Human pregnancy is a delicate and complex process where multiorgan interactions between two independent systems, the mother, and her fetus, maintain pregnancy. Intercellular interactions that can define homeostasis at the various cellular level between the two systems allow uninterrupted fetal growth and development until delivery. Interactions are needed for tissue remodeling during pregnancy at both fetal and maternal tissue layers. One of the mechanisms that help tissue remodeling is via cellular transitions where epithelial cells undergo a cyclic transition from epithelial to mesenchymal (EMT) and back from mesenchymal to epithelial (MET). Two major pregnancy-associated tissue systems that use EMT, and MET are the fetal membranes (amniochorion) amnion epithelial layer and cervical epithelial cells and will be reviewed here. EMT is often associated with localized inflammation, and it is a well-balanced process to facilitate tissue remodeling. Cyclic transition processes are important because a terminal state or the static state of EMT can cause accumulation of proinflammatory mesenchymal cells in the matrix regions of these tissues and increase localized inflammation that can cause tissue damage. Interactions that determine homeostasis are often controlled by both endocrine and paracrine mediators. Pregnancy maintenance hormone progesterone and its receptors are critical for maintaining the balance between EMT and MET. Increased intrauterine oxidative stress at term can force a static (terminal) EMT and increase inflammation that are physiologic processes that destabilize homeostasis that maintain pregnancy to promote labor and delivery of the fetus. However, conditions that can produce an untimely increase in EMT and inflammation can be pathologic. These tissue damages are often associated with adverse pregnancy complications such as preterm prelabor rupture of the membranes (pPROM) and spontaneous preterm birth (PTB). Therefore, an understanding of the biomolecular processes that maintain cyclic EMT-MET is critical to reducing the risk of pPROM and PTB. Extracellular vesicles (exosomes of 40-160 nm) that can carry various cargo are involved in cellular transitions as paracrine mediators. Exosomes can carry a variety of biomolecules as cargo. Studies specifically using exosomes from cells undergone EMT can carry a pro-inflammatory cargo and in a paracrine fashion can modify the neighboring tissue environment to cause enhancement of uterine inflammation. [BMB Reports 2022; 55(8): 370-379]

INTRODUCTION

Human pregnancy and parturition are complex systems to understand. During pregnancy, two independent biological and physiological systems co-exist, namely the mother and the fetus, to maintain pregnancy that will aid fetal growth and development (1). Parturition is a unique process that reverses all balanced states of pregnant uterine tissues in a synchronized way to ensure normal delivery at term (2-6). Preterm birth (PTB), <37 weeks) contributing to 1 million neonatal deaths around the world/year is a major complication impacting ≈12% of all pregnancies (7-10). Survivors of PTB face lifelong challenges, and mothers who deliver preterm have many later medical complications (11-13). Therefore, reducing PTB risk is a global healthcare priority (9, 14). PTB is not just an early initiation of labor resulting in delivery, but a syndrome initiated by failures in any of the feto-maternal uterine systems that maintain pregnancy (1, 15). Currently, it is thought that the mechanisms of delivery in normal parturition and PTB are different, but the true answer remains unclear. Reducing PTB risk remains a challenge as this condition may arise with a feto-maternal medical indication for early delivery or could be spontaneous with no known etiology (1, 6). Similarly, preterm prelabor rupture of the membranes (pPROM) leading to PTB is another complication of pregnancy and it accounts for ~40% of all PTB. Even with improvements in prenatal care over the past three decades, rates of pPROM and subsequent PTB have worsened.

Keywords: Cervix, Fetal membranes, pPROM, Pregnancy, Progesterone

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While several tests, such as pooling, fern tests, nitrazine, and Amnisure, are available to confirm that pPROM has occurred after the fact, there are no reliable methods to predict pPROM before it occurs. This is primarily since the underlying causes of pPROM are unknown, and attempts to develop screening or interventions without this information have been largely unsuccessful. Risk factors of pPROM are including reproductive tract infections, behavioral (e.g. cigarette smoking), and obstetrical complications (e.g. polyhydramnios). Other potential contributors to pPROM include toxic environmental exposures, genetic predisposition, and biochemical signals from the fetus that promote fetal membrane apoptosis. Tremendous advances in medical research have improved our knowledge of the feto-maternal uterine organ system and its contribution to pregnancy and parturition at term and preterm. Pregnancy and fetal development are dynamic processes characterized by temporal and cell-specific changes in feto-maternal uterine tissues. As mentioned above, a better understanding of the cellular and molecular biological changes within the feto-maternal uterine system and how they maintain homeostasis while promoting normal pregnancy growth and development may provide insights into how disruption of these processes can cause adverse pregnancy events. This review will focus on the structural changes associated with one fetal and one maternal tissue that are essential for pregnancy maintenance and promoting parturition. The organs that will be described here are fetal membranes (amniochorion) and maternal cervix whose cellular and extracellular matrix remodeling and functional integrity are critical for pregnancy maintenance. Pathologies associated with these tissues are associated with pPROM and spontaneous PTB. Current interventions strategies are not designed to treat disease states specifically associated with these tissues and therefore difficult to diagnose and or prevent these organ-specific disorders. After discussing the structure and function of these tissues, this review will focus on the remodeling mechanisms of these tissues and how pregnancy maintenance hormone regulates these processes. Additionally, the role of extracellular vesicles as communication channels between the feto-maternal system will also be discussed.

**HUMAN FETAL MEMBRANES**

Fetal membranes are distinct from the placenta and serve as a barrier between the feto-placental and the maternal compartments. Fetal membranes consist of the amnion layer and the chorion layer and are connected by collagen-rich extracellular matrix (ECM). The amnion layer is composed of a single layer of amnion epithelial layer connected to the amnion extracellular matrix by a type IV collagen-rich basement membrane. These layers are attached to the maternal decidua. Amnion epithelial cells are constantly bathed in amniotic fluid, signifying its importance as a primary responder to changes in the intrauterine (amniotic) cavity. The chorion trophoblast layer, which is distinctly different from placental cytotrophoblast cells is attached to the maternal decidua and maintains the immune tolerance at the maternal-fetal interface. The amnion epithelial layer, chorion trophoblast cells are connected to the chorionic extracellular matrix by another layer of type IV collagen-rich basement membranes. These ECM spaces also contain amniotic and chorionic mesenchymal cells. Mesenchymal cells are ~10% of the total amnion and chorion layer.

