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REVIEW ARTICLE

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The role of fetal membranes during gestation, at term, and preterm labor

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Abstract

During pregnancy, the fetal membranes (*i.e.*, amniochorionic membranes) surround the intrauterine cavity and provide mechanical, immune, and endocrine support to protect the fetus. Though they are a vital component of the intrauterine cavity, the fetal membranes are largely overlooked as an extension of the placenta, leading to a poor understanding of their role during gestation, parturition, or preterm birth. The fetal membranes are comprised of fetal cellular and stromal layers and line up with maternal decidua forming the feto-maternal interface during pregnancy. This interface plays a large role during pregnancy and the induction of term or preterm parturition (*e.g.*, labor). Here we summarize the function of the fetal membranes focusing on their role during gestation at term, and during preterm births.

Key words: amnion, chorion, membrane integrity, preterm rupture of the membrane, labor signaling

THE FUNCTION OF THE FETAL MEMBRANE DURING GESTATION

The fetal membrane is a thick, collagenous bilayer comprised of a fetal amnion and chorion epithelium connected to the maternal decidua capsularis. Acting as the feto-maternal interface, one of the fetal membranes' primary functions during gestation is to compartmentalize potential maternal exposures to various risk factors (*e.g.*, maternal infection, environmental pollutants, behavioral risks) that may induce a fetal inflammatory response that can lead to adverse pregnancy outcomes if not contained. The amnion component of the fetal membrane predominantly contributes to this barrier function by: (1) supporting fetal growth and amniotic fluid load, (2) withstanding local deformation and mechanical stretch,[1–6] (3) preserving an intact amnion epithelial layer,[7] (4) testing the environment and responding to local insults (*e.g.*, infection, reactive oxygen species), and (5) undergoing cellular and collagen remodeling to maintain extracellular matrix homeostasis. All of these roles are critical for maintaining membrane integrity during pregnancy (Figure 1). Conversely, the multi-layered chorion is responsible for modulating the choriodecidual (feto-maternal) interface immune environment by producing anti-inflammatory hormones and cytokines that buffer maternal immune cell invasion. Chorion expression of human leukocyte antigens (HLA-G) provides immune tolerance at this interface. These endocrine (a major source of progesterone) and paracrine signalers (anti-inflammatory interleukin-10) help to maintain immune cell homeostasis at the choriodecidual interface during gestation (Figure 1).

Additionally, the fetal membrane acts as a critical site of communication between the mother and the fetus.[8,9] This is evident by the roles it plays in maintaining immune homeostasis and transporting nutrients, water, and drugs during gestation.[10–13] Inflammatory balance

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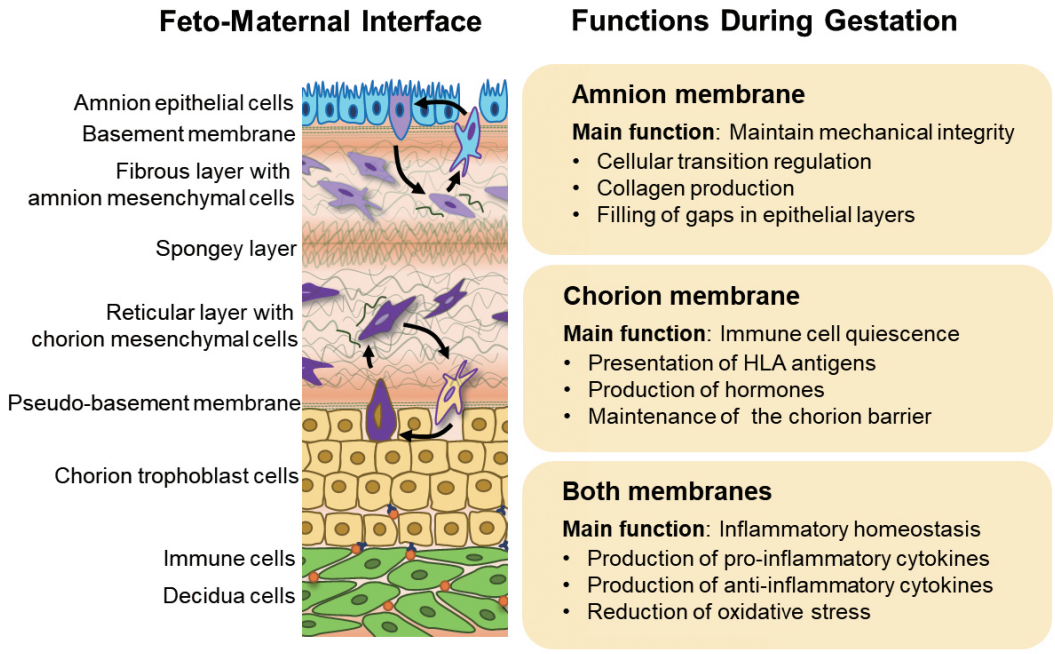

