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Cervical Remodeling during Pregnancy and Parturition

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Abstract

Appropriate and timely cervical remodeling is key for successful birth. Premature cervical opening can result in preterm birth which occurs in 12.5% of pregnancies. Research focused on the mechanisms of term and preterm cervical remodeling is essential to prevent prematurity. This review highlights recent findings that better define molecular processes driving progressive disorganization of the cervical extracellular matrix. This includes studies that redefine the role of immune cells and identify diverse functions of the cervical epithelia and hyaluronan in remodeling. New investigations proposing that infection-induced premature cervical remodeling is distinct from the normal process are presented. Recent advances in our understanding of term and preterm cervical remodeling provide new directions for investigation and compel investigators to reevaluate currently accepted models.

Overview

The transformation of the cervix from a closed rigid structure to one that opens sufficiently for birth is an active dynamic process that begins long before the onset of labor. Better understanding of the molecular process of cervical remodeling is critical for the development of therapies to treat preterm birth and postterm pregnancies due to cervical malfunction. In this review, recent insights gained from studies in rodent models will be presented and contrasted with human studies. Although the mechanisms used to achieve the appropriate hormonal environment for each phase of cervical remodeling differ between human and rodent (**Box 1**), the end result is a similar endocrine environment; further, there is a growing body of evidence that molecular mechanisms of cervical remodeling are well conserved between these two species. This review highlights some of the recent findings in this area.

Distinct phases of remodeling

Cervical remodeling can be loosely divided into four distinct but overlapping phases termed softening, ripening, dilation and postpartum repair (Table 1) [1,2]. Softening can be defined as the first measurable decline in the tensile strength or tissue compliance compared to nonpregnancy. Biomechanical studies in mice or digital exam in women indicate softening begins by day 12 of a 19 day gestation in mice and in the first trimester of pregnancy in women [1,3]. This phase is unique from the subsequent two phases in that softening is a relatively slow and incremental process taking place in a progesterone rich environment. Despite the progressive increase in compliance, tissue competence is maintained. Following softening, cervical ripening is a more accelerated phase characterized by maximal loss of tissue

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compliance and integrity. Ripening occurs in the hours preceding birth in mice and in the weeks or days preceding birth in women. Upon initiation of uterine contractions, the ripened cervix can dilate sufficiently to allow passage of a term fetus. The final phase of remodeling termed postpartum repair ensures recovery of tissue integrity and competency. Each phase of remodeling is orchestrated within a unique endocrine environment affecting epithelial, stromal, immune and endothelial cell function as well as the composition and structure of the extracellular matrix (ECM). Although each of these cell types plays an important function in this process, this review focuses primarily on epithelial and immune cells given recent advances in these areas.

Contribution of Immune Cells to Cervical Remodeling

Normal cervical remodeling

In 1981, Mont Liggins first proposed a model in which inflammatory cells mediate changes in the cervical ECM leading to cervical ripening [4]. This was an attractive model leading to the hypothesis that infection-mediated preterm birth was simply an acceleration of the inflammatory response that occurred during normal physiological cervical ripening. Leukocytes infiltrating the cervix at birth were proposed to secrete proteases that contribute to the destruction, loss and disorganization of the collagen rich matrix to allow cervical dilation [5–7]. Inflammatory cells that secrete proinflammatory cytokines are present in the cervix prior to birth in women and animals [5,6,8–12]. Although immunohistochemical (IHC) studies reported an increase in macrophage numbers during ripening, interpretation of these data is complicated in some cases by evaluation of tissues collected after delivery in human studies [9,13]. More recent studies comparing term cervical tissues collected before and during cervical ripening to after vaginal delivery have led investigators to reevaluate the role of inflammatory cells in the initiation of cervical ripening [14–17]. Interleukin 8 expression and neutrophil numbers increased in the cervix after vaginal delivery rather than during ripening [16]. Consistent with these human studies, neutrophil numbers do not increase in the mouse or sheep until after birth. Moreover, administration of the monoclonal antibody RB6-8C5 (GR1), which depletes neutrophils and also weakly binds monocytes, does not affect parturition in mice. Myeloperoxidase activity, a functional marker of neutrophils, was also not increased until postpartum [18,19].

