Novel Pathways of Inflammation in Human Fetal Membranes Associated with Preterm Birth and Preterm Pre-labor Rupture of the Membranes

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Abstract

Spontaneous preterm birth (PTB) and preterm pre-labor rupture of the membranes (pPROM) are major pregnancy complications. Although PTB and pPROM have common etiologies, they arise from distinct pathophysiologic pathways. Inflammation is a common underlying mechanism in both conditions. Balanced inflammation is required for feto-placental growth; however, overwhelming inflammation (physiologic at term and pathologic at preterm) can lead to term and preterm parturition. A lack of effective strategies to control inflammation and reduce the risk of PTB and pPROM suggest that there are several modes of the generation of inflammation which may be dependent on the type of uterine tissue. The avascular fetal membrane (amniochorion), which provides structure, support and protection to the intrauterine cavity, is one of the key contributors of inflammation. Localized membrane inflammation helps tissue remodeling during pregnancy. Two unique mechanisms that generate balanced inflammation are the progressive development of senescence (aging) and cyclic cellular transitions: epithelial to mesenchymal (EMT) and mesenchymal to epithelial (MET). The intrauterine build-up of oxidative stress at term or in response to risk factors (preterm) can accelerate senescence and promote a terminal state of EMT, resulting in the accumulation of inflammation. Inflammation degrades the matrix and destabilizes membrane function. Inflammatory mediators from damaged membranes are

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propagated via extracellular vesicles (EV) to maternal uterine tissues and transition quiescent maternal uterine tissues into an active state of labor. Membrane inflammation and its propagation are fetal signals that may promote parturition. This review summarizes the mechanisms of fetal membrane cellular senescence, transitions, and the generation of inflammation that contributes to term and preterm parturitions.

Keywords

Amniochorion; fetal membranes; EMT; mesenchymal cells; progesterone; cytokines; inflammation; p38MAPK; exosome

1. Background/Introduction

Factors that maintain human pregnancy and signals that can initiate labor and delivery (parturition) are being investigated by many laboratories. Several reports have expanded knowledge on various topics related to pregnancy and childbirth in the past decade but still several 'unknowns' exist. These knowledge gaps can be considered one of the major reasons for adverse pregnancy outcomes that complicate pregnancies around the globe, specifically, preterm births (gestation <37 weeks). The World Health Organization estimated the global preterm birth rate for singleton gestation at 10.5% (Beck et al., 2010; Muglia & Katz, 2010; Rubens et al., 2014). A recent report by the March of Dimes, USA showed that the United States experienced an almost 3-decade rise in the preterm birth rate, beginning in 1980 and peaking in 2006 at 12.8%. Although this rate declined in recent years to 11.4%, preterm birth still accounts for 1 in 9 neonates born every year (McCabe, Carrino, Russell, & Howse, 2014). Approximately 65% of preterm births are 'spontaneous' (designated as PTB in the rest of this manuscript), while the other 35% are 'indicated' preterm births with known maternal or fetal clinically indicated risks that force early delivery (Andrews et al., 2006; Beck et al., 2010; Di Renzo & Roura, 2006; Goldenberg, Culhane, Iams, & Romero, 2008; Kramer et al., 2012; R. Romero, S.K. Dey, & S.J. Fisher, 2014a; Villar et al., 2012). Preeclampsia, gestational diabetes, fetal growth restrictions, multiple fetal pregnancies, and fetal anomalies are some of the indications for early delivery (Ananth & Vintzileos, 2006). PTB has an unknown etiology and presents with two distinct phenotypes: PTB with intact fetal membranes and PTB that follows preterm pre-labor rupture of the membranes (pPROM) (Goldenberg et al., 2008; Villar et al., 2012). A reduction in preterm births, regardless of whether spontaneous or iatrogenic, is extremely important, not only to reduce the rate of neonatal mortality (~1 million/year globally) or morbidity, but also to minimize the societal impact of prematurity (low birth weight) as preterm babies may experience several adult-onset diseases earlier in life (Beck et al., 2010). Adult-onset diseases seen in preterm babies are partly linked to epigenetic changes due to improper programming of the fetus (D.J. Barker et al., 2010; Devaskar & Thamotharan, 2007; Garg et al., 2012; Robins, Marsit, Padbury, & Sharma, 2011). *In utero* fetal growth is a timed event (~40 weeks of gestation) until delivery and epigenetically driven developmental programming occurs at each stage of growth (D.J. Barker et al., 2010). This growth and programming is properly nourished by an *in utero* environment where any disturbances can alter fetal developmental programming impacting organ maturation and predisposing

individuals to adult-onset diseases (D.J. Barker et al., 2010; Barker et al., 2013; Barker, Thornburg, Osmond, Kajantie, & Eriksson, 2010; Thornburg, 2015; Wallack & Thornburg, 2016). Therefore, it is important to know the mechanisms and pathways leading to PTB as it is critical to reduce PTB risk.