Figure 1. Feto-maternal interfaces' functions during gestation. The fetal membrane lines the intrauterine cavity and creates a protective barrier around the fetus throughout gestation. It is divided into two components: the amnion membrane (amnion epithelial cells [blue] – stroma [green lines] – amnion mesenchymal cells [light purple]) and the chorion membrane (chorion mesenchymal cells [dark purple] – stroma [green lines] – chorion trophoblast [yellow]), that are connected to the maternal decidua to create the feto-maternal interface. During gestation, it is critical that the mechanical, immune, and inflammatory properties of the membranes stay in homeostasis to allow for normal gestation progression. Both the amnion and chorion membranes play a role in these functions by contributing to cellular transitions (epithelial-to-mesenchymal and mesenchymal-to-epithelial transitions [black arrows]) to replace shed cells in both layers and to produce collagen (dark green lines). Additionally, the chorion regulates the quiescence of resident decidua immune cells (orange) by expressing HLA receptors (dark blue) (*i.e.*, HLA-G) and by secreting progesterone. To maintain inflammatory homeostasis, all cell layers in both membranes regulate the amount of pro-inflammatory cytokines and oxidative stress within the intrauterine cavity by producing anti-inflammatory cytokines and breaking down free oxygen radicles. HLA: human leukocyte antigen.

and the regulation of immune homeostasis at the fetal membrane decidual interface are essential components in preventing membrane dysfunction. Progesterone is a key anti-inflammatory gestational hormone that mediates inflammatory homeostasis by acting through an inducible negative feedback system that exists locally at the feto-maternal interface.[9,14] During gestation, the placenta and fetal membrane cells produce progesterone resulting in endocrine signaling through its nuclear (*i.e.*, progesterone receptor isoform A [PRA] and progesterone receptor isoform B [PRB][15]) and membrane-bound progesterone receptors (*i.e.*, PGRMC1/2 and MPα/MPβ[16]) across both feto-maternal interfaces. To note, the amnion and chorion layers do not contain nuclear progesterone receptors; however, they do express PGRMC1 and 2. Progesterone signaling during gestation is critical for the amnion epithelium to maintain its barrier function of sequestering the amniotic fluid in the intrauterine cavity. It does this by promoting mesenchymal cells within the fetal membrane collagen layers to undergo cellular transitions (*i.e.*, mesenchymal-to-epithelial transition [MET[17]]) (Figure 1) to refill weak points in the epithelium. Progesterone also plays a critical role in inhibiting the function of pro-inflammatory cytokines (*i.e.*, granulocyte-macrophage colony-stimulating factor [GM-CSF], tumor necrosis factor α [TNF-α], Interleukin [IL]-6, IL-8) that can weaken collagen in the fetal membranes.[6,14] Anti-inflammatory cytokine IL-10, produced locally by fetal membrane cells, has also been shown to be functionally similar to progesterone in its ability to regulate pro-inflammatory cytokine production and HLA-G expression contributing to the maintenance of immune homeostasis.[8,18]

Water and nutrient transport during gestation are essential for maintaining normal growth and development of the fetus[9,11] Aquaporins are plasma membrane proteins existing at more than eleven different isoforms whose levels in the endometrium and fetal-derived cells are altered during the implantation process.[11] Aquaporins have been shown to play a role in myometrial contractions and cervical ripening; However, they do contribute to the following process at the feto-maternal interface: (1) transport of water across the placenta to support the placentation, (2) maintenance of amniotic fluid volume, (3) and embryonic/fetal survival, growth, and development.[9–11] It has also been proposed that fetal membrane cells might express amnio acids, fatty acids, and vitamin transport proteins, similar to the placenta, suggesting fetal membranes could also play a role in nutrient transport to the fetus during gestation.