A second complication in the interpretation of IHC assessment of macrophages within the cervix is that antibodies used in IHC studies recognize other cell types and do not identify the phenotype and activation status of macrophages in the cervix microenvironment. For example, macrophages are reported to be increased in the cervix during ripening in women [9,17,20] and mice [12] but the antibodies used in human, e.g.CD14 and CD68, also bind monocytes; in mouse, F4/80 (BM8) weakly binds monocytes whereas CD54 recognizes many cell types including endothelial cells [21–24]. Moreover, these antibodies cannot distinguish macrophage phenotype (proinflammatory M1 versus alternatively activated M2 phenotype). To overcome this limitation, recent assessment of multiple cell surface markers by flow cytometry along with gene expression studies distinguish neutrophil, monocyte or macrophage numbers or phenotype. Using cervices collected before, during or after cervical ripening, these studies confirm a primary role of inflammatory cells in postpartum tissue repair rather than the initiation of cervical ripening [25,26]. Eosinophils are increased in number at the time of labor. In addition, tissue monocytes are increased in the cervix but not peripheral blood during ripening and are distinct from the resident macrophage population. It was anticipated that macrophage numbers would increase as monocytes differentiate into macrophages, yet macrophage numbers were unchanged during cervical ripening or postpartum. This paradox might be explained by the fact that as macrophages become activated, there is an increased turnover of these cells leading to no net change in macrophage numbers. In contrast to numbers, there is a dramatic change in the macrophage gene expression profile postpartum. As early as

two hours after birth in mice, macrophage markers are expressed with phenotypes of M1 classically activated proinflammatory macrophages [interleukin 1 alpha (*Il1a*), tumor necrosis factor alpha (*Tnfα*), monocyte chemotactic protein 1] as well as M2 alternatively activated macrophages [chitinase 3-like, arginase 1, interleukin 1 receptor antagonist] which are important in immunosuppression and tissue repair [26,27]. This data supports a model in which newly recruited macrophages and/or resident macrophages display a heterogeneous phenotype to facilitate efficient recovery and repair of the cervix postpartum. A mixed population of macrophages with diverse phenotypes would serve two purposes: 1) M1 macrophages protect the reproductive tract from the threat of microbial invasion and allow postpartum clearance of ECM molecules required during ripening, and 2) M2 macrophages suppress overactivation of proinflammatory responses and promote repair of the cervix to the nonpregnant state. These studies suggest that ECM changes necessary for cervical ripening are not mediated through an inflammatory response and that matrix cleanup and tissue repair in the postpartum stage of recovery is regulated by immune cells. Furthermore, the increase in cervical but not peripheral blood monocytes in mice highlights the importance of assessing myeloid cell phenotype and activation within the cervical microenvironment in addition to peripheral blood during cervical ripening and postpartum. Although recent studies in women report leukocytes in peripheral blood are primed for activation during term and preterm labor [28], further studies are required to determine if these changes in peripheral blood mimic leukocyte phenotype and activation within the cervical microenvironment.

The role of inflammation in cervical remodeling in women is predicted to be similar to the mouse due to recent microarray studies using cervical tissue collected prior to birth that distinguish unripe versus ripe cervix based on clinical assessment [15]. This work reports little increase in proinflammatory gene expression during cervical ripening, although both proinflammatory and immunosuppressive signaling was upregulated in the cervix after delivery [14,15]. Increased cervical proinflammatory gene expression after vaginal delivery is confirmed in other recent studies as well [29].

Infection-induced preterm cervical remodeling

Despite the proposed paradigm shift in the mechanism of normal cervical ripening, there is clear evidence that infection is a causative agent of premature cervical ripening and a significant risk factor for preterm birth. Infection-mediated preterm birth roughly accounts for 25–40% of all preterm births [30]. Additionally, the ability of inflammatory processes to induce prematurity is supported by the finding that administration of a Toll-like receptor 4 antagonist to a nonhuman-primate model suppresses the incidence of infection-induced preterm birth [31]; furthermore, mice deficient in IL1a and TNFα receptors or those treated with TNFα blockers are more resistant to LPS-induced preterm birth [32,33]. Thus, understanding the mechanisms and causes of preterm birth along with normal birth are critical for developing therapies. In the past few years, advances in our understanding of the normal remodeling process has provided a foundation upon which to identify similarities and differences between preterm and normal cervical remodeling. Recent studies in mouse models reveal significant differences in the mechanism of cervical remodeling in preterm birth models versus normal ripening [34]. Diverse gene pathways were upregulated in an infection preterm birth model as compared to term cervical ripening, leading the authors to propose that premature opening of the cervix with infection does not occur by acceleration of the normal pathway. The finding that inflammatory cells are sufficient but not necessary for cervical ripening reveals that there is more than one mechanism to achieve cervical ripening. It also shows that the model proposed by Dr. Liggins twenty-nine years ago, i.e. that inflammatory cells mediate changes in the cervical ECM leading to ripening, is likely correct for infection-mediated but not term cervical ripening.

Cervical Epithelia: Barrier Properties, Immune Surveillance and More

The cervical epithelia and mucus have important protective roles during pregnancy and parturition that include prevention of infection and protection against mechanical insult during delivery. In order to fulfill these roles, epithelia undergo marked proliferation during pregnancy (Figure 1) [35]. In recent years, the observation that the expression of specific proteins are induced and regulated during cervical ripening and dilation in mice further highlight additional roles of epithelia in regulation of the parturition process. Some examples include aquaporins, gap junction proteins connexin 26 and 43, hyaluronan synthase 2 (HAS2), steroid 5 alpha reductase type 1 (SRD5a1) and desmogleins (1 alpha and 1 beta) [25,36–38].

