA and aromatized within the placenta to estrogens, which oppose the action of progesterone [7]. In addition, the rise in CRH expression also induces synthesis of surfactant protein, surfactant protein A (SP-A), by the fetal lung [8]. SP-A gene expression is also increased by proinflammatory stimuli such as interleukine-1 (IL-1) via activation of NF-κB [9]. Therefore, augmented surfactant production by the maturing fetal lung may serve as a fetal signal for the initiation of labor. Lockwood et al. observed an exponential increase in maternal levels of CRH during gestation, peaking at the time of delivery, and this maternal serum CRH is placental origin [5]. When maternal plasma CRH level around 16–20 weeks of gestation is high, women are destined to experience preterm delivery, whereas when maternal CRH is low, women go into post-term delivery [10]. Thus, placental secretion of CRH decides the timing of delivery, working as “placental clock” [10]. Increased adrenal release of glucocorticoids from maternal stress further releases placental CRH by positive feedback loop, which promotes preterm delivery. Maternal stress is derived from

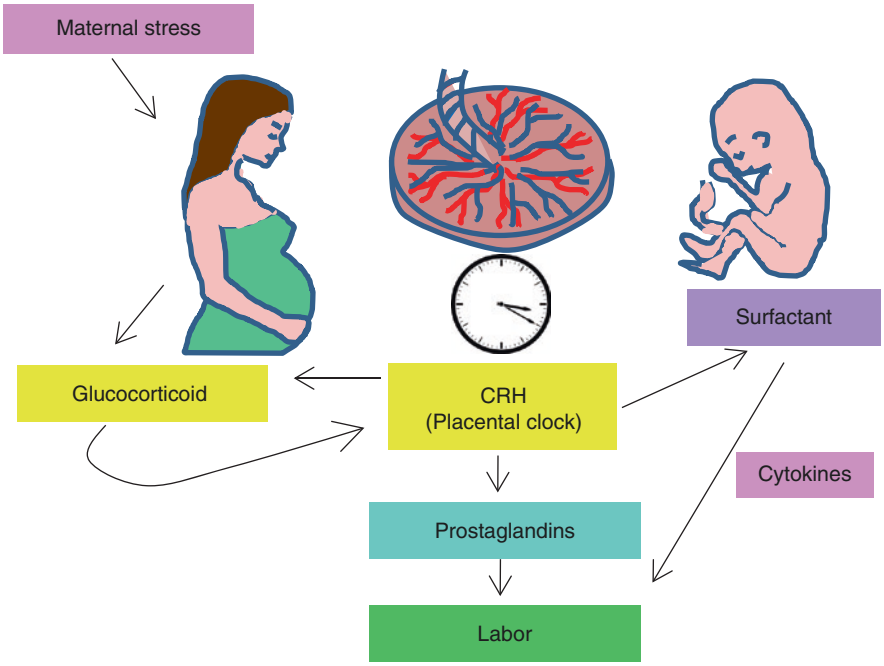

Fig. 15.1 Placental CRH decides the timing delivery as "placental clock". Maternal plasma CRH levels exponentially increase during pregnancy, peaking at term. This reflects the enhanced CRH synthesis in placenta. In women of preterm delivery, this increase is more rapid. Placental synthesis of CRH further increases glucocorticoid production in both mother and fetus in a manner of positive feed-forward loop. CRH stimulates prostaglandin production, which leads to cervical softening and myometrial contraction. The increase of CRH and glucocorticoid in fetus causes fetal lung maturation and surfactant synthesis. These surfactant proteins derived from fetus induce labor via upregulation of inflammatory cytokines

social factors, so it might be possible to decrease the preterm delivery rate by improving the environment of low socioeconomic individuals, reducing working hours for pregnant women, and providing psychological assistance to alleviate maternal mental stress.

15.3 Infection-Related Preterm Delivery and Vaginal Microbiome

Intrauterine infection is an important cause leading to preterm delivery, which occupies approximately 25% of preterm delivery cases [11]. Infection can occur between maternal decidua and fetal chorion (choriodecidual space) by bacteria ascending from vagina [12]. The most commonly identified bacteria are *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Gardnerella vaginalis*, *Peptostreptococcus*, and *Bacteroides* species—all vaginal organisms of relatively low virulence [12].

Prevalence of a *Lactobacillus*-poor vaginal community is inversely correlated with gestational age at delivery [13]. Nearly one-third of women with *Lactobacillus*-poor vaginal community delivered very preterm infants. In contrast, at least three-quarters of women who carried their pregnancies to term had *Lactobacillus*-dominant vaginal microbiota. Risk for preterm birth was more prominent for subjects with elevated *Gardnerella* or *Ureaplasma* abundances [13]. Interestingly, hyaluronic acids play an important role in epithelial barrier protection of the lower reproductive tract from bacteria. Depletion of hyaluronic acids in the cervix and vagina resulted in increased epithelial and mucosal permeability of bacteria and increased preterm delivery rates in mice [14]. Thus, keeping healthy vaginal microbiota is a key for successful pregnancy.

The mechanisms by which intrauterine infections lead to preterm delivery are related to activation of the innate immune system (Fig. 15.2). Microorganisms are recognized by pattern recognition receptors—such as toll-like receptors (TLRs).

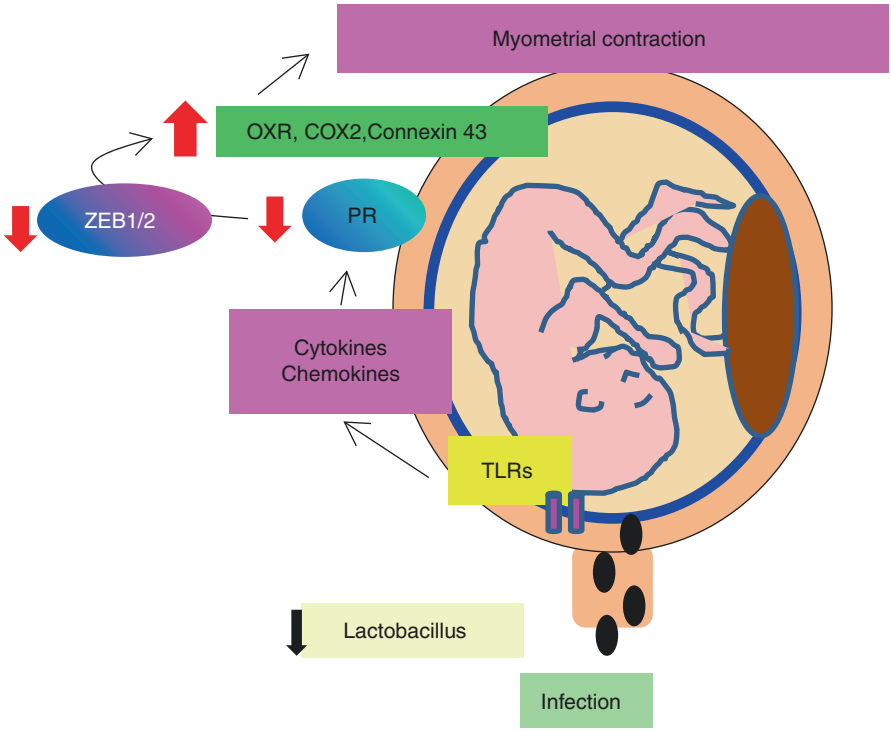

Fig. 15.2 Bacterial infections and myometrial contractions. Bacterial infections are recognized by pattern recognition receptors, such as toll-like receptors (TLRs). TLRs signaling activate inflammatory pathway as NFκB, which leads to induction of proinflammatory cytokines and chemokines. The inflammation induces decline of progesterone receptor (PR) and increases contraction-associated genes of uterus, oxytocin receptor, cylooxygenase-2 (COX2), and connexin 43 through downregulation of transcriptional factors, ZEB1 and ZEB2, which eventually leads to myometrial contraction and labor

