Cervical remodeling in term and preterm birth: insights from an animal model

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Abstract

Proper cervical function is essential for a normal pregnancy and birth to occur. Understanding the mechanisms that take place in normal pregnancy will allow a better comprehension of the complications involved in premature cervical remodeling and lead to better methods of diagnostics and prevention for preterm birth. Unfortunately, human samples are not easily available, and samples that are collected are often confounded by variations in timing and region of cervix from which sample is collected. Animal models, specifically the mouse, have facilitated a great deal of exploration into the mechanisms of cervical function and pathways of preterm birth. This review highlights some of the groundbreaking discoveries that have arisen from murine research including 1) the identification of early pregnancy changes in collagen fibril processing and assembly that result in progressive modifications to collagen architecture with subsequent loss of tissue stiffness during pregnancy, 2) the determination that immune cells are not key to cervical ripening at term but have diverse phenotypes and functions in *postpartum* repair, and 3) the finding that the process of preterm cervical ripening can differ from term ripening and is dependent on the etiology of prematurity. These findings, which are relevant to human cervical biology, provide new insights that will allow targeted studies on the human cervix as well as identify potential biomarkers for early detection of premature cervical ripening and development of improved therapies to prevent premature ripening of the cervix and subsequent preterm birth.

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Introduction

During pregnancy, the cervix remains closed and firm in order to prevent passage of an immature infant through the birth canal. Conversely, at term the cervix must become pliable and open sufficiently to allow delivery of a term fetus. While functionally distinct from the uterus, remodeling of the cervix must be appropriately coordinated with the onset of uterine contractility to ensure successful parturition. Investigations aimed at understanding these essential yet unique processes are required in order to understand the causes of preterm birth, which occurs at an alarming rate of 12.7% of all births (Martin 2011).

In recent years, investigations focused on the molecular mechanisms of cervical remodeling in the murine model have advanced our understanding of this dynamic process. The advantages of this model in the cervical biology field are numerous and include 1) the ability to obtain well-timed cervical tissue at all time points in the 19-day pregnancy, 2) the availability of mice with targeted mutations, 3) the fact that loss of progesterone (P₄) function culminates in the onset of labor in human

and mouse, and 4) a growing body of evidence that many of the processes that occur during stages of cervical remodeling are similar in mouse and human. This review will highlight studies on the pregnant rodent cervix and provide a rationale as to how they contribute insights into human cervical biology.

Cervical architecture

The cervical stroma comprised primarily fibroblasts and to a lesser extent smooth muscle cells (Leppert 1995). These cells secrete an extracellular matrix (ECM) rich in fibrillar collagen, matricellular proteins, elastin, glycosaminoglycans, and proteoglycans. Changes in the ECM composition over the course of pregnancy and labor determine flexibility and mechanical strength of the cervix. Both the inner os (endocervix) and outer region of the cervix (exocervix) are lined with squamous mucosal epithelia. The uppermost region of the endocervix is lined with columnar epithelia, and this squamocolumnar junction delineates the boundary between uterine and cervical tissue. Resident immune cells

including neutrophils, eosinophils, and macrophages are present in the cervix though their localization and relative numbers change over the course of pregnancy (Timmons & Mahendroo 2006, Read *et al.* 2007). All these cell types have specific functions during pregnancy and parturition that are in part regulated by the steroid hormone environment. The maintenance of pregnancy requires that P_4 levels are high and estrogen (E_2) levels are relatively low resulting in an increased P_4/E_2 ratio. Increased E_2 levels in the latter third of gestation and a decline in P_4 function in the day or two preceding parturition in mice results in a decline in the P_4/E_2 ratio. The reduced P_4/E_2 ratio is a hallmark of parturition and begins the cascade of accelerated cervical changes responsible for the final loss of structural integrity necessary for the safe passage of the fetus.

The initiation of cervical remodeling was once considered a passive process that was initiated and induced with the onset of uterine contractions at term. Since that time, research by many investigators has confirmed that cervical remodeling is a dynamic process that begins long before the onset of uterine contractions. The cervical remodeling process can be divided into four overlapping phases termed softening, ripening, dilation, and *postpartum* repair (Read *et al.* 2007).