The fetal membranes are fetal tissues in origin. They play major roles in maintaining pregnancy by providing multi-level protection to the growing fetus. Fetal membranes accommodate constant challenges. Stretches experienced due to fetal growth or increasing amniotic fluid volume do not impact the structural or functional properties of the membranes. Fetal membranes have very few immune cells, partly attributable to the avascular nature of this tissue. Fetal membranes remodel throughout pregnancy. One of the well-reported remodeling processes is a balanced collagenolytic process where ECM collagen is broken down, but it is replaced with nascent collagen produced by stromal mesenchymal cells and amnion and chorion cells.

**HUMAN CERVIX**

The cervix, the maternal reproductive tissue that will be discussed here, plays an important role in protecting the developing fetus and helps to maintain the pregnancy until term delivery. It is composed of two cellular compartments: the epithelial and the stromal layer. The epithelial layer lining the cervical canal is divided into three distinct regions: the ectocervix, the transformation zone, and the endocervix. While the stromal layer is composed mainly of the extracellular matrix (ECM) incorporating fibroblasts, immune, and smooth muscle cells. The cervix remains firm and closed throughout pregnancy and undergoes cervical ripening and dilation during labor and delivery. The cervix functions as a barrier to prevent ascending microorganisms from the vaginal canal from reaching the intrauterine cavity. The mucus produced by cervical epithelium serves as a barrier to prevent infection as well as protect from mechanical and other exogenous insults. Cervical cells are a rich source of antimicrobial peptides that can reduce the invasion of microbes as well as produce chemokine and other inflammatory mediators to reduce the spread of infection, aid immune protection, and assist in the rebuilding of damaged tissue. Compared to fetal membrane remodeling during pregnancy, cervical remodeling is better studied. Cervical remodeling occurs throughout pregnancy and is divided into four distinct but overlapping phases as detailed in this reference. The cervix undergoes remodeling to maintain its intactness and to remain closed throughout pregnancy. This process also helps to maintain pregnancy by keeping the fetus within the uterus and (3). Remodeling is characterized by changes in the epithelial, stromal, immune, and endothelial cell function in the cervix as...
well as changes in the composition and structure of the ECM (4). Cervical ripening at term is associated with inflammatory changes that include vascular permeability, cytokine increase and MMP activities (51-53) causing weakening of the cervix resulting in labor and childbirth.

**CELLULAR REMODELING OF FETAL MEMBRANE AND CERVIX**

The collagen-rich matrix remodeling is enabled by a balanced activity between matrix-degrading enzymes and their specific inhibitors (54, 55). This balanced activity remodels the collagen matrix to strengthen both fetal membranes and the cervix (36, 56-61). Multiple reports have shown that collagen degradation by matrix metalloproteinases (MMPs) either at the membrane level or cervix or both is a predisposing factor for preterm birth (56, 58, 62-71). However, understudied are cellular remodeling that is an essential process required for both membrane and cervical remodeling. Both tissues are multicell layered and cells undergo constant remodeling. The process of cellular remodeling is elucidated recently in our lab as well as by others.

**FETAL MEMBRANE CELLULAR REMODELING**

Discovery of biological microfractures in fetal membranes allowed us to explore the process of its formation, significance, and association with normal and adverse pregnancy events. Microfractures are areas of biological and structural changes (32, 72, 73). Microfractures are characterized by 1) altered amnion epithelial layer where cells are vacated, 2) deterioration of basement membrane layer, 3) presence of migrating cells in the ECM, and 4) migrating cells develop tunnels in the ECM that extend from the basement membrane through the spongy layer (32). Microfractures are likely developed due to amnion epithelial cell shedding or topographically altered due to senescence, apoptosis, or necrosis. Microfractures can develop from both amnion and chorion trophoblast layers and invade through the ECM. They can connect between the two layers and can be considered as channels of crosstalk between amnion and chorion layers throughout gestation and likely get resealed to facilitate tissue remodeling. The number of microfractures and their dimensions is significantly higher in fetal membranes from preterm birth than gestational age matched PTB with no rupture of the membranes. These microfractures are areas where tissue remodeling was insufficient or ineffective due to underlying pathological reasons or premature senescence. These regions are also associated with the large amounts of collagen degradation suggesting that localized MMP activity or inflammation is associated with microfracture formation or their repair process. Persistent microfractures can act as channels for amniotic fluid leak and inflammatory cell infiltration. Microfractures are higher and their morphometry is distinct in pROM compared to gestational age matched PTB with no rupture suggesting that microfractures play a role in pROM pathology (32).