Epithelia that line the female reproductive tract including the cervix have functions that contribute to innate and adaptive immunity. They secrete cytokines and chemokines that recruit and activate inflammatory cells and antimicrobial factors that eliminate invading pathogens. They have been described as “sentinels of immune protection” [39]. Indeed during most of the mouse pregnancy, leukocytes within the cervix line the subepithelial region of the cervix. In addition, epithelia themselves also produce pattern recognition receptors for bacteria and viruses (Toll-like receptors 2–5, TLR2-5), antimicrobials and protease inhibitors. Indeed, premature delivery in mice due to bacterial infection requires TLR4 and its downstream signaling molecule, myeloid differentiation primary response gene 88 (MYD88) [40].

The barrier properties of the mucosal epithelia are regulated in part by junction proteins which seal off intercellular space between adjacent cells as well as maintain apical and basolateral polarity. In the mouse, temporal changes in the tight junction proteins, claudin 1 and 2, as well as a shift in cellular localization occurs with progression of pregnancy [41]. The junction protein desmoglein and markers of terminally differentiated epithelia such as keratin proteins are upregulated during cervical ripening with maximal expression shortly postpartum [34, 41]. Although specific functions of junctional proteins and the role of epithelial differentiation in cervical remodeling are unclear, the temporal shift in normal pregnancy and aberrant expression in preterm and postterm birth models suggests that changes in permeability barrier properties are regulated through pregnancy [41,42].

Recently, additional proteins that might contribute to barrier protection have been reported. Trefoil factor 1 (TFF1) and serine protease inhibitor Kazal type 5 (SPINK5) are induced during cervical softening with maximal expression at birth [1]. TFF1 is a secreted protein expressed in the gastric mucosa whose functions include protection and restitution of gut mucosal epithelia [43]. SPINK5 encodes a protease inhibitor expressed in stratified epithelia of the skin which prevents degradation of desmogleins and other proteins involved in barrier formation [44]. Based on their functions in gut and skin, respectively, TFF1 and SPINK5 might contribute to a “heightened level of security” in the pregnant cervix in order to better maintain a barrier capable of fighting infection and other insults to the reproductive tract. Reduction or absence in expression of these protective factors might be a contributing factor in susceptibility to infection-mediated preterm birth and is an important question for future investigations. These proteins are expressed in the reproductive tract of women along with another recently described protease inhibitor, elafin [1,45].

In summary, the cervical epithelia appear to have specific functions at each stage of remodeling (Figure 1). During softening, the major role of the epithelia is in protection via increased expression of surveillance factors (e.g. TFF1, SPINK5). At the end of pregnancy, the epithelia also express proteins that promote ripening (e.g. SRD5a1 and HAS2) and alter barrier properties of the cervix (e.g. claudin 1 and 2, desmogleins) [34,37,38,41]. Expression of proteins involved in terminal differentiation pathways (e.g. keratins) are maximal during postpartum repair and proposed to facilitate this stage of remodeling. Future studies to highlight

the mechanisms by which the epithelia regulate cervix function throughout remodeling will be important.

Collagen and Tissue Biomechanics

Characteristic changes of the female cervix in early pregnancy was described by Hegar in 1895 as “softening” [46]. These studies were the first hint that changes in the structural organization of the tissue occur in the first trimester of pregnancy resulting in increased tissue compliance (the physical yielding of tissue to a mechanical force). Biomechanical measurements confirm the progressive increase in tissue compliance that begins in early pregnancy and ends with maximal loss of tensile strength at the time of birth [47]. The gradual change in mechanical properties during normal pregnancy along with the increase in tissue volume allows progressive softening to take place; yet, the cervix remains closed maintaining the fetus *in utero* [48].

Collagen is the most abundant protein in the cervix, and fibrillar collagen is the main structural protein that influences the tensile properties of the cervix [3]. Collagen's properties are influenced in part by changes in synthesis, posttranslational modifications, assembly of fibers and degradation of fibers. Conflicting data exists in the literature regarding the importance of collagen degradation versus changes in collagen structure to the cervical ripening phase as discussed in **Box 2**. Future studies to better understand the mechanisms by which collagen tensile strength is modulated over the course of pregnancy as well as the timing of these changes are critical. Current understanding confirm that changes in collagen structure precede cervical softening and contribute to the progressive decline in the tensile strength of the cervix which is maximal at birth and rapidly regained in the postpartum period.

