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Human Fetal Membranes at Term: Dead Tissue or Signalers of Parturition?

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Abstract

Various endocrine, immune, and mechanical factors produced by feto-maternal compartments at term increase intrauterine inflammatory loads to induce labor. The role of fetal (placental) membranes (amniochorion) as providers of parturition signals has not been well investigated. Fetal membranes line the intrauterine cavity and grow with and protect the fetus. Fetal membranes exist as an entity between the mother and fetus and perform unique functions during pregnancy. Membranes undergo a telomere-dependent p38 MAPK-induced senescence and demonstrate a decline in functional and mechanical abilities at term, showing signs of aging. Fetal membrane senescence is also allied with completion of fetal maturation at term as the fetus readies for delivery, which may also indicate the end of independent life and longevity of fetal membranes as their functional role concludes. Fetal membrane senescence is accelerated at term because of oxidative stress and increased stretching. Senescent fetal membranes cells produce senescence-associated secretory phenotype (SASP-inflammation) and also release proinflammatory damage-associated molecular patterns (DAMPs), namely HMGB1 and cell-free fetal telomere fragments. In a feedback loop, SASP and DAMPs increase senescence and enhance the inflammatory load to promote labor. Membranes increase the inflammatory load to disrupt homeostatic balance to transition quiescent uterine tissues toward a labor phenotype. Therefore, along with other well-described labor-promoting signals, senescent fetal membranes may also contribute to human term parturition.

Normal term human parturition is initiated when fetal organ systems are matured around 37 – 40 weeks' gestation. Conventional theories of parturition initiation signaling are primarily linked to feto-maternal endocrine and immune changes in the intrauterine cavity, correlating with fetal growth and development [1,2,3]. Homeostatic imbalances produced by these changes lead to an inflammatory overload that disrupts the maintenance of pregnancy, resulting in labor-related changes [4,5]. Nonetheless, the signature of these signals and their precise mechanism in initiating parturition are still unclear. Interventional strategies to reduce the risk of preterm labor have been designed primarily based on our understanding of

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signaling effects by maternal compartments. However, higher rates of spontaneous preterm births (PTB) warrant further investigation of these signals and their mechanisms in normal term pregnancies; additional studies can provide insight into pathologic activation of signals associated with preterm parturition. Fetal endocrine signals as well as signals generated as a result of fetal organ maturation have been well reported in the literature as signalers of parturition [4]. A new type of fetal-derived signaling that occurs at term through senescent fetal (placental) membranes (amniochorion) and placental cells has been reported [6,7]. Senescence of the membranes generates “sterile inflammation” that can potentially increase the inflammatory load required to trigger parturition [8,7,9]. Since the concepts of fetal membrane senescence-associated aging, aging-induced sterile inflammation, and their roles in parturition are novel and being developed, this review summarizes the development of fetal membrane senescence and how senescent fetal cells may function as signalers of human parturition.

Fetal membrane structure and function

Human fetal membranes, the innermost tissue layers that form the intrauterine cavity, are fetal in origin and serve as a barricade between the feto-placental and maternal compartments. Fetal membranes comprise the amnion (innermost layer of the intraamniotic cavity) and the chorion (fetal tissue connected to maternal decidua) and are connected by the collagen-rich extracellular matrix (ECM) [10]. ECM, which is made up of fibrous proteins embedded in a polysaccharide gel and various types of collagen, provides the architectural and structural framework of fetal membranes [11]. The amnion is constantly bathed in amniotic fluid, signifying its importance as a primary responder to changes in the amniotic cavity. The chorion is in close proximity to maternal decidua and maintains the immune tolerance at the maternal-fetal interface [12,13,14].

The amnion and chorion, fetal tissues in origin, function as a single unit and can potentially be conceptualized as an organism that maintains its own homeostatic balance. This organism plays a major role in maintaining pregnancy by providing multilevel protection to the growing fetus. Fetal membranes accommodate constant challenges (immune, structural, mechanical, and endocrine) during pregnancy, continue to grow, and mechanistically and biochemically maintain elasticity to the stretches experienced during fetal growth. Despite the fact that membranes overlying the placenta and cervix face distinctly different environments and insults during pregnancy, they still maintain the homeostatic balance necessary to sustain fetal growth without interruption. This companionship between the fetus and membranes continues until term, when the fetus reaches maturity and the membranes reach longevity. As fetal membrane cells multiply and grow during pregnancy, they undergo a telomere-dependent cellular senescence, resulting in an aging phenotype indicative of its life expectancy. Histologic and biochemical changes associated with senescence and senescence-associated secretory phenotype (SASP), a unique inflammatory signature, have been documented in term fetal membranes at delivery [6,15]. This review describes the function of fetal membranes and proposes a novel mechanistic model, wherein aging but viable and inflamed fetal membrane cells at term may act to signal fetal maturity and their own dysfunctional status and, in turn, prompt parturition.