**CELLULAR TRANSITION AS A MECHANISM OF REMODELING**

Examination of cells in microfractures revealed predominantly a mesenchymal morphology and staining of membrane cells from normal term labored/delivered and pROM also showed dual cell type specific markers. Cytokeratin -18, a classic epithelial cell marker, and its co-localization with vimentin (mesenchymal marker) in cells of the amnion epithelial cell layer suggested that cells are in an in between state of transition termed ‘mesostate’. This dual staining was also observed when amnion epithelial cells were in a 2D culture system where CK-18+ cells partially transitioned to dual staining (CK-18+/Vimentin+) cells. Towards the end of a five-day culture period, cells were predominantly vimentin+ and exhibited mesenchymal morphological changes that enable it to assume a mesenchymal cell phenotype, which includes enhanced migratory capacity, invasiveness, elevated resistance to apoptosis, and greatly increased production of ECM components (74, 75). EMT often results in the degradation of the basement membrane and the formation of a mesenchymal cell that can migrate away from the epithelial layer in which it originated. These observations were made in cancer cells; however, a similar mechanism is observed in human amniocchorion. In our *in vitro* models, microfracture studies, and *in vivo* animal models, classic signs of EMT were observed in fetal membranes (33). This is more pronounced in term and pROM membranes (33). Similar findings were also reported by others in human fetal membranes at term or membranes undergoing artificial rupture and remodeling. This suggests that multiple pathophysiological changes in fetal membranes can lead to EMT (76, 77). Investigation of mechanisms behind EMT lead us to identify the following changes in human fetal membrane tissues and helped us to define the cellular mechanisms of remodeling: [1] amnion epithelial cells of the membranes are constantly shed, and microfractures are created. The microfractures are channels of migration for shed cells [2] migration is aided by the cellular transition to more migratory mesenchymal cells [3] transitioned cells change their morphology from classic epithelial to mesenchymal shape and express vimentin, N-cadherin, pro-EMT transcription factors (e.g., SNAIL, SLUG, ZEB 1), [4] migration is facilitated by basement membrane degradation with the increased production of active MMP9, and [5] epithelial cells (CK-18+/E-cadherin+) predominantly are often in metastate in amnion membrane expressing both epithelial/mesenchymal markers and they are transitioning to remodel. This raises the question of factors controlling transitions. Based on *in vitro* models and *in vivo* animal studies, we have determined that transition is not a one-way process but recycling of mesenchymal cells in the ECM is an essential process to limit
their numbers to ~10% of the epithelial cells (72). Mesenchymal cells do not accumulate as they transition back to epithelial cells (mesenchymal-epithelial cell transition [MET]) and help to fill the gaps of shed cells and rebuild the tissues. This cyclic process of transition minimizes the accumulation of mesenchymal cells in the ECM that are prone to pro-inflammation. Transforming growth factor (TGF)-β mediated signaling is one of the key mechanisms of EMT in human amnion epithelial cells (33, 75). TGF-β is a constituent of amniotic fluid during pregnancy and it can function through TGF-β receptors expressed on amnion cell surfaces (78). Silencing of TGF-β receptor can ameliorate TGF-β mediated EMT in amnion epithelial cells. TGF-β is under the influence of changes in redox radicals in the cells of the intraamniotic cavity and in the amniotic fluid. Hyperoxic environment increases TGF-β concentration in the cells as well as in the amniotic fluid, and its levels are at the highest at term before delivery, and TGF-β’s impact on amnion cell EMT is dominant at term compared to any other stages of gestation. EMT is associated with localized inflammation as it increases proinflammatory cytokines and matrix degrading enzymes.

An additional factor that balances this cyclic transition process is the function of progesterone (P4). Progesterone binding through progesterone receptor membrane components (PGRMCs), specifically PGRMC2, activate protooncogene c-MYC in the mesenchymal cells and promotes their transition back to amnion epithelial cells via MET. Constitutive expression of PGRMC2 and constant supply of P4 during pregnancy recycles mesenchymal cells and prevents their accumulation in the ECM. Balanced and localized collagen degradation and nascent collagen production by cells are also seen during this process to rebuild tissue matrix or reseal microfractures.

THE TERMINAL STATE OF EMT AT TERM CAUSES ACCUMULATION OF MESENCHYMAral CELLS, INFLAMMATION, AND PROPELS MEMBRANE WEAKENING

At term, the recycling process is stalled due to increased oxidative stress experienced in the intraamniotic cavity. Oxidative stress is increased at term as fetal maturation is completed. Oxidative stress impacted by the increased presence of reactive oxygen species causes the following: [1] induces stress signaling p38 mitogen activated kinases (MAPK) in amnion epithelial cells (79, 80); [2] p38MAPK performs multiple functions in these cells; it causes senescence and increases TGF-β production using an alternate pathway (78); [3] increased EMT and accumulation of mesenchymal cells in the ECM; [4] oxidative stress also reduces PGRMC2 expression on mesenchymal cells forcing a functional progesterone withdrawal at term, prevents cellular recycling causing accumulation of mesenchymal cells; [5] mesenchymal cells are prone to an enhanced response to oxidative stress and other inflammatory stimuli; [6] increased localized inflammation during EMT is further enhanced by newly transitioned mesenchymal cells; and [7] cytokine production and MMP activities are increased; and [8] enhances membrane matrix degradation, weakening and dysfunction. Membrane dysfunction is associated with the generation of inflammation, which is characterized by the release of damage associated molecular pattern markers (DAMPs) including HMGB1, cell free fetal tissue DNA fragments, IL-33 among others. Gene-expression of inflammatory mediators by fetal membrane cells due to a terminal state of EMT along with its natural senescence is considered as one of the signaling mechanisms for initiation of the labor process. These data are supportive of cellular derailments besides matrix degradation, which can contribute to term labor.

PREMATURE ACTIVATION OF EMT

It is noted that EMT is not restricted to term laboring membranes. Membranes from pPROM and a subset of PTB with no ROM also showed signs of EMT (33, 81, 82). pPROM membranes had pronounced EMT, and it was like that seen in term labor membranes that rupture spontaneously. OS is one of the major risk pathophysiology associated with pPROM (73). Multitudes of risk factors (intrauterine and cervicovaginal infections, high BMI, nutritional and behavioral issues, genetic and environmental factors) can all contribute to OS increase and reactive oxygen species increase in the amniotic fluid as well as in the membranes (83, 84). This pathophysiologic state that occurs before term and prematurely in response to various pregnancy-associated risk factors can cause membrane destabilization and rupture (85). OS induced PGRMC2 downregulation prevents MET and causes to accumulation of proinflammatory mesenchymal cells in pPROM membrane ECM (82). Activation of p38 MAPK, senescence, and EMT in pPROM membranes due to OS inducing risk factors is like that seen at term (85, 86). Senescence activation and EMT are two distinct but overlapping mechanisms that can generate inflammation in the fetal membranes. Although both senescence and EMT are natural physiologic processes, they can be activated prematurely in cases of preterm, and both these processes develop independently. This can be risk factor specific response (87). Management of OS associated pPROM and PTB should include reduction of OS induced cellular damages, activation of a stress signaling pathway (e.g., p38MAPK), senescence, EMT and MET, and senescence associated inflammation are critical.