Hyaluronan and Proteoglycans

Alterations in collagen structure and packing are influenced by the composition of glycosaminoglycans (GAGs) in the ECM (Figure 1 and 2). Cervical total GAG content increases with progression of pregnancy and is accompanied by a dramatic change in composition [49]. GAGs include the unsulfated GAG, hyaluronan (HA), as well as proteins containing sulfated GAG chains (proteoglycans). Proteoglycans have diverse functions in signal factor binding and modulate collagen fibril size, spacing and access to proteases [50–52]. Numerous proteoglycans such as versican, decorin, biglycan, fibromodulin, and asporin are expressed abundantly in the cervix with no change in mRNA expression during pregnancy [1,53]. Proteoglycan function is regulated not only by levels of the core protein encoded by these genes but also by the composition, length and degree of sulfation of the GAG chain that is posttranslationally attached to the core protein. Thus, changes in GAG chains might regulate proteoglycan function in the cervix given the potential role of proteoglycans such as decorin, to modulate collagen fibril size and regulate growth factor binding and versican, to influence structural disorganization the ECM. With improved tools to study GAGs now available, such as the ability to measure GAG chain composition, length and sulfation by fluorophore assisted carbohydrate electrophoresis [38], ^{23}NaNMR to evaluate proteoglycan abundance in tissue [54] and mouse knockout models [51], a greater emphasis on research in this understudied area of cervical biology is required.

Increased hyaluronan synthase 2 expression and the subsequent increase in HA is a distinct feature of cervical ripening and dilation [38]. The functions of HA in other biological processes are influenced by HA size and HA binding proteins. In the mouse cervix, HA is predominantly high molecular weight (MW) before birth and low MW shortly after birth [55]. These studies suggest HA might regulate a two-step process during ripening and dilation (Figure 2). In the first step (during ripening), large MW HA and its association with the proteoglycan versican is required for increased viscoelasticity, tissue distensibility, hydration and disorganization of collagen matrix. We propose that just prior to or during the onset of labor, the increased

expression of hyaluronidase and ADAMTS1 (a disintegrin and metalloprotease with thrombospondin-like repeats-1) leads to disruption of HA-versican cross-links due to breakdown of HA and versican, respectively. The increased breakdown to smaller size products might be a second step in the loss of cervical tensile strength and integrity required for cervical dilation and birth. This hypothesis is supported by reported increases in hyaluronidase activity in cervical mucus of women at term [56]. In addition, studies report the efficacy of hyaluronidase treatment in reducing the length of labor and frequency of cesarean delivery due to cervical malfunction in women [57]. The increase in low MW HA and its colocalization with the HA binding protein inter alpha trypsin inhibitor heavy chain (IaI), might concomitantly influence activation of the proinflammatory process important during postpartum tissue repair similar to described functions of HA in other biological processes [55,58]. Although direct experimental evidence is required, the timing of HA and IaI heavy chain association in the cervix correlates with increased activation of both classical (M1) and alternatively activated (M2) macrophages in the postpartum period [26]. Such a model would link changes in the ECM to activation of immune cell responses during postpartum tissue repair (Figure 2). To summarize, although functions of specific proteoglycans remain an important area of investigation, hyaluronan appears to modulate matrix structure and perhaps inflammatory processes during cervical ripening and postpartum repair.

Other Extracellular Matrix Components

Additional ECM components influence the biomechanical properties of the cervix. Elastin fibers provide recoil to tissues that undergo repeated stretch; in the human cervix, elastin fibers are located in defined regions of the stromal matrix and comprise 0.9–2.4% of the total amount of connective tissue without significant change in content over the course of pregnancy [59]. The importance of elastin fibers in mediating reversible extensibility or elasticity is suggested by the decline in elastin fiber content in women with cervical insufficiency and the increased incidence of cervical insufficiency in women with a genetic mutation in fibrillin, a component of elastin microfibrils [60].

Matricellular proteins are associated with the extracellular matrix but are not considered structural components of the ECM. Matricellular proteins such as SPARC (secreted protein acidic and rich in cysteine), thrombospondin 1, thrombospondin 2, and tenascin C modulate interactions of cells with the ECM during development, remodeling and wound healing [61]. Consistent with their described roles in wound healing and tissue repair, transcripts encoding tenascin C, thrombospondin 1 and 2, and fibrillin are induced several-fold in the postpartum mouse or human cervix [2,14,25]. Mice deficient in each of these proteins have numerous phenotypic abnormalities resulting from altered ECM production and assembly [62–64]. For example, mice deficient in thrombospondin 2 undergo premature cervical softening but not preterm birth as determined by tissue biomechanical studies [65]. In summary, matricellular proteins are expressed and regulated during cervical remodeling, but their specific function in this process remains to be elucidated.

Working Model based on Animal Studies

Recent advances highlighted in this review allow the development of a working model in mice which distinguish processes important during softening versus those occurring during latter phases of ripening and dilation. Softening is characterized by increased collagen turnover and reduced collagen cross-linking. As mature, cross-linked collagen is depleted from the matrix, it is replaced with the less mature collagen resulting in a progressive decline in tissue stiffness. Concomitant with changes in the ECM is the localization of leukocytes to the subepithelial region, increased proliferation and surveillance capability of epithelia by expression of repair and barrier maintenance factors. These changes take place in a high progesterone and low

estrogen environment. The transition to the accelerated phase of cervical ripening is mediated by a decline in progesterone synthesis, increased cervical progesterone metabolism and increased synthesis of estradiol and relaxin. The ongoing processes initiated during the softening phase are then joined during ripening by increased vascularization [66], a change in GAG composition and alteration in tissue hydration which together result in increased tissue volume. The best understood GAG change is an increase in hyaluronan which facilitates further disorganization of collagen fibers and provides increased viscoelasticity to the cervix. The maximal loss of tissue tensile strength might result from disruption of stable high molecular weight hyaluronan cross-linked to versican due to increased metabolism of both GAGs. Disruption of these cross-links might culminate in loss of integrity, allowing maximal dilation during labor upon initiation of uterine contractions. Changes in barrier properties of the cervical epithelia via changes in expression of tight junction, aquaporin and gap junction proteins might also accompany this phase of remodeling.

Ripening is also characterized by an influx of tissue monocytes to the cervical stroma prior to birth; during labor or shortly postpartum, these monocytes are differentiated to produce macrophages with diverse phenotypes. The postpartum activation of M1 macrophages and neutrophils generate proinflammatory molecules that are important in matrix cleanup, whereas the alternatively activated M2 macrophages prevent overactivation of the inflammatory process and promote tissue repair. Finally, postpartum repair is characterized by a significant increase in transcription of genes involved in matrix repair (e.g. matricellular proteins, collagen assembly) and epithelial barrier function. Although much of the proposed model remains to be tested experimentally in humans, animal studies have advanced our understanding of the mechanisms of cervical remodeling during pregnancy and parturition. The ability to obtain carefully timed tissues at each phase of remodeling has been critical in advancing our knowledge, in particular in our ability to investigate processes in place early in pregnancy during softening and especially to discern processes required for the initiation of cervical ripening from processes that are important during labor or postpartum. As has been pointed out in other reviews, differences in the mechanism(s) by which progesterone function is abrogated to initiate cervical ripening in the rodent model differs from human; advances in our understanding of this important question will require studies in women or a more appropriate animal model (**Box 1**) [67]. In addition, continued studies in the human are necessary to confirm similarities and differences in each step of cervical remodeling as compared to animal models. The recent appreciation by investigators to utilize human term cervical tissue that is clinically evaluated by the Bishop's score (a clinical score determined by a vaginal exam commonly used to predict the degree or stage of cervical ripening or dilation) as unripe versus ripe, and the comparison to cervix collected after vaginal delivery is likely to advance our understanding of human cervical remodeling at a rapid pace in the coming years [14–17].

As our understanding of the normal process of cervical remodeling is advanced, a number of critical questions can now be addressed as highlighted in **Box 3**. In closing, cervical remodeling is a dynamic progressive process resulting primarily from changes in the extracellular matrix. Significant advances in our understanding of the normal and preterm remodeling process provide great optimism that this knowledge will be translated into clinical advances in treatment and prevention of prematurity.

Box 1. Hormone Regulation of Cervical Remodeling

Hormonal regulation is perhaps the most studied aspect of cervical remodeling. Loss of progesterone function is mediated during the accelerated phases of cervical ripening and dilation at the end of pregnancy. There are clear differences between species in the mechanism of decreased progesterone function, and this has led some investigators to question the usefulness of animal models for parturition studies [67]. Recent reviews

provide a concise summary of evidence supporting the requirement for loss of progesterone function during ripening in women and animal models as well as some mechanisms by which this is achieved [2].

Each phase of cervical remodeling takes place in a distinct endocrine environment. The mechanisms in place to achieve these unique hormonal microenvironments vary between species, and recent studies highlight the distinct steroid hormone pathways between women and rodent that lead to a similar steroid hormone microenvironment in the pregnant cervix. In the mouse, this results from a loss of progesterone synthesis in the ovary as well as an increase in the metabolism of progesterone within the cervix due to increased expression of steroid 5 alpha reductase type 1 (*Srd5a1*). Characterization of *Srd5a1* knockout mouse first highlighted the important role of local steroid hormone metabolism in cervical ripening and parturition [37,68].