TLRs are a family of transmembrane receptors that are involved in the regulation of the innate immune system [15]. TLR4 exists in both epithelial cells and mesenchymal cells of amnion [16]. TLR4-deficient mice are resistant to preterm delivery by intrauterine inoculation of heat-killed bacteria or LPS [17]. Activation of TLRs elicits the release of inflammatory chemokines and cytokines—such as IL-8, IL-1β, and tumor necrosis factor (TNF) α. Microbial endotoxins and proinflammatory cytokines stimulate the production of prostaglandins and matrix-degrading enzymes (matrix metalloproteinases, MMPs), which lead to preterm rupture of membrane. In the myometrium and cervix, proinflammatory cytokines activate inflammation-associated transcriptional factors such as NFκB and AP-1, inhibiting progesterone receptor (PR) function, which induces the expression of myometrial contractile genes [18]. In the cases of preterm delivery, concentrations of proinflammatory cytokines increase in amniotic fluid and migration of neutrophils and macrophages into the myometrium, cervix, and fetal membranes is observed [8, 19, 20]. Thus, inflammation plays an important role in both term and preterm delivery. In addition, intra-amniotic infection also attacks a fetus, causing a fetal systemic inflammatory response (FIRS). The concept of FIRS is determined by elevated fetal plasma IL-6 level [21]. FIRS is a risk factor for severe neonatal morbidity such as respiratory distress syndrome, neonatal sepsis, pneumonia, chronic lung disease, necrotizing enterocolitis, intraventriclular hemorrhage, and cerebral palsy [21]. Thus, although it is important to prevent intrauterine infection, immediate medical intervention to delivery is required once the sign of intra-amniotic infection appears to prevent a fetus from damage.

15.4 Myometrial Quiescence and Contraction

Throughout most of pregnancy, uterine quiescence is maintained by elevated progesterone acting through progesterone receptor (PR) [18]. In human, serum progesterone concentrations do not fall as labor approaches, so a decrease in local progesterone concentrations or number of receptors is a plausible mechanism of decline in PR function [22]. Progesterone antagonizes the inflammatory pathway such as NFκB and AP-1 by acting nuclear progesterone receptor (PR) and suppresses proinflammatory cytokines and chemokines. When pregnancy comes close to term, circulating estradiol-17β (E2) levels increases [23, 24], and enhanced estrogen receptor α (ERα) activity is enhanced [25, 26], which promote a proinflammatory cascade that contribute to the decline in PR function and initiate myometrial contraction (Fig. 15.2). Estrogens also induce an influx of macrophages and neutrophils into the uterus and further enhance proinflammatory event [27]. ERα activation facilitates myometrial contraction by enhancing transcription of the contraction-associated genes of the uterus, such as oxytocin receptor, connexin-43, and COX2 [25, 28–30]. The expressions of these contraction-associated genes are low throughout most of pregnancy but are highly upregulated at term.

A microRNA is a small noncoding RNA molecule (containing about 22 nucleotides) found in plants, animals, and some viruses, which functions in RNA silencing and posttranscriptional regulation of gene expression [31]. Recently, miR-200

family is found to be closely associated with labor. In both mouse and human uterus, miR-200 family (miR-200b and miR-429) is highly induced at term, whereas its target genes, ZEB1 and ZEB2, zinc finger E-box binding homeobox proteins, are downregulated [32]. ZEB1 and ZEB2 are transcriptional factors that are associated with epithelial mesenchymal transition. ZEB1 is directly upregulated by the action of P4/PR. ZEB1 and ZEB2 not only inhibit expression of the contraction-associated genes, oxytocin receptor and connexin-43, but also block oxytocin-induced contractility in human myometrial cells. The downregulation of ZEB1 and ZEB2 was observed in LPS- or RU486- induced mouse preterm delivery model. Thus, the miR-200 family and their targets, ZEB1 and ZEB2, are P4/PR-mediated regulators of uterine quiescence and contractility during pregnancy and labor.

15.5 Structure of Fetal Membrane and Preterm Premature Rupture of Membrane (pPROM)

Preterm premature rupture of membrane (pPROM) is associated with about one-third of preterm delivery cases and occurs in 1–3% of all pregnancies. The primary load-bearing structure of the fetal membranes is the amnion, which comprises a single layer of epithelial cells and an underlying layer of mesenchymal cells [33]. Mesenchymal cells are the primary source of collagen and matrix support in the amnion. Interstitial collagens (types I, III, and V) maintain the mechanical integrity of the amnion. Fetal membrane rupture is preceded by the degradation of collagen that is mediated primarily by matrix metalloproteinase (MMPs) in the amnion. Interstitial collagenase, MMP1, cleaves the triple helix of fibrillar collagen, which is then further degraded by the gelatinases, MMP2 and MMP9. pPROM and intera-uterine MMPs activity is closely correlated. MMP1 in amniotic fluid and MMP9 in amniotic membranes are elevated in women with pPROM [34–37]. Ehlers-Danlos syndrome, inheritable connective tissue disorder, is a risk factor of PROM by a defect in the structure, production, or processing of collagen or proteins that interact with collagen [38, 39].