CERVICAL TISSUE AND OXIDATIVE STRESS

Cervix is composed of the epithelial and the stromal layer. The epithelial layer lining the cervical canal is divided into three distinct regions: the ectocervix, the transformation zone, and the endocervix, while the stromal layer is composed mainly of the extracellular matrix (ECM) incorporating fibroblasts, immune, and smooth muscle cells (44, 88-91). As mentioned above, cervical remodeling is a required process to maintain pregnancy.
and like fetal membranes, multitudes of cellular matrix remodeling are needed to maintain cervical integrity, maintenance of intact tissue, and natural production of antimicrobial peptides to reduce the influx of vaginal microbiome and provide the barrier functions. It is clear from previous studies that OS has detrimental effects on placental, uterine, and fetal tissues, which can lead to preterm birth (47, 57, 92-97). The impact of OS on cervical function, specifically remodeling, during pregnancy is not well reported. Several studies have suggested that OS may be involved in cervical remodeling during pregnancy (98-100). Multiple reports have highlighted the importance of a balanced oxidative stress reaction in the cervix (similar to that reported for membranes) as a mechanism for remodeling and suppression of antioxidants is linked to the cervical ripening at term preceding parturition (100). To alleviate any ambiguity regarding the role of oxidative stress and cervical function, we have recently conducted several studies using isolated cervical epithelial and stromal cells. Data summary reported in reference # (90) is summarized again here: [1] OS increased ROS production and activated the p38MAPK pathway in all three cervical cells; [2] OS promoted cell cycle arrest in ectocervical epithelial cells; [3] OS induced necrosis in cervical cells; [4] high level of senescence and low level of autophagy were observed in cervical stromal cells under OS. Conversely, a low level of senescence and high level of autophagy was observed in endocervical epithelial cells; and [5] OS increased p38MAPK-mediated sterile inflammation in cervical cells. As we have reported (90), ectocervical and stromal cells are more resistant to OS with minimal pathologic changes, which is expected from a tissue undergoing remodeling throughout its existence to provide the foundation for transition zone and endocervical tissue. These cells can proliferate and cause localized inflammation for remodeling. Constant exposure of ectocervical epithelial cells to vaginal microbiota forces them to attain heightened endogenous immune tolerance to prevent damage from exogenous factors such as infection and OS (101), a process likely aided by the resident immune cells (49). On the other hand, the cervical stromal cells and endocervical epithelial cells are usually protected from the vaginal microbiota due to their location and their production of mucus which serve as physical barriers with antimicrobial peptides (102). However, they are prone to damage if exposed. Excessive damage of the cervical endocervical epithelial barrier by oxidative stress may impact remodeling of the cervical stroma. These damages can compromise the mechanical properties of the cervix.

**OXIDATIVE STRESS EFFECT DURING PREGNANCY OR ADVERSE PREGNANCY EVENTS**

Using ecto, endo and stromal cells derived from cervical tissues, we have recently reported the effect of oxidative stress on cervical tissue remodeling (90). As p38MAPK activation was one of the signaling mechanisms observed in our prior studies, the role of this signaler was further explored in cervical tissue as well (90). As reported already in our prior publication (90), the following were our reported observations when cervical cells were exposed to oxidative stress inducer: [1] increased ROS production and activated the p38MAPK pathway in all three cervical cells; [2] promoted cell cycle arrest in ectocervical epithelial cells; [3] induced necrosis in cervical cells; [4] high level of senescence and low level of autophagy were observed in cervical stromal cells under oxidative stress. Conversely, a low level of senescence and high level of autophagy was observed in endocervical epithelial cells; and [5] oxidative stress increased p38MAPK-mediated sterile inflammation in cervical cells (90). Cervical cells exhibit a cell type dependent response, and this provides distinct mechanisms to remodel the tissue during pregnancy as needed based on their architecture, cellularity, environment, and intercellular interactions. Fluctuations in the redox environment are expected during pregnancy and the adaptability of cells to these changes and remodeling potential is critical to maintaining tissue homeostasis.

**EMT OF CERVICAL CELLS AND ITS CONTROL BY PROGESTERONE**

As mentioned in the fetal membrane section above, progesterone plays a critical role in the cervical cellular transition. Vaginal progesterone is a well reported intervention approach to reduce the risk of preterm birth (103-106). The mechanism of progesterone action on cervical cells is well reported although the antiinflammatory properties of progesterone are well known. Progesterone is well known for the regulation of EMT and MET in cancer cells. Based on the data that progesterone may play a role in cellular transition that can generate localized inflammation in fetal membranes, we have tested the effect of progesterone on cervical cells (56, 88). In response to an infectious environment, the cervix is highly vulnerable to pathological changes and ascending infections by pathologic vaginal microbiome (107-110). Our recent reports using endocervical epithelial and stromal cells exposed to lipopolysaccharide (LPS) revealed the following and repeated here (56): [1] human endocervical epithelial cells maintain a meta state but predominantly maintained an epithelial morphology; [2] Cervical stromal cells expressed mesenchymal markers and fibroblastoid morphology; [3] Progesterone alone did not alter the cell shapes and expression of EMT markers either in endocervical or in stromal cells; [4] LPS induced EMT in endocervical cells, caused inflammation in both endocervical and stromal cells but P4 prevented this LPS-induced transition and inflammation. This suggests that infection can potentially cause the static state of EMT and inflammation to facilitate matrix degradation; [5] P4 did not promote MET in stromal cells; [6] LPS slowed down but P4 induced wound healing in both cell types (56). Extensive collagen and cellular turnover help to remodel the cervix during pregnancy (89). The role of resident cervical macrophages has been reviewed in detail during the remodeling process by Steve Yellon detailing of the role of
immune cells is not attempted here (49). Cervix, although structurally and functionally different from fetal membranes, also undergo a cyclic remodeling process to maintain tissue homeostasis during pregnancy. MET is not pronounced in the cervix and accumulation of mesenchymal cells is seen in the stromal region. The consequence of this accumulation, if any, is unclear.

EMT GENERATES LOCALIZED INFLAMMATION

In both fetal membranes and the cervix, localized inflammation promotes collagen degradation or cell migration. This is a balanced inflammation as the tissue environment exhibits normalcy after remodeling. However, the static state of EMT induced by infectious agents or other oxidative stress-inducing conditions can cause an overwhelming inflammatory response that can cause collagenolysis of both tissues, weakening them and imbalancing tissue homeostasis. pPROM and PTB are conditions where such imbalances are often observed.