Recent studies in women reveal that local steroid hormone metabolism within the cervix is key to pregnancy maintenance and initiation of parturition [69]. Although circulating progesterone and estrogen levels are high during pregnancy in women, increased 17β-hydroxysteroid dehydrogenase (17βHSD) type 2 expression in the cervical epithelia [69] maintains elevated progesterone levels with a decrease in estradiol, similar to the steroid hormonal environment of the mouse cervix during the softening phase of remodeling. During cervical ripening and dilation, 17βHSD type 2 is downregulated, leading to increased estradiol synthesis. In addition, the activity of the reductive 20α-hydroxy-steroid dehydrogenase, 20αHSD enzyme (aldo-keto reductase 1C1) is maintained resulting in a net loss of local progesterone.

Taken together, the described findings contribute two important concepts to our understanding of cervical biology and parturition studies in general. First, circulating steroid hormone levels are not always reflective of the local steroid concentration in the cervical microenvironment as was described in the *Srd5a1* null mouse [37,68]. Second, although the pathways differ between human and mouse, local steroid hormone metabolism is a common mechanism to achieve a distinct hormonal environment within the cervix during pregnancy and parturition in both mouse and human [68,69]. Further studies in the human cervix are required to confirm that the temporal change in 17βHSD type 2 activity lead to measurable changes in estrogen and progesterone concentration.

Box 2. Do the major structural changes in cervical remodeling result from collagen degradation, altered collagen structure or both?

Studies in women and animal models report a decline in collagen solubility during the softening phase of remodeling compared to the nonpregnant cervix [1,47]. Collagen that is soluble in acetic acid and pepsin reflects newly synthesized collagen or collagen that is less organized. Studies in the mouse cervix report a decline in activity of lysyl oxidase in early pregnancy [70]. This enzyme catalyzes the formation of strong collagen cross-links. Loss of this activity might be responsible in part for early changes in collagen structure resulting in a decline in collagen solubility. The importance of progressive changes in collagen assembly during cervical softening is further supported by the finding that preterm birth is increased in women with inherited defects in collagen and elastin synthesis or assembly [60].

In contrast to observed changes in collagen during softening, studies focused on ripening and dilation at the end of pregnancy are conflicting. Loss of cervical collagen as a result of increased protease expression by cervical cells and invading leukocytes has long been considered a primary mechanism for loss of tissue integrity at term. The review by House *et. al*. includes publications that reported a decline in collagen content at the time of labor

or shortly postpartum in women and animal models [47]. Other investigations refute changes in collagen content during pregnancy or labor [1,71]. Collagen mRNA synthesis is upregulated during pregnancy [3]. Changes in collagen organization and structure are supported by polarized light microscopy and transmission electron microscopy studies [72]. Finally, biomechanical changes in a rat cervix treated with collagenase are not consistent with biomechanical changes during normal ripening [73]. Taken together, current data in the field supports a model in which changes in collagen processing, assembly and structure contribute to the progressive changes in tensile strength of the cervix through pregnancy. We predict that the structural reorganization of collagen by the end of pregnancy likely allows access of proteases to active sites allowing more efficient removal of less mature collagen. The observed upregulation of collagen assembly genes in the postpartum period would rapidly replenish the matrix with mature collagen resulting in little change in total collagen content. The relative contribution of collagen degradation versus structural reorganization remains controversial, and further investigations will be required to clarify this inconsistency.

Box 3. Outstanding questions in the field

- Although endocrine events that initiate parturition are distinct in human versus mouse, downstream processes such as increased HA synthesis, expression of proinflammatory markers postpartum, etc., appear conserved. What additional aspects of cervical remodeling during pregnancy, parturition and postpartum are conserved between human and animal models?
- Cervical remodeling is characterized by increased tissue vascularization allowing delivery of leukocytes and soluble factors. The role and regulation of microvasculature development in the pregnant cervix is not understood. What changes in cervical vasculature are important for cervical remodeling?
- High MW HA synthesis is upregulated during cervical ripening with increased breakdown to low MW products postpartum. Are hyaluronan (HA)/versican cross-links required for increased viscoelasticity during cervical ripening, and is the breakdown of HA from high to low MW required for maximal loss of tensile strength during dilation and labor? Does low MW HA influence leukocyte activation during postpartum repair?
- Administration of hyaluronidase to women at term reduces time of labor and frequency of cesarean section. How is HA synthesis and breakdown regulated, and is this altered in preterm and/or postterm birth?
- The cervical epithelia have multiple functions during pregnancy that include innate immunity, barrier protection, repair and expression of proteins that initiate ripening. Would reductions in cervical epithelial repair, barrier properties and defense molecules lead to a greater susceptibility to preterm birth?
- Roughly 50% of preterm births (PTB) have a defined etiology (e.g. infection, cervical insufficiency, premature rupture of membranes, previous PTB). What other factors are involved in the initiation of preterm cervical ripening in the remaining 50% or PTB?
- As our understanding of normal cervical remodeling is increased, research focused on identifying mechanisms by which the cervix opens prematurely during PTB will be enhanced. Are there some causes of PTB in which cervical ripening is an acceleration of the normal process whereas other cases (i.e. recently reported infection mediated PTB) might occur by a distinct mechanism?

