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Epithelial to Mesenchymal Transition (EMT) of Feto-Maternal Reproductive Tissues Generates Inflammation: A Factor Aiding Term and Preterm Births

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ABSTRACT

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 define homeostasis at the various cellular levels between the two systems allow uninterrupted fetal growth and development until delivery. Further, interactions are needed for tissue remodeling during pregnancy on both the fetal and maternal tissue layers. One of the mechanisms that help tissue remodeling is cellular transition where epithelial cells undergo a cyclic epithelial to mesenchymal transition (EMT) and back to mesenchymal to epithelial transition (MET). Herein, we reviewed two major pregnancy-associated tissue systems that use EMT and MET, the fetal membrane (amniochorion) amnion epithelial layer and cervical epithelial cells. EMT is often associated with localized inflammation, and it is a well-balanced process that facilitates 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, which increases localized inflammation that can cause tissue damage. Interactions that determine homeostasis are often controlled by both endocrine and paracrine mediators. The 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 static (terminal) EMT and increase inflammation, which are physiologic processes that destabilize homeostasis that maintains pregnancy to promote labor and delivery. However, conditions that can cause an untimely increase in EMT and inflammation are pathologic. This tissue damage is often associated with adverse pregnancy complications, such as preterm prelabor rupture of membranes (pPROM) and spontaneous preterm birth (PTB). Therefore, an understanding of the biomolecular processes that maintain cyclic EMT-MET is critical in 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. Specifically, exosomes from cells that have undergone EMT carry pro-inflammatory cargo and modify the neighboring tissue environment in a paracrine fashion to cause enhancement of uterine inflammation.

Human pregnancy and parturition are complex phenomena; during pregnancy, two independent biological and physiological systems which involve the fetus and the mother co-exist to maintain pregnancy, and aid fetal growth and development¹. Parturition is a unique physiologic process that reverses all homeostatic states of uterine tissues during pregnancy in a synchronized way to ensure normal delivery at term²⁻⁶. Preterm birth ([PTB], < 37 weeks), contributing to 1 million neonatal deaths worldwide every year, is a major complication impacting ~ 12% of all pregnancies⁷⁻¹⁰. Survivors of PTB face lifelong challenges, and mothers who deliver preterm are predisposed to a wide range of medical complications later in life¹¹⁻¹³. Therefore, reducing the risk of PTB is a global healthcare priority^{9,14}. PTB is not only an early initiation of labor resulting in preterm delivery but also a syndrome initiated by failures in any of the fetomaternal uterine systems that maintain pregnancy^{1,15}. Currently, although it is still unclear, it is thought that the mechanisms of delivery in normal parturition and PTB are different. Reducing the risk of PTB remains a challenge, as this condition may result from a fetomaternal medical indication for early delivery or could be spontaneous with unknown etiology^{1,6}. Preterm prelabor rupture of membranes (pPROM) leading to PTB is another complication of pregnancy, and it accounts for ~ 40% of all the PTBs. Even with improvements in prenatal care over the past three decades, the rates of pPROM and subsequent PTB have increased^{10,11}. This could be attributed to the lack of reliable pPROM predicting methods; while several tests, such as pooling, fern tests, nitrazine, and Amnisure®, are available to confirm that pPROM has occurred, there are no reliable methods to predict pPROM before it occurs¹³⁻¹⁷. This is primarily because the underlying causes of pPROM are unknown and attempts to develop screening or interventions without this information have been largely unsuccessful¹⁸. Epidemiological and clinical studies have identified some factors that increase the risk of pPROM¹³⁻¹⁷, including reproductive tract infections, behavioral (e.g., cigarette smoking), and obstetrical complications (e.g., polyhydramnios). Other potential pPROM risk factors include toxic environmental exposures, genetic predisposition, and biochemical signals from the fetus that promote fetal membrane apoptosis^{13-17,19}.