EXTRACELLULAR VESICLES SPREAD INFLAMMATORY MEDIATORS FROM THE CERVIX AND FETAL MEMBRANES

Exosomes (30-160 nm natural cellular particles) generated from the fetal membrane and cervical cells can carry inflammatory mediators (111). These exosomes can be considered as paracrine signalers as they can be received by neighboring cells/tissues or distant tissues (112, 113). The fate of inflammatory cargo carrying exosomes derived from both fetal membrane and cervical cells has been examined (114, 115). Exosomes from fetal membrane cells after exposure to either infection or oxidative stress can reach maternal tissues where they can cause inflammatory changes associated with parturition (113, 116-119). These are some of the fetal signals of parturition (120). Similarly, cervical cell derived exosomes can go towards the fetal tissues and increase inflammation at the feto-maternal interface (decidua/fetal membranes) (115). Although not as pronounced as fetal inflammatory exosome response, maternal cell derived exosomes can also trigger inflammatory response (116).

CONCLUSIONS

All collagenolytic processes and matrix turnover mechanisms have been detailed in the literature for both fetal membranes and cervix. Cell-mediated events that can lead to collagenolysis have not been discussed in detail previously; however, cellular mechanisms involved in tissue remodeling during pregnancy is poorly understood. An attempt is made here to describe how cellular transitions are critical in maintaining homeostasis and how static state or terminal state of specific transition process can deter cellular remodeling and generate inflammation that can destabilize tissue integrity and compromise its functions. Both fetal and maternal tissues are prone to these issues to cause adverse pregnancy outcomes.

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CONFLICTS OF INTEREST

The authors have no conflicting interests.

REFERENCES

1. Romero R, Dey SK and Fisher SJ (2014) Preterm labor: one syndrome, many causes. Science 345, 760-765
2. Tal R and Taylor HS (2000) Endocrinology of Pregnancy; in Endotext, Feingold KR, Anawalt B, Boyce A et al. (eds.), South Dartmouth (MA)
3. Rokas A, Mesiano S, Tamam O, Labella A, Zhang G and Muglia L (2020) Developing a theoretical evolutionary framework to solve the mystery of parturition initiation. Elife 9, e58343
4. Smith R (1998) Alterations in the hypothalamic pituitary adrenal axis during pregnancy and the placental clock that determines the length of parturition. J Reprod Immunol 39, 215-220
5. Menon R, Bonney EA, Condon J, Mesiano S and Taylor RN (2016) Novel concepts on pregnancy clocks and alarms: redundancy and synergy in human parturition. Hum Reprod Update 22, 535-560
6. Mendelson CR, Montalbano AP and Gao L (2017) Fetal-to-maternal signaling in the timing of birth. J Steroid Biochem Mol Biol 170, 19-27
7. Beck S, Wojdyla D, Say L et al (2010) The worldwide incidence of preterm birth: a systematic review of maternal mortality and morbidity. Bull World Health Organ 88, 31-38
8. Shapiro-Mendoza CK and Lackritz EM (2012) Epidemiology of late and moderate preterm birth. Semin Fetal Neonatal Med 17, 120-125
9. Simmons LE, Rubens CE, Darmstadt GL and Gravett MG (2010) Preventing preterm birth and neonatal mortality: exploring the epidemiology, causes, and interventions. Semin Perinatal 34, 408-415
10. Blencowe H, Cousens S, Oestergaard MZ et al (2012) National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet 379, 2162-2172
11. Dammann O and Leviton A (1997) Maternal intrauterine infection, cytokines, and brain damage in the preterm newborn. Pediatr Res 42, 1-8
12. Jacobsson B (2004) Infectious and inflammatory mechanisms in preterm birth and cerebral palsy. Eur J Obstet Gynecol Reprod Biol 115, 159-160
13. Muglia LJ and Katz M (2010) The enigma of spontaneous preterm birth. N Engl J Med 362, 529-535
14. McCabe ER, Carrino GE, Russell RB and Howse JL (2014) Fighting for the next generation: US Prematurity in 2030. Pediatrics 134, 1193-1199

