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COMPARISON OF THE MECHANISMS RESPONSIBLE FOR CERVICAL REMODELING IN PRETERM AND TERM LABOR

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Abstract

Understanding the mechanisms of term and preterm cervical remodeling is essential to prevent prematurity. Is preterm cervical remodeling caused by the same mechanisms that cause cervical remodeling at term, and are these changes accelerated in time? This question has been pondered by obstetricians seeking for strategies to prevent preterm labor for many years. Mice represent an informative model of preterm birth. Thus, in this review we will discuss the recent findings from mouse models that identify and characterize the initiators and cellular effectors of cervical remodeling at term and preterm delivery. These studies suggest that similarities and differences exist between term and preterm cervical remodeling. Complement is an initiator or mediator in preterm delivery but is not involved in the physiological process that leads to term delivery. Therefore, complement constitutes a specific and selective target for potentially preventing preterm delivery, thus improving neonatal health.

Keywords

Mouse model; inflammation; preterm labor; cervical remodeling complement activation; pregnancy

INTRODUCTION

Successful parturition in mammalian species is characterized by two essential processes. First, the myometrium changes from a quiescent contractile state. Second, the uterine cervix must undergo a substantial remodeling process, during which a closed, rigid cervix turns into a distensible and elastic ring that stretches to allow passage of the fetus. Then, the cervix undergoes rapid repair in order to ensure protection of the reproductive tract from microbial infection. Appropriate and timely cervical remodeling, which results in dilation and effacement of the cervix, can lead to preterm birth, a leading cause of perinatal mortality and morbidity. More than 15 million neonates are born too soon worldwide annually. Preterm neonates are at risk for serious medical problems, such as cerebral palsy, infection, respiratory, blindness, hearing loss, learning and developmental problems (Lawn et al., 2005; Oestergaard et al., 2011). Unfortunately, for the majority of cases of premature births, there is no procedure or test to predict premature birth. A recently published meta-analysis showed that none of the biomarkers evaluated meet the criteria to be considered a clinically useful test for predicting spontaneous preterm birth (Agudelo et al., 2011). Unfortunately,

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treatment is rarely successful. There are only a few applicable preventative and therapeutic solutions for preterm delivery – a condition that contributes directly and indirectly to an estimated 1 million deaths that occur worldwide every year.

Many observations in humans highlight the crucial and causative role of cervical remodeling in preterm delivery (PTD). Drugs that arrest or inhibit uterine contractions, known as tocolytics, can delay delivery, but do not reliably reduce or prevent preterm birth (Anotayanonth et al., 2004; Crowther et al., 2011; King et al., 2001; Papatsonis et al., 2005). Additionally, assessment of contraction frequency is not a useful test for predicting preterm birth (Iams et al., 2001). Extensive evidence is available that a short cervix by transvaginal ultrasound is a predictor of preterm parturition (Hassan et al., 2000; Iams et al., 1996; Vaisbuch et al., 2010a). A short cervix is suggestive of cervical ripening (Romero, 2007). Vaginal progesterone has been shown to reduce preterm birth in patients with a short cervix (DeFranco et al., 2007; Fonseca et al., 2007; Hassan et al., 2011). Finally, that cervical cerclage reduces recurrent preterm birth in women with prior preterm birth and a short cervix constitutes compelling evidence of the important role of the cervix in PTD (Berghella et al., 2005; Owen et al., 2009).

Research focused on the mechanisms of term and preterm cervical is important for the development of a strategy to prevent preterm birth. Is preterm cervical remodeling caused by the same mechanism(s) that cause(s) cervical remodeling at term but these changes are accelerated in time? This question has been pondered by obstetricians seeking for strategies to prevent preterm labor for many years. Mice constitute an informative model of preterm birth (Elovitz and Mrinalini, 2004; Jenkin and Young, 2004). Thus, in this review we will discuss the recent findings that identify and characterize the initiators and cellular effectors of cervical remodeling at term and preterm in mice. These studies suggest that similarities and differences exist between term and preterm cervical remodeling. The identification of initiators and/or mediators in PTD not involved in the physiological process that leads to term delivery could represent specific and selective targets for preventing PTD.