Cervical softening: progressive, incremental changes in ECM structure

Cervical softening is defined as the first measurable increase in tissue distensibility when compared with the nonpregnant cervix and occurs by gestation day 12 in the mouse and rat (Harkness & Harkness 1959, Read *et al.* 2007). Once initiated, there is a slow incremental decline in the tissue stiffness that is maximal at the time of birth (Mahendroo *et al.* 1999, Word *et al.* 2005). The P_4/E_2 ratio remains high during this phase and the tissue integrity is maintained despite the increased tissue compliance.

Fibrillar collagen types I and III are the main structural proteins in the cervix responsible for mechanical strength (Leppert 1995). Changes in the ratio of type I to III collagen, alterations in the multistep process of post-transcriptional processing of collagen monomers, and/or the assembly of collagen monomers into collagen fibrils can affect collagen structure and tissue mechanical strength (Kadler *et al.* 1996, Liu *et al.* 1997, Myers *et al.* 2010). Previous studies on the murine pregnant cervix documented an increase in collagen solubility during cervical softening with no change in total collagen content. This suggested the potential for regulated alterations in collagen processing during cervical softening (Read *et al.* 2007). While collagen solubility is an indirect indicator of collagen structure, evidence to support downregulation of specific steps in the collagen-processing pathway is mounting (Fig. 1).

This evidence includes a decline in cervical expression and/or activity of lysyl hydroxylase and lysyl oxidase (LOX), respectively, early in pregnancy and a subsequent decline in collagen containing the stronger pyridinoline cross-links in the second half of gestation (Ozasa *et al.* 1981, Akins *et al.* 2011). Cross-links between collagen molecules are essential in providing strength to the resulting collagen fibers and tissues (Eyre *et al.* 1984). Lysyl hydroxylase is an ER-resident enzyme that adds hydroxyl groups to lysine residues in the procollagen chain. The degree of hydroxylation determines the type and strength of potential cross-links that will be formed extracellularly. In the extracellular space, LOX catalyzes the formation of cross-links that occur between hydroxylated and/or nonhydroxylated lysine residues in collagen, resulting in the stronger pyridinoline or weaker non-pyridinoline cross-links respectively. A reduction in collagen cross-linking has been shown to reduce mechanical strength of the resulting tissue (Eyre *et al.* 1984, Canty & Kadler 2005). This suggests that reduced cross-linking in the cervix during softening plays a role in the reduced mechanical strength of the cervix seen at this time. Equally important in this study was the finding that the ratio of the fibrillar collagens, type I and type III, remains constant throughout pregnancy, and thus alterations in I to III ratio is unlikely to be an important mechanism for the progressive decline in tissue stiffness (Akins *et al.* 2011). Furthermore, collagen turnover appears upregulated throughout pregnancy. In support of this idea, collagen I and III synthesis is robust, steps in processing appear upregulated to accommodate increased collagen turnover (e.g. C-terminal and N-terminal processing of procollagen), and collagen content remains constant throughout pregnancy and parturition. While the activity of matrix metalloproteases (MMPs) that breakdown collagen have not been reported in the cervix during the softening period, it is likely that there is a constant breakdown of collagen to maintain steady collagen levels and facilitate progressive replacement of mature collagen fibrils with the newly synthesized, poorly cross-linked collagen.

In addition to collagen processing, collagen fibril structure and tensile strength are regulated by the composition of additional molecules within the ECM. Matricellular proteins are a family of nonstructural proteins that modulate collagen function and cell–matrix interactions (Bornstein & Sage 2002). Genes encoding two members of this family, thrombospondin 2 (*Thbs2*) and tenascin-C (*Tnc*), are downregulated early in gestation and remain low until just before birth (Akins *et al.* 2011). Numerous studies including the evaluation of mice deficient in *Thbs2* indicate that this matricellular protein plays a role in cervical softening and in the formation of appropriately assembled collagen fibrils, regulates activity of *Mmp2*, and is an inhibitor of angiogenesis in a variety of tissues (Kyriakides *et al.* 1998, Kokenyesi *et al.* 2004). The decline in cervical

Figure 1 Progressive declines in cervical collagen cross-linking, thrombospondin 2 (THBS2), and tenascin C (TNC) that begin early in pregnancy contribute to formation of larger collagen fibers with reduced mechanical strength. The synthesis and assembly of collagen fibers is a multistep process in which procollagen monomers are synthesized intracellularly followed by N- and C-terminal processing and assembly into fibrils and fibers in the extracellular space. During pregnancy, both collagen synthesis and metabolism by proteases is elevated suggesting increased collagen turnover. Compared with the nonpregnant cervix, transcripts encoding enzymes important in cross-link formation, lysyl hydroxylase (*Plod2*) and lysyl oxidase (*Lox*) are downregulated by gestation day 8 of pregnancy. In addition, the expression of two matricellular proteins, THBS2 and TNC, declines in a similar timeframe. In an environment of increased collagen turnover, these early pregnancy changes contribute to the progressive replacement of mature collagen with poorly cross-linked collagen resulting in the gradual increase in cervical collagen fibril diameter and fiber size and the subsequent gradual decline in mechanical strength of the tissue, which is lowest at the time of labor.