http://bmbreports.org
15. Villar J, Papageorghiou AT, Knight HE et al (2012) The preterm birth syndrome: a prototype phenotypic classification. Am J Obstet Gynecol 206, 119-123
16. Parry S and Strauss JF, 3rd (1998) Premature rupture of the fetal membranes. N Engl J Med 338, 663-670
17. Shubert PJ, Diss E and Iams JD (1992) Etiology of preterm premature rupture of membranes. Obstet Gynecol Clin North Am 19, 251-263
18. Naeye RL and Peters EC (1980) Causes and consequences of premature rupture of fetal membranes. Lancet 1, 192-194
19. French JL and McGregor JA (1996) The pathobiology of premature rupture of membranes. Semin Perinatol 20, 344-368
20. Menon R and Fortunato SJ (2007) Infection and the role of inflammation in preterm premature rupture of the membranes. Best Pract Res Clin Obstet Gynaecol 21, 467-478
21. Elovitz MA and Mrinkalni C (2004) Animal models of preterm birth. Trends Endocrinol Metab 15, 479-487
22. Chauhan SP and Ananth CV (2013) Periviable births: epidemiology and obstetrical antecedents. Semin Perinatol 37, 382-388
23. Lawn JE, Kinney MV, Belizan JM et al (2013) Born too soon: accelerating actions for prevention and care of 15 million newborns born too soon. Reprod Health 10 Suppl 1, S6
24. Pavlicev M and Norwitz ER (2018) Human parturition: nothing more than a delayed menstruation. Reprod Sci 25, 166-173
25. Hay ED (1981) Extracellular matrix. J Cell Biol 91, 205s-255s
26. Bourne GL and LACY D (1960) Ultra-structure of human amnion and its possible relation to the circulation of amniotic fluid. Nature 186, 952-954
27. Castillo-Castrejon M, Meraz-Cruz N, Gomez-Lopez N et al (2014) Choriocarcinoma cells from term human pregnancies show distinctive functional properties related to the induction of labor 2. Am J Reprod Immunol 71, 86-93
28. King AE, Kelly RW, Sallenave JM, Bocking AD and Challis JR (2007) Innate immune defences in the human uterus during pregnancy. Placenta 28, 1099-1106
29. Vora S, Abbas A, Kim CJ et al (2010) Nuclear factor-kappa B localization and function within intrauterine tissues from term and preterm labor and cultured fetal membranes. Reprod Biol Endocrinol 8, 8
30. Montenegro D, Romero R, Kim SS et al (2009) Expression patterns of microRNAs in the chorioamniotic membranes: a role for microRNAs in human pregnancy and parturition 3. J Pathol 217, 113-121
31. Menon R, Richardson LS and Lappas M (2019) Fetal membrane architecture, aging and inflammation in pregnancy and parturition. Placenta 79, 40-45
32. Richardson LS, Vargas G, Brown T et al (2017) Discovery and characterization of human amniochorionic membrane microfractures. Am J Pathol 187, 2821-2830
33. Richardson LS, Taylor RN and Menon R (2020) Reversible EMT and MET mediate amnion remodeling during pregnancy and labor. Sci Signal 13, 1486
34. Richardson LS, Radhna E, Urribarz-Garza R, Lavu N and Menon R (2020) Stretch, scratch, and stress: suppressors and supporters of senescence in human fetal membranes. Placenta 99, 27-34
35. Jacobs SO, Sheller-Miller S, Richardson LS, Urrabaz-Garza R, Radhna E and Menon R (2020) Characterizing the immune cell population in the human fetal membrane. Am J Reprod Immunol 85, e13368
36. Vadillo-Ortega F, Gonzalez-Avila G, Furth EE et al (1995) 92-kd type IV collagenase (matrix metalloproteinase-9) activity in human amniochorion increases with labor. Am J Pathol 146, 148-156
37. Vadillo-Ortega F, Hernandez A, Gonzalez-Avila G, Bermejo L, Iwata K and Strauss JF, III (1996) Increased matrix metalloproteinase activity and reduced tissue inhibitor of metalloproteinases-1 levels in amniotic fluids from pregnancies complicated by premature rupture of membranes. Am J Obstet Gynecol 174, 1371-1376
38. Vadillo-Ortega F, Sadowsky DW, Haluska GJ et al (2002) Identification of matrix metalloproteinase-9 in amniotic fluid and amniochorion in spontaneous labor and after experimental intrauterine infection or interleukin-1 beta infusion in pregnant rhesus monkeys. Am J Obstet Gynecol 186, 128-138
39. Bryant-Greenwood GD (1998) The extracellular matrix of the human fetal membranes: structure and function. Placenta 19, 1-11
40. Strauss JF 3rd (2013) Extracellular matrix dynamics and fetal membrane rupture. Reprod Sci 20, 140-153
41. Mahendroo MS (2012) Cervical remodeling in term and preterm birth: insights from an animal model. Reproduction 143, 429-438
42. Yellon SM (2017) Contributions to the dynamics of cervix remodeling prior to term and preterm birth. Biol Reprod 96, 13-23
43. Read CP, Word RA, Ruscheinsky MA, Timmons BC and Mahendroo MS (2007) Cervical remodeling during pregnancy and parturition: molecular characterization of the softening phase in mice. Reproduction 134, 327-340
44. Word RA, Li XH, Hnat M and Carrick K (2007) Dynamics of cervical remodeling during pregnancy and parturition: mechanisms and current concepts. Semin Reprod Med 25, 69-79
45. Becher N, Adams Waldorf K, Hein M and Uldbjerg N (2009) The cervical mucus plug: structured review of the literature. Acta Obstet Gynecol Scand 88, 502-513
46. Critchfield AS, Yao G, Jaishankar A et al (2013) Cervical mucus properties stratify risk for preterm birth. PLoS One 8, e69528
47. Hansen LK, Becher N, Bastholm S et al (2014) The cervical mucus plug inhibits, but does not block, the passage of ascending bacteria from the vagina during pregnancy. Acta Obstet Gynecol Scand 93, 102-108
48. Frew L, Makieva S, McKinlay AT et al (2014) Human cathelicidin production by the cervix. PLoS One 9, e103434
49. Yellon SM (2019) Immunobiology of cervix ripening. Front Immunol 10, 3156
50. Styras D, Wang H, Vladic YS, Ekman G, Eriksson H and
Sahlin L (2002) Increased level of matrix metalloproteinases 2 and 9 in the ripening process of the human cervix. Biol Reprod 67, 899-894

52. Iwahashi M, Muragaki Y, Oshimia A and Umesaki N (2003) Decreased type I collagen expression in human uterine cervix during pregnancy. J Clin Endocrinol Metab 88, 2231-2235

53. Myers DA (2012) The recruitment and activation of leukocytes into the immune cervix: further support that cervical remodeling involves an immune and inflammatory mechanism. Biol Reprod 87, 107

54. Zucker S, Hyamotoz M, Conner C et al (1999) Measurement of matrix metalloproteinases and tissue inhibitors of metalloproteinases in blood and tissues. Clinical and experimental applications. Ann N Y Acad Sci 878, 212-227

55. Matrisian LM, Wright J, Newell K and Witty JP (1994) Matrix-degrading metalloproteinases in tumor progression. Princess Takamatsu Symp 24, 152-161

56. Tantengco OAG, Richardson LS, Vink J et al (2021) Progesterone alters human cervical epithelial and stromal cell transition and migration: implications in cervical remodeling during pregnancy and parturition. Mol Cell Endocrinol 529, 111276

57. Chai M, Barker G, Menon R and Lappas M (2012) Increased oxidative stress in human fetal membranes overlying the cervix from term non-labouring and post labour deliveries. Placenta 33, 604-610

58. Gonzalez JM, Franzke CW, Yang F, Romero R and Girardi G (2011) Complement activation triggers metalloproteinases release inducing cervical remodeling and preterm birth in mice. Am J Pathol 179, 838-849

59. Gonzalez JM, Dong Z, Romero R and Girardi G (2011) Cervical remodeling/ripening at term and preterm delivery: the same mechanism initiated by different media tors and different effector cells. PLoS One 6, e26877