- Can our understanding of "cervical biology" during normal mouse pregnancy and parturition be translated to improved clinical outcomes in women? Can we identify new risk factors for premature cervical ripening, with the hope of developing therapies to prevent prematurity and clinical tools to better identify women at risk of preterm birth with greater accuracy?

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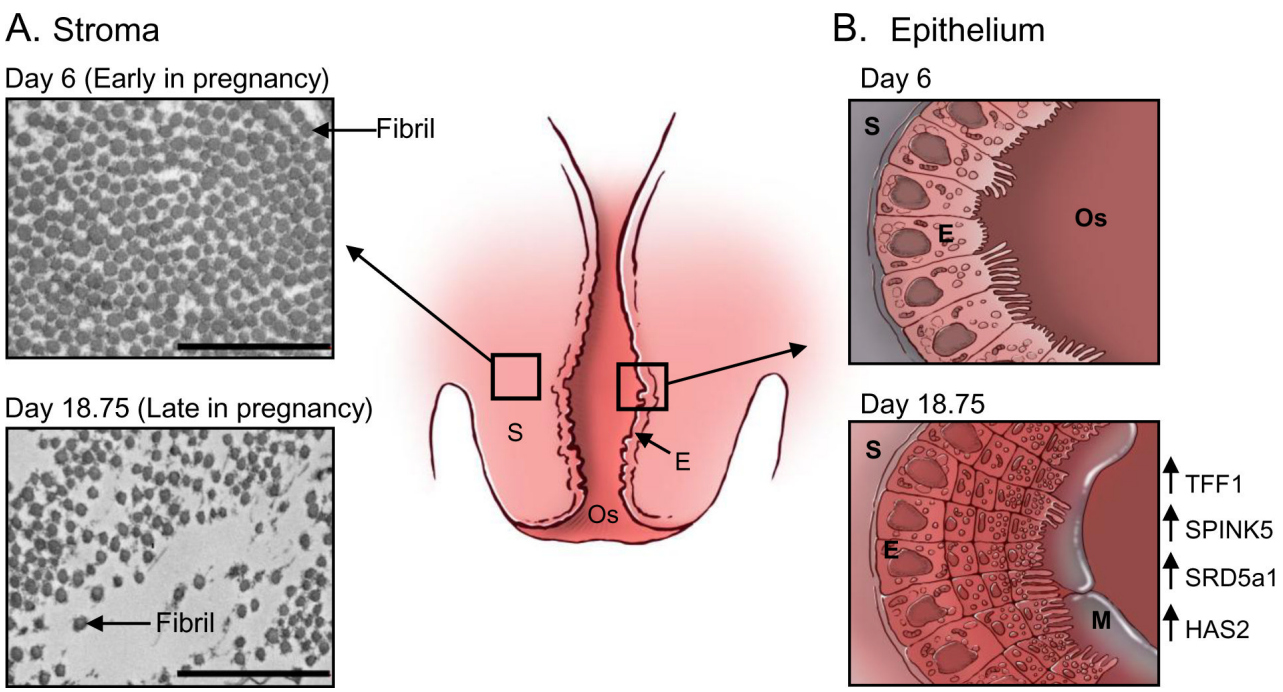

Figure 1.

Changes in the Cervical Stroma and Epithelia During the Remodeling Process. (a) Transmission electron microscopy (20,500X) of a cross section of mouse cervical collagen fibrils. These images provide a dramatic illustration of the change in matrix organization from early to late pregnancy that result in maximal loss of cervical tensile strength at birth. On day 6 of gestation (upper), fibrils are smaller and form a tightly packed organized structure. By late on gestation (day 18; lower), the fibrils are slightly larger, most likely due to the loss of collagen cross-linking and changes in matricellular proteins. Packing is disorganized with large spaces between fibrils and the increase in glycosaminoglycans (GAGs) produced at term. This disorganization of the extracellular matrix leads to a loss of tissue integrity allowing the cervix to open during birth. Bar, 1μm. (b) The cervical epithelia play important functions to protect the weakened stromal matrix. To meet these demands, the epithelia (E) proliferate and differentiate through pregnancy. In addition, changes in barrier properties as well as increased expression of repair and surveillance proteins function to protect the cervix (e.g. TFF1 and SPINK5). The increased production of cervical mucus provides both immune and physical protection. Additionally, enzymes upregulated during cervical ripening, such as HAS2 (synthesizes HA) and SRD5a1, are produced in the epithelium. SRD5a1 is required for metabolism of progesterone in the cervix. The loss of progesterone function is a key step in initiating cervical ripening at term. S, Stroma, M, Mucus, Os- cervical opening.