15.6 Fibronectin

Fibronectin (FN) is a large extracellular glycoprotein that helps cells attach to the matrix. Fetal FN (fFN) is one of the FN proteins produced by fetal cells. It is diffusely distributed in the fetal membrane, from the amnion to decidua, providing structural support and adhesion of the fetal membranes to the uterine lining, and fFN in cervical and vaginal secretions has been used as a clinical marker of preterm delivery [40]. In vitro, fFN treatment results in increased expression of MMP1 and MMP9, mRNA, and enzymatic activity, as well as COX2 mRNA and PGE_2 synthesis in amnion mesenchymal cells, activating both NFκB and MAPK pathway [41] (Fig. 15.3). fFN has a unique alternatively spliced exon encoding extra domain-A (EDA) [42]. The treatment of amnion mesenchymal cells with recombinant EDA

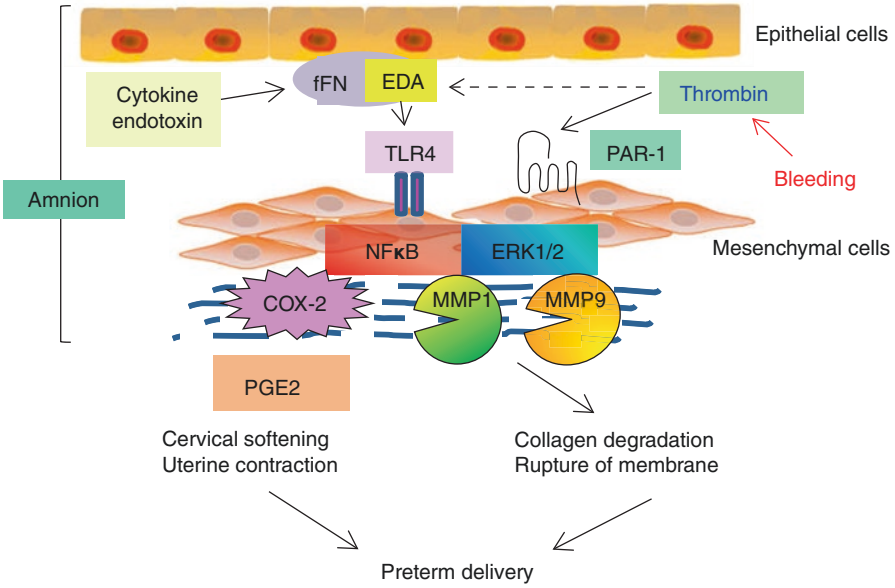

Fig. 15.3 fFN and thrombin signaling in the pathogenesis of pPROM and preterm delivery. LPS and proinflammatory cytokines such as TNF-α effect increased amounts of free fFN from amnion epithelial cells. Thrombin generated by intrauterine bleeding also increases free fFN in extracellular matrix of amnion. Released fFN activates TLR4 receptor on mesenchymal cells through its EDA. Activation of TLR4 leads to intracellular signaling through NFκB and ERK1/2 to induce expression of COX2 and MMPs, thereby leading to cervical ripening, uterine contractions, and collagenolytic degradation of the fetal membranes. Thrombin also directly activates PAR-1 signaling, which upregulates MMP9

also resulted in increases in MMP1 and MMP9 mRNA levels and enzymatic activity, as well as in the COX2 mRNA level and PGE_2 synthesis, indicating that EDA is a functional domain of fFN, and function of EDA was mediated via TLR4 [41]. Thus, neutralization of fFN-EDA domain or antagonism of TLR4 may have therapeutic potential for preterm delivery and pPROM.