60. Moore RM, Mansour JM, Redline RW, Mercers BM and Moore JJ (2006) The physiology of fetal membrane rupture: insight gained from the determination of physical properties. Placenta 27, 1037-1051

61. Becher N, Hein M, Danielesen CC and Uldbjerg N (2004) Matrix metalloproteinases and their inhibitors in the cervical mucus plug at term of pregnancy. Am J Obstet Gynecol 191, 1232-1239

62. Vadillo-Ortega F and Estrada-Gutierrez G (2005) Role of matrix metalloproteinases in preterm labour. BJOG 112 Suppl 1, 19-22

63. Wang H, Parry S, Macones G et al (2004) Functionally significant SNP MMP8 promoter haplotypes and preterm premature rupture of membranes (PPROM). Hum Mol Genet 13, 2659-2669

64. Jeong HC, Kim HY, Kim HY et al (2021) Changes in gene expression of cervical collagens, metalloproteinases, and tissue inhibitors of metalloproteinases after partial cervical excision-induced preterm labor in mice. PLoS One 16, e0250108

65. Watari M, Watari H, DiSanto ME, Chacko S, Shi GP and Strauss JF 3rd (1999) Pro-inflammatory cytokines induce expression of matrix-metabolizing enzymes in human cervical smooth muscle cells. Am J Pathol 154, 1735-1762

66. Rath W, Winkler M and Kemp B (1998) The importance of extracellular matrix in the induction of preterm delivery. J Perinat Med 26, 437-441

67. Akins ML, Lubly-Phelps K, Bank RA and Mahendroo M (2011) Cervical softening during pregnancy: regulated changes in collagen cross-linking and composition of matricellular proteins in the mouse. Biol Reprod 84, 1053-1062

68. Menon R and Fortunato SJ (2004) The role of matrix-degrading enzymes and apoptosis in rupture of membranes. J Soc Gynecol Invest 11, 427-437

69. Fortunato SJ and Menon R (2002) Screening of novel matrix metalloproteinases (MMPs) in human fetal membranes. J Assist Reprod Genet 19, 483-486

70. Maymon E, Romero R, Pacora P et al (2001) A role for the 72 kDa gelatinase (MMP-2) and its inhibitor (TIMP-2) in human parturition, premature rupture of membranes and intraamniotic infection. J Perinat Med 29, 308-316

71. Fortunato SJ, Menon R and Lombardi SJ (1999) MMP/TIMP imbalance in amniotic fluid during PROM: an indirect support for endogenous pathway to membrane rupture. J Perinat Med 27, 362-368

72. Richardson L and Menon R (2018) Proliferative, migratory, and transition properties reveal metastate of human amnion cells. Am J Pathol 188, 2004-2015

73. Menon R and Richardson LS (2017) Preterm prelabor rupture of the membranes: a disease of the fetal membranes. Semin Perinatol 41, 409-419

74. Kalluri R and Weinberg RA (2009) The basics of epithelial-mesenchymal transition. J Clin Invest 119, 1420-1428

75. Kalluri R (2009) EMT: when epithelial cells decide to become mesenchymal-like cells. J Clin Invest 119, 1417-1419

76. Janzen C, Sen S, Lei MY, Gagliardi de Assumpcao M, Chullis J and Chaudhuri G (2017) The role of epithelial to mesenchymal transition in human amniotic membrane rupture. J Clin Endocrinol Metab 102, 1261-1269

77. Mogami H, Hari Kishore A, Akgul Y and Word RA (2017) Healing of preterm ruptured fetal membranes. Sci Rep 7, 13139

78. Richardson L, Dixon CL, Aguilera-Aguirre L and Menon R (2018) Oxidative stress-induced TGF-beta/TAB1-mediated p38MAPK activation in human amnion epithelial cells. Biol Reprod 99, 1100-1112

79. Menon R, Boldogh I, Urrabaz-Garza R et al (2013) Senescence of primary amniotic cells via oxidative DNA damage. PLoS One 8, e33416

80. Lavu N, Richardson L, Radnaa E et al (2019) Oxidative stress-induced downregulation of glycogen synthase kinase 3 beta in fetal membranes promotes cellular senescence-dagger. Biol Reprod 101, 1018-1030

81. Omere C, Richardson L, Saade GR, Bonney EA, Kechchian T and Menon R (2020) Interleukin (IL)-6: a friend or foe of pregnancy and parturition? evidence from functional studies in fetal membrane cells. Front Physiol 11, 891

82. Lozovyy V, Richardson L, Saade G and Menon R (2021) Progesterone receptor membrane components: key regulators of fetal membrane integrity. Biol Reprod 104, 445-
EMT during pregnancy
Ramkumar Menon

Menon R and Moore JJ (2020) Fetal membranes, not a mere appendage of the placenta, but a critical part of the fetal-maternal interface controlling parturition. Obstet Gynecol Clin North Am 47, 147-162

Murtha AP and Menon R (2015) Regulation of fetal membrane inflammation: a critical step in reducing adverse pregnancy outcome. Am J Obstet Gynecol 213, 447-448

Dutta EH, Behnha F, Boldogh I et al (2016) Oxidative stress damage-associated molecular signaling pathways differentiate spontaneous preterm birth and preterm premature rupture of the membranes. Mol Hum Reprod 22, 143-157

Poletti J, Dutta EH, Behnha F, Saade GR, Torloni MR and Menon R (2015) Aging of intrauterine tissues in spontaneous preterm birth and preterm premature rupture of the membranes: a systematic review of the literature. Placenta 36, 969-973

Menon R, Behnha F, Poletti J and Richardson LS (2020) Novel pathways of inflammation in human fetal membranes associated with preterm birth and preterm prelabor rupture of the membranes. Semin Immunopathol 42, 431-450

Tantengco OAG, Richardson LS and Menon R (2021) Effects of a gestational level of estradiol on cellular transition, migration, and inflammation in cervical epithelial and stromal cells. Am J Reprod Immunol 85, e13370

Tantengco OAG and Menon R (2020) Contractile function of the cervix plays a role in normal and pathological pregnancy and parturition. Med Hypotheses 145, 110336

Tantengco OAG, Vink J, Medina PMB and Menon R (2021) Oxidative stress promotes cellular damages in the cervix: implications for normal and pathologic cervical function in human pregnancy dagger. Biol Reprod 105, 204-216