Recent studies have also identified the presence of transport proteins (involved primarily in the transport

of various molecules, including drugs from the mother) within fetal membrane cells and in fetal membrane cell-derived vesicles (*i.e.*, exosomes—extracellular vesicles of 30–160 nm released from these cells).[13,19] A few of these transporter proteins include permeability-glycoprotein (P-gp), breast cancer resistance protein-1 (BCRP-1), and the organic anion transporting polypeptide 2B1 (OATP2B1) were primarily localized in the chorion with limited expression in the amnion.[12,13] Their expression and function in the fetal membranes are similar to that observed in the placenta, changing the dogma that the placenta is the only intrauterine organ responsible for molecule transport.[12] Further studies and a better understanding of this drug transportation route may lead to novel therapeutic approaches and innovative drug therapies during pregnancy. This is critical, as a breakdown of the homeostatic processes of the fetal membranes described above leads to catastrophic membrane failure. This failure and dysfunction leading to fetal membrane rupture is a physiologic requirement at term but pathologic and consequential if it happens prematurely.

FETAL MEMBRANES' CONTRIBUTION TO LABOR INDUCTION

While the placenta is critical for fetal survival during gestation and at term, it is reported to not play a prominent role in the induction of labor signaling as unhindered placental function is required until fetal delivery. Conversely, the fetal membranes induce multiple parallel signaling pathways described below to shift immune homeostasis to a pro-inflammatory environment that contributes to labor onset. Discovered in 1976 by Keirse *et al.*, and verified in 1978 by Duchesne *et al.*, it was initially thought that the membranes' sole contribution to labor onset was prostaglandin production.[20,21] Prostaglandins are a group of lipid autacoids derived from arachidonic acid synthesized as a response to local tissue damage or infection that has been well known to control inflammation, vascular flow, and the induction of labor.[22] Labor-inducing prostaglandins produced by the fetal membranes and decidua, such as prostaglandin E_2 (PGE_2) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), are vital mediators of parturition that act primarily through collagen degradation to alter membrane structure, contract the myometrium, and ripen the cervix.[20,23] These lipids are found at elevated amounts in the amniotic fluid at both term and preterm parturition.[20,22–24] These elevated levels may result from decreased prostaglandin metabolism, increased prostaglandin production, and or changes in the expression of different prostaglandin receptors.[24] The multifactorial regulation of prostaglandins is a crucial component of labor signaling. However, it is only one of the many pathways fetal membrane cells induce to ensure the delivery of the fetus. Various pathways will be discussed in greater detail in the following paragraphs.

Close to term, the growing fetus ensues increased aerobic respiration and metabolic demands. These changes, accompanied by low levels of fetal and maternal antioxidants (*i.e.*, selenium, copper, zinc, manganese, vitamin C, and vitamin E[25]), yield elevated amounts of reactive oxygen species (ROS) in the amniotic cavity (Figure 2). The imbalance between ROS production and levels of antioxidants leads to intraamniotic oxidative stress (OS) buildup. Increased ROS induces cellular, organelle, and DNA damage within fetal membrane cells, causing them to undergo telomere alteration. Telomere alteration activates stress signaler p38 mitogen-activated protein kinase activation (MAPK) that promotes cellular senescence (*i.e.*, aging). While the rapid and abundant indication of fetal membrane senescence may also be induced pathologically, this process is usually physiologic and coincides with fetal maturation at term. In parallel, intrauterine OS and cytokines (*i.e.*, transforming growth factor [TGF] β) accelerate fetal membrane weakening by inducing cellular transition dysfunction (*i.e.*, increased epithelial-to-mesenchymal transition [EMT] and inhibition of MET through PGRMC functional withdrawal)[17] (Figure 2) in the amnion cells that leads to an increase in pro-inflammatory mesenchymal cells, collagen breakdown, and a lower mechanical threshold for rupture.[26] Senescent fetal membrane cells also exhibit senescence-associated secretory phenotype (SASP), a unique group of inflammatory mediators comprising cytokines, chemokines, and various pro-inflammatory markers. SASPs precede the release of pro-inflammatory damage-associated molecular patterns (DAMPs) such as high mobility group box 1 and cell-free fetal telomere fragments.[27–29] SASP and DAMPS promote labor by propagating senescence across the feto-maternal interface and by increasing the inflammatory load (Figure 2). Changes in inflammatory homeostasis trigger the uterine tissues to transition toward a pro-labor phenotype. These pathways have been demonstrated within term labor[29,30] and preterm birth[29,30] fetal membranes, as well as *in vitro* cell models treated with OS inducers.[31] Additionally, there are other parallel signaling cascades (*i.e.*, toll-like receptors, the receptor for advanced glycation end products,[32] nucleotide oligomerization domain,[33] AP-1,[34] and NF-κB,[35]) that play roles in promoting a pro-inflammatory environment within the intrauterine cavity at term.