Parturition is initiated when fetal growth is completed. Biochemicals released from matured fetal organs indicate the completion of pregnancy and transition quiescent uterine tissues to an active state of labor (Gao et al., 2015; Mendelson, Montalbano, & Gao, 2017). Maternal decidua, myometrium and cervix serve as various pregnancy clocks and their coordinated activation sets off an alarm in response to fetal signals, resulting in parturition (Gonzalez, Dong, Romero, & Girardi, 2011; Menon, Bonney, Condon, Mesiano, & Taylor, 2016; Menon, Mesiano, & Taylor, 2017; Mesiano et al., 2002; Mesiano, Wang, & Norwitz, 2011; Norwitz et al., 2015; Park, Park, Lockwood, & Norwitz, 2005; Smith, Mesiano, & McGrath, 2002). On the fetal side, placental clock signals change in the endocrine milieu as the placenta ages (Smith, 1998; Smith & Nicholson, 2007). Untimely events, initiated either by the mother or the fetus in response to various risk exposures, preset these alarms, leading to premature activation of the labor cascade, ending in PTB. Understanding the mechanisms by which various feto-maternal tissues maintain pregnancy and molecular and physiologic signals that can lead to parturition at term are essential to decipher the mechanisms leading to PTB and pPROM. Based on current knowledge, PTB in a given subject may be considered as a disease of the placenta (Faye-Petersen, 2008), endometrium/ decidua (Bukowski et al., 2017; Pavlicev & Norwitz, 2018; Petraglia, Arcuri, de Ziegler, & Chapron, 2012; Rosen, Kuczynski, O'Neill, Funai, & Lockwood, 2001; Sinkey et al., 2020; Snegovskikh et al., 2009), myometrium (Bukowski et al., 2017; Huszar & Naftolin, 1984; Olson et al., 2003; Tattersall et al., 2008) and/or cervix (Keelan, 2018; Mercer et al., 2000; Vink et al., 2016; Yellon, 2017). In a classic review by Brosens et al, placental bed vascular diseases, classification of defective deep placentation associated with different obstetrical syndromes based on salient features of placentation defects have been described (Brosens, Pijnenborg, Vercruysse, & Romero, 2011). Multitudes of risk factors can impact development of feto-placental units and predispose them to inflammatory activation early on during pregnancy. These classifications are based on clinical and basic research evidences. The majority of current interventions for controlling preterm labor target these organs based on their pathologic involvement. However, systematic reviews and meta-analysis have shown limited success in delaying PTB in specific subsets of subjects by targeting maternal uterine tissues (Conde-Agudelo, Romero, & Nicolaides, 2020; Dodd, Flenady, Cincotta, & Crowther, 2008; Fernandez-Macias, Martinez-Portilla, Cerrillos, Figueras, & Palacio, 2019; Miyazaki et al., 2016; Vogel, Nardin, Dowswell, West, & Oladapo, 2014). This is due to the complexity of pathways leading to PTB and it is also unlikely that PTB is a single organ or single individual (maternal) disorder. Limited clinical and diagnostic success achieved in the past decades has been overshadowed by the high rate of PTB around the globe. Disparity in the PTB rate among different ethnic groups (Manuck et al., 2015; Menon, 2008), and the occurrence of pPROM in a major subset, further complicates our understanding of the pathophysiology of PTB. There are few clinical trials of pPROM leading to PTB (Berghella & Saccone, 2019; Di Sarno, Raffone, & Saccone, 2019; Langen et al., 2018), even though pPROM is associated with an excessive risk of neonatal morbidity and mortality, early

neonatal infections, bronchopulmonary dysplasia, and necrotizing enterocolitis (Schmitz et al., 2019) .