Advances in reproductive biology research have improved our knowledge on the fetomaternal uterine organ system and its contribution to improvements in pregnancy, and parturition at term and preterm care²¹⁻²⁴. Pregnancy and fetal development are dynamic processes characterized by temporal and cell-specific changes in fetomaternal uterine tissues³²⁻³⁴. As mentioned above, a better understanding of the cellular and molecular biological changes within the fetomaternal 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 the fetal membranes (amniochorion) and maternal cervix whose cellular and extracellular matrix remodeling and functional integrity are critical for pregnancy maintenance and promotion of parturition. Pathologies associated with these tissues are associated with pPROM and spontaneous PTB. Current interventions are not designed to treat disease states specifically associated with these tissues and it is therefore difficult to prevent these organs-specific disorders. After discussing the structure and function of these tissues, this review will focus on their remodeling mechanisms and how pregnancy maintenance hormones regulate these processes. Additionally, the role of extracellular vesicles as communication channels between the feto-maternal system will be discussed.

Human fetal membranes: Human fetal membranes, also referred to as the amniochorionic membrane, are the innermost lining of the uterine cavity. Fetal membranes are distinct from the placenta and serve as a barrier between the feto-placental and maternal compartments. Fetal membranes are comprised of the amnion layer and chorion layer (fetal tissue connected to maternal decidua) and are connected by a collagen-rich extracellular matrix (ECM)²⁵. The ECM, which is made up of fibrous proteins along with various types of collagens, provides the architectural and structural framework of the fetal membranes.^{25,26} The amnion layer is composed of a single layer of amnion epithelial cells connected to the amnion ECM by a type IV collagen-rich basement membrane. Amnion epithelial cells are constantly bathed in amniotic fluid, and therefore strategically placed to serve as important primary responders to changes in the intrauterine (amniotic) cavity. The chorion trophoblast layer, which is distinctly different from the placental cytotrophoblast cells, is attached to the maternal decidua and maintains the immune tolerance at the maternal-fetal interface.²⁷⁻³⁰ Like the amnion epithelial cell layer, chorion trophoblast cells are connected to the chorionic ECM by another layer of type IV collagen-rich basement membrane. These ECM spaces also contain amniotic and chorionic mesenchymal cells; mesenchymal cells are ~ 10% of the total amnion and chorion layers.³¹⁻³³

The amnion and chorion are fetal tissues and play major roles in maintaining pregnancy by providing multi-level protection to the growing fetus. Fetal membranes accommodate constant challenges during pregnancy, continue to grow, and maintain elasticity to the stretch forces experienced during fetal growth. Stretches experienced due to fetal growth or increasing amniotic fluid volume do not have an impact on the structural or functional properties of the membranes.³⁴ Even though membranes overlaying the placenta and cervix face distinctly different environments and insults during pregnancy, the membranes still maintain the

homeostatic balance necessary to sustain fetal growth without interruption. Fetal membranes have very few immune cells, which is partly attributable to the avascular nature of their tissue.³⁵ This partnership between the fetus and the membranes continues until term, when the fetus reaches maturity and the membranes reach their longevity. Fetal membranes are remodeled 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 the stromal mesenchymal, amnion, and chorion cells³⁶⁻⁴⁰.

Human cervix: The cervix, the maternal reproductive tissue, plays an important role in protecting the developing fetus and maintaining pregnancy until term delivery. It is composed of two cellular compartments: the epithelial layer 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 of the ECM, incorporating fibroblast, immune, and smooth muscle cells.^{41,42} The cervix remains firm and closed throughout pregnancy and undergoes cervical ripening and dilation during labor and delivery.^{43,44} It functions as a barrier to prevent ascending microorganisms in the vaginal canal from reaching the intrauterine cavity. The mucus produced by the cervical epithelia serves as a barrier for preventing infection and protecting from mechanical and other exogenous insults.^{45,46,47} Cervical cells are a rich source of antimicrobial peptides that can reduce the invasion of microbes and produce chemokines and other inflammatory mediators, therefore, reducing the spread of infection, aiding immune protection, and assisting in the rebuilding damaged tissue.^{48,49}

Compared to fetal membrane remodeling during pregnancy, cervical remodeling is better studied. 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.³ The remodeling occurs throughout pregnancy and is divided into four distinct but overlapping phases: softening, ripening, dilation, and postpartum cervical repair⁵⁰. Remodeling is characterized by changes in the epithelial, stromal, immune, and endothelial cervix cell functions, as well as changes in the composition and structure of the ECM.⁴ Cervical ripening at term is associated with increased vascular permeability; production of inflammatory cytokines, and collagen-degrading enzymes; chemotaxis and infiltration of leukocytes; and activation of proteolytic enzymes, which further degrade the collagen in the cervix⁵¹⁻⁵³. This weakens the cervix, resulting in the disappearance of its morphological features to allow effacement of the uterus, which facilitates labor and childbirth.