A question is how fFN is increased and released in preterm delivery. Fibronectin-1 (FN1) protein and mRNA were dose-dependently increased by lipopolysaccharide (LPS) or TNF-α treatment in epithelial cells. This data show that epithelial cells of the amnion function as a sensor to harmful inflammatory stimuli and sends a "danger signal" by releasing fFN in the extracellular matrix. Then, mesenchymal cells receive the fFN danger signal from epithelial cells and begin producing MMPs and PGE_2. In other words, fFN "amplifies" the dangerous signal produced by endotoxins and proinflammatory cytokines in order to cause the rupture of the membrane or a preterm delivery via activation of MMPs and PGE2. This amplification of inflammation by fFN function would be evolutionally important because once infection has occurred, a fetus should be immediately released from harmful intrauterine inflammation by

delivery. Moreover, the delivery of an already infected fetus is the only way to protect a mother from fatal inflammation such as maternal sepsis.

15.7 Intrauterine Bleeding, Thrombin, and Risk of Preterm Delivery

Intrauterine bleeding or hematoma during early pregnancy is correlated with an increased risk for adverse maternal and neonatal complications. Nagy et al. reported a 2-fold increase in preterm delivery in the hematoma group [43]. Moreover, pregnancy-induced hypertension, preeclampsia, placental abruption, and fetal growth restriction were also frequent in this group. Similarly, Tuuli et al. reported that subchorionic hematoma was associated with a 1.5-fold increase in preterm delivery and pPROM, a 2-fold increase in spontaneous abortion and stillbirth, and a 5-fold increase in placental abruption [44]. These reports indicate that intrauterine bleeding during pregnancy is a strong risk factor of perinatal complications, especially of preterm delivery.

Thrombin is a trypsin-like serine proteinase, the most abundant enzyme associated with blood coagulation. In addition to its role in hemostasis, thrombin also influences normal and pathological processes, such as inflammation, tissue repair, embryogenesis, angiogenesis, and tumor invasion [45]. There is considerable clinical evidence pointing to a role of thrombin in preterm delivery. Thrombin-antithrombin complexes, markers of in vivo generation of thrombin, are increased in the plasma [46, 47] and amniotic fluid [46] of women in preterm labor or pPROM. Placental abruption-induced thrombin generation has been associated with fetal membrane weakening and pPROM [47, 48], and treatment of amnion explants with thrombin results in increased levels of MMP9 and mechanical weakening [49]. In an animal model, intrauterine administration of whole blood to pregnant rats stimulates myometrial contractility, whereas blood containing heparin or a thrombin inhibitor does not [50].

Thrombin activity was significantly increased in amniotic membranes from women who delivered preterm [51]. Considering that the decidua is the primary source of thrombin [52], increased thrombin activity is probably due to the bleeding from the decidua in the early stage of pregnancy, and thrombin activity would remain in the amnion for several months until the preterm delivery finally occurs. In primary amnion cells, thrombin treatment resulted in an increase of MMP1 and MMP9 mRNA and enzymatic activity, conversion of MMP2 to its active form, and COX2 mRNA and PGE_2 synthesis in amnion mesenchymal cells (Fig. 15.3). These activations were mediated by G protein-coupled thrombin receptor, protease-activated receptor-1 (PAR-1), and TLR4 [51]. When thrombin or PBS was locally injected into the uterus of pregnant mice, all thrombin-injected mice delivered preterm, whereas PBS did not [51]. In these mice, *collagenase-2 (MMP8)* and *collagenase-3 (MMP13)*, gelatinase *MMP9* mRNA as well as PGE_2 synthesis were all increased in fetal membranes. Thus, thrombin weakens the membrane by degrading collagen through upregulation of MMPs

and stimulates cervical ripening and myometrial contraction through the production of PGE_2.

Conclusion

Bacterial infection, presumably due to *Lactobacillus*-poor vaginal community, activates pattern recognition receptors, which induces the release of inflammatory chemokines and cytokines. Chemokines and cytokines result in decline of progesterone receptor (PR) function, and enhancement of estrogen receptor activity in uterus initiates myometrial contraction. Placental CRH exponentially increases during pregnancy, serving as a "placental clock," which is further increased by maternal and fetal glucocorticoid as positive feedback loop. CRH enhances prostaglandin synthesis. Fetal fibronectin or thrombin increases matrix metalloproteinases and PGE2 synthesis in amnion mesenchymal cells. Together, these molecular events converge to membrane rupture, cervical ripening, and myometrial contraction of preterm delivery and pPROM. Continuing basic research is necessary to reduce the preterm delivery.