pPROM is associated with 30-35% of all PTB and the number of cases of pPROM in the United States is close to 175,000/year (Menon & Moore, 2020). These numbers exceed all indicated PTBs and have been steady for decades. pPROM is a very poorly studied syndrome. While several tests are available to confirm a diagnosis of pPROM *post facto* (e.g. pooling, fern tests, nitrazine, and Amnisure®), there is no reliable method to predict pPROM *a priori* because the precise pathophysiologic causes or biomarkers for pPROM are unknown (Elci, Gunes Elci, & Sayan, 2020; Kim et al., 2007; Mercer & Lewis, 1997; Simhan & Canavan, 2005). A limited number of studies have been done on pPROM to clearly understand its etiology, pathophysiology, and early diagnostic markers to provide timely interventions to minimize the incidence of rupture and subsequent PTB (Menon & Moore, 2020). PTB and pPROM share similar causal factors, show a redundancy in biomarkers and exhibit similarities in clinical presentations. However, the mechanisms and pathways leading to PTB and pPROM are different and should therefore be considered distinct phenotypes for clinical management (Menon, 2008, 2019; Menon & Fortunato, 2004). Clinicians are often dealt management dilemmas due to similarities in the clinical course of these syndromes and/or due to limited management strategies. Therefore, it will be beneficial to understand the mechanisms leading to these conditions that can provide a better diagnosis and interventions.

The high rate of PTB and pPROM can partly be blamed on the availability of limited or reliable basic science data (mechanisms, pathways, and biomarkers) to synthesize evidence that is needed to conduct clinical trials or provide proper management. As mentioned above, many current interventions target maternal uterine tissues as they are the timekeepers and final promoters of labor and delivery. Fetal signals (endocrine, paracrine, immune etc.) that can offset maternal clocks by transitioning them from a quiescent to an active state are critical contributors of parturition at term and preterm. Therefore, current research has been focused heavily on the fetal side, especially on fetal-derived intrauterine tissues such as the placenta and placental membranes (the amniochorionic membrane or fetal membrane). Fetal signals (their tissue source, pathways of generation, characteristics, propagation and maternal tissue targets) are currently being investigated. Understanding communication signals regarding fetal readiness for delivery and the facilitation of delivery after a normal pregnancy and parturition, will immensely benefit our pursuit in designing strategies to reduce the premature development of fetal signals leading to preterm labor.

This review is focused on certain novel mechanistic aspects of fetal tissue-based signals, their generation and propagation to maternal tissues, and how this indicates parturition. The placenta is a well-studied fetal organ during pregnancy and parturition (Andrews et al., 2006; Cao, Stout, Lee, & Mysorekar, 2014; Challis et al., 2009; Faye-Petersen, 2008; Mor & Kwon, 2015; Seferovic et al., 2019). However, the fetal membranes are often ignored or poorly studied, as their contributions are not regarded as anything beyond the sac that forms the cavity (Menon, 2016). Recent advances in cell biology (Abrahams et al., 2013; Arechavaleta-Velasco, Ogando, Parry, & Vadillo-Ortega, 2002; Canzoneri et al., 2013; Dunand et al., 2014; Feng, Allen, Marinello, & Murtha, 2019; Gomez-Lopez,

Hernandez-Santiago, Lobb, Olson, & Vadillo-Ortega, 2013; C.E. Kendal-Wright, 2007; Lappas, 2013; Malak et al., 1993; Myatt & Sun, 2010; Sato, Collier, Vermudez, Junker, & Kendal-Wright, 2016; Sun, He, & Yang, 2002), organ-on-chip (Gnecco et al., 2017; L. Richardson et al., 2019), and mechanistic models (El et al., 2006; Kumar et al., 2009; Kumar et al., 2011) have made tremendous advances in fetal membrane biology research. There are multiple reasons for using fetal membranes for our studies: 1) they are fetal in origin (Fox, 1981); 2) they are structurally, mechanically and functionally different to the placenta, an organ that has been well studied (Menon, Richardson, & Lappas, 2018); 3) fetal membranes are a rich source of functionally relevant biochemicals that help the pregnancy and promote parturition; 4) membranes start their growth along with the fetus and provide mechanical, structural, immune, antimicrobial and endocrine functions to the uterine cavity that are distinct from the placenta (Fox, 1981; Menon, 2016); 5) membranes are a rich source of stem cells, a property that has been widely used in regenerative medicine (Nogami et al., 2016; Saito, Lin, Murayama, Hashimoto, & Yokoyama, 2012); and 6) pPROM is considered a "disease of the fetal membranes" (Murtha & Menon, 2015). Therefore, we have been using fetal membranes, membrane-derived cells, and the membrane extracellular matrix (ECM) as a model to better understand the contributions to the mechanisms of this tissue, as well as to use it as a proxy to understand fetal contributions to pregnancy and parturition at both term and preterm. We are not attempting to describe the structure and development of fetal membranes here as it has been done in several other recent reviews (Martin, Richardson, da Silva, Sheller-Miller, & Menon, 2019; Menon et al., 2018). We have been studying fetal membranes using an explant culture approach that we developed in the early 1990s (Fortunato, Menon, Swan, & Lyden, 1994), and modified over time or by using cells from the fetal membranes (amnion epithelium, amnion mesenchyme, chorion mesenchyme and chorion trophoblast) (Jin, Richardson, Sheller-Miller, Zhong, & Menon, 2018; L. Richardson & Menon, 2018). By using fetal membrane tissues and cells as a model system, we have developed two independent mechanistic pathways that can contribute to normal term birth and also how membrane cells continue down the PTB and pPROM pathways. The rest of the review will focus on how fetal membrane cells help to maintain pregnancy and will detail two novel mechanistic pathways that send signals to the mother to promote parturition, senescence and cellular transitions.