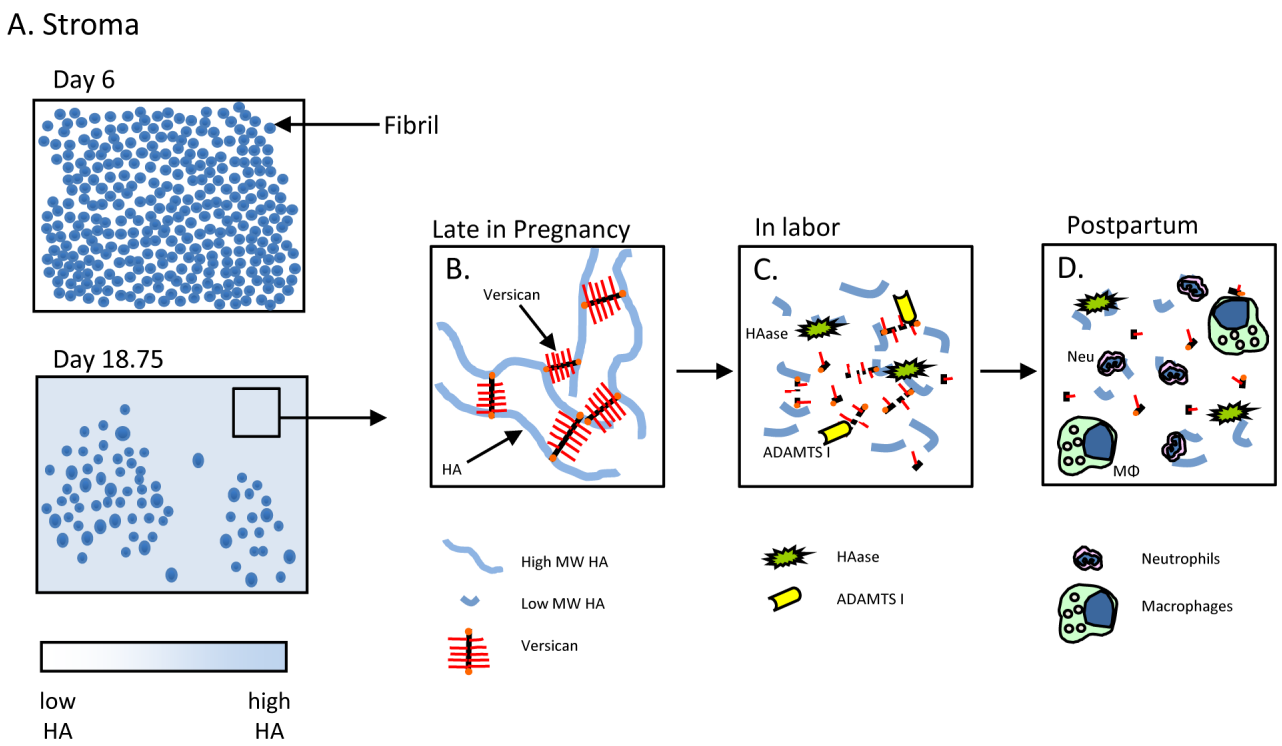

Figure 2.

Proposed model for hyaluronan's functions in cervical remodeling. (a) A cartoon representing the stromal cross section from Figure 1a illustrates the increase in high MW hyaluronan (HA) in the stromal matrix from early to late pregnancy. (b) As HA synthesis is increased at the end of pregnancy, we propose it forms cross-links with versican leading to increased tissue compliance, viscoelasticity and collagen disorganization. (c) During labor, hyaluronidase (HAase) and ADAMTS enzymes cleave HA and versican, respectively, causing a complete loss of integrity and cervical dilation. (d) During postpartum repair, low MW HA, versican fragments and damaged collagen must be removed with the help of immune cells such as neutrophils (Neu) and macrophages (MΦ).

Table 1

Distinct Features During Phases of Cervical Remodeling

Softening	Ripening/Dilation	Postpartum Repair
Slow, progressive phase	Rapid phases	↑ Synthesis of ECM molecules
↑ Progesterone function	↓ Progesterone function	↑ Metabolism of high MW HA to low MW HA
↓ Estrogen levels	↑ Estrogen levels	↑ Matricellular proteins
Changes in collagen structure (↓ cross-linking, ↑ solubility)	↑ Tissue hydration	↑ Proinflammatory gene expression
↑ Tissue growth	↑ high MW Hyaluronan	↑ Neutrophils
↑ Epithelial repair and protective factors (Tff1, Spink 5)	Maximal ↓ of tensile strength	Activation of M1 and M2 macrophages
↓ Tensile strength	↑ Tissue monocytes	
	↑ Vascularization	