Published in final edited form as:

Matrix Biol. 2019 May ; 78-79: 24–31. doi:10.1016/j.matbio.2018.03.002.

Cervical hyaluronan biology in pregnancy, parturition and preterm birth

Mala Mahendroo

Department of Obstetrics and Gynecology and Green Center for Reproductive Biological Sciences, 5323 Harry Hines Blvd, Dallas, TX, USA 75390-9032

Abstract

Cervical hyaluronan (HA) synthesis is robustly induced in late pregnancy in numerous species including women and mice. Recent evidence highlights the diverse and dynamic functions of HA in cervical biology that stem from its expression in the cervical stroma, epithelia and immune cells, changes in HA molecular weight and cell specific expression of HA binding partners. Mice deficient in HA in the lower reproductive tract confirm a structural role of HA to increase spacing and disorganization of fibrillar collagen, though this function is not critical for pregnancy and parturition. In addition, cervical HA depletion via targeted deletion of HA synthase genes, disrupts cell signaling required for the differentiation of epithelia and their mucosal and junctional barrier, resulting in increased susceptibility to ascending infection-mediated preterm birth. Finally the generation of HA disaccharides by bacterial hyaluronidases as made by Group B streptococcus can ligate toll like receptors TLR2/4 thus preventing appropriate inflammatory responses as needed to fight ascending infection and preterm birth. This review summarizes our current understanding of HA's novel and unique roles in cervical remodeling in the process of birth.

Keywords

hyaluronan; cervical ripening; preterm birth; ascending infection; parturition; cervix

INTRODUCTION

Hyaluronan (HA) is a key player in diverse aspects of female reproductive biology that span folliculogenesis and oocyte fertilization to birth. In the mammalian ovarian follicle, HA synthesis by the cumulus cells is critical to form a hydrated and viscoelastic matrix around the oocyte [1–3]. Formation of an extracellular matrix (ECM)-rich complex for which HA is the backbone is required for mammalian fertilization as evidenced by defects in ovulation and fertilization upon disruption of this HA-dependent matrix. At the other end of the reproductive spectrum, HA has long been proposed to play a significant role in pregnancy and parturition, the processes by which the reproductive tract prepares for and initiates birth

Corresponding author: Mala Mahendroo, Mala.mahendroo@utsouthwestern.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

of young. Two aspects of this process for which HA synthesis is likely critical is in the function of the fetal membranes and remodeling of the cervix in preparation for birth[4, 5]. In both tissues, HA is proposed to facilitate alterations in fibril collagen architecture to promote rupture of fetal membranes and increased cervical compliance as required for normal parturition. The focus of this review will highlight recent insights that expand HA's role in the physiology of cervical remodeling and identify pathophysiology associated with disruption of HA in the cervix.

ROLE OF CERVIX IN PREGNANCY AND PARTURITION

The function of the cervix during pregnancy is to provide a structural barrier to keep the developing fetus in the uterus for 37-40 weeks of human gestation until it is prepared for extra uterine life. During pregnancy in women, the cervix is gradually remodeled from a pinhole opening to one capable of dilating 10 cm to allow delivery. Cervical remodeling is a gradual process that begins in early pregnancy and can be divided into 3 overlapping phases during pregnancy termed softening, ripening and dilation followed by a repair phase postpartum [6]. Through pregnancy the cervical material properties are altered to allow for increased flexibility (referred to as compliance) and for a loss of structural integrity (referred to as competence).

Consistent between humans and mice, cervical softening is defined as a period in which the cervix is structurally competent yet gradually increases in compliance [6]. In contrast, cervical ripening and dilation phases are defined as a period in which tissue loses its structural integrity and thus competence is lost while tissue compliance is maximal. In mice which have a 19 day pregnancy, softening encompasses most of pregnancy (gestation days 6-17). Ripening and dilation occur in rapid succession between gestation days 18-19 and thus these two phases are studied as a single unit in mice.

Phase specific changes in the composition, assembly and structure of the ECM

The cervical stroma, comprised of fibroblasts and smooth muscle cells, is the primary source of the structural proteins, fibrillar collagens I and III and as well as elastic fibers. Other cervical ECM components include small leucine rich proteoglycans (eg decorin, biglycan, and fibromodulin), matricellular proteins (SPARC, thrombospondin 1, thrombospondin 2, tenascin C), versican and HA [7].

Cervical Softening—A key hallmark of the softening phase is the turnover of mature cross-linked collagen and replacement with immature poorly cross-linked collagen fibrils [8, 9]. Genes encoding lysyl hydroxylase (*Plod2*) and lysyl oxidase (*Lox*) that determine the type and degree of collagen cross-links are downregulated by gestation day 6[8, 10]. Marked changes in fibrillar collagen structure, reductions in mature cross-link density and increased collagen synthesis and turnover directly correspond to the gradual decline in tissue stiffness from gestation day 12 onwards. The expression of several matricellular proteins with potential to impact collagen structure and function decline during softening. These include thrombospondin 1, thrombospondin 2 and tenascin C. In contrast and consistent with the potential increased turnover of ECM during pregnancy, proteoglycans required for assembly of collagen and elastic fibers (e.g. decorin) and matricellular proteins required for collagen

deposition in the ECM (e.g. SPARC) remain abundantly expressed throughout gestation [6]. Elastic fiber ultrastructure is also changed during softening and recent studies in decorin null mice identify the contribution of elastic fibers to tissue mechanical function [10].

Cervical Ripening—Towards the end of pregnancy in the cervical ripening phase the cervix moves towards a maximal increase in compliance and a loss of tissue competence. A hallmark of this phase is the increased synthesis of the glycosaminoglycan, HA [11-13]. The observation that hyaluronan synthesis precedes or accompanies the remodeling of the cervix shortly before birth was noted over 50 years ago in multiple species that include the human and mouse [5, 11]. In the 50+ years since the observation that HA may be a component of the “ground substance” that increases in the cervical stroma prior to birth, we can now appreciate that HA has numerous and diverse roles in the cervix that overlap with its established function in other biological systems. HA’s ability to form large molecular weight, space filling, hydrodynamic structures and promote tissue viscoelasticity are proposed to facilitate the increased tissue hydration and marked disruption of collagen organization in the cervical stroma during cervical ripening. The increased synthesis of HA by the cervical epithelia and its secretion into cervical mucus during ripening was considered to provide increased lubrication required for passage of a term fetus through the birth canal. As discussed later, the roles of HA in the cervical epithelia likely extend beyond a structural role to include pathogen sensing and cell signaling.

ROLE OF HA IN THE CERVIX DURING PREGNANCY AND PARTURITION

HA Synthesis

The enzyme responsible for HA synthesis, hyaluronan synthase (Has), is encoded by three genes termed *Has1*, *Has2*, and *Has3* [14, 15]. The expression of *Has2* is critical in development as mice with a global targeted mutation in the *Has2* gene die on gestation day 10.5 due to cardiac defects [16]. In contrast mice lacking *Has1* and *Has3* are viable with no essential role in development [17]. The development of a floxed *Has2* allele provides a valuable tool for assessment of *Has2*’s role in the adult mouse [18]. *Has1* and *Has3* expression is low to negligible in the nonpregnant and pregnant cervix of women and mice [11]. In contrast, *Has2* expression is induced at term in women and in mice on gestation day 15 with peak expression during cervical ripening on gestation day 18. Consistent with the expression of *Has2*, the HA content in the mouse cervix increases from 7.4 nmol/mg dry wt in nonpregnant to 24.6 nmol/mg dry wt on gestation day 18 [19]. Thus during term ripening on gestation day 18 the total GAG content more than doubles with HA comprising 71% of total GAGs as compared to nonpregnant in which HA comprises 51% of total GAGs. While an understanding of the transcriptional regulators of cervical *Has2* remains incomplete, its expression is in part regulated by the steroid hormone estrogen and the peptide hormone relaxin. The administration of an estrogen receptor antagonist will reduce *Has2* expression in late pregnancy while administration of exogenous estrogen to an ovariectomized mouse results in increased *Has2* expression and HA content in the mouse cervix [19]. Mice with a targeted mutation in the relaxin gene have reduced *Has2* transcripts and reduced HA content on gestation day 18 as compared to WT controls [20].

HA Metabolism

The breakdown of HA to smaller molecular weight fragments is regulated by the enzyme hyaluronidase which is encoded by five genes, *Hyal* 1-5. *Hyal1* and *Hyal2* are the predominant genes expressed in somatic tissues and both are expressed in the human and mouse cervix [11]. Hyaluronidase activity in the mouse cervix as measured by fluorescent resonance energy transfer (FRET) demonstrates an increase in hyaluronidase activity at the time of labor and shortly postpartum[19]. Similarly, cervical hyaluronidase activity is significantly increased at term in women in labor and not in labor as compared to nonpregnant. The temporal increase in hyaluronidase activity corresponds to the shift from predominantly high molecular weight HA during cervical ripening to an increase in lower molecular weight HA fragments postpartum. In human vaginal epithelial cells, low molecular weight HA has been demonstrated to promote the synthesis of anti-microbial peptides and promotes wound healing by increasing the migratory capacity of epithelia[21]. These findings suggest that low MW-HA is a key modulator of innate host defense by the lower reproductive tract epithelia.

HA binding partners in the cervix

The association of HA with specific binding partners, termed hyaladherins, can modulate the physical structure and properties of HA. A diverse array of hyaladherins have been described in the cervix that include inter alpha trypsin inhibitor protein (ITI), proteoglycans and receptors. The association of HA with large proteoglycans such as versican, aggrecan and brevican is essential to the stabilization of HA structure and assembly of several tissues during development and postnatal life [22]. HA interactions with proteoglycans are further strengthened by associations with members of the link protein family (HAPLN 1-4). Versican is the predominant HA-binding large proteoglycan expressed in the cervical stroma and likely plays a collaborative role with HA to facilitate structural reorganization of collagen fibers during cervical ripening.

HA binding receptors expressed in the cervix include CD44 and toll like receptors (TLRs) [23, 24]. The prototypic HA receptor, CD44 is specifically expressed by immune cells and the basal cervical epithelia but not in terminally differentiated epithelia. The pattern recognition TLR receptors recognize pathogen-associated molecular patterns and growing evidence suggests that HA may be an endogenous ligand of TLR2 and TLR4 [21]. In the lower reproductive tract epithelia, low MW HA-dependent wound healing and anti-microbial activities require functional TLR2 or TLR4. The TLR signaling pathway activated by low MW-HA is likely distinct from a pathogen-derived ligand such as lipopolysaccharide since nuclear translocation of NF κ B is not initiated in response to low MW-HA in vaginal epithelial cells. HA also binds to the heavy chain of ITI with increased co-localization noted in the postpartum cervix. While the diversity of HA's functions remains to be understood, novel insights into HA's role in cervical biology has been elucidated in mice with targeted mutations in the genes encoding HA synthases.

Lack of HA does not affect cervical distensibility

To assess the function of HA in cervical remodeling, mice with a global loss of Has1 and Has3 (Has 1/3 KO) were bred to mice harboring a Has2 floxed gene and progesterone

receptor (PR) Cre knockin transgene (Has 2 KO)[25]. Crossing the Has1/3 KO mice with the Has2 KO mouse resulted in mice with depletion of HA in the reproductive tract as PR is expressed in the uterus and cervix. The long held hypothesis that HA is required to achieve maximal increase in tissue compliance and tissue hydration was not supported by the observation that these mice have normal and on time parturition, a normal decline in cervical tissue stiffness as measured by tissue mechanics and a normal increase in tissue wet weight. Despite a normal decline in tissue stiffness, the characteristic increase in spacing of collagen fibers in the cervical stroma was absent in the term HA depleted cervix as visualized by Masson's trichrome stain suggesting that HA may have a role in ECM reorganization that remains to be elucidated. Studies in mice lacking HA in the cervix provide insights into additional functions of HA in the cervical epithelia during pregnancy.

Lack of HA disrupts cervical epithelial barrier

The cervical epithelia provide an immunologic barrier and a structural barrier against pathogenic bacteria and viruses. These cells can sense pathogens through expression of toll like receptors (TLRs) and prevent ascension of pathogens via expression of antimicrobials, cytokines, chemokines and via recruitment of immune cells. The epithelia also provide mucosal and junctional barriers that play an immunomodulatory role to protect against ascending infection. These functions are uniquely regulated during pregnancy and critical for the prevention of preterm birth resulting from an ascending infection.

Unexpectedly, studies in the HA depleted cervix revealed a critical role of HA in organization, differentiation, mucosal and junctional barrier function of the cervical epithelia during pregnancy. The ordered progression of basal epithelia near the basement membrane to terminally differentiated polarized mucus-laden cells at the apical surface was lost in the absence of HA (Figure 1). Cells appeared in disarray and mucus secretions appeared reduced in the terminal layers. While cell proliferation was unperturbed as determined by KI67 immunofluorescence (IF) staining, cell permeability assays support a role of HA in both epithelial tight junction barrier function and in establishment of a normal mucosal barrier. Consistent with a role for HA in tight junction barrier function, IF staining for the tight junction protein, occludin, was diminished compared to WT controls. The ability of HA to regulate epithelial tight junctional barrier has also been described in the intestinal epithelia [26, 27]. In these studies, size specific HA (35kDa) induces synthesis of the tight junction protein, ZO-1, and protects mice from *Citrobacter rodentium* infection.

While the epithelial defects had no noted impact on cervical function in normal pregnancy, when faced with an ascending infection with *E. Coli*, these mice rapidly succumbed to premature birth at a significantly greater rate than wild type controls (Figure 1)[25]. HA depletion did not impact either epithelial cell proliferation or apoptosis thus further investigations are required to understand the mechanisms by which cervical epithelial barrier function is regulated as well as the hyaladherin that collaborates with HA to ensure the epithelia are capable of limiting pathogen infiltration. These findings uncover a novel and critical function of HA and identify a compromise in epithelia barrier function as a new risk factor for susceptibility to ascending infection and premature birth.

LACK OR DEPLETION OF HA PREDISPOSES TO ASCENDING INFECTION AND PRETERM BIRTH

Preterm Birth

Understanding the molecular mechanisms by which the cervix remodels is an important line of investigation as premature opening of the cervix can result in premature birth of a baby that is not well-prepared for extra uterine life[28]. Premature birth, defined as birth before gestation week 37, impact 15 million children worldwide annually [29]. In the United States, 1 in every 10 babies is born premature and health care costs for these infants exceed 31 billion dollars each year[30]. Premature birth is the primary cause of infant mortality in the first year of life and those that survive are at increased risk of lifelong health complications that include neurological deficits, respiratory and gastrointestinal deficits. Early detection and prevention of preterm birth is complicated by the fact that the etiology is diverse and in roughly 60% of spontaneous preterm births the underlying etiology is unknown [31]. Irrespective of the cause, premature cervical changes precede preterm birth, thus understanding mechanisms of remodeling is likely to aid in development of clinical tools for accurate detection and prevention of preterm birth.

Loss of HA synthesis

Infection or inflammation contribute to 25-40% of spontaneous births with an identifiable etiology[28]. Although systemic infections may account for some of these PTBs, the majority of cases arise from ascension of bacteria through the lower reproductive tract into the uterus and amniotic fluid. Research efforts in the last decade have established that the vaginal microbe diversity is reduced in pregnancy as compared to nonpregnancy and that the bacterial species *Lactobacillus* predominates during pregnancy [32, 33]. However to date, no specific microbe or microbiome profile has been defined as causative of PTB. Mice lacking cervical HA have a greater susceptibility to ascending infection and preterm birth as compared to wild type mice thus highlighting the importance of the cervical epithelia and mucus to form a protective barrier against ascending infection in pregnancy. We suggest that not only microbe composition but also the ability of the epithelial barrier to defend against ascension of pathogens are collectively a key determinant of risk for infection-mediated PTB[25]. The unanticipated role of HA as a protective factor against pathogen mediated PTB suggests that insufficient HA levels could be a susceptibility factor that predisposes some women to infection-mediated preterm birth.

Pathogen mediated breakdown of HA

Elegant studies recently described in mice identify a mechanism by which pathogen-facilitated loss of HA may be achieved in the lower reproductive tract and contribute to increased susceptibility of ascending infection and preterm birth. Group B streptococcus (GBS) is a gram positive bacteria and approximately 30% of women have heavy GBS vaginal colonization [34, 35]. Transmission of GBS to the fetus can occur during vaginal delivery or by ascending infection that leads to inflammation of the fetal membranes and subsequently preterm birth or still birth. Understanding the maternal and bacterial

determinants that regulate the ability of GBS to ascend into the upper reproductive tract is critical for prevention.

Current understanding of bacterial factors that allow GBS to evade clearance include 1) ECM factors that promote the adherence to host epithelial cells 2) hemolytic activity that facilitates immune cell evasion and 3) hyaluronidase activity capable of breaking down host HA. The hyaluronidase made by GBS is encoded by the HylB gene. HylB breaks down high molecular weight HA into disaccharide components which bind toll like receptors, TLR2 and TLR4. In contrast to pathogen derived TLR ligands such as lipopolysaccharide or peptidoglycan, the binding of HA disaccharides to TLR2/4 limits proinflammatory TLR signaling cascades thus facilitating GBS evasion of host immunity (Figure 2) [36]. Recent studies by Vornhagen, J et al demonstrate elevated HylB activity in GBS strains isolated from women in preterm labor compared to strains isolated from women at term but not in labor [37, 38]. GBS strains with genetic loss of HylB in a mouse model of ascending infection have a reduced ability for ascension into the upper reproductive tract and lower rates of preterm birth. The maternal uterine inflammatory response and leukocyte invasion is lower with exposure to WT GBS as compared to GBS strain with depleted HylB activity. These findings implicate HyalB as a virulence factor and provides mechanistic insight as to how GBS can avoid immune detection in the lower reproductive tract and ascend into the upper reproductive tract to elicit placental and fetal inflammation and preterm birth.

FUTURE RESEARCH QUESTIONS AND DIRECTIONS

Current evidence has established that HA has multiple roles in the cervix during pregnancy and parturition. This includes its presence in both the epithelial and stromal extracellular matrix, its temporal shift from high to low molecular weight and the cell specific-expression of HA binding partners [23]. Furthermore, not only a lack of cervical HA but also increased generation of low molecular weight HA increase the risk of preterm birth resulting from an ascending infection. These findings are just the tip of the iceberg in our understanding of the mechanisms by which this fascinating molecule mediates its cell and temporal specific actions in the cervix during pregnancy and parturition. Key priorities to further our understanding include 1) defining the TLR signaling cascades regulated by low molecular weight HA and/or HA disaccharides, 2) delineation of the mechanism by which HA regulates appropriate epithelial cell differentiation and/or mucosal and junctional barrier protection 3) uncovering the role of HA in the cervical stroma ECM that was not revealed in the conditional HA knockout mouse studies 4) defining mechanisms of HA pathophysiology in cervical remodeling that may predispose to cervical dysfunction and preterm birth in women and 5) determining the overall hyaluronidase activity of pathogens in addition to GBS that increase risk of ascending infection. Focused studies in animal models, human cervical epithelial cell lines and limited human tissue is likely to reveal novel and dynamic functions of HA over the course of pregnancy, onset of labor and postpartum tissue repair. These in turn will provide the necessary information to understand how HA metabolism may be hijacked by pathogens or misregulated in cervical cells resulting in increased risk of premature birth. We suggest such advancements consequently may motivate the application of developed hyaluronidase inhibitors [39] or HA of a defined size as a therapeutic or allow for development of new therapeutics that will aid in prevention of preterm birth.

Acknowledgments

I thank Shanmugasundaram Nallasamy for critical reading of the manuscript and previous members of my laboratory who have contributed to our understanding of HA in cervical biology.

Funding: This work was supported by the National Institutes of Health [1 P01 HD087150-01A1 and HD011149]

References

1. Russell DL, Salustri A. Extracellular matrix of the cumulus-oocyte complex. *Semin Reprod Med.* 24(4)2006; :217–27. [PubMed: 16944419]
2. Salustri A, Yanagishita M, Hascall VC. Synthesis and accumulation of hyaluronic acid and proteoglycans in the mouse cumulus cell-oocyte complex during follicle-stimulating hormone-induced mucification. *J Biol Chem.* 264(23)1989; :13840–7. [PubMed: 2503506]
3. Salustri A, Campagnolo L, Klinger FG, Camaioni A. Molecular organization and mechanical properties of the hyaluronan matrix surrounding the mammalian oocyte. *Matrix Biol.* 2018
4. Skinner SJ, Liggins GC. Glycosaminoglycans and collagen in human amnion from pregnancies with and without premature rupture of the membranes. *Journal of developmental physiology.* 3(2)1981; : 111–21. [PubMed: 7299102]
5. Danforth DN. The fibrous nature of the human cervix, and its relation to the isthmic segment in gravid and nongravid uteri. *Am J Obstet Gynecol.* 53(4)1947; :541–60. [PubMed: 20291226]
6. Nallasamy S, Mahendroo M. Distinct Roles of Cervical Epithelia and Stroma in Pregnancy and Parturition. *Semin Reprod Med.* 35(2)2017; :190–200. [PubMed: 28278536]
7. Mahendroo M. Cervical remodeling in term and preterm birth: insights from an animal model. *Reproduction.* 2012
8. Akins ML, Luby-Phelps K, Bank RA, Mahendroo M. Cervical Softening During Pregnancy-Regulated Changes in Collagen Cross-Linking and Composition of Matricellular Proteins in the Mouse. *Biology of Reproduction.* 84(5)2011; :1053–62. [PubMed: 21248285]
9. Yoshida K, Jiang H, Kim M, Vink J, Cremers S, Paik D, Wapner R, Mahendroo M, Myers K. Quantitative evaluation of collagen crosslinks and corresponding tensile mechanical properties in mouse cervical tissue during normal pregnancy. *PloS one.* 9(11)2014; :e112391. [PubMed: 25397407]
10. Nallasamy S, Yoshida K, Akins M, Myers K, Iozzo R, Mahendroo M. Steroid Hormones Are Key Modulators of Tissue Mechanical Function via Regulation of Collagen and Elastic Fibers. *Endocrinology.* 158(4)2017; :950–962. [PubMed: 28204185]
11. Straach KJ, Shelton JM, Richardson JA, Hascall VC, Mahendroo MS. Regulation of hyaluronan expression during cervical ripening. *Glycobiology.* 15(1)2005; :55–65. [PubMed: 15317739]
12. Golichowski AM, King SR, Mascaro K. Pregnancy-related changes in rat cervical glycosaminoglycans. *Biochem J.* 192(1)1980; :1–8. [PubMed: 7305889]
13. Danforth DN, Veis A, Breen M, Weinstein HG, Buckingham JC, Manalo P. The effect of pregnancy and labor on the human cervix: changes in collagen, glycoproteins, and glycosaminoglycans. *Am J Obstet Gynecol.* 120(5)1974; :641–51. [PubMed: 4278606]
14. Spicer AP, Augustine ML, McDonald JA. Molecular cloning and characterization of a putative mouse hyaluronan synthase. *J Biol Chem.* 271(38)1996; :23400–6. [PubMed: 8798545]
15. Spicer AP, Olson JS, McDonald JA. Molecular cloning and characterization of a cDNA encoding the third putative mammalian hyaluronan synthase. *J Biol Chem.* 272(14)1997; :8957–61. [PubMed: 9083017]
16. Camenisch TD, Spicer AP, Brehm-Gibson T, Biesterfeldt J, Augustine ML, Calabro A Jr, Kubalak S, Klewer SE, McDonald JA. Disruption of hyaluronan synthase-2 abrogates normal cardiac morphogenesis and hyaluronan-mediated transformation of epithelium to mesenchyme. *The Journal of Clinical Investigation.* 106(3)2000; :349–360. [PubMed: 10930438]
17. Kiene LS, Homann S, Suvorava T, Rabausch B, Muller J, Kojda G, Kretschmer I, Twarock S, Dai G, Deenen R, Hartwig S, Lehr S, Kohrer K, Savani RC, Grandoch M, Fischer JW. Deletion of