2. Inflammatory response at term and in PTB

Prior to detailing the mechanisms by which fetal membranes maintain pregnancy and promote parturition by controlling inflammation, we will summarize the overall role of inflammation in pregnancy and parturition. Feto (placenta, membranes, umbilical cord) maternal (decidua, myometrium, and cervix) reproductive tissues maintain immune homeostasis during pregnancy and tolerate the semi-allogeneic fetus until parturition (Alijotas-Reig, Llurba, & Gris, 2014; Chavan, Griffith, & Wagner, 2017; Mor, Cardenas, Abrahams, & Guller, 2011; Negishi, Takahashi, Kuwabara, & Takeshita, 2018b; Schumacher, Sharkey, Robertson, & Zenclussen, 2018; Svensson-Arvelund et al., 2015). Balanced immune interactions by feto-maternal units ensure pregnancy maintenance and feto-placental growth (Gomez-Lopez, StLouis, Lehr, Sanchez-Rodriguez, & Arenas-Hernandez, 2014; Kshirsagar et al., 2012; Negishi et al., 2018b). Pregnancy success

is determined by immune regulatory mechanisms at the feto-maternal interface tissues, ensuring that both innate and adaptive immune cells aptly support feto-placental development by regulating localized inflammation while remodeling uterine tissues (Erlebacher, 2013; Lash, 2015; S. Liu et al., 2017; Nancy & Erlebacher, 2012; Schliefsteiner et al., 2017). On the contrary, parturition in both humans and animals is associated with a physiologic inflammatory process (Dudley, 1999; Goldenberg et al., 2008; Gomez-Lopez et al., 2011; R. Romero, S. K. Dey, & S. J. Fisher, 2014b; Romero, Gotsch, Pineles, & Kusanovic, 2007). This inflammation required to overcome immune balance at the feto-maternal interface tissues and to induce parturition associated changes in maternal tissues is characterized by the infiltration and activation of immune cells (both innate and adaptive immune cells) into the feto-maternal interface, along with the increased production of pro-inflammatory mediators (e.g. IL- 1β, IL-6, IL-8, TNF-α, GM-CSF etc.) and decreased levels of anti-inflammatory mediators (e.g. IL-10, TGF-β etc.) (Boonkasidecha, Kannan, Kallapur, Jobe, & Kemp, 2017; Bukowski et al., 2017; Cappelletti et al., 2017; Edey et al., 2016; Gomez-Lopez et al., 2014; Hamilton et al., 2012; Osman, Young, Jordan, Greer, & Norman, 2006; Peltier, 2003; Rinaldi, Makieva, Saunders, Rossi, & Norman, 2017; Xu et al., 2018; Zhang et al., 2017). Events contributing to physiologic immune imbalances and inflammation at term include, but are not limited to, signals of fetal organ maturation (Mendelson et al., 2017; Montalbano, Hawgood, & Mendelson, 2013), fetal membrane stretch (Mohan, Sooranna, Lindstrom, Johnson, & Bennett, 2007), stress- induced damage to the uterine tissues promoting immune cell chemotaxis (C. S. Buhimschi et al., 2009; Jauniaux, Poston, & Burton, 2006; Sharp, Heazell, Crocker, & Mor, 2010; Wadhwa, Culhane, Rauh, & Barve, 2001), and fetal membrane-placental-decidual senescence (Behnia, Sheller, & Menon, 2016a; Behnia, Taylor, et al., 2015b; Bonney et al., 2016; Hirota et al., 2010a; Menon, Behnia, et al., 2016; Polettