Cervix and myometrium: role in preterm and normal term birth in mare

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Abstract

Myometrial activation and cervical remodeling during term and preterm labor involve complex molecular mechanisms. Progress in elucidating these mechanisms has been slow and based on classical molecular techniques such as realtime quantitative reverse transcription polymerase chain reaction. Recently, using ribonucleic acid sequencing, we have elucidated key regulators and molecular mechanisms triggering these events. Objective is to summarize changes in myometrial and cervical transcriptome during placentitis and normal prepartum period in comparison to normal pregnancy.

Keywords: Mare, cervix, myometrium, pregnancy, progestins, parturition, placentitis

Introduction

Placentitis induced preterm labor and term labor share a common pathway that includes 3 major events: myometrial activation, cervical remodeling and chorioallantois activation (separation and rupture).[1-4] Understanding molecular mechanisms of these 3 events holds potential for development of new diagnostic tools and therapies to forestall placentitis induced preterm labor. Recently, using a transcriptomic approach (i.e. ribonucleic acid sequencing), we elucidated key regulators and molecular mechanisms, triggering these events in an experimental model of acute ascending placentitis. Objective is to summarize our recent findings, with a focus on mechanisms underlying myometrial activation and cervical remodeling during equine placentitis and normal prepartum period in comparison to normal pregnancy.

Myometrial activation

Key event in placentitis induced preterm labor and term labor is myometrial activation with subsequent initiation of labor.[1,5] Myometrial activation involves complex myriad of coordinated changes involving immune and hormonal factors, upregulation of several contraction-associated proteins (CAPs), and arrangement of cytoskeletal machinery that provides uterus capacity to generate force and contract[1,5] Mechanisms underlying myometrial activation during equine placentitis and normal prepartum period are summarized (Figures 1 and 2).

Progestins and progesterone receptor signaling (ligand receptor signaling)

In mammals studied,[6-8] progestins, acting through its nuclear receptor (PR, also known as PGR), plays a central role in maintaining myometrial quiescence during pregnancy through blockage of inflammatory cascade and suppression of CAPs. Recently, we reported that myometrial tissue concentrations of 5_α dihydroprogesterone (5_α DHP, also known as DHP), allopregnanolone (3_α DHP) and 20_α hydroxy 5_α dihydroprogesterone (20_α DHP) were lower (progestin withdrawal) in mares with experimentally induced acute placentitis compared to age- and pregnancy-matched controls.[1] This local reduction in 5_α DHP and its downstream metabolites is attributed to a decline in enzymes responsible for synthesis of these progestins, such as 5_α reductase type 1 (5_α R1 also known as SRD5A1) and aldo-keto reductase family 1 member C23 (AKR1C23, also known as AKR1C1).[1] Moreover, expression of nuclear PR is downregulated (functional progestin withdrawal) in equine myometrium during placentitis. A closer look at PR-isoforms proteins (i.e. PR-A and PR-B) revealed a decrease in PR-B to PR-A ratio.[1] It is worth noting that in primates, progesterone (P_4) promotes myometrial quiescence through PR-B-mediated antiinflammatory actions.[9] At labor, PR-A becomes more predominant and inhibits antiinflammatory actions of PR-B and stimulates proinflammatory gene expression.[9] Downregulation in progestin-PR signaling is in turn associated with activation of NF-$_\kappa$B pathway and upregulation of proinflammatory cytokines (e.g. IL1$_\beta$), as well as upregulation

of transcripts coding for CAPs (e.g. PTGS2 and GJA4).[1] Together, these findings suggest that placentitis induces localized progestin withdrawal and progestin functional withdrawal in myometrium that lead to myometrial activation through activation of inflammatory cascade and upregulation of CAPs.

In prepartum myometrial transcriptome, although we did not identify any significant changes in geneses coding for SRD5A1, AKR1C1 and PR, we identified upregulation of aldehyde dehydrogenases (ALDH1A1, ALDH1A2, and ALDH1A3). It is worth noting that ALDH1 family is involved in conversion of retinaldehyde to retinoic acid, which in turn decreases PR transcription.[10] Therefore, upregulation of ALDHs during prepartum period might contribute to myometrial preparation for labor.

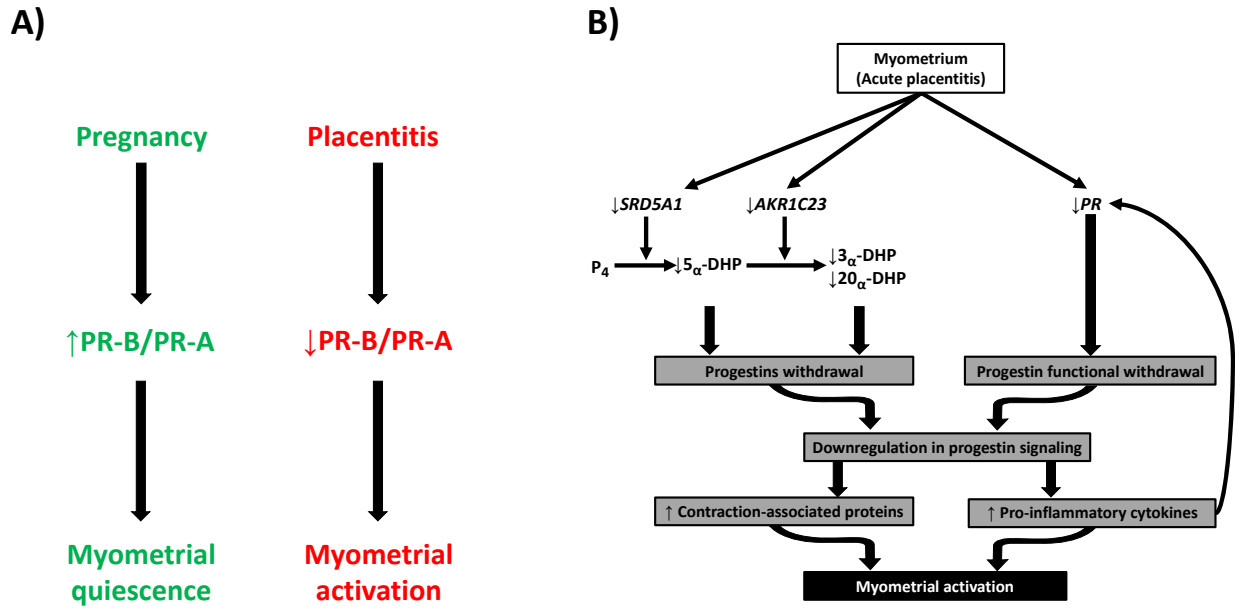

Figure 1. Progestin-progesterone receptor signaling during equine placentitis. A) PR-B/ PR-A ratio in myometrium during pregnancy and placentitis. B) Molecular mechanisms underlying progestin withdrawal and progestin functional withdrawal in equine myometrium during placentitis.

Activation of inflammatory cascade in myometrium

During inflammatory reaction in pathogenic infection, inflammatory cascade is initiated by immune recognition of pathogen mediated through toll-like receptors (TLRs), which are primary and earliest detection mechanisms for pathogens.[11] Among known TLRs, TLR2 and TLR4 are responsible for recognition of gram positive and negative bacteria, respectively.[12] Recently, we identified *Streptococcus* induced placentitis to be associated with upregulation of TLR2 in myometrial samples retrieved from placentitis group in comparison to control group.[2] Moreover, this upregulation is associated with upregulation of a wide array of TLR2-dependent downstream molecules in inflammatory cascade.[2] These findings highlight central role of TLR2 in triggering inflammatory signals in myometrium during *Streptococcus* induced placentitis. Therefore, targeting TLR2 through therapeutic inhibition (antagonism) might be beneficial for prevention and/or treatment of *Streptococcus* induced placentitis. This notion is supported by reports in primates that treatment of amniotic infection using TLR antagonists (TLRA) resulted in a downregulation of proinflammatory cytokines with subsequent delay or prevention of preterm birth.[13]

In prepartum myometrium, we identified upregulation of several inflammation related genes (e.g.

TNFAIP6, *ICAM1*, *SOCS3, CXCR4*).[2] Upregulation of these genes might reflect presence of sterile inflammatory signaling in myometrium during prepartum stage in mare.

Myometrial infiltration with leukocytes

In women, myometrial infiltration with leukocytes is hallmark of switching myometrium from a quiescent to a contractile state during term and preterm labor.[14-16] Similarly, we reported that equine placentitis is associated with increased myometrial infiltration with leukocytes.[1,2] Moreover, we elucidated chemokine signaling mechanisms implicated in upregulation of several chemotactic factors including; C-C Motif Chemokine Ligand (CCL2, 4, and 8), C-X-C Motif Chemokine Ligand (CXCL1, 2, 3, 6, 8, and 9) and calgranulins (S100A8 and S100A9).[1,2] In equine prepartum myometrium (330 days GA), although we identified upregulation of CCL2, CXCL1, CXCL3, and CXCL6, we did not observe marked leukocytic infiltration in myometrium.[2] This might reflect an early chemotaxis event taking place in prepartum myometrium in preparation for labor.

Myometrial Apoptosis

During placentitis, equine myometrium is associated with a significant upregulation of apoptosis related transcripts, including: caspases (CASP3, CASP4, CASP7), activating transcription factor 3 (ATF3), fas cell surface death receptor (FAS), fos proto-oncogene subunit (FOS), activator protein 1 (AP-1), and baculoviral IAP repeat containing 3 (BIRC3).[2] Myometrial apoptosis occurred during chorioamnionitis in women[17,18] and infection-induced labor in mice.[19] Additionally, apoptosis by itself might be a key event in switching myometrial cells from quiescent to contractile status.[17,18,20]

Uterine contraction associated genes

Placentitis induced myometrial inflammation is associated with upregulation of several contraction-associated transcripts, including prostaglandin endoperoxide synthase 2 (PTGS2, also cyclooxygenase 2; COX2), prostaglandin E Receptor 3 (PTGER3), gap junction alpha 4 (GJA4, also known as connexin-37; CXN37 or CX37), matrix metallopeptidases (MMP1 and MMP8) plus downregulation relaxin (RLN).[2] Role of these genes in myometrial activation during term and preterm labor is well established in women and mice.[21] For example, PTGS2 is essential for synthesis of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), which is a potent uterotonic (ecbolic).[21] Contrarily, GJAs are believed to play a critical role in preterm and term labor by forming gap junctions in myometrium, which increase myometrial cell coupling with subsequent generation of synchronous myometrial contractions.[21-24]

Cervical remodeling

Cervical remodeling is transformation of cervix from a rigid, tightly closed structure into a flaccid and open one to permit fetal delivery during term and preterm labor.[25-27] Cervical remodeling consists of 4 overlapping phases (i.e. softening, ripening, dilation, and postpartum repair).[25-27] Cervical remodeling requires decreases in cervical collagen concentrations (i.e. extracellular matrix (ECM) degradation) and wide dispersing of collagen fibers through increasing cervical water content (i.e. cervical hydration), with subsequent decrease of cervical tensile strength to allow cervical dilation.[28] Mechanisms underlying cervical remodeling events during equine placentitis and normal prepartum period are summarized below.

Extracellular matrix (ECM) degradation

During placentitis, we reported upregulation of several proteases, including MMPs (e.g. MMP1, 8, 13 and 14).[29] These MMPs are believed implicated in cervical collagen degradation during equine placentitis.[29] Contrarily, cervix from prepartum mares (330 d GA) was not associated with significant change in MMPs expression.

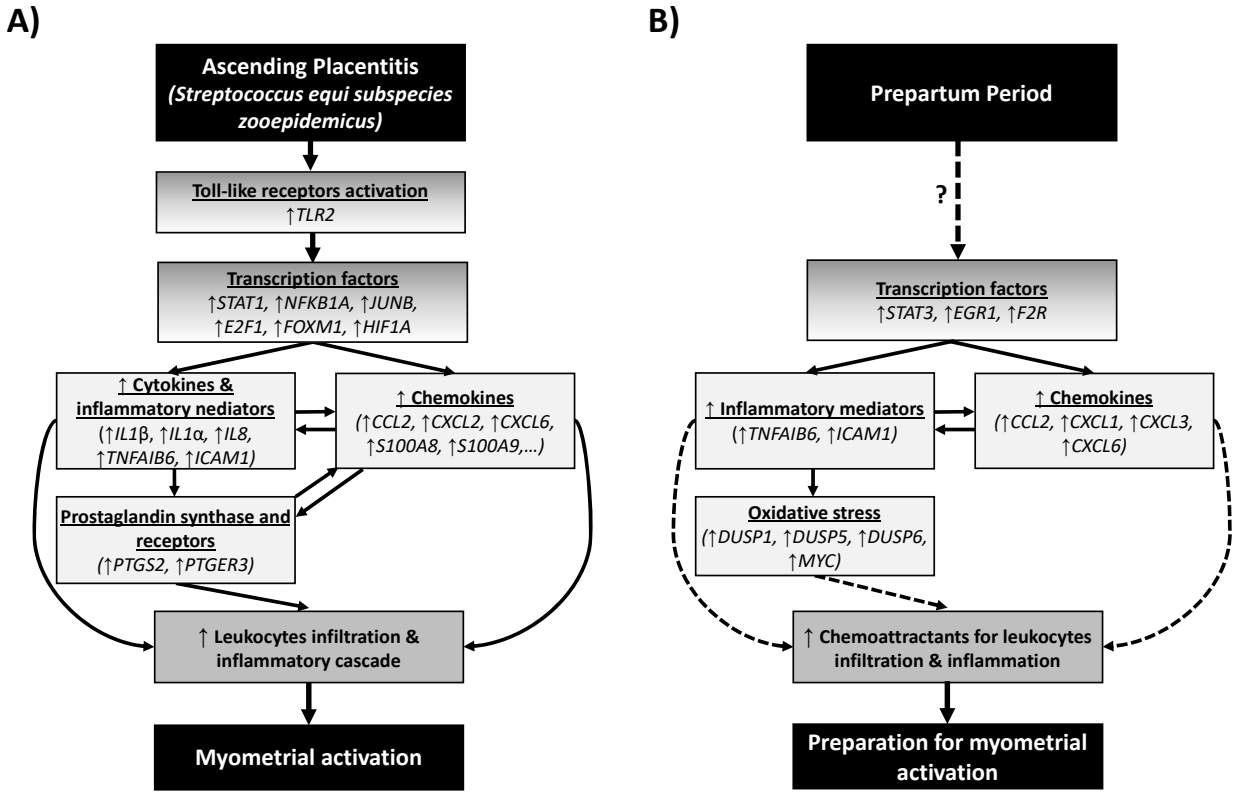

Figure 2. Inflammatory events and associated molecular mechanisms in equine myometrium during placentitis (A) and prepatum period (B).

Cervical hydration

Along with ECM degradation, cervical hydration is another important component in cervical remodeling in which water content increases in cervical tissue, reducing collagen density.[29] Cervical hydration could be achieved through various mechanisms, e.g. upregulation of aquaporins (AQPs) water channels and hydrophilic proteoglycans, as well as increased vascular permeability.[25-27,29] For instance, cervical remodeling during placentitis is associated with upregulation of AQP9 (water transporter), aggrecan (ACAN; a hydrophilic proteoglycan), plus vascular permeability-related genes such as Rac family small GTPase (RAC) and nitric oxide synthase (eNOS, also known as NOS3).[29] Altogether, these findings highlight possible molecules implicated in cervical ECM degradation and cervical hydration during placentitis.

Conclusion

This review provides a brief overview of key regulators and molecular mechanisms underlying myometrial activation and cervical remodeling during placentitis and prepartum period. Strategies to block identified key regulators and associated pathways (e.g. using TLRAs) hold potential for therapies to forestall placentitis-induced preterm birth.

Acknowledgement

Supported by Albert G. Clay Endowment of University of Kentucky.

Conflict of interest

None to report.

Abbreviations

20αDHP; 20α-hydroxy-5α dihydroprogesterone
3αDHP; allopregnanolone
5α-DHP; 5α-dihydroprogesterone
AKR1C23; aldo-keto reductase family 1 member C23
ALDH1; aldehyde dehydrogenases
AP-1; Activator protein 1
AQPs; aquaporins
ATF3; activating transcription factor 3
BIRC3; baculoviral IAP repeat containing 3
CAPs; contractions-associated proteins
CASP; caspase
CCL; C-C Motif Chemokine Ligand
CXCL; C-X-C Motif Chemokine Ligand
CXCR4; C-X-C motif chemokine receptor 4
DUSP; Dual Specificity Phosphatase
ECM; Extracellular matrix
eNOS/NOS3; nitric oxide synthase
FAS; Fas Cell Surface Death Receptor
GJA4/CXN37; gap junction alpha 4/connexin-37
ICAM1; intercellular adhesion molecule 1
IL; Interleukin
MMP; matrix metallopeptidases
MYC; MYC Proto-Oncogene, BHLH Transcription Factor
NF-κB; Nuclear factor-κB
P4; progesterone
$PGF_{2\alpha}$; Prostaglandin $F_{2\alpha}$
PR/PGR; Progesterone receptor
PTGER3; Prostaglandin E Receptor 3
PTGS2/COX2; prostaglandin-endoperoxide synthase 2/Cyclooxygenase 2
RAC; Rac family small GTPase
RLN; relaxin
S100A; calgranulins
SRD5A1; 5α reductase type 1
TLR; Toll-like receptors
TLRA; TLR antagonists
TLRs; toll-like receptors
TNFAIP6; TNF alpha induced protein 6

References

1. El-Sheikh Ali H, Legacki EL, Loux SC, et al: Equine placentitis is associated with a downregulation in myometrial progestin signaling. Biol Reprod 2019;101:162-176.
2. El-Sheikh Ali H, Legacki EL, Loux SC, et al: Transcriptomic analysis reveals the key regulators and molecular mechanisms underlying myometrial activation during equine placentitis. Biol Reprod 2020; in press.
3. El-Sheikh Ali H, Legacki EL, Scoggin KE, et al: Steroid synthesis and metabolism in the equine placenta during placentitis. Reproduction 2020;159:289-302.
4. Fernandes CB, Ball BA, Loux SC, et al: Uterine cervix as a fundamental part of the pathogenesis of pregnancy loss associated with ascending placentitis in mares. Theriogenology 2019;45:167-175.
5. Lyle SK: Immunology of infective preterm delivery in the mare. Equine Vet J 2014;46:661-668.
6. Wu S-P, DeMayo FJ: Progesterone receptor signaling in uterine myometrial physiology and preterm birth. In: Forrest D, Tsai S: editors. Current topics in developmental biology. Cambridge; Academic Press, 2017: p. 171-190.
7. Merlino AA, Welsh TN, Tan H, et al: Nuclear progesterone receptors in the human pregnancy myometrium: Evidence that parturition involves functional progesterone withdrawal mediated by increased expression of progesterone receptor-a. J Clin Endocrinol Metab 2007;92:1927-1933.
8. Mesiano S, Wang Y, Norwitz ER: Progesterone receptors in the human pregnancy uterus: Do they hold the key to birth timing? Reprod Sci 2011;18:6-19.

9. Tan H, Yi L, Rote NS, et al: Progesterone receptor-a and-b have opposite effects on proinflammatory gene expression in human myometrial cells: Implications for progesterone actions in human pregnancy and parturition. J Clin Endocrinol Metab 2012;97:E719-E730.
10. Chwalisz K, Fahrenholz F, Hackenberg M, et al: The progesterone antagonist onapristone increases the effectiveness of oxytocin to produce delivery without changing the myometrial oxytocin receptor concentrations. Am J Obstet Gynecol.1991;165:1760-1770.
11. Medzhitov R: Toll-like receptors and innate immunity. Nat Rev Immunol 2001;1:135-145.
12. Akira S, Takeda K: Toll-like receptor signalling. Nat Rev Immunol 2004;4:499.
13. Adams Waldorf KM, Persing D, Novy MJ, et al: Pretreatment with toll-like receptor 4 antagonist inhibits lipopolysaccharide-induced preterm uterine contractility, cytokines, and prostaglandins in rhesus monkeys. Reprod Sci 2008;15:121-127.
14. Thomson AJ, Telfer JF, Young A, et al: Leukocytes infiltrate the myometrium during human parturition: Further evidence that labour is an inflammatory process. Hum Reprod 1999;14:229-236.
15. Shynlova O, Nedd-Roderique T, Li Y, et al: Myometrial immune cells contribute to term parturition, preterm labour and post-partum involution in mice. J Cell Mol Med 2013;17:90-102.
16. Mackler AM, Green LM, McMillan PJ, et al: Distribution and activation of uterine mononuclear phagocytes in peripartum endometrium and myometrium of the mouse. Biol Reprod 2000;62:1193-1200.
17. Lirussi F, Rakotoniaina Z, Madani S, et al: Adrb3 adrenergic receptor is a key regulator of human myometrial apoptosis and inflammation during chorioamnionitis. Biol Reprod 2008;78:497-505.
18. Leroy M-J, Dallot E, Czerkiewicz I, et al: Inflammation of choriodecidua induces tumor necrosis factor alpha-mediated apoptosis of human myometrial cells1. Biol Reprod 2007;76:769-776.
19. Muhle RA, Pavlidis P, Grundy WN, et al: A high-throughput study of gene expression in preterm labor with a subtractive microarray approach. Am J Obstet Gynecol 2001;185:716-724.
20. Shynlova O, Oldenhof A, Dorogin A, et al: Myometrial apoptosis: Activation of the caspase cascade in the pregnant rat myometrium at midgestation. Biol Reprod 2006;74:839-849.
21. Arthur P, Taggart MJ, Zielnik B, et al: Relationship between gene expression and function of uterotonic systems in the rat during gestation, uterine activation and both term and preterm labour. J Physiol 2008;586:6063-6076.
22. Sakai N, Tabb T, Garfield RE: Modulation of cell-to-cell coupling between myometrial cells of the human uterus during pregnancy. Am J Obstet Gynec 1992;167:472-480.
23. Balducci J, Risek B, Gilula NB, et al: Gap junction formation in human myometrium: A key to preterm labor? Am J Obstet Gynec 1993;168:1609-1615.
24. Cook JL, Zaragoza DB, Sung DH, et al: Expression of myometrial activation and stimulation genes in a mouse model of preterm labor: Myometrial activation, stimulation, and preterm labor. Endocrinology 2000;141:1718-1728.
25. Mahendroo M: Cervical remodeling in term and preterm birth: Insights from an animal model. Reproduction 2012;143:429-438.
26. Liggins G: The cervix in pregnancy and labor, clinical and biochemical investigation. Edinburgh. Cuurchill Livingrtone.1981:1-9.
27. Leppert PC: Anatomy and physiology of cervical ripening. Clin Obstet Gynecol 1995;38:267-279.
28. Read CP, Word RA, Ruscheinsky MA, et al: Cervical remodeling during pregnancy and parturition: Molecular characterization of the softening phase in mice. Reproduction 2007;134:327-340.
29. El-Sheikh Ali H, Legacki EL, Loux SC, et al: Transcriptome analysis reveals the key regulators and molecular mechanisms underlying equine cervix remodeling during placentitis and prepartum period. Proc Annu Conf Plant Anim Genome 2019; PO0311.