Thbs2 expression during pregnancy may contribute to alterations similar in collagen architecture and/or protease activity, which could also aid in the progressive decline in mechanical strength. The mechanism by which THBS2 regulates collagen architecture in the cervix as well as hormonal regulation of this process remains to be determined. *Tnc* is upregulated during tissue injury and has numerous reported roles that mediate both proinflammatory and fibrotic responses to mediate tissue repair (Midwood *et al.* 2011). Thus, downregulation of *Tnc* during pregnancy and marked induction shortly *postpartum* is consistent with *Tnc*'s proposed functions in tissue injury. Taken together, the gradual replacement of mature cross-linked collagen with collagen harboring reduced cross-links along with the decline in THBS2 and TNC in the cervix is key to modulating collagen architecture within the ECM during softening and initiating the incremental fall in cervical mechanical strength while maintaining tissue integrity.

Along with the extensive changes in the cervical ECM during softening, the cervical epithelia may also play an important role during this time though our understanding of their roles in cervical remodeling remains to be defined. Epithelial functions include immunoprotection and maintenance of a barrier, which are likely important in the protection of the stromal compartment as the matrix remodels. During softening, these functions are

exemplified by localization of immune cells to the epithelial/subepithelial regions of the cervix as well as the induction of proteins involved in restitution and repair of the cervical mucosal epithelia (Read *et al.* 2007). These include trefoil factor 1 (TFF1) and serine protease inhibitor Kazal type 5 (SPINK5). TFF1 is a secreted peptide first described in the gastric mucosa where its functions include protection and restitution of gut mucosal epithelia. *Spink5* encodes a protease inhibitor expressed in stratified epithelia of the skin and functions to prevent degradation of proteins involved in barrier formation. While further studies are needed to define a function of TFF1 and SPINK5 in the cervix, the observation that transcripts encoding these proteins are upregulated during cervical softening suggests that the epithelia play an important function in protection and maintenance of cervical competency.

Cervical ripening and dilation: accelerated phases of remodeling

Given the short duration of the ripening and dilation phases, it is difficult to identify processes that distinguish these two overlapping phases of remodeling and thus they are presented jointly in this section. Unlike cervical softening, the ripening process is very rapid. Ripening is

initiated on the last day of murine pregnancy (gestation day 18) and results in maximal loss of tissue compliance by late day 18 (Mahendroo *et al.* 1999). This phase occurs in a low P_4/E_2 environment and is accompanied by a loss of structural integrity. The importance of collagen structure to the biomechanical properties of the cervix has been appreciated for many years yet the mechanisms by which accelerated changes during ripening occur have been greatly debated. Numerous studies report a decline in collagen content resulting from increased activity of proteases as a key mechanism for loss of tissue integrity at term (Rajabi *et al.* 1991). Other experimental findings call this model into question including the observations that 1) collagen synthesis is upregulated during pregnancy, 2) biomechanical properties of cervixes treated exogenously with collagenase differ from term ripening, and 3) changes in collagen structure and organization rather than abundance are observed by polarized light microscopy and transmission electron microscopy (Yu *et al.* 1995, Buhimschi *et al.* 2004, Akins *et al.* 2011). Studies on mice and rats indicate that while total collagen content is constant throughout pregnancy and parturition, collagen structure undergoes dynamic changes as determined by second harmonic generation (SHG), electron microscopy, and light-induced fluorescence (Yu *et al.* 1995, Maul *et al.* 2003, Akins *et al.* 2011). Collagen ultrastructure as observed in electron micrographs reveals a progressive increase in collagen fibril diameter in the cervix from early to late gestation along with increased spacing between fibrils (Fig. 1). Evaluation of collagen fiber structure by SHG microscopy reveals progressive changes in the morphological features of collagen type I fibers over the course of pregnancy (Akins *et al.* 2010). Most notable in SHG images is the transition from long thin collagen fibers to thicker curved fibers at term. These striking visual observations emphasize the progressive changes in collagen structure that likely begin with the reduction in cross-linked collagen and matricellular proteins during cervical softening. These changes are further built upon during ripening to allow for a maximal increase in the tissue compliance and loss of integrity of the cervix seen during dilation.