- Hyaluronan Synthase 3 Inhibits Neointimal Hyperplasia in Mice. *Arterioscler Thromb Vasc Biol.* 36(2)2016; :e9–16. [PubMed: 26586662]
18. Matsumoto K, Li Y, Jakuba C, Sugiyama Y, Sayo T, Okuno M, Dealy CN, Toole BP, Takeda J, Yamaguchi Y, Kosher RA. Conditional inactivation of Has2 reveals a crucial role for hyaluronan in skeletal growth, patterning, chondrocyte maturation and joint formation in the developing limb. *Development.* 136(16)2009; :2825–35. [PubMed: 19633173]
 19. Akgul Y, Holt R, Mummert M, Word A, Mahendroo M. Dynamic changes in cervical glycosaminoglycan composition during normal pregnancy and preterm birth. *Endocrinology.* 153(7)2012; :3493–503. [PubMed: 22529214]
 20. Soh YM, Tiwari A, Mahendroo M, Conrad KP, Parry LJ. Relaxin regulates hyaluronan synthesis and aquaporins in the cervix of late pregnant mice. *Endocrinology.* 153(12)2012; :6054–64. [PubMed: 23087172]
 21. Dusio GF, Cardani D, Zanobbio L, Mantovani M, Luchini P, Battini L, Galli V, Diana A, Balsari A, Rumio C. Stimulation of TLRs by LMW-HA induces self-defense mechanisms in vaginal epithelium. *Immunology and cell biology.* 89(5)2011; :630–9. [PubMed: 21102537]
 22. Wight TN. Provisional matrix: A role for versican and hyaluronan. *Matrix Biol.* 60–612017; :38–56.
 23. Ruschinsky M, De la Motte C, Mahendroo M. Hyaluronan and its binding proteins during cervical ripening and parturition: dynamic changes in size, distribution and temporal sequence. *Matrix Biol.* 27(5)2008; :487–97. [PubMed: 18353623]
 24. Gonzalez JM, Xu H, Ofori E, Elovitz MA. Toll-like receptors in the uterus, cervix, and placenta: is pregnancy an immunosuppressed state? *Am J Obstet Gynecol.* 197(3)2007; :296 e1–6. [PubMed: 17826427]
 25. Akgul Y, Word RA, Ensign LM, Yamaguchi Y, Lydon J, Hanes J, Mahendroo M. Hyaluronan in cervical epithelia protects against infection-mediated preterm birth. *J Clin Invest.* 124(12)2014; :5481–9. [PubMed: 25384213]
 26. Kim Y, West GA, Ray G, Kessler SP, Petrey AC, Fiocchi C, McDonald C, Longworth MS, Nagy LE, de la Motte CA. Layilin is critical for mediating hyaluronan 35kDa-induced intestinal epithelial tight junction protein ZO-1 in vitro and in vivo. *Matrix Biol.* 2017
 27. Kim Y, Kessler SP, Obery DR, Homer CR, McDonald C, de la Motte CA. Hyaluronan 35kDa treatment protects mice from *Citrobacter rodentium* infection and induces epithelial tight junction protein ZO-1 in vivo. *Matrix Biol.* 622017; :28–39. [PubMed: 27845198]
 28. Cunningham, FG, Leveno, KJ, Bloom, SL, Spong, CY, Dashe, JS, Hoffman, BL, Casey, BM, Sheffield, JS. *Preterm Labor, Williams Obstetrics, 24e.* McGraw-Hill Education; New York, NY: 2013.
 29. Blencowe H, Cousens S, Chou D, Oestergaard M, Say L, Moller AB, Kinney M, Lawn J. Born too soon: the global epidemiology of 15 million preterm births. *Reproductive health.* 10(Suppl 1)2013; :S2. [PubMed: 24625129]
 30. Caughey AB, Zupancic JA, Greenberg JM, Garfield SS, Thung SF, Iams JD. Clinical and Cost Impact Analysis of a Novel Prognostic Test for Early Detection of Preterm Birth. *AJP reports.* 6(4)2016; :e407–e416. [PubMed: 27917307]
 31. Ferrero DM, Larson J, Jacobsson B, Di Renzo GC, Norman JE, Martin JN Jr, D'Alton M, Castelazo E, Howson CP, Sengpiel V, Bottai M, Mayo JA, Shaw GM, Verdenik I, Tul N, Velebil P, Cairns-Smith S, Rushwan H, Arulkumaran S, Howse JL, Simpson JL. Cross-Country Individual Participant Analysis of 4.1 Million Singleton Births in 5 Countries with Very High Human Development Index Confirms Known Associations but Provides No Biologic Explanation for 2/3 of All Preterm Births. *PloS one.* 11(9)2016; :e0162506. [PubMed: 27622562]
 32. Romero R, Hassan SS, Gajer P, Tarca AL, Fadrosch DW, Nikita L, Galuppi M, Lamont RF, Chaemsathong P, Miranda J, Chaiworapongsa T, Ravel J. The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. *Microbiome.* 2(1)2014; :4. [PubMed: 24484853]
 33. DiGiulio DB, Callahan BJ, McMurdie PJ, Costello EK, Lyell DJ, Robaczewska A, Sun CL, Goltsman DS, Wong RJ, Shaw G, Stevenson DK, Holmes SP, Relman DA. Temporal and spatial