Complement activation is required for delivery but not term delivery

Activation of the complement system, an important component of innate immunity, has been associated with several complications of pregnancy including spontaneous pregnancy loss (Girardi et al., 2006, 2006) and pre-eclampsia (Haeger et al., 1991; Derzy et al., 2010). Several studies have also suggested that complement activation plays a crucial role in PTD (Lynch et al., 2008; Vaisbuch et al., 2009). Importantly, complement activation initiated by the alternative pathway has been associated with preterm labor and delivery, but not term delivery (Vaisbuch et al., 2010b). The association between complement activation and pregnancy complications led us to investigate the role of complement in PTD in mice. To test the role of complement in the pathogenesis of cervical remodeling that leads to preterm labor, we used a mouse model of PTD induced by intravaginal administration of LPS (Gonzalez et al., 2011a, 2011b). This model mimics a clinical or subclinical genital infection that can ascend through the vagina to the cervix and initiate the process of cervical remodeling leading to premature labor. No maternal morbidity or mortality was observed in this model. This is a unique model of inflammation-induced preterm labor that to our knowledge has not previously been reported.

Increased complement component C3 deposition was observed in the cervical columnar epithelium in samples collected antepartum and intrapartum in LPS-treated mice compared with mice with term labor (Figure 1) (Gonzalez et al., 2011a,b). A moderate increase in the complement split products, C3a and C5a, was observed in serum of LPS-treated mice antepartum and intrapartum compared with that of mice which deliver at term (Gonzalez et al., 2011a). However, these values were within the normal serum levels for a mouse and

were much lower than the serum C3a and C5a values reported in inflammatory processes like recurrent miscarriages and in animal or human sepsis (Girardi et al., 2004; Smedegard et al., 1989). These data indicate that the model of intravaginal LPS-induced PTD is characterized by a localized inflammatory process in the cervix and not systemic inflammation.

While trying to identify the complement components and receptors required for cervical remodeling induced by LPS, we discovered that C5a-C5aR interaction is a critical mediator of LPS-induced preterm delivery. We arrived to this conclusion after observing that C5aR-deficient mice did not deliver preterm in response to LPS when compared to wild type mice (Gonzalez et al., 2011a). Increased metalloproteinases activity and collagen degradation were not observed in cervical tissue of C5aR-deficient mice treated with LPS compared to wild-type mice (Figure 2) (Gonzalez et al., 2011a). These findings correlated well with the results of biomechanical studies of the cervix. Decreased distensibility was observed in the cervix of C5aR-deficient mice treated with LPS compared with that of wild-type mice (Gonzalez et al., 2011a). These data suggest that C5aR is required for preterm cervical remodeling and PTD in LPS-treated mice (Gonzalez et al., 2011a). The finding that mice deficient in C5aR (Gonzalez et al., 2011a) and other complement components (unpublished observations) deliver at term emphasizes that complement is not required for cervical remodeling at term.

The observation that the absence of C5aR prevents PTD in mice has important therapeutic implications. Complement inhibitors are being tested in patients with inflammatory, ischemic and autoimmune diseases. Identifying complement-related markers that predict high risk for PTD would allow the design of interventions that prevent or arrest this pathological process.

Inflammatory cells are present in the cervix in preterm but not term delivery

In addition, the presence of inflammatory cells in the cervix was also reported in the intrauterine LPS mouse model of preterm delivery. On the other hand, conflicting results can be found in the literature regarding the role of inflammatory cells in cervical remodeling at term (Elovitz and Mrinalini, 2004; Yellon et al., 2009; Gonzalez et al., 2009). Mackler et al. (1999) demonstrated that macrophage trafficking to the cervix contributes to the ripening process, in preparation for parturition in mice at term. In addition, increased infiltration of macrophages was associated with reduced density of collagen in the cervix late in pregnancy (Yellon et al., 2008).