The composition of the membrane and its ability to produce a broad spectrum of biomarkers at different gestational stages illustrate the fetal membrane's contribution to the growing fetus, as well as its potential role in adverse pregnancy outcomes [16]. Fetal membrane biomarker responses are difficult to determine until after placental delivery, and therefore their contribution to normal or abnormal parturition is always subject to the 'cause or effect' quandary. Growth of fetal membrane cells during pregnancy plateaus at term, retaining the capacity for DNA replication [17]. The presence of stem cells further depicts the contribution of fetal membranes during in utero fetal life. Delivery of fetal membranes along with the fetus ends their organismal life.

Fetal membranes in adverse pregnancy outcomes

A hostile intrauterine environment can force a homeostatic imbalance on various fetal membranes' physiologies, leading to their dysfunction and contributing to adverse outcomes. Fetal membranes are often discussed in association with preterm premature rupture of the membranes (pPROM) with imminent preterm birth (PTB) or in normal term pregnancies experiencing spontaneous rupture or requiring artificial rupture of the membranes prior to delivery. Histologic chorioamnionitis (neutrophil infiltration of the membranes) is a pathologic state of the membranes often associated with adverse pregnancy outcomes and is used to assess the status of the preterm neonate [18]. Fetal membrane disturbances [19] at various levels, including epigenetic [20,21], immunologic [22,23,24,25,26,27,28,29], endocrine [30,31,32,33], and mechanical [34,35,36,37], have been associated with spontaneous preterm labor and delivery and pPROM in response to various risk exposures during pregnancy. Disturbances to multitudes of interacting factors that maintain the fetal membrane's functional integrity during pregnancy can lead to heightened inflammation and oxidative stress, both of which are detrimental to maintain normal pregnancy. Biochemical and mechanical responses from fetal membranes are also decisive in delineating the pathways of spontaneous onset of labor with intact membranes or a subset of women with pPROM [38,34,39,40]. This author has characterized "*pPROM as a disease of the fetal membranes*" based on multiple pieces of evidence that are unique to fetal membranes from pPROM when compared to gestational age-matched membranes from PTB with intact membranes [15,41]. Thus, membranes play a major role in determining adverse pregnancy outcomes, at least in a major subset of PTB.

Physiologic aging of fetal membranes in normal term deliveries, and pathophysiologic activation of premature aging in early pPROM

A systematic review recently published by Polettini et al [42] on aging of intrauterine tissues and adverse pregnancy outcomes identified 2 major knowledge gaps: 1) although aging of uterine tissues is mentioned in several studies as a factor associated with adverse pregnancy outcomes, no studies have reported any biomarkers or clinical indicators of premature aging as pathologies of PTB and pPROM; and 2) no studies have provided evidence of mechanistic pathways contributing to physiologic aging of uterine tissues or how risk factors can contribute to premature activation of aging and aging-associated pathologies. The review also highlighted the need for more studies to understand the mechanisms of uterine tissue

aging. Our report is an extension of our prior systematic review and provides a summary of ongoing work. We have summarized recent fetal membrane studies in which mechanistic pathways and activation of physiologic (normal parturition) and pathophysiologic (PTB and pPROM) aging are reported.

Premature activation of fetal membrane inflammatory and oxidative stress pathways have been implicated in PTB with intact membranes and pPROM [43,44,15,45]. Several biomarker studies provide evidence for the proinflammatory/oxidative stress status of fetal membranes. These studies are normally conducted using normal term fetal membranes, either from not in labor (collected from cesarean sections) or from term labor (vaginal deliveries) as a control group to assess various changes associated with PTB and pPROM. However, several lines of evidence now imply similarities between fetal membranes from women at term after spontaneous onset of labor and delivery and early pPROM (< 34 weeks) ending in preterm birth, questioning the usefulness of term membranes as appropriate controls for studying adverse pregnancies. Comparisons between various markers in fetal membranes from normal term birth, early PTB with intact membranes, and pPROM revealed 1) shortened fetal leukocyte and fetal membrane DNA telomere length (a marker of cellular senescence) [43], 2) oxidative stress and antioxidant deficiency as indicated by increased protein and lipid peroxidation [46,15], 3) activation of p38, a prosenescence and stress-activated mitogen activated kinase (MAPK), 4) morphologic changes to cellular elements (mitochondria and endoplasmic reticulum) demonstrating senescence-associated changes [15], 5) development of senescence-associated β -galactosidase activity [15], and 6) loss of nuclear envelope protein lamin B, a sign of senescence. These findings contributed to the concept of '*in utero aging of fetal membranes*' through replicative senescence mediated by telomere reduction.