A notable feature of ripening that likely facilitates matrix disorganization is the increased synthesis of the glycosaminoglycan and hyaluronan (HA; Straach *et al.* 2005). Early in pregnancy cervical HA represents just 19% of total cervical glycosaminoglycans; however, at term this figure rises to 71% (Akgul *et al.* 2012). HA's functions are dependent on its size as well as its interactions with other HA-binding molecules. Large molecular weight HA is a hydrophilic, space-filling molecule that provides increased viscoelasticity to tissues while low-molecular weight HA has proinflammatory functions (Powell & Horton 2005). During ripening, large molecular weight HA is elevated to facilitate increased spacing between collagen fibers and may

contribute to the modest increase in tissue hydration (Ruscheinsky *et al.* 2008). As highlighted in the next section, low-molecular weight HA predominates during *postpartum* repair. HA is synthesized by HA synthases encoded by the genes *Has1*, *Has2*, and *Has3* and broken down by the enzyme hyaluronidase (HYAL) encoded by five genes (*Hyal1–5*) (Itano & Kimata 1996, Spicer *et al.* 1996, Stern 2003). Cervical transcripts encoding *Has2* are induced during ripening with peak expression on gestation day 18 (Straach *et al.* 2005). HA matrices are stabilized by interaction with other molecules such as the proteoglycan, versican, the cell surface receptor, CD44, and the serum protein, inter-alpha trypsin inhibitor (Ruscheinsky *et al.* 2008). Versican is localized to the cervical stromal matrix while CD44 is expressed in cervical epithelia as well as in immune cells. The presence of HA-rich matrices surrounding both the cervical mucosal epithelia and the stroma as well as cell-specific expression of HA-interacting molecules suggest that HA has multiple cell-specific functions during cervical ripening.

There is a marked proliferation of the mucosal epithelia in the latter half of rat pregnancy that is dependent on the actions of the hormones relaxin and E_2 (Lee *et al.* 2005). By gestation day 16 and 17 as the softening phase merges into the ripening phase, the epithelium becomes laden with mucin-secreting vacuoles important in immune surveillance and lubrication. During this time, the epithelia also express steroid 5 alpha reductase type 1 (*Srd5a1*) important in local P_4 metabolism (Mahendroo *et al.* 1999). The loss of P_4 function resulting from the decline in P_4 synthesis in the ovary along with increased P_4 metabolism by SRD5A1 facilitates the transition to the cervical ripening phase of remodeling. During ripening, regulated changes in the barrier properties, hydration, and cell-to-cell communications are suggested by regulated expression of tight junction proteins (claudin 1 and 2), aquaporins, gap junction proteins (Connexin 26 and 43), and desmogleins (Anderson *et al.* 2006, Timmons & Mahendroo 2007, Timmons *et al.* 2007, Gonzalez *et al.* 2009). Further studies are required to understand the important roles that the epithelia play in protecting the softened cervix and in orchestration of remodeling events that occur in the cervical stroma.

As discussed in Box 1, the overall labor process is considered to be an inflammatory process though recent investigations in the cervical biology field suggest that some aspects of parturition may not be regulated by an inflammatory response. Immune cells that infiltrate the cervix during cervical ripening have long been considered to play a key role in ECM degradation and remodeling via activation of proinflammatory signals and the release of MMPs that breakdown collagen (Liggins 1981). Numerous studies based on immunohistochemical analysis of immune cells suggest a functional role of macrophages or neutrophils in the process of

Box 1 Is term labor an inflammatory response?