Propagation of these signals from fetal to the maternal compartments could occur in two different ways: diffusion through (1) microfractures (*i.e.*, fissures in fetal membrane collagen layers connecting the feto-maternal compartments), or (2) exosomes released from fetal membrane cells (Figure 2). Microfractures have been shown to be in higher quantity and contain deeper features within term or preterm fetal membranes.[26,36,37] These findings have been recreated experimentally *in vitro*, suggesting their relevance in the propagation of parturition signals.[37] In parallel, fetal membrane cell-derived exosomes are capable of carrying contents from the cell of their origin to distant cell layers.[19,28,38] It is reported that exosome cargo from OS

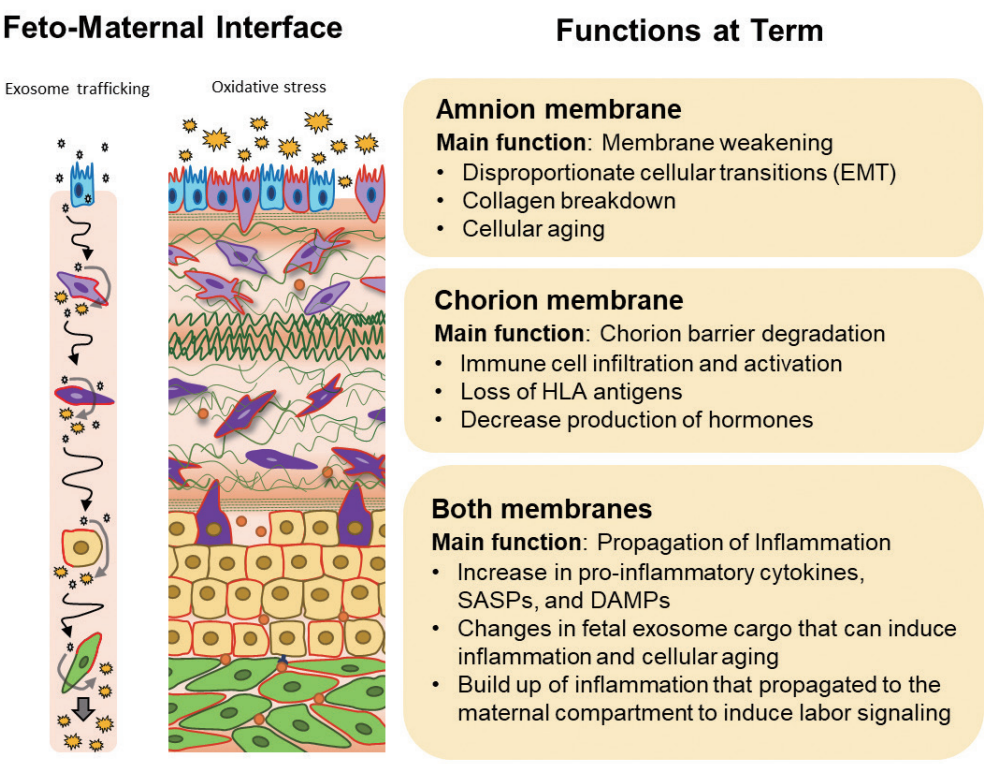

Figure 2. Labor-associated changes at the feto-maternal interface. Cartoon schematic showing fetal membrane anatomy (amnion epithelial cells [blue] – stroma [green lines] – amnion mesenchymal cells [light purple]) and the chorion membrane (chorion mesenchymal cells [dark purple] – stroma [green lines] – chorion trophoblast [yellow] – maternal decidua [green]) and processes that contribute to parturition signaling. At term, oxidative stress (yellow stars) contributes to the induction of amnion membrane weakening (*i.e.*, EMT [red lined cells], collagen break down, senescence) and chorion barrier degradation (*i.e.*, HLA downregulation [dark blue receptors], immune cell infiltration, and decreased hormone production). Both membrane components increase the production of inflammation and propagate inflammatory signals from the fetal to maternal tissue. These signals can be propagated through migrating cells or exosomes (grey dots) that carry cellular cargo to neighboring cells/tissues that then induce oxidative stress. HLA: human leukocyte antigen; EMT: epithelial-to-mesenchymal transition; SASP: senescence-associated secretory phenotype; DAMP: damage-associated molecular patterns.