At term, fetal membrane senescence is a normal physiologic mechanism correlated with the longevity of fetal membranes. However, in pPROM, it is a pathophysiologic activation leading to premature or accelerated aging, inflammation, and proteolytic collapse of membrane ECM, which causes structural and mechanical damage and dysfunction and leads to rupture. Senescence machinery is activated in both incidents by oxidative stress [47,15]. In vitro models of primary amnion cells (from normal, not-in-labor membranes exposed to oxidative stress) have recapitulated the pathway of induction of senescence [44]. Differences do exist between membranes functioning at term and pPROM, which are likely dependent on the type of oxidative stress, the strength and type of oxidative stress signals, and the nature of secondary signals generated by oxidative stress-induced cellular damage [41]. The premature activation of oxidative stress and senescence at pPROM is rather intense in response to various risk stimuli [41]. The pathologic outcome is likely due to the extent of cell death, tissue damage, and specific signaling cascade initiated by oxidative stress-induced damages.[41] Failure of antioxidant supplementation trials to reduce the incidence of adverse pregnancy outcomes can partly be attributed to our lack of knowledge of antioxidant status, oxidative stress damages, and damage-induced pathways, such as premature senescence activation.

Mechanisms of biologic aging of human fetal membranes at term

Fetal membrane cells are capable of growth and DNA replication at term; however, the process is stalled as it reaches longevity and demonstrates structural, functional, and biomolecular changes characteristic of advanced biologic aging. Two key function-based definitions proposed by Masoro et al [48] and Finch et al [49] may aid the understanding of mechanisms of biologic aging of fetal membranes: 1) fetal membranes are expected to deteriorate during their gestational life, once their maturation is complete around 12th week of pregnancy, and they become vulnerable to subtle changes in the intrauterine environment, decreasing their ability to survive; and 2) senescence is a mechanism associated with the deterioration process of the membranes, which alters their function and decreases vitality. In normal pregnancies, methodical progression of senescence is under physiologic control and is an inevitable process. Fetal membranes attain an irreversible senescent phenotype due to increased oxidative stress prior to the initiation of labor at term [15]. This oxidative stress can cause senescence of fetal membranes, increasing SASP, a unique inflammatory signature [6]. Senescence of fetal membranes, therefore, can be detrimental to the continuation of pregnancy, and the signals from senescent fetal membranes could trigger parturition [6].

Besides SASP, senescence-associated cellular injury causes release of damage-associated molecular patterns (DAMPs, HMGB1 [8], uric acid, histone (H)3, Interleukin (IL)-33, heat shock protein (HSP) 70, and cell-free fetal DNA fragments and telomere fragments) from term membranes. My laboratory has reported that DAMPs (HMGB1 and telomere fragments [components of cell free fetal DNA]) induce p38 MAPK (stress-associated mitogen activated kinase)-mediated senescence of fetal cells (term, not-in-labor amnion cells) to enhance the development of aging phenotype and inflammatory load in the fetal tissue, which in turn is likely to promote labor-associated changes. The studies on fetal membranes reported here are mostly done using membranes collected from the mid-zone portion, away from the placental bed or the portion overlying the cervix, to avoid any confounding cells in those regions (placental infiltrates and inflammatory cells) that can potentially impact the outcome and focus our studies primarily to amnion and chorion cells. However, our unpublished data suggest that these regions also have similar senescence-associated changes, suggesting that regional variations in the fetal membranes are minimal when membranes undergo senescence.

Active p38 MAPK can cause functional progesterone withdrawal in myometrial cells

In myometrial cells, progesterone withdrawal and subsequent myometrial activation (ie, increased contractility and excitability) are key events in the initiation of labor. Unlike most viviparous species, human parturition is triggered by a functional (rather than systemic) progesterone withdrawal, which is mediated, in part, by switching of the PR-A:PR-B ratio in myometrial cells from PR-B-dominance (which mediates antiinflammatory and relaxing actions of progesterone) to PR-A-dominance (which inhibits the antiinflammatory actions of progesterone and increases contractility) [50]. The shift to PR-A dominance is thought to increase NF- κ B-mediated induction of major prolabor genes whose products promote key

changes in myometrial cell function, including increased response to proinflammatory stimuli, increased COX-2-mediated prostaglandin F_{2α} (PGF_{2α}) production, and increased responsiveness to uterotonins, such as oxytocin (OT) and PGF_{2α}.