Cellular remodeling of fetal membranes and cervix: The remodeling of the collagen-rich matrix is facilitated by a balanced activity between matrix-degrading enzymes and their specific inhibitors^{54,55}. This balanced activity remodels the collagen matrix to strengthen both the 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 PTB^{56,58,62-71}. However, cellular remodeling that is an essential process required for both membrane and cervical remodeling is understudied. Both tissues are multicell layered, and cells undergo constant remodeling. The process of cellular remodeling has been recently elucidated in our lab, and by other studies.

Fetal membrane cellular remodeling: The discovery of biological microfractures in fetal membranes allowed us to explore the process of their formation, significance, and association with normal and adverse pregnancy events. Microfractures are structural changes that are likely initiated by multitudes of disturbances of the cellular layers^{32,72,73}. Microfractures are characterized by 1) alterations in the amnion epithelial layer where cells are vacated, 2) deterioration of the basement membrane layer, 3) presence of migrating cells in the ECM, and 4) migrating cells developing tunnels in the ECM that extend from the basement membrane through the spongy layer³². Microfractures are likely extensions of areas often vacated by amnion cells shedding or topographically altered due to senescence (mechanism of aging), apoptosis, or necrosis. Microfractures can develop from both amnion and chorion trophoblast layers and invade the ECM. They can connect between the two layers and can be considered 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 pPROM than gestational age-matched PTB with no ROM. 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 large amounts of collagen degradation, suggesting that localized MMP activity or inflammation is associated with microfracture formation or repair processes. Persistent microfractures can act as channels for amniotic fluid leakage and inflammatory cell infiltration. Microfractures are higher with morphometry is distinct in pPROM compared to gestational age-matched PTB with no ROM, suggesting that microfractures play a role in the pathogenesis of pPROM³².

Cellular transition as a mechanism of remodeling: Examination of cells in microfractures revealed a mesenchymal morphology predominantly, and the staining of cells from the

membrane of normal term labored/delivered and pPROM 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 layer suggested that cells are in-between state of transition termed as “metastate.” 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 5-day culture period, cells were predominantly vimentin+ and exhibited mesenchymal morphology. This is indicative of epithelial-mesenchymal transition (EMT). Kalluri and Weinberg defined EMT as a biologic process that allows a polarized epithelial cell, which normally interacts with the basement membrane via its basal surface, to undergo multiple biochemical changes that enable it to assume a mesenchymal cell phenotype with enhanced migratory capacity, invasiveness, elevated resistance to apoptosis, and greatly increased production of ECM components^{74,75}. The completion of EMT is signaled by the degradation of the underlying 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 has been observed in human amniochorion. In our in vitro models, microfracture studies, and in vivo animal models, classic signs of EMT were observed in the fetal membranes³³ and were more pronounced in term and pPROM membranes³³. Similar findings were also reported by others in human fetal membranes at term or membranes undergoing artificial rupture and remodeling. This suggests that multiple pathophysiologic changes in fetal membranes can lead to EMT^{76,77}. Investigations of the mechanisms behind EMT revealed the following changes in human fetal membrane tissues and helped us to define the following cellular remodeling mechanisms: (1) amnion epithelial cells of the membranes are constantly shed and microfractures are created (the microfractures are channels of migration for shedding 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 increased production of active MMP9; and (5) epithelial cells (CK-18+/E-cadherin+) are often predominantly in a metastate in the amniotic membrane, expressing both epithelial and 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 the recycling of mesenchymal cells in the ECM is an essential process to limit their numbers to ~10% of the epithelial cells⁷². Mesenchymal cells do not accumulate as they transition back to

epithelial cells (mesenchymal-epithelial transition [MET]) and help to fill the gaps of shedding cells and rebuild the tissues. This cyclic transition process minimizes the accumulation of mesenchymal cells in the ECM that are prone to pro-inflammation and oxidative stress stimuli. 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 the surface of amnion cells⁷⁸. Silencing of the 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 intra-amniotic cavity and in the amniotic fluid. A hyperoxic environment increases the concentration of TGF- β in the cells and in the amniotic fluid, with the highest levels at term before delivery. The impact of TGF- β on amnion cell EMT is dominant at term compared to any other stage of gestation. EMT is associated with localized inflammation, as it increases pro-inflammatory 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 a 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 the process of rebuilding tissue matrix or reseat microfractures.

The terminal state of EMT at term causes accumulation of mesenchymal cells, inflammation, and propels membrane weakening. At term, the recycling process is stalled due to the increased oxidative stress experienced in the intra-amniotic cavity. Oxidative stress is increased at term as fetal maturation is completed. It is impacted by the increased presence of reactive oxygen species (ROS) causing the following: (1) induces stress signaler p38 mitogen-activated kinases (MAPK) in amnion epithelial cells^{79,80}; (2) p38 MAPK performs multiple functions in these cells, as it causes senescence and increases TGF- β production through an alternate pathway⁷⁸; (3) increased EMT and accumulation of mesenchymal cells in the ECM; (4) oxidative stress also reduces the expression of PGRMC2 on mesenchymal cells, forcing functional progesterone withdrawal at term, preventing cellular recycling, and 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; (7) cytokine production and MMP activities are increased; and (8) enhanced membrane matrix degradation, weakening and dysfunction. Membrane dysfunction is associated with inflammation, which is characterized by the release of damage-associated molecular pattern markers (DAMPs), including HMGB1, cell-free fetal tissue DNA fragments, and IL-33. Generation of inflammatory mediators by fetal membrane cells due to a terminal state of EMT, along with its natural senescence, is considered one of the signaling mechanisms for initiation of the labor process. These data are supportive of cellular derangements besides matrix degradation, which can contribute to term labor.

Premature activation of EMT: It has been reported 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 had pronounced EMT, and it was like that seen in term labor membranes that rupture spontaneously. OS is one of the major risks associated with pPROM⁷³. Multiple risk factors (intrauterine and cervicovaginal infections, high BMI, nutritional and behavioral issues, and genetic and environmental factors) contribute to an increase in OS and an increase in ROS 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 rupture⁸⁵. OS-induced PGRMC2 downregulation prevents MET and causes the accumulation of pro-inflammatory mesenchymal cells in pPROM ECM⁸². Activation of p38 MAPK, senescence, and EMT in pPROM 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 are natural physiologic processes, they can be activated prematurely in cases of PTB. This can be a risk factor-specific response⁸⁷. Management of OS-associated pPROM and PTB should include reduction of OS-induced cellular damage, activation of a stress signaling pathway (e.g., p38 MAPK), senescence, EMT, and senescence-associated inflammation are critical.

Cervical tissue and oxidative stress: The cervix is composed of epithelial and stromal layers. 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 of the extracellular matrix (ECM), incorporating fibroblast, immune, and smooth muscle cells^{44,88-91}. As mentioned above, cervical remodeling is an essential process in maintaining pregnancy, and like fetal membranes, cellular matrix remodeling is needed to maintain cervical integrity, maintain intact tissue, and natural production of antimicrobial peptides to reduce the influx of the vaginal microbiome and provide barrier functions. It is clear from previous studies that OS has