fetal membrane cells contains active forms of p38MAPK and SASPs capable of promoting labor signaling. Thus, fetal membranes at term undergo senescence and cellular transitions, weaken the collagen layers, and produce powerful pro-inflammatory signals sent through various channels to convey to the maternal side the readiness for labor and delivery of the fetus (Figure 2).

FETAL MEMBRANE PATHOLOGIES CORRELATED WITH PRETERM BIRTH

Similar labor induction pathways can be activated physiologically at term or pathologically preterm. Preterm birth (PTB) is defined as birth before the 37th week of complete gestation. Preterm labor and delivery can be medically induced or spontaneous. Spontaneous preterm birth can be subdivided into two categories: preceded by preterm labor with intact membranes or preceded by preterm premature rupture of membranes (pPROM). Among spontaneous PTBs, pPROM or failure of the fetal membrane is the initiating cause of 40% of preterm births and affects 3% of all total pregnancies.[39,40] pPROM is classified as a rupture of the membranes before the onset of labor and before 37 weeks of gestation[1,2,39,41,42] (Figure 3). Fetal membrane rupture, at any stage of gestation, can be induced by both physical stretch or oxidative stress that biochemically weakens the collagen layers of the membranes leading to catastrophic mechanical failure. Rupture of the membranes increases the risk of ascending intraamniotic infection and adverse fetal outcomes such as hypoxia, pulmonary hypoplasia, respiratory distress syndrome, neonatal sepsis, umbilical cord prolapse, necrotizing enterocolitis, neurosensory impairment, cerebral palsy, developmental delay, and even fetal death.[39,43,44] The etiologies, pathways, and mechanisms of spontaneous PTB and pPROM are still unclear. However, it has been shown that spontaneous PTB is associated with minimal DNA damage, extracellular signal-regulated kinases and c-Jun N-terminal kinases activation, which does not lead to premature cellular senescecne.[45] While in cases of pPROM, OS and inflammation (*i.e.*, ROS, thrombin, and pro-inflammatory cytokines)[14,46,47] arising from fetal membrane signaling cascades (*i.e.*, Activator protein 1, NF-κB, p38MAPK),[34,45,48] induce senescence,[49] cellular transitions (*i.e.*, EMT and microfractures),[17,37,50] and paracrine or endocrine exosomal signaling tht can play critical roles in premature labor onset. Further investigation is needed to delineate the synergistic and differential roles