Several components make up the pregnant reproductive system: the uterus, cervix placenta, and fetal membranes. All have distinct compositions and functions but work together at term with a common goal birth. While these tissues are under the influence of the same steroid hormones, progesterone (P_4) and estrogen, the processes that allow successful parturition at term (e.g. uterine contractions, fetal membrane breakdown, and cervical remodeling) occur by distinct mechanisms. Current evidence supports a model in which some aspects of parturition are mediated by activation of inflammatory responses while other key components are not. P_4 is an anti-inflammatory agent and loss of P_4 function at term results in activation of proinflammatory responses. This leads to activation of NF- κ B and subsequent increases in contractile-associated proteins (e.g. oxytocin receptor and connexin-43) in the uterus, thus inducing myometrial contractility. Increased proinflammatory signals in the amniotic fluid also promote increased prostaglandin production in fetal membranes. Prostaglandins in turn increase expression of MMPs, allowing fetal membrane breakdown and rupture. In the term cervix, proinflammatory responses are not activated during ripening. Both proinflammatory and immunosuppressive responses are activated after onset of labor and thus likely to facilitate *postpartum* repair of the cervix. While unnecessary for term ripening, upregulation of proinflammatory cytokines and prostaglandin synthesis are sufficient to promote premature cervical ripening during infection-mediated preterm birth. After birth, cervical recovery and repair are necessary to protect the reproductive tract from environmental insults and ensure subsequent pregnancies. However, the functional use of the fetal membrane is completed at term and thus processes that induce degradation without repair are beneficial. We suggest a physiological advantage for these differing mechanisms. Current evidence suggests that some but not all aspects of labor is an inflammatory response.

cervical ripening (Junqueira *et al.* 1980, Luque *et al.* 1998, Mackler *et al.* 1999, Young *et al.* 2002, Osman *et al.* 2003). The fact that antibodies used for assessment of macrophages and neutrophils can also recognize monocytes and other myeloid cells and the recent appreciation that a single type of immune cell (e.g. macrophage) can have diverse phenotypes dependent on the microenvironment limits the conclusions that can be drawn from these reports. Evidence from our group and others suggest that activation of immune cells is not required for physiological ripening at term (Hirsch *et al.* 2006, Timmons & Mahendroo 2006, Gonzalez *et al.* 2009, Timmons *et al.* 2009). During ripening there is an influx of monocytes into the cervix that are dependent on the loss of P_4 function. In addition, resident macrophages and neutrophils are present in the cervix at this time. Despite the presence of these leukocytes, mRNA expression of proinflammatory genes such as *Il1a*, *Il6*, *Tnf*, *Cxcl1*, *Ptgs1* (*Cox1*), *Ptgs2* (*Cox2*), and *Cxcl2* (*Mip2*) as well as MMPs such as *Mmp2*, *Mmp8*, and *Mmp9* are not induced during ripening though a few are increased shortly *postpartum*. Furthermore, depletion of neutrophils has no effect on timing of parturition and myeloperoxidase activity, a marker of functional neutrophils, is not detectable until *postpartum*. These data suggest that immune cells are present but not activated during cervical ripening, thus leading our group and others to suggest that physiological ripening is not mediated by an inflammatory cascade. One notable exception is the increase in transcripts encoding the chemoattractant, eotaxin1 (*Ccl10*) on gestation day 18 followed by a significant increase in eosinophils in the cervix during labor. *Il5* deficiency in mice leads to a significant decrease in eosinophils in the cervix during

cervical ripening though it has no effect on the timing of parturition (Robertson *et al.* 2000). While the function of eosinophils in the cervix remains to be determined, recent studies suggest that eosinophils promote the differentiation of monocytes into alternatively activated macrophages (M2) in adipose tissue, which are key in maintenance of glucose homeostasis (Wu *et al.* 2011). Eosinophils may have a similar function in the cervix to regulate monocyte differentiation and macrophage polarization during *postpartum* repair. As will be discussed in the next section, evidence to support activation of monocytes during labor or *postpartum* repair is accumulating.

Postpartum recovery of the cervix: tissue repair

The recovery of the cervix following birth must occur rapidly to ensure protection of the reproductive tract from environmental insults as well as to ensure the success of subsequent pregnancies. Gene microarray comparisons between ripening and shortly *postpartum* emphasize the dramatic and rapid changes that drive this transition (Timmons & Mahendroo 2007, Gonzalez *et al.* 2009). These include changes in epithelial specific genes such as desmogleins and keratin 16 as well as inflammatory associated genes and genes involved in ECM synthesis, processing, and repair. In contrast to the cervical ripening phase, myeloid-derived immune cells likely play an important role in this phase of remodeling. Evidence in support of this hypothesis is the observation that neutrophil numbers and myeloperoxidase activity are increased as is expression of genes associated with classical proinflammatory M1 macrophages and alternatively activated M2 macrophages as early as 2 h

postpartum. While further experimentation is required to confirm this hypothesis, we propose that monocytes entering the cervix during ripening are differentiated to macrophages and polarized to both M1 and M2 phenotypes with onset of labor or *postpartum*. This macrophage diversity allows removal of matrix 'debris' (e.g. HA, versican, and poorly assembled collagen), yet ensures rapid suppression of inflammatory responses that, if uncontrolled, would lead to further tissue damage. Consistent with the activation of proinflammatory responses during this phase is the increased activity of the HA metabolizing enzyme, hyaluronidase 1, and subsequent increase in low-molecular weight HA, which has reported functions in activation of proinflammatory responses (Ruscheinsky *et al.* 2008, Akgul *et al.* 2012). In tandem with matrix cleanup by immune cells, expression of genes that function in collagen synthesis and cross-linking (e.g. *Col1a*, *Col3a*, *Plod2*, and *Lox*) and metabolism of proteoglycans such as versican (e.g. *Adamts1*) and wound healing (e.g. *Tnc* and *Thbs1/2*) are upregulated in the *postpartum* cervix. The combined action of these molecules promotes tissue repair and inhibition of angiogenesis (Bornstein & Sage 2002, Timmons & Mahendroo 2007, Midwood *et al.* 2011).

Premature cervical ripening

Effective therapies and tools for accurate detection of preterm birth is complicated by the fact that while there are a multitude of established causes for prematurity such as infection, environment, genetics, in roughly 50% of cases the cause is unknown. In most cases, premature cervical ripening precedes onset of preterm labor. This knowledge emphasizes the need to define the molecular processes that drive term and preterm cervical remodeling. While preterm birth was once considered an acceleration of the normal physiological process, evidence to support diverse mechanisms by which preterm birth can occur is mounting (Gross *et al.* 2000, Hirsch *et al.* 2006). Recent studies using two established mouse models of preterm birth have provided novel insights into distinct processes by which cervical ripening can occur. Animals administered lipopolysaccharide (LPS), found in the outer membrane of Gram-negative bacteria, elicit a proinflammatory response similar to infection and undergo preterm labor without a loss in circulating P_4 concentration several hours after administration (Hirsch & Muhle 2002, Elovitz & Mrinalini 2004). Premature loss of P_4 function by administration of a P_4 receptor antagonist (RU486/mifepristone) similarly results in onset of preterm labor within several hours after treatment. Gene microarray and expression comparisons between term and the infection-induced premature ripening model indicate that the activation of proinflammatory cascades is sufficient for infection-mediated preterm ripening but not necessary for term cervical ripening (Gonzalez *et al.* 2009).

Studies from our group (summarized in Table 1) are in agreement with these findings and confirm that cervical ripening can be initiated by more than one mechanism and is not necessarily an acceleration of the physiological process at term (Holt *et al.* 2011). Specifically, premature ripening induced by the P_4 receptor antagonist, mifepristone, results from an acceleration of processes in place during term ripening as well as partial activation of proinflammatory and tissue repair processes observed during *postpartum* repair. In contrast to the mifepristone induced or term remodeling, premature ripening induced in an infection model occurs by a distinct mechanism, which is dominated by an influx of neutrophils in the cervix, a marked proinflammatory response, and increased expression of prostaglandin cyclooxygenase endoperoxide synthase 2 (*Ptgs2*). Interestingly, while the neutrophil collagenase MMP8 is upregulated in the infection model, total collagen content does not decline. The complement system is reported to play a role in activation of inflammatory responses in both preterm birth models as mice deficient in the complement receptor C5a do not deliver preterm in response to LPS or mifepristone treatment (Gonzalez *et al.* 2011). Taken together, these studies confirm that multiple mechanisms exist to facilitate cervical ECM remodeling and loss of tensile strength and that the mechanism is dependent on the etiology of prematurity.

Relevance to cervical remodeling in women

There are clearly notable distinctions between human and mouse physiology of pregnancy and parturition that suggest the molecular process of cervical remodeling is not identical in the two species. The fact that humans are bipedal compared with the quadruped mouse suggests the mechanisms to maintain cervical competence in the human may be more complex. In addition, the continued synthesis of P_4 by the human placenta indicates that other mechanisms predominate in abrogating P_4 function at the end of pregnancy in contrast to mice and other species in which luteolysis is key to loss of P_4 function at term. There is potential for differences in epithelial cell function between species as the human cervix is composed of glandular epithelia and a columnar and squamous epithelia that line the endocervix and ectocervix, respectively, while the mouse does not have glandular epithelia and the endocervix and ectocervix is primarily composed of squamous epithelia with a small region of columnar epithelia in the endocervix. Given the recent appreciation of the important roles of epithelia in the maintenance of barrier properties and immune surveillance in mouse and human, a better understanding of these differences is needed (Timmons *et al.* 2010, Nold *et al.* 2012).