- variation of the human microbiota during pregnancy. *Proc Natl Acad Sci U S A*. 112(35)2015; : 11060–5. [PubMed: 26283357]
34. Allen U, Nimrod C, Macdonald N, Toye B, Stephens D, Marchessault V. Relationship between antenatal group B streptococcal vaginal colonization and premature labour. *Paediatrics & child health*. 4(7)1999; :465–9. [PubMed: 20212961]
35. Katz J, Lee AC, Kozuki N, Lawn JE, Cousens S, Blencowe H, Ezzati M, Bhutta ZA, Marchant T, Willey BA, Adair L, Barros F, Baqui AH, Christian P, Fawzi W, Gonzalez R, Humphrey J, Huybregts L, Kolsteren P, Mongkolkeha A, Mullany LC, Ndyomugenyi R, Nien JK, Osrin D, Roberfroid D, Sania A, Schmiedel C, Silveira MF, Tielsch J, Vaidya A, Velaphi SC, Victora CG, Watson-Jones D, Black RE. Mortality risk in preterm and small-for-gestational-age infants in low-income and middle-income countries: a pooled country analysis. *Lancet*. 382(9890)2013; : 417–425. [PubMed: 23746775]
36. Kolar SL, Kyme P, Tseng CW, Soliman A, Kaplan A, Liang J, Nizet V, Jiang D, Murali R, Arditi M, Underhill DM, Liu GY. Group B Streptococcus Evades Host Immunity by Degrading Hyaluronan. *Cell host & microbe*. 18(6)2015; :694–704. [PubMed: 26651945]
37. Vornhagen J, Adams Waldorf KM, Rajagopal L. Perinatal Group B Streptococcal Infections: Virulence Factors, Immunity, and Prevention Strategies. *Trends in microbiology*. 25(11)2017; : 919–931. [PubMed: 28633864]
38. Vornhagen J, Quach P, Boldenow E, Merrill S, Whidbey C, Ngo LY, Adams Waldorf KM, Rajagopal L. Bacterial Hyaluronidase Promotes Ascending GBS Infection and Preterm Birth. *mBio*. 7(3)2016;
39. Scotti L, Singla RK, Ishiki HM, Mendonca FJ, da Silva MS, Barbosa Filho JM, Scotti MT. Recent Advancement in Natural Hyaluronidase Inhibitors. *Current topics in medicinal chemistry*. 16(23)2016; :2525–31. [PubMed: 27086786]

Highlights

- Hyaluronan synthase 2 is upregulated in the cervix in late pregnancy
- HA has diverse roles in the stromal ECM and the epithelial pericellular matrix
- Lack of HA does not affect cervical distensibility
- Lack of HA disrupts the cervical epithelial mucosal and junctional barrier
- The absence or inappropriate metabolism of HA predisposes to ascending infection and preterm birth

Figure 1.

Top Panel Schematic to illustrate disruption in cervical epithelial cell differentiation in the absence of HA. As a result there is a breach of both the mucosal barrier and the cell junctional barriers resulting in the ability of pathogens to move into the cervix as well as ascend into the upper reproductive tract. *Lower panel.* Loss of the Has2 gene and not Has1 and Has3 in the cervix results in an increased susceptibility to ascending E. coli induced preterm birth rates. Blue bars indicate PTB rates when 10⁷ CFUs of E. coli are placed in the vagina on gestation day 16 and purple bars indicate PTB rates when 10⁵ CFUs are placed in the vagina on gestation day 16. PTB is birth is defined as delivery that occurs within 48 hours of E. coli exposure. **WT**-wild type, **Has2**-Has2^{fl/fl}PR^{Cre+}, **Has1/3**-Has1^{-/-}Has3^{-/-} and **Has1/2/3**-Has1^{-/-}, Has2^{fl/fl}PR^{Cre+}, Has3^{-/-}.

Figure 2.

Potential mechanism by which Group B streptococcus elicits hyaluronidase-mediated breakdown of epithelial HA to evade immune-detection. (Left panel) High molecular weight HA does not bind TLR receptors thus in the presence of high molecular weight HA pathogen derived TLR ligands (eg peptidoglycans, lipopolysaccharide) can bind TLR2 or TLR4 respectively to activate signaling cascades in the cervical epithelia and/or recruited immune cells that can deploy a proinflammatory immune response to fight ascending infection. (Right panel) Pathogens such as Group B streptococcus (GBS)) secrete hyaluronidase that breakdown HA to disaccharides which ligate TLR2 or TLR4 thus prevent binding of pathogen derived TLR ligands that elicit proinflammatory responses as required to prevent ascending infection and preterm birth.