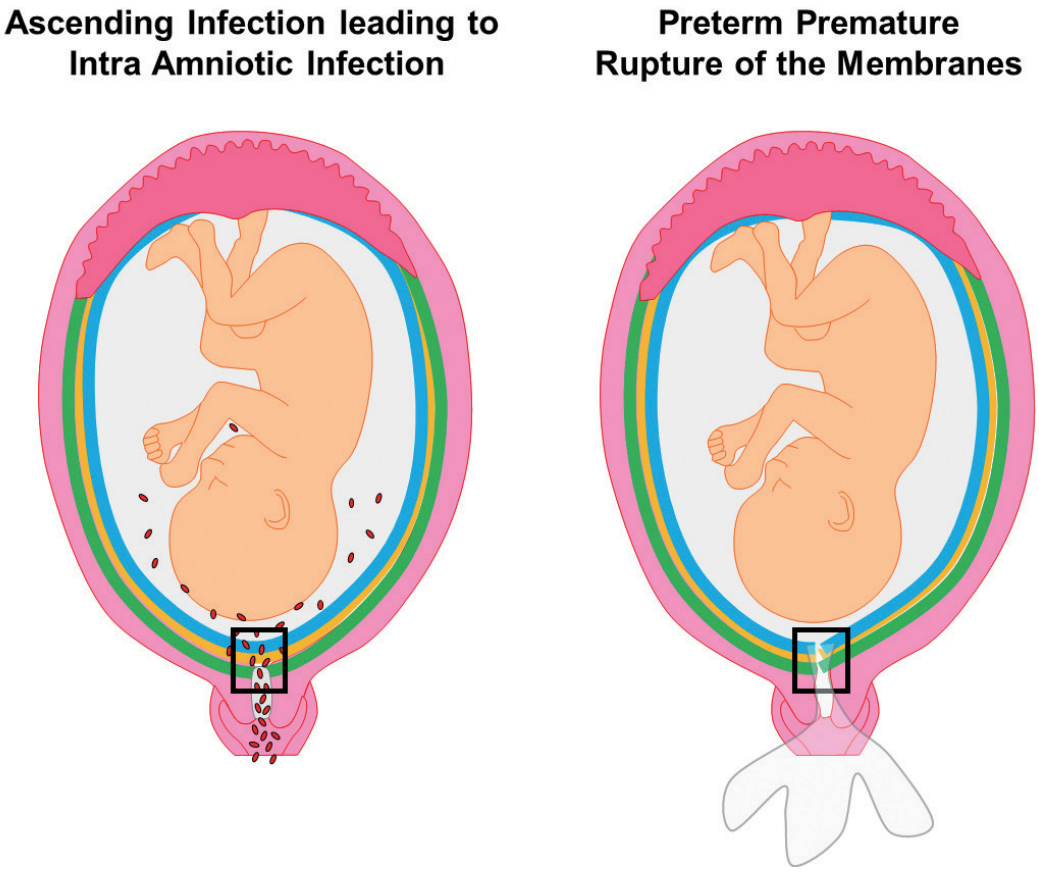

Figure 3. Schematic of the feto-maternal interface during adverse pregnancy outcomes. The intrauterine cavity (pink) is surrounded by maternal (decidua [green]) and fetal (amnion [blue] and chorion [yellow]) layers that form the feto-maternal interface during pregnancy. During adverse pregnancy complication that results in ascending vaginal infection (*i.e.*, bacteria/viruses [red dots]), infectious agents transverse the decidua-fetal membrane interface inducing chorioamnionitis. This results in an infiltration of immune cells, specifically neutrophils, that migrate through the feto-maternal interface and induce large amounts of inflammation. As the infection progresses, infectious agents that reach the amnionic cavity create what is called an intraamniotic infection which is correlated with preterm birth. Infection-induced preterm birth is also associated with pPROM. This occurs due to infectious or oxidative stress-induced cell disruption and collagen degradation leading to weakening and rupture of the membranes. pPROM: preterm premature rupture of the membranes.

of each step in the labor cascade between physiological term parturition and pathologic preterm labor.

Histologic chorioamnionitis (HCA) is a relatively common pregnancy complication that can lead to adverse maternal and infant outcomes. Clinically HCA presents as maternal fever, uterine fundal tenderness, maternal tachycardia (> 100 beats/min), fetal tachycardia (> 160 beats/min), and purulent or malodorous amniotic fluid.[51,52] Additionally, it has become increasingly linked to preterm delivery, with 40%–70% of HCA births complicated by pPROM or spontaneous PTB.[53] HCA is often associated with intraamniotic polymicrobial bacterial infection (*i.e.*, Group B streptococcus, Mycoplasma pneumoniae, Ureaplasma, Gardnerella vaginalis, Escherichia coli, and Bacteroides) and or increased sterile inflammation (*i.e.*, ROS) within the intrauterine cavity. HCA presents as a gradient promotion of migrating immune cells, dominantly neutrophils, from the maternal circulation or decidua capsularis stimulated into the fetal membranes by chemokines (*i.e.*, IL-8 and GM-CSF). These chemokines, along with bacteria antigens, activate neutrophils which produce pro-inflammatory cytokines that activate prostaglandins and collagen-degrading enzymes (*i.e.*, MMPs) as they migrate from the chorion to the amnion. In parallel, within the collagen of the fetal membranes, neutrophils release neutrophil extracellular traps (*i.e.*, NETs), which are web-like structures formed of DNA fibers, histones, and antimicrobial proteins that trap pathogens. These NETs immobilize the bacterial threat but adversely induce NETosis, a very specific type of NET-induced cell death that can promote collagen degradation through laminin cleavage and remodeling.[54] Pro-inflammatory cytokine production and NET formation lead to fetal membrane weakening and subsequent rupture preterm. The high prevalence of HCA, especially compared to the rarer incidence of placentitis (*i.e.*, immune cell infiltration of the placenta), makes this condition, and hence the fetal membrane, an essential subject of study for PTB onset.