A key finding in breast cancer cell lines is that the PR-A:PR-B ratio is largely determined by the stability of the PR-A and PR-B proteins, which is caused by posttranslational modifications, especially phosphorylation by MAPKs at specific serine residues in the N-terminal domain [51,52]. PR-A stability is increased by MEKK1-induced p38 MAPK activation, which increased the PR-A:PR-B ratio, whereas PR-B stability was increased by activation of ERK1/2, leading to a decrease in the PR-A:PR-B ratio. Our ongoing studies have noted that p38 MAPK from senescent fetal cells can cause functional progesterone withdrawal in myometrial cells, and DAMPs released from senescent fetal cells can provide inflammatory milieu required for p38 MAPK activation in myocytes. Thus, senescent fetal membrane cells release DAMPs and SASPs that in a feedback loop, can increase their own damage as well as cause labor-associated changes in the myometrium. This effect is indicative of a signaling mechanism caused by aging fetal membranes to promote their own delivery in addition to the fetus.

Fetal membranes are not dead tissues at term labor but are one of the signal callers in the initiation of parturition

Multitudes of mediators and pathways have been reported as initiators of term parturition. Inflammatory overload triggering parturition is a well-documented mechanism, and inflammation is contributed by both fetal and maternal compartments. These fetomaternal signals cause transition of quiescent myometrium to an active stage (contraction/labor) through functional progesterone withdrawal as described above [39,53,54]. The contribution of fetal membranes in this process is unclear. Fetal membranes are not dead tissues at term or after delivery. At term, they can send sterile inflammatory signals, enhancing the overall load of inflammation in the decidua, myometrium, and cervix to initiate parturition. The proposed signaling model is depicted in Figure 1. Oxidative stress in the intrauterine cavity is expected to increase at term prior to labor. This increase may be due to increased fetal metabolic activity, decreased maternal supply of substrates to support fetal growth demands, depletion of antioxidants, stretching of the cavity by the fetus [55], or other yet unclear mechanisms. Oxidative stress-induced activation of p38 MAPK and its downstream effectors accelerates the development of an “aging fetal membrane phenotype,” which makes fetal membranes dysfunctional, accelerates damage, and generates signals like DAMPs and SASP that can be propagated to maternal side, as described above.

This review presents a role for fetal membranes in human parturition and shows that signals from fetal membranes alone will not trigger human parturition. Parturition is a well-orchestrated activity between both fetomaternal compartments, and each one of the tissue-organ systems has its own contribution. A synergy between various compartments finally leads to parturition. This review highlights the role and contribution of fetal membranes. They are individual organisms with a life span of approximately 9 months that have a defined function during pregnancy. Once implanted, they undergo cellular division, perform

specific functions, and eventually reach telomere-dependent senescence, a mechanism promoting an aging phenotype and eventual dysfunction. This aging results in specific signals that will culminate in parturition. Therefore, fetal membrane senescence and senescence-associated biochemical and functional changes can be considered as signalers of parturition.

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Figure 1. A model depicting different stages of transformation of fetal amnion membranes from normal to senescent phenotype in response to reactive oxygen species (ROS) at term generating signals that can initiate labor-associated changes

1. ROS is increased in the amniotic fluid at term, and these molecules can accelerate a telomere-dependent senescence process that is functional in fetal amniotic membranes.
2. ROS induces p38 MAPK-mediated senescence of amnion cells.
3. Senescent cells are inflammatory and produce SASP that consists of cytokines, chemokines, growth factors, matrix degrading enzymes, and other biochemicals.
4. SASP further accelerates progression of senescence in amnion cells
5. Tissue injury due to senescence also produces DAMP marker release. DAMPs include HMGB-1, HSP-70, H-3, IL-33, cell-free fetal telomere fragments, and degraded DNA.
6. SASP and DAMPs can further increase inflammatory load by chemotaxis and activation of leukocytes in the membranes and in other feto-maternal uterine compartments.
7. The overall increase of inflammation due to SASP, DAMPs, and immune cell activation contributes to sterile inflammation.
8. The sterile inflammatory markers can be propagated to other feto-maternal tissues (chorion, decidua, myometrium, and cervix), causing labor-associated changes and primarily increasing the inflammatory load to imbalance the quiescence status to transform into an active form. These changes could include

1) decidual immune activation, chemotaxis, and leukocyte and NK cell activations; 2) myometrial functional progesterone withdrawal (and inflammation; and 3) cervical ripening by inflammatory activation. Increasing inflammation in each compartment causes labor-associated changes.