While well-designed studies using limited pregnant human cervix tissue will be required for our ultimate understanding of term and preterm cervical remodeling

Table 1 Salient features of preterm and term ripening and *postpartum* repair compared with gestation day 15.

	RU486 (d15)	LPS (d15)	Term (d18)	Postpartum (2–4 h)
Myeloid cells numbers	↑ Mo ↔ Mac ↔ Neu ↑ Eo	↔ Mo ↔ Mac ↑ Neu ↔ Eo	↑ Mo ↔ Mac ↔ Neu ↔ Eo	↑ Mo ↔ Mac ↑ Neu ↑ Eo
Macrophage phenotype ^a	M1 and M2	M1 and M2	Macphages present but no expression of M1 and M2 markers	M1 and M2
Induced proinflammatory genes	<i>Il1a</i> , <i>Ptgs1</i>	<i>Il1a</i> , <i>Il6</i> , <i>Tnf</i> , <i>Cxcl2</i> , <i>Ptgs2</i>	<i>Ccl11</i> (eotaxin-1)	<i>Il1a</i> , <i>Tnf</i> , <i>Ccl2</i> (<i>Mcp1</i>), <i>Cxcl2</i>
Protease gene expression	<i>Mmp8</i> <i>Adamts1</i> <i>Adamts4</i>	<i>Mmp8</i> <i>Adamts1</i> <i>Adamts4</i>	<i>Adamts1</i> <i>Adamts4</i>	<i>Mmp8</i> <i>Adamts1</i> <i>Adamts4</i> (ND)
Total collagen content	No change	No change	No change	No change
Hyaluronan synthase	↑ <i>Has1</i>	↑ <i>Has1</i>	↑ <i>Has2</i>	↑ <i>Has2</i>
HA localization	Stromal matrix	Stromal matrix	Epithelial and stromal matrix	Epithelial and stromal matrix
Average tissue ^b wet weight (mg)	25.9	16.4	26.2	35.4
Progesterone function/levels	↓	↔	↓	↓

ND, not determined.

^aBased on gene expression of classical M1 macrophages (*Il6*, *Il1a*, *Tnf*, and *Cxcl2*) and alternatively activated M2 macrophages (*YM1* and *Il1rn*).^bPreterm birth induced on gestation day 15. Untreated day 15 cervix average wet weight is 15.8 mg.

in women, the advances in our understanding of molecular events that modulate murine cervical remodeling provide insights that are likely to be relevant to human cervical biology. Similar to the mouse, early pregnancy changes in cervical compliance as determined by digital examination was noted over 100 years ago and was the basis for the Hegar score used to determine pregnancy before more sophisticated measurements. Furthermore, the importance of collagen processing and assembly in early pregnancy in women is highlighted by the fact that women with genetic defects in collagen and elastin synthesis or assembly (e.g. Ehlers–Danlos and Marfan syndromes) have an increased incidence of cervical insufficiency (Anum *et al.* 2009). These studies further suggest that polymorphisms associated with genes important for connective tissue synthesis and metabolism may predispose women to preterm birth due to cervical insufficiency or preterm premature rupture of membranes. On the other end of the spectrum, the failure to activate genes important in connective tissue remodeling could be an underlying cause of postterm pregnancies due to failure of cervical remodeling (Kistka *et al.* 2007). Another important similarity between mouse and human at this stage of remodeling is the fact that these changes occur during a period in which the P₄ to E₂ ratio is high in both species, thus hormonal regulation of these processes may be conserved. Future targeted studies are needed to evaluate the contribution of reductions in collagen cross-linking and matricellular proteins to physiological softening as well as the identification of specific mutations or polymorphisms in genes involved in connective tissue synthesis or degradation, which may contribute to either cervical insufficiency or postterm pregnancy due to failure of cervical ripening. Aberrant regulation of described processes important in cervical

softening may also contribute to premature cervical shortening as determined by sonography, which is a risk factor for women with a previous history of preterm birth (Owen *et al.* 2001).