detrimental effects on placental, uterine, and fetal tissues, which can lead to PTB^{47,57,92-97}. However, 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 pregnancy⁹⁸⁻¹⁰⁰. Multiple reports have highlighted the importance of a balanced OS 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 parturition¹⁰⁰. To alleviate any ambiguity regarding the role of OS and cervical function, we have recently conducted several studies using isolated cervical epithelial and stromal cells. The data summary reported in reference # 90 is as follows: (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) a high level of senescence and a low level of autophagy were observed in the cervical stromal cells under OS, while, conversely, a low level of senescence and a high level of autophagy were observed in endocervical epithelial cells; and (5) OS increased p38MAPK-mediated sterile inflammation in cervical cells. As we previously reported⁹⁰, 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 the transition zone and endocervical tissue. These cells can proliferate and cause localized inflammation for remodeling. Constant exposure of ectocervical epithelial cells to vaginal microbiota induces a heightened endogenous immune tolerance to prevent damage from exogenous factors, such as infection and OS¹⁰¹, a process likely aided by resident immune cells⁴⁹. On the other hand, the cervical stromal and endocervical epithelial cells are usually protected from the vaginal microbiota due to their location and production of mucus, which serves as physical barriers with antimicrobial peptides¹⁰². 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. This damage can compromise the mechanical properties of the cervix.

The effects of oxidative stress on pregnancy: Using ecto, endo, and stromal cells derived from cervical tissues, we recently reported the effect of oxidative stress on cervical tissue remodeling⁹⁰. As p38 MAPK activation was one of the signaling mechanisms observed in our prior studies, the role of this signaler in cervical tissue was further explored as well⁹⁰. As reported already in our previous publication⁹⁰, the following were observed when cervical cells were exposed to an oxidative stress inducer: (1) increased ROS production and activation of the p38MAPK pathway in all the three cervical cells; (2) promoted cell cycle arrest in ectocervical epithelial cells; (3) induced cervical cells necrosis; (4) a high level of senescence

and a low level of autophagy were observed in the cervical stromal cells, while conversely, a low level of senescence and a high level of autophagy were observed in endocervical epithelial cells; and (5) oxidative stress increased p38MAPK-mediated sterile inflammation in cervical cells⁹⁰. 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 to reduce the risk of PTB¹⁰³⁻¹⁰⁶ and the progesterone's mechanism of action on cervical cells is well reported, although the anti-inflammatory 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 pathologic vaginal microbiome infections¹⁰⁷⁻¹¹⁰. Our recent reports after exposing endocervical epithelial and stromal cells to lipopolysaccharide (LPS) following were revealed⁵⁶: (1) human endocervical epithelial cells maintain a metastate but predominantly maintained an epithelial morphology; (2) cervical stromal cells expressed mesenchymal markers and fibroblastoid morphology; (3) progesterone alone did not alter the shapes of cells and expression of EMT markers neither in endocervical nor in stromal cells; 4) LPS induced EMT in endocervical cells, caused inflammation in both endocervical and stromal cells, but P4 prevented LPS-induced transition and inflammation (This suggests that infection can potentially cause a static state of EMT and inflammation to facilitate matrix degradation.); 5) P4 did not promote MET in stromal cells; and 6) LPS slowed down wound healing, but P4 induced wound healing in both cell types⁵⁶. Extensive collagen and cellular turnover help to remodel the cervix during pregnancy⁸⁹. The role of resident cervical macrophages has been reviewed in detail during the remodeling process by Steve Yellon, but a detailed role of immune cells is not attempted here⁴⁹. The cervix, although structurally and functionally different from the fetal membranes, also undergoes 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 the fetal membranes and the cervix, localized inflammation promotes collagen degradation or cell migration. This is a balanced inflammation, as the tissue's environment exhibits normalcy after remodeling. However, the static state of EMT induced by infectious agents or other oxidative stress-inducing conditions can induce an overwhelming inflammatory response that can cause collagenolysis of both tissues, weakening them and causing an imbalance in 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 as cargo¹¹¹ and can be considered as paracrine signalers, as they can be received by the 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 parturition¹²⁰. 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 the fetal inflammatory exosome response, maternal cell-derived exosomes can also trigger inflammatory responses¹¹⁶.

Conclusions: All collagenolytic processes and matrix turnover mechanisms for both the fetal membranes and cervix have been detailed in the literature. However, cell-mediated events that can lead to collagenolysis have not been discussed in detail so far and cellular mechanisms involved in tissue remodeling during pregnancy are poorly understood. In this review, we described how cellular transitions are critical in maintaining homeostasis and how a static state or terminal state of the 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, causing adverse pregnancy outcomes.

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