Some studies in humans also suggest the involvement of inflammatory cells, neutrophils, and macrophages in cervical ripening (Bokstrom et al., 1997). Nevertheless, these cervical samples at term were collected after the delivery of the neonate and placenta by Cesarean section. Because surgical interventions induce leukocyte demargination and infiltration into tissues these results should be interpreted cautiously. Another study showed the involvement of macrophages in cervical ripening at term by immunohistochemistry (Sakamoto et al., 2005). In these studies, the authors used CD14 – a marker also present in neutrophils and dendritic cells – to identify macrophages. In addition, Bishop scores instead of histological studies –the ultimate confirmation of cervical remodeling – were used to evaluate cervical ripening. Of note, the term samples in this study were obtained after vaginal delivery when the repair process of the cervix, which is likely mediated by inflammation, had already started (Timmons and Mahendroo, 2007). Cervical cytology performed postpartum demonstrated evidence of inflammation supporting its role in the repair process (Price et al., 1992). Studies from our laboratory suggest that cervical ripening at term does not require inflammatory cells. Neither macrophages nor neutrophils were present in the cervical tissue of mice both antepartum and intrapartum (Gonzalez et al.,

2011a). These data are in agreement with other studies. Timmons and colleagues showed that macrophages and neutrophils are not found during cervical remodeling at term in mice (Timmons and Mahndroo, 2007). The studies demonstrating that steroid 5 α -reductase type 1 null mice (Srd5a1 $^{-/-}$) which do not recruit inflammatory cells in the cervix give birth normally buttress the concept that inflammatory cells are not required for term parturition (Timmons and Mahndroo, 2007). In addition, granulocyte depletion with monoclonal antibodies LyG (Gr1) before birth has no effect on the timing or outcome of parturition in mice (Timmons and Mahndroo, 2007). A case report on Chediak-Higashi syndrome characterized by decreased leukocyte chemotaxis showed that pregnancy, labor, and delivery were not affected (Price et al., 1992). Human and mouse data suggest that labor at term is a nonleukocyte dependent process (Timmons and Mahndroo, 2007; Hassan et al., 2009). Furthermore, a human study underscored the idea that there is no correlation between inflammation and cervical remodeling at term. In this study, the authors reported that inflammation-related genes did not emerge as differentially expressed with cervical ripening (Hassan et al., 2009).

Even though human studies are required for the understanding of the mechanisms of cervical ripening, these studies are challenging. Certain variables, like the site from which the biopsies are collected and the time of collection, might influence the interpretation of results. Human biopsies represent a small area of the cervix and when the biopsies are obtained; intrapartum may overlap with postpartum and the cervical repair process might have already started. Indeed many studies demonstrate the crucial role of inflammatory cells in the cervical tissue repair after delivery.

Preterm and term cervical remodeling share a common final effector pathway that involves metalloproteinases activation and collagen degradation

Collagen type I (70%) and type III (30%) are the predominant components of the extracellular matrix of the cervix and give it tensile strength. Collagenases of the matrix metalloproteinase (MMP) family degrade collagen, softening the cervix (a process called cervical remodeling), and preparing it for dilation and delivery (Word, 2007). For many years it was believed that MMP-2 degrades collagen to gelatin and subsequently MMP-9 degrades gelatin. However, recent studies demonstrated that MMP-9 is also a collagenase with the capacity to cleave collagens type I and III (Gonzalez et al., 2011a). Thus, MMP-2 and MMP-9 are both capable of degrading collagens type I and III, the main constituents of the cervix.

There is some disagreement in the literature regarding the role of MMPs in collagen degradation and cervical remodeling. Substantial evidence that MMPs degrade collagen, increasing cervical distensibility and leading to parturition, has been described (Gonzalez et al., 2011a,b; Sennstrom et al., 2003; Stygar et al., 2002). Our studies demonstrate that changes in collagen distribution/organization by MMPs precede cervical ripening both in preterm and term delivery (Gonzalez et al., 2011a,b). During cervical remodeling at term and preterm, collagen packing was disorganized, collagen fiber dispersion increased, and greater spacing between fibers was observed (Gonzalez et al., 2011a,b). These changes were correlated with changes in the biomechanical properties of the cervix, whose main characteristic was increased distensibility. The crucial role of MMPs in the cervical changes that precede parturition is illustrated in functional zymography tests (Gonzalez et al., 2011a,b). Decreased density in collagen fibers was associated with increased cervical MMP activity. In situ zymography using DQ gelatin and DQ collagen I as substrate revealed an increase in activity of MMPs with gelatinolytic and collagenolytic activity in the cervix of mice that deliver preterm and term. Altogether, these data suggest that MMP-9 and/or MMP-2 might be responsible for the degradation of the cervical matrix. Immunohistochemical studies identified MMP-9 as the main collagenase involved in

cervical remodeling in term and preterm delivery. Increased MMP-9 (but not MMP-2) staining was observed in the stroma and epithelium of the cervixes of mice during term and preterm delivery (Gonzalez et al., 2011a,b).