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References

1. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008;371(9606):75–84. doi:10.1016/S0140-6736(08)60074-4.
2. Juang CM, Chou P, Yen MS, Twu NF, Horng HC, Hsu WL. Adenomyosis and risk of preterm delivery. BJOG. 2007;114(2):165–9. doi:10.1111/j.1471-0528.2006.01186.x.
3. Hedegaard M, Henriksen TB, Sabroe S, Secher NJ. Psychological distress in pregnancy and preterm delivery. BMJ. 1993;307(6898):234–9.
4. Jones SA, Brooks AN, Challis JR. Steroids modulate corticotropin-releasing hormone production in human fetal membranes and placenta. J Clin Endocrinol Metab. 1989;68(4):825–30. doi:10.1210/jcem-68-4-825.
5. 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(3):243–51.
6. Challis JR, Sloboda DM, Alfaidy N, Lye SJ, Gibb W, Patel FA, et al. Prostaglandins and mechanisms of preterm birth. Reproduction. 2002;124(1):1–17.
7. Mendelson CR. Minireview: fetal-maternal hormonal signaling in pregnancy and labor. Mol Endocrinol. 2009;23(7):947–54. doi:10.1210/me.2009-0016.
8. 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 U S A. 2004;101(14):4978–83. doi:10.1073/pnas.0401124101.
9. Islam KN, Mendelson CR. Potential role of nuclear factor kappaB and reactive oxygen species in cAMP and cytokine regulation of surfactant protein-A gene expression in lung type II cells. Mol Endocrinol. 2002;16(6):1428–40. doi:10.1210/mend.16.6.0856.
10. 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(5):460–3.
11. Challis JR, Lockwood CJ, Myatt L, Norman JE, Strauss 3rd JF, Petraglia F. Inflammation and pregnancy. Reprod Sci. 2009;16(2):206–15. doi:10.1177/1933719108329095.