Gestational diseases, such as pre-eclampsia and gestational diabetes, play a role in many adverse maternal outcomes by inducing systemic stress (*i.e.*, high ROS, downregulation of NADPH oxidases)[55,56] and inflammation (*i.e.*, Interluken-6,

Tumor necrosis factor, Leptin).[57] Pre-eclampsia is a leading cause of maternal and fetal morbidity and mortality defined by stage 2 hypertension with onset after 20 weeks of gestation in a woman with previously normal blood pressure. Gestational diabetes, characterized by a diagnosis of diabetes mellitus for the first time during pregnancy, has been identified as a risk factor for pre-eclampsia. Though the pathophysiology of pre-eclampsia has not been fully understood, it has been suggested that systemic maternal endothelial dysfunction resulting from abnormal placental development, preexisting maternal risk factors (*i.e.*, pre-pregnancy obesity, advanced maternal age, insulin resistance, chronic hypertension, *etc.*),[58] and the physiological activation of the inflammatory cascade caused by normal pregnancy may lead to OS, inflammation, and vascular dysfunction. Though these diseases typically affect the placenta, and hence are maternally targeted, systemic inflammation induced by pre-eclampsia could negatively affect fetal membrane function leading to preterm rupture. Assessing the biological function of fetal membranes and or fetal membrane cellular layers will provide a more thorough understanding of the mechanisms by which the fetal membranes functions in normal pregnancy environment and its potential contributions to the onset preterm birth. This knowledge is expected to provide novel strategies for treating or diagnosing adverse pregnancy outcomes.

DIAGNOSTIC POTENTIAL OF FETAL MEMBRANE COMPONENTS

Since the early 19th century, fetal membrane tissue and its cellular components have been utilized in a variety of clinical fields to treat ocular surface defects and heal diabetic ulcers, surgical incisions, burns, deep wounds, and leg ulcers.[59] However, it was not until 1956 that fetal membrane components were used as diagnostics, when Fuchs and Riis *et al.* isolated fetal trophoblast cells (*e.g.*, fetal membrane and placenta in origin) from amniocytes to identify fetal sex and genetic disorters.[60] This invasive technique has now been predominantly replaced with isolations of fetal trophoblasts from the maternal blood in the 1990's to diagnose chromosomal abnormalities (*i.e.*, trisomy 21, trisomy 18, and Klinefelter syndrome), fetal sex, mendelian disorders, and fetal rhesus blood type.[61] The future of prenatal diagnosis lies in merging the information known about labor-associated biological processes with a non-invasive sampling of maternal blood, leading to fetal membrane component isolation. Such tools would allow clinicians to sample fetal membrane cells and or exosomes from maternal blood to (1) detect biomarkers of adverse pregnancy outcomes, (2) assess fetal membrane cellular and inflammatory status, and (3) identify fetal genetic and epigenetic changes due to maternal environmental exposures.

SUMMARY

The fetal membranes play a vital role in the labor cascade by undergoing tissue and cellular-specific changes that lead to the propagation of labor-initiating signals from the fetus to the mother at term and preterm. As the feto-maternal interface, if studied properly, the fetal membrane-decidua could answer many of the field's questions regarding term labor initiation, inflammation, infection, and diseases that lead to preterm birth. To better understand the mechanism behind the labor cascade, all aspects of fetal membrane origin, as well as its cellular characteristics, need to be taken into account. Therefore, providing a global understanding of the role of fetal membranes during gestation and labor could provide insight into diagnosing and treating adverse pregnancy outcomes.

DECLARATIONS

Author contributions

Nina Truong: Reviewed the literature, and drafted the manuscript. Ramkumar Menon: Editing and reviewing the manuscript. Lauren Richardson: Conceptualization, created the figures, and draft the manuscript.

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Conflict of interest

Ramkumar Menon and Lauren Richardson are the Editorial Board Member of the journal. The article was subject to the journal's standard procedures, with peer review handled independently of these members and their research group.

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