In both women and mice, cervical ripening and dilation are preceded by a decline in P₄ function. In the mouse, loss of P₄ function at term is achieved by a decline in ovarian P₄ synthesis (Virgo & Bellward 1974), increased local metabolism of P₄ (Mahendroo *et al.* 1996, 1999, Ishida *et al.* 2007), and a change in expression of P₄ receptor isoforms and coactivators (Fang *et al.* 2002, Condon *et al.* 2003). In the human, circulating levels of P₄ do not decline before birth due to its synthesis in the placenta. However, the remaining documented mechanisms for loss of P₄ function, such as local P₄ metabolism, changes in P₄ receptor isoform and coactivator expression are conserved (Mesiano *et al.* 2002, Condon *et al.* 2003, Stjernholm-Vladic *et al.* 2004, Andersson *et al.* 2008). Furthermore, administration of P₄ receptor antagonists such as mifepristone to women will induce cervical ripening and preterm birth, emphasizing the need for P₄ to maintain pregnancy and loss of P₄ function to initiate parturition (Elliott *et al.* 1998).

Processes that govern ripening and dilation appear common between mice and women. In both species, the glycosaminoglycan composition is altered over the course of pregnancy and likely modulates matrix organization (Maillot & Zimmermann 1976, Osmers *et al.* 1993, Myers *et al.* 2009, Akgul *et al.* 2012). This includes increased concentration of HA in the stroma and mucus that ranges from high to low molecular weight and increased transcription of hyaluronidase 2 (Akgul *et al.* 2012). These observations suggest a similar function of high-molecular weight HA to support increased tissue hydration and collagen disorganization

during ripening. The presence of low-molecular weight HA with onset of labor or *postpartum* may have specific functions in activation of proinflammatory responses as described in other systems (Jiang *et al.* 2005). The importance of HA breakdown to low-molecular weight HA is supported by reports that women treated with intracervical injections of hyaluronidase at term had a shorter duration of labor and increased rate of vaginal delivery compared with the placebo group (Spallicci *et al.* 2007). Similar findings are described in animal models (Byers *et al.* 2010). The size-specific functions of HA in ripening, dilation, and *postpartum* repair require further investigation as does the contribution of aberrant HA function or processing to preterm and postterm birth.

While a large body of literature suggests a key role of inflammation in mediating cervical ripening in women, many of these studies utilize tissues collected after prolonged labor or post-vaginal delivery at which time the gene expression profile is markedly altered compared with ripening (Young *et al.* 2002, Osman *et al.* 2003). Moreover, immunohistochemical analysis is complicated as most of the identifying surface markers such as CD68 in human or F4/80 in mice are found on multiple myeloid-derived cells (macrophages, monocytes, and eosinophils) as well as nonhematopoietic cells and thus the antibodies used cannot distinguish specific myeloid cell subtypes (Gordon & Taylor 2005). In addition, the recent insight that a single type of myeloid cell can have a spectrum of phenotypes that range from proinflammatory to immunosuppressive (e.g. M1 and M2 macrophages), further muddies interpretation of immunohistochemical studies on human and animal models. The limited studies that utilize well-characterized tissues collected during ripening before birth suggest no increase in the proinflammatory chemoattractant, *Il8*, while microarray studies that compare gene expression patterns at term before ripening to *postpartum* reveal increased expression of proinflammatory genes after vaginal delivery but not during cervical ripening (Sakamoto *et al.* 2004, 2005, Hassan *et al.* 2006, 2009). Studies on mice in which precisely timed cervical tissue can be obtained before, during, and after term cervical ripening, the use of more sophisticated technologies to allow identification of myeloid cells via multiple surface markers and identification of markers that distinguish macrophages of varying phenotypes have been instrumental in clarifying the contribution of immune cells to both physiological and pathophysiological remodeling and emphasize the need to reevaluate more critically the role of immune cells in human term cervical ripening. Further investigations are warranted using available human tissues from term- and infection-induced preterm birth samples in order to develop targeted therapies or identify biomarkers that may be predictive of this subset of PTB.

Summary

While much work remains, our understanding of the molecular processes that drive cervical remodeling has advanced in recent years. Incremental and cumulative changes in the ECM occur in a P₄-rich environment during softening with further rapid and more aggressive changes to the ECM upon loss of P₄ function during cervical ripening and dilation. To complete the loop of remodeling and allow subsequent pregnancy, proinflammatory and immunosuppressive processes modulate tissue repair *postpartum*. Dynamic, cumulative changes in the cervical ECM are initiated early in pregnancy and culminate in the formation of a highly disorganized ECM of unique composition that allows for the loss of tissue integrity resulting in cervical opening during parturition.

Declaration of interest

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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