Mackler AM, Iezza G, Akin MR, McMillan P and Yellon SM (1999) Macrophage trafficking in the uterus and cervix precedes parturition in the mouse. Biol Reprod 61, 879-883

Poletti J, Dutta EH, Behnha F, Saade GR, Torloni MR and Menon R (2015) Aging of intrauterine tissues in spontaneous preterm birth and preterm premature rupture of the membranes: a systematic review of the literature. Placenta 36, 969-973

Martin LF, Moco NP, de Lima MD et al (2017) Histo logic chorioamnionitis does not modulate the oxidative stress and antioxidant status in pregnancies complicated by spontaneous preterm delivery. BMC Pregnancy Childbirth 17, 376

Burton GJ and Jauhiaux E (2004) Placental oxidative stress: from miscarriage to preeclampsia. J Soc Gynecol Invest 11, 342-352

Myatt L (2010) Review: reactive oxygen and nitrogen species and functional adaptation of the placenta. Placenta 31 Suppl, S66-S69

Woods JR Jr (2001) Reactive oxygen species and preterm premature rupture of membranes-a review. Placenta 22 Suppl A, S38-S44

Denny PA (2010) Oxidative stress in development: nature or nurture? Free Radic Biol Med 49, 1147-1151

Sahlin L, Wang H, Stjernholm Y et al (2000) The expression of glutaredoxin is increased in the human cervix in term pregnancy and immediately post-partum, particularly after prostaglandin-induced delivery. Mol Hum Reprod 6, 1147-1153

Ryu HK, Moon JH, Heo HJ, Kim JW and Kim YH (2017) Maternal C-reactive protein and oxidative stress markers as predictors of delivery latency in patients experiencing preterm premature rupture of membranes. Int J Gynaecol Obstet 136, 145-150

Heng YJ, Di Quinzio MK, Permezel M, Rice GE and Georgiou HM (2010) Temporal expression of antioxidants in human cervicovaginal fluid associated with spontaneous labor. Antioxid Redox Signal 13, 951-957

Barrios De Tomasi J, Opata MM and Mowa CN (2019) Immunity in the cervix: interphase between immune and cervical epithelial cells. J Immunol Res 2019, 7693183

Yarbrough VL, Winkle S and Herbst-Kralovetz MM (2015) Antimicrobial peptides in the female reproductive tract: a critical component of the mucosal immune barrier with physiological and clinical implications. Hum Reprod Update 21, 353-377

da Fonseca EB, Damiao R and Nicholsonikes K (2009) Prevention of preterm birth based on short cervix: progesterone. Semin Perinatol 33, 334-337

Romero R, Nicolades K, Conde-Agudele A et al (2012) Vaginal progestosterone in women with an asymptomatic sonographic short cervix in the midtrimester decreases preterm delivery and neonatal morbidity: a systematic review and metaanalysis of individual patient data. Am J Obstet Gynecol 206, 124-119

Romero R, Yeo L, Chaemsaiithong P, A (2002) Cervical uteraplasma urealyticum colonization: predictors of delivery latency in patients experiencing preterm delivery. Ultrasound Obstet Gynecol 28, 768-774

Romero R, Jacobson B, Haagberg H and Wenerholm UB (2006) Cervical length in women in preterm labor with intact membranes: relationship to intra-amniotic inflammation/microbial invasion, cervical inflammation and preterm delivery. Ultrasound Obstet Gynecol 28, 768-774

Romero R, Mattsby-Baltzer I, Wenerholm UB, Hagberg H and Jacobsson B (2005) Interleukin-6 and interleukin-8 in cervical fluid in a population of Swedish women in preterm labor: relationship to microbial invasion of the amniotic fluid, intra-amniotic inflammation, and preterm delivery. Acta Obstet Gynecol Scand 84, 551-557

Naltone R, Jalaia J, Laurikainen E, Karlkainen U and Alanten A (2002) Cervical ureaplasma urealyticum colonization: comparison of PCR and culture for its detection and association with preterm birth. Scand J Infect Dis 34, 35-40

Menon R and Shahin H (2020) Extracellular vesicles in spontaneous preterm birth. Am J Reprod Immunol 85, e13353
112. Shepherd MC, Radnaa E, Tantengco OA et al (2021) Extracellular vesicles from maternal uterine cells exposed to risk factors cause fetal inflammatory response. Cell Commun Signal 19, 100

113. Hadley EE, Sheller-Miller S, Saade G et al (2018) Amnion epithelial cell derived exosomes induce inflammatory changes in uterine cells. Am J Obstet Gynecol 219, 478

114. Monsivais LA, Sheller Miller S, Russell W et al (2020) Fetal membrane extracellular vesicle profiling reveals distinct pathways induced by infection and inflammation in vitro. Am J Reprod Immunol 84, e13282

115. Tantengco OAG, Radnaa E, Shahin H, Kechichian T and Menon R (2021) Cross talk: trafficking and functional impact of maternal exosomes at the Feto-maternal Interface under normal and pathologic states. Biol Reprod 105, 1562-1576

116. Shahin HI, Radnaa E, Tantengco OAG et al (2021) Microvesicles and exosomes released by amnion epithelial cells under oxidative stress cause inflammatory changes in uterine cells. Biol Reprod 105, 464-480

117. Sheller-Miller S, Choi K, Choi C and Menon R (2019) Cyclic-recombinase-reporter mouse model to determine exosome communication and function during pregnancy. Am J Obstet Gynecol 221, 502 e501-502 e512

118. Sheller-Miller S, Lei J, Saade G, Salomon C, Burd I and Menon R (2016) Feto-maternal trafficking of exosomes in murine pregnancy models. Front Pharmacol 7, 432

119. Sheller-Miller S, Trivedi J, Yellon SM and Menon R (2019) Exosomes cause preterm birth in mice: evidence for paracrine signaling in pregnancy. Sci Rep 9, 608

120. Menon R, Mesiano S and Taylor RN (2017) Programmed fetal membrane senescence and exosome-mediated signaling: a mechanism associated with timing of human parturition. Front Endocrinol (Lausanne) 8, 196