Conversely, recent studies suggest that there is no correlation between MMP activity, collagen degradation, and cervical ripening. In one of these studies, incubation of cervical tissue with MMP-1 did not affect the tissue's biomechanical properties (Buhimschi et al., 2004). Nevertheless, because these studies were performed in vitro, the extrapolation of these results to an in vivo physiologic process has limitations.

Conflicting results also exist regarding the cervical collagen content during ripening. Shi et al. demonstrated that cervical resistance and collagen content decreased progressively during pregnancy (Shi et al., 1999). In addition, these authors found that the collagen content determines cervical resistance (Shi et al., 1999). On the other hand, recent studies have reported that total collagen content does not change during cervical ripening (Read et al., 2007). Measurement of total collagen content does not reflect the organization/distribution of the collagen fibers in the cervix. Increased disorganization and dispersion of cervical collagen fibers can increase the distensibility of the cervix without changes in total collagen content.

Women with genetic disorders that affect fibrillar collagen synthesis or structure (Ehlers-Danlo syndrome and osteogenesis imperfecta) are at increased risk for preterm delivery (Anum et al., 2009). The fact that preterm birth is associated with defects in collagen synthesis or assembly constitutes the ultimate confirmation that a correlation exists between changes in collagen structure and organization and cervical ripening.

Different sources of MMPs in term and preterm delivery

It has been extensively reported that inflammatory cells, macrophages and neutrophils, are important sources of MMPs. As previously described (Gonzalez et al., 2011a), macrophages release MMP-9 in LPS-treated mice that deliver preterm. In contrast, no inflammatory cells were observed in the cervical tissue of mice at term, suggesting that other sources of MMPs might be responsible for collagen digestion and increased cervical distensibility during parturition at term.

Within the cervical tissue, cervical columnar epithelial cells and fibroblasts could be an alternative source of MMPs. Studies from our laboratory showed that End-1 – a cell line from normal human endocervical epithelia – is capable of synthesizing MMPs. Quantitative real-time polymerase chain reaction (qRT-PCR) experiments showed the presence of MMP-2 and MMP-9 mRNA in these cells. In addition, in situ zymography and immunohistochemistry studies demonstrated the presence of active MMP-2 and MMP-9 in End-1 cells. Moreover, positive staining for MMP-9 and robust activity against collagen I was also observed in the cervical columnar epithelium in mice at term (Gonzalez et al., 2011a).

Cervical fibroblast might also contribute to MMP production and cervical ripening at term. Positive staining for MMP-9 was found in the cervix of mice during term parturition (Gonzalez et al., 2011a).

Human studies reinforce the idea of fibroblasts as a source of MMPs (Malmstrom et al., 2007). Cervical fibroblasts obtained from pregnant women are capable of releasing MMPs in culture (Malmstrom et al., 2007).

Combining mouse and human data, we conclude that cervical fibroblasts and columnar epithelial cells might be responsible for the production of MMPs in cervical remodeling at term.

MMPs release and cervical collagen degradation are triggered by different mechanisms in term and preterm delivery

Massive complement C3 deposition has been observed in the cervical tissue of LPS-treated mice during antepartum and intrapartum (Gonzalez et al., 2011a). In addition, in vitro studies demonstrated that complement component C5a triggers the release of MMPs from macrophages, thus initiating cervical remodeling, dilation, and preterm birth in inflammation-induced preterm delivery (Gonzalez et al., 2011a,b). On the other hand, complement activation is not required in the physiological ripening of the cervix at term. The absence of C5aR on End-1 cells, a source of MMPs during term parturition, suggests that MMP release from columnar epithelial cells might not be initiated by C5a. In an attempt to identify other molecules and/or receptors that might initiate MMP secretion and collagen degradation at term, we looked for progesterone receptors. The presence of progesterone receptors has been demonstrated in the cervical columnar epithelia in humans and rabbits (Cano et al., 1990; Chilton and Geis, 1990). We then studied progesterone receptors in columnar epithelial End-1 cells and indeed found progesterone receptor (PR) at the RNA level in these cells. In agreement with these results, we were able to detect progesterone receptors (PR) on days 19 and 20 in the mouse cervical columnar epithelium by immunohistochemistry (Gonzalez et al., 2011a). We previously demonstrated that columnar epithelial End-1 cells secrete MMP-9. Interestingly, progesterone, which inhibited MMP release from macrophages in PTD, also inhibited the release of MMP-9 in these cells (Figure 3). The finding that progesterone suppresses the release of MMPs from columnar epithelial cells suggests that a decline in progesterone action can induce the release of MMPs and thus initiate cervical ripening and parturition at term. Knowing that in most subprimate placental mammals progesterone withdrawal before the initiation of term labor is manifested by a significant decrease in circulating progesterone levels (Zakar and Hertelendy, 2007), we next investigated the levels of progesterone in pregnant mice that delivered preterm and those that gave birth at term.

We found that progesterone levels do not diminish close to preterm parturition induced by intravaginal LPS (Gonzalez et al., 2011a). Systemic administration of LPS causes progesterone diminution by interfering with the hypothalamic pituitary gonadal axis, which results in luteolysis (Erlebacher et al., 2004). This has also been observed in mouse models of PTD in which systemic inflammation/infection develops in the mother (Elovitz and Wang, 2004). However, our model of LPS induced localized inflammation in the cervix is not associated with a decrease of progesterone levels. On the contrary, a dramatic diminution in serum progesterone levels was observed intrapartum in mice that delivered at term (Gonzalez et al., 2011a). Progesterone diminution can activate the secretion of MMPs from columnar epithelial cells and thus degrade collagen, leading to increased cervical distensibility and parturition at term. It is also known that fibroblasts express progesterone receptors (Roberson et al., 2012). Thus, we propose that a similar mechanism driven by progesterone withdrawal can result in the release of MMPs from cervical fibroblasts during term parturition.

CONCLUSION

We used a mouse model of inflammation-induced preterm delivery, which closely resembles the clinical scenario in humans. In this model, a low dose of LPS administered intravaginally induces a localized cervical inflammatory response that triggers cervical remodeling and PTD. We characterized the process of parturition in inflammation-induced

PTD and at term with the goal of finding possible differences that might result in the identification of biomarkers and/or targets for therapy. Figure 4 summarizes the differences and similarities in term and preterm labor. The first and crucial observation was that preterm delivery is initiated by complement activation, but complement activation is not required for term parturition. Second, term delivery was found to be nonleukocyte-dependent, while on the other hand, PTD was shown to be mediated by macrophages. Macrophages are the source of MMPs in PTD, while cervical stromal fibroblasts and columnar epithelial cells seem to be responsible for the production of MMPs in cervical remodeling at term. The trigger for MMP release is C5a in PTD, while our studies suggested that progesterone withdrawal might be the initiator of cervical ripening at term.

Regardless the different initiators and cellular mediators, a common downstream pathway has been identified that involves MMP release and collagen degradation in both term and PTD.

In conclusion, preterm and term cervical remodeling occur through the same mechanisms, but are initiated by different stimuli and effector cells. Thus, we demonstrated that preterm labor is not simply an acceleration of the normal physiological cervical processes that lead to term parturition. The complement system might be a potential biomarker and possible specific and selective target for therapy and prevention of preterm labor/delivery.

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References

1. Anotayanonth S, et al. Betamimetics for inhibiting preterm labour. *Cochrane Database Syst Rev*. 2004; 4:CD004352. [PubMed: 15495104]
2. Anum EA, et al. Connective tissue and related disorders and preterm birth: clues to genes contributing to prematurity. *Placenta*. 2009; 30 (3):207–215. [PubMed: 19152976]
3. Berghella V, et al. Cerclage for short cervix on ultrasonography: meta-analysis of trials using individual patient-level data. *Obstet Gynecol*. 2005; 106(1):181–189. 461. [PubMed: 15994635]
4. Bokstrom H, et al. Leukocyte subpopulations in the human uterine cervical stroma at early and term pregnancy. *Hum Reprod*. 1997; 12(3):586–590. 464. [PubMed: 9130764]
5. Buhimschi IA, et al. Physical and biomechanical characteristics of rat cervical ripening are not consistent with increased collagenase activity. *Am J Obstet Gynecol*. 2004; 191 (5):1695–1704. [PubMed: 15547544]
6. Cano A, et al. Expression of estrogen receptors, progesterone receptors, and an estrogen receptor-associated protein in the human cervix during the menstrual cycle and menopause. *Fertil Steril*. 1990; 54 (6):1058–1064. [PubMed: 1700956]
7. Chilton BS, Geis LS. Steroid receptors in the developing and the adult rabbit endocervix and in endocervical epithelial cells isolated by flow cytometry. *J Steroid Biochem Mol Biol*. 1990; 37 (5): 649–659. [PubMed: 2278850]
8. Conde-Agudelo A, et al. Novel biomarkers for the prediction of the spontaneous preterm birth phenotype: a systematic review and meta-analysis. *BJOG*. 2011; 118(9):1042–1054. 477. [PubMed: 21401853]
9. Crowther CA, Hiller JE, Doyle LW. Magnesium sulphate for preventing preterm birth in threatened preterm labour. *Cochrane Database Syst Rev*. 2002; 4:CD001060. [PubMed: 12519550]
10. DeFranco EA, et al. Vaginal progesterone is associated with a decrease in risk for early preterm birth and improved neonatal outcome in women with a short cervix: a secondary analysis from a randomized, double-blind, placebo-controlled trial. *Ultrasound Obstet Gynecol*. 2007; 30 (5): 697–705.

11. Derzsy Z, et al. Activation of the complement system in normal pregnancy and preeclampsia. *Mol Immunol*. 2010; 47 (7–8):1500–1506. [PubMed: 20181396]
12. Elovitz MA, Mrinalini C. Animal models of preterm birth. *Trends Endocrinol Metab*. 2004; 15 (10):479–487. [PubMed: 15541647]
13. Elovitz M, Wang Z. Medroxyprogesterone acetate, but not pro- gesterone, protects against inflammation-induced parturition and intrauterine fetal demise. *Am J Obstet Gynecol*. 2004; 190 (3):693–701. [PubMed: 15042001]
14. Erlebacher A, et al. Ovarian insufficiency and early pregnancy loss induced by activation of the innate immune system. *J Clin Invest*. 2004; 114 (1):39–48. [PubMed: 15232610]
15. Fonseca EB, et al. Progesterone and the risk of preterm birth among women with a short cervix. *N Engl J Med*. 2007; 357 (5):462–469. [PubMed: 17671254]
16. Girardi G, et al. Complement C5a receptors and neutrophils mediated fetal injury in the antiphospholipid syndrome. *J Clin Invest*. 2003; 112 (11):1644–1654. [PubMed: 14660741]
17. Girardi G, Redecha P, Salmon JE. Heparin prevents antiphospho-lipid antibody-induced fetal loss by inhibiting complement activation. *Nat Med*. 2004; 10 (11):1222–1226. [PubMed: 15489858]
18. Girardi G, et al. Complement activation induces dysregulation of angiogenic factors and causes fetal rejection and growth restriction. *J Exp Med*. 2006; 203 (9):2165–2175. [PubMed: 16923853]
19. Gonzalez JM, et al. Preterm and term cervical ripening in CD1 Mice (*Mus musculus*): similar or divergent molecular mechanisms? *Biol Reprod*. 2009; 81 (6):1226–1232. [PubMed: 19684330]
20. Gonzalez JM, et al. Cervical remodeling/ripening at term and preterm delivery: the same mechanism initiated by different mediators and different effector cells. *PLoS ONE*. 2011a; 6(11):pe26877.
21. Gonzalez JM, et al. Complement activation triggers metalloproteinases release inducing cervical remodeling and preterm birth in mice. *Am J Pathol*. 2011b; 179 (2):838–849. [PubMed: 21801872]
22. Haeger M, Unander M, Bengtsson A. Complement activation in relation to development of preeclampsia. *Obstet Gynecol*. 1991; 78 517 (1):46–49. [PubMed: 2047067]
23. Hassan SS, et al. Patients with an ultrasonographic cervical length ≤ 15 mm have nearly a 50% risk of early spontaneous preterm delivery. *Am J Obstet Gynecol*. 2000; 182 (6):1458–1467. [PubMed: 10871466]
24. Hassan SS, et al. Vaginal progesterone reduces the rate of preterm birth in women with a sonographic short cervix: a multicenter, randomized, double-blind, placebo-controlled trial. *Ultrasound Obstet Gynecol*. 2011; 38 (1):18–31. [PubMed: 21472815]
25. Iams JD, et al. The length of the cervix and the risk of spontaneous premature delivery, National Institute of Child Health and Human Development Maternal Fetal Medicine Unit Network. *N Engl J Med*. 1996; 334 (9):567–572. [PubMed: 8569824]
26. Iams JD, et al. Frequency of uterine contractions and the risk of spontaneous preterm delivery. *N Engl J Med*. 2002; 346 (4):250–255. [PubMed: 11807149]
27. Jenkin G, Young IR. Mechanisms responsible for parturition; the use of experimental models. *Anim Reprod Sci*. 2004; 82–83:567–581.
28. King JF, et al. Calcium channel blockers for inhibiting preterm labour. *Cochrane Database Syst Rev*. 2002; 2:CD002255. [PubMed: 12076443]
29. Lawn JE, Cousens S, Zupan J. 4 million neonatal deaths: when? Where? Why? *Lancet*. 2005; 365 (9462):891–900.
30. Lynch AM, et al. Complement activation fragment Bb in early pregnancy and spontaneous preterm birth. *Am J Obstet Gynecol*. 2008; 199(4):354.e1–354.e8. [PubMed: 18928972]
31. Mackler AM, et al. Macrophage trafficking in the uterus and cervix precedes parturition in the mouse. *Biol Reprod*. 1999; 61 (4):879–883. [PubMed: 10491619]
32. Malmstrom E, et al. The importance of fibroblasts in remodeling of the human uterine cervix during pregnancy and parturition. *Mol Hum Reprod*. 2007; 13 (5):333–341. [PubMed: 17337476]
33. Oestergaard MZ, et al. Neonatal mortality levels for 193 countries in 2009 with trends since 1990: a systematic analysis of progress, projections, and priorities. *PLoS Med*. 2011; 8(8):pe1001080.

34. Owen J, et al. Multicenter randomized trial of cerclage for preterm birth prevention in high-risk women with shortened midtrimester cervical length. *Am J Obstet Gynecol.* 2009; 201(4):375.e1–375.e8. [PubMed: 19788970]
35. Papatsonis D, et al. Oxytocin receptor antagonists for inhibiting preterm labour. *Cochrane Database Syst Rev.* 2005; 3:CD004452. [PubMed: 16034931]
36. Price FV, et al. Chediak Higashi syndrome in pregnancy. *Obstet Gynecol.* 1992; 79(5 Pt 2):804–806. 5. [PubMed: 1565370]
37. Read CP, et al. Cervical remodeling during pregnancy and parturition: molecular characterization of the softening phase in mice. *Reproduction.* 2007; 134 (2):327–340. [PubMed: 17660242]
38. Roberson AE, et al. Interleukin 1beta regulates progesterone metabolism in human cervical fibroblasts. *Reprod Sci.* 2012; 19 (3):271–281. [PubMed: 22064385]
39. Romero R. Prevention of spontaneous preterm birth: the role of sonographic cervical length in identifying patients who may benefit from progesterone treatment. *Ultrasound Obstet Gynecol.* 2007; 30 (5):675–686. [PubMed: 17899585]
40. Sakamoto Y, et al. Macrophages and not granulocytes are involved in cervical ripening. *J Reprod Immunol.* 2005; 66 (2):161–173. [PubMed: 16045998]
41. Sennstrom MB, et al. Matrix metalloproteinase-8 correlates with the cervical ripening process in humans. *Acta Obstet Gynecol Scand.* 2003; 82 (10):904–911. [PubMed: 12956839]
42. Shi L, et al. Changes in cervical resistance and collagen fluorescence during gestation in rats. *J Perinat Med.* 1999; 27 (3):188–194. [PubMed: 10503180]
43. Smedegard G, Cui LX, Hugli TE. Endotoxin-induced shock in the rat. A role for C5a *Am J Pathol.* 1989; 135 (3):489–497.
44. Stygar D, et al. Increased level of matrix metalloproteinases 2 and 9 in the ripening process of the human cervix. *Biol Reprod.* 2002; 67 (3):889–894. [PubMed: 12193399]
45. Timmons BC, Mahendroo M. Processes regulating cervical ripening differ from cervical dilation and postpartum repair: insights from gene expression studies. *Reprod Sci.* 2007; 14 (Suppl 8):53–62. [PubMed: 18089611]
46. Vaisbuch E, et al. Fragment Bb in amniotic fluid: evidence for complement activation by the alternative pathway in women with intra-amniotic infection/inflammation. *J Matern Fetal Neonatal Med.* 2009; 22 (10):905–916. [PubMed: 19603351]
47. Vaisbuch E, et al. The risk of impending preterm delivery in asymptomatic patients with a nonmeasurable cervical length in the second trimester. *Am J Obstet Gynecol.* 2010a; 203(5):446.e1–446.e9. [PubMed: 20659728]
48. Vaisbuch E, et al. Activation of the alternative pathway of complement is a feature of pre-term parturition but not of spontaneous labor at term. *Am J Reprod Immunol.* 2010b; 63 (4):318–330. [PubMed: 20163401]
49. Word, RA. Cervical function during pregnancy and parturition in Preterm Birth: Mechanisms, mediators, prediction, prevention and interventions. In: Petraglia, F., editor. *Informa Healthcare Lond.* London: Informa Healthcare; 2007. p. 19-34.
50. Yellon SM, Ebner CA, Sugimoto Y. Parturition and recruitment of macrophages in cervix of mice lacking the prostaglandin F receptor. *Biol Reprod.* 2008; 78 (3):438–444. [PubMed: 18003949]
51. Yellon SM, Ebner CA, Elovitz MA. Medroxyprogesterone acetate modulates remodeling, immune cell census, and nerve fibers in the cervix of a mouse model for inflammation-induced preterm birth. *Reprod Sci.* 2009; 16 (3):257–264. [PubMed: 19087974]
52. Zakar T, Hertelendy F. Progesterone withdrawal: key to parturition. *Am J Obstet Gynecol.* 2007; 196 (4):289–296. [PubMed: 17403397]

Figure 1. Complement activation in preterm and term delivery

(A) Complement C3 deposition in the cervixes of mice that delivered preterm. Samples were collected antepartum and intrapartum. Black arrows indicate complement deposition in the cervical columnar epithelium (CE).

(B) Complement C3 deposition in the cervixes of mice that deliver at term. C3 deposition was not observed antepartum (days 17, 18 and 19) or intrapartum.

Figure 2. Absence of C5aR prevents cervical remodeling and preterm delivery in lipopolysaccharide (LPS)-treated mice

In situ zymography using DQ gelatin, DQ collagen I, and DQ collagen IV. In contrast to the increased fluorescence (increased gelatin and collagen degradation) observed in the cervix of LPS-treated wild-type mice (C5aR+/+), gelatin and collagen degradation was not increased in LPS-treated C5aR -/- mice. Gelatin and collagen degradation in these mice was comparable to control cervical tissue. The bottom panel shows collagen detection by IHC. A dense network of collagen fibers was observed in the cervix of LPS-treated C5aR -/- mice in contrast to the dispersion of thin fibers of collagen observed in the cervix of LPS-treated wild-type C5aR+/+ mice.

Figure 3. Cervical columnar epithelial cells and stromal cells are possible sources of matrix metalloproteinase (MMP) at term parturition

(A) Immunohistochemical determination of MMP-9 in cervical stromal fibroblasts at term.

(B) In situ zymography using substrates DQ-gelatin and DQ-collagen I and immunohistochemical detection of MMP-2 and MMP-9 on cervical columnar epithelial End-1 cells incubated with and without progesterone. The green fluorescence indicates the substrate has been digested. MMP-2 and MMP-9 were detected by immunohistochemistry using specific antibodies followed by fluorescein isothiocyanate (FITC)-labeled secondary antibodies.

Figure 4. Distinct and similar features that characterize cervical remodeling at preterm and term in mice.