12. Goldenberg RL, Hauth JC, Andrews WW. Intrauterine infection and preterm delivery. N Engl J Med. 2000;342(20):1500–7. doi:10.1056/NEJM200005183422007.
13. DiGiulio DB, Callahan BJ, McMurdie PJ, Costello EK, Lyell DJ, Robaczewska A, et al. Temporal and spatial variation of the human microbiota during pregnancy. Proc Natl Acad Sci U S A. 2015;112(35):11060–5. doi:10.1073/pnas.1502875112.
14. Akgul Y, Word RA, Ensign LM, Yamaguchi Y, Lydon J, Hanes J, et al. Hyaluronan in cervical epithelia protects against infection-mediated preterm birth. J Clin Invest. 2014;124(12):5481–9. doi:10.1172/JCI78765.
15. Akira S, Takeda K. Toll-like receptor signalling. Nat Rev Immunol. 2004;4(7):499–511. doi:10.1038/nri1391.
16. Adams KM, Lucas J, Kapur RP, Stevens AM. LPS induces translocation of TLR4 in amniotic epithelium. Placenta. 2007;28(5–6):477–81. doi:10.1016/j.placenta.2006.08.004.
17. Wang H, Hirsch E. Bacterially-induced preterm labor and regulation of prostaglandin-metabolizing enzyme expression in mice: the role of toll-like receptor 4. Biol Reprod. 2003;69(6):1957–63. doi:10.1095/biolreprod.103.019620.
18. Renthal NE, Williams KC, Mendelson CR. MicroRNAs—mediators of myometrial contractility during pregnancy and labour. Nat Rev Endocrinol. 2013;9(7):391–401. doi:10.1038/nrendo.2013.96.
19. Osman I, Young A, Ledingham MA, Thomson AJ, Jordan F, Greer IA, et al. Leukocyte density and pro-inflammatory cytokine expression in human fetal membranes, decidua, cervix and myometrium before and during labour at term. Mol Hum Reprod. 2003;9(1):41–5.
20. Thomson AJ, Telfer JF, Young A, Campbell S, Stewart CJ, Cameron IT, et al. Leukocytes infiltrate the myometrium during human parturition: further evidence that labour is an inflammatory process. Hum Reprod. 1999;14(1):229–36.
21. Gomez R, Romero R, Ghezzi F, Yoon BH, Mazor M, Berry SM. The fetal inflammatory response syndrome. Am J Obstet Gynecol. 1998;179(1):194–202.
22. Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. Science. 2014;345(6198):760–5. doi:10.1126/science.1251816.
23. Buster JE, Chang RJ, Preston DL, Elashoff RM, Cousins LM, Abraham GE, et al. Interrelationships of circulating maternal steroid concentrations in third trimester pregnancies. II. C18 and C19 steroids: estradiol, estriol, dehydroepiandrosterone, dehydroepiandrosterone sulfate, delta 5-androstenediol, delta 4-androstenedione, testosterone, and dihydrotestosterone. J Clin Endocrinol Metab. 1979;48(1):139–42. doi:10.1210/jcem-48-1-139.
24. Challis JR. Sharp increase in free circulating oestrogens immediately before parturition in sheep. Nature. 1971;229(5281):208.
25. 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(6):2924–30. doi:10.1210/jcem.87.6.8609.
26. Welsh T, Johnson M, Yi L, Tan H, Rahman R, Merlino A, et al. Estrogen receptor (ER) expression and function in the pregnant human myometrium: estradiol via ERalpha activates ERK1/2 signaling in term myometrium. J Endocrinol. 2012;212(2):227–38. doi:10.1530/JOE-11-0358.
27. Tibbetts TA, Conneely OM, O'Malley BW. Progesterone via its receptor antagonizes the pro-inflammatory activity of estrogen in the mouse uterus. Biol Reprod. 1999;60(5):1158–65.
28. Murata T, Narita K, Honda K, Matsukawa S, Higuchi T. Differential regulation of estrogen receptor alpha and beta mRNAs in the rat uterus during pregnancy and labor: possible involvement of estrogen receptors in oxytocin receptor regulation. Endocr J. 2003;50(5):579–87.
29. Piersanti M, Lye SJ. Increase in messenger ribonucleic acid encoding the myometrial gap junction protein, connexin-43, requires protein synthesis and is associated with increased expression of the activator protein-1, c-fos. Endocrinology. 1995;136(8):3571–8. doi:10.1210/endo.136.8.7628395.
30. Tsuboi K, Sugimoto Y, Iwane A, Yamamoto K, Yamamoto S, Ichikawa A. Uterine expression of prostaglandin H2 synthase in late pregnancy and during parturition in prostaglandin F receptor-deficient mice. Endocrinology. 2000;141(1):315–24. doi:10.1210/endo.141.1.7236.

31. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116(2):281–97.
32. Renthal NE, Chen CC, Williams KC, Gerard RD, Prange-Kiel J, Mendelson CR. miR-200 family and targets, ZEB1 and ZEB2, modulate uterine quiescence and contractility during pregnancy and labor. Proc Natl Acad Sci U S A. 2010;107(48):20828–33. doi:10.1073/pnas.1008301107.
33. Parry S, Strauss 3rd JF. Premature rupture of the fetal membranes. N Engl J Med. 1998;338(10):663–70.
34. Athayde N, Edwin SS, Romero R, Gomez R, Maymon E, Pacora P, et al. A role for matrix metalloproteinase-9 in spontaneous rupture of the fetal membranes. Am J Obstet Gynecol. 1998;179(5):1248–53. doi:S0002937898701413 [pii].
35. Draper D, McGregor J, Hall J, Jones W, Beutz M, Heine RP, et al. Elevated protease activities in human amnion and chorion correlate with preterm premature rupture of membranes. Am J Obstet Gynecol. 1995;173(5):1506–12. doi:0002-9378(95)90640-1 [pii].
36. Maymon E, Romero R, Pacora P, Gervasi MT, Bianco K, Ghezzi F, et al. Evidence for the participation of interstitial collagenase (matrix metalloproteinase 1) in preterm premature rupture of membranes. Am J Obstet Gynecol. 2000;183(4):914–20. doi:10.1067/mob.2000.108879.
37. Vadillo-Ortega F, Gonzalez-Avila G, Furth EE, Lei H, Muschel RJ, Stetler-Stevenson WG, et al. 92-kd type IV collagenase (matrix metalloproteinase-9) activity in human amniochorion increases with labor. Am J Pathol. 1995;146(1):148–56.
38. Barabas AP. Ehlers-Danlos syndrome: associated with prematurity and premature rupture of foetal membranes; possible increase in incidence. Br Med J. 1966;2(5515):682–4.
39. Yen JL, Lin SP, Chen MR, Niu DM. Clinical features of Ehlers-Danlos syndrome. J Formos Med Assoc. 2006;105(6):475–80. doi:10.1016/S0929-6646(09)60187-X.
40. Lockwood CJ, Senyei AE, Dische MR, Casal D, Shah KD, Thung SN, et al. Fetal fibronectin in cervical and vaginal secretions as a predictor of preterm delivery. N Engl J Med. 1991;325(10):669–74. doi:10.1056/NEJM199109053251001.
41. Mogami H, Kishore AH, Shi H, Keller PW, Akgul Y, Word RA. Fetal fibronectin signaling induces matrix metalloproteases and cyclooxygenase-2 (COX-2) in amnion cells and preterm birth in mice. J Biol Chem. 2013;288(3):1953–66. doi:10.1074/jbc.M112.424366.
42. Kornblihtt AR, Vibe-Pedersen K, Baralle FE. Human fibronectin: molecular cloning evidence for two mRNA species differing by an internal segment coding for a structural domain. EMBO J. 1984;3(1):221–6.
43. Nagy S, Bush M, Stone J, Lapinski RH, Gardo S. Clinical significance of subchorionic and retroplacental hematomas detected in the first trimester of pregnancy. Obstet Gynecol. 2003;102(1):94–100.
44. Tuuli MG, Norman SM, Odibo AO, Macones GA, Cahill AG. Perinatal outcomes in women with subchorionic hematoma: a systematic review and meta-analysis. Obstet Gynecol. 2011;117(5):1205–12. doi:10.1097/AOG.0b013e31821568de.
45. Coughlin SR. Thrombin signalling and protease-activated receptors. Nature. 2000;407(6801):258–64. doi:10.1038/35025229.
46. Chaiworapongsa T, Espinoza J, Yoshimatsu J, Kim YM, Bujold E, Edwin S, et al. Activation of coagulation system in preterm labor and preterm premature rupture of membranes. J Matern Fetal Neonatal Med. 2002;11(6):368–73.
47. Rosen T, Kuczynski E, O'Neill LM, Funai EF, Lockwood CJ. Plasma levels of thrombin-antithrombin complexes predict preterm premature rupture of the fetal membranes. J Matern Fetal Med. 2001;10(5):297–300.
48. Mackenzie AP, Schatz F, Krikun G, Funai EF, Kadner S, Lockwood CJ. Mechanisms of abruption-induced premature rupture of the fetal membranes: Thrombin enhanced decidual matrix metalloproteinase-3 (stromelysin-1) expression. Am J Obstet Gynecol. 2004;191(6):1996–2001. doi:10.1016/j.ajog.2004.08.003.

49. Kumar D, Schatz F, Moore RM, Mercer BM, Rangaswamy N, Mansour JM, et al. The effects of thrombin and cytokines upon the biomechanics and remodeling of isolated amnion membrane, in vitro. Placenta. 2011;32(3):206–13. doi:10.1016/j.placenta.2011.01.006.
50. Balbin M, Fueyo A, Knauper V, Pendas AM, Lopez JM, Jimenez MG, et al. Collagenase 2 (MMP-8) expression in murine tissue-remodeling processes. Analysis of its potential role in postpartum involution of the uterus. J Biol Chem. 1998;273(37):23959–68.
51. Mogami H, Keller PW, Shi H, Word RA. Effect of thrombin on human amnion mesenchymal cells, mouse fetal membranes, and preterm birth. J Biol Chem. 2014;289(19):13295–307. doi:10.1074/jbc.M114.550541.
52. Lockwood CJ, Krikun G, Papp C, Toth-Pal E, Markiewicz L, Wang EY, et al. The role of progestationally regulated stromal cell tissue factor and type-1 plasminogen activator inhibitor (PAI-1) in endometrial hemostasis and menstruation. Ann N Y Acad Sci. 1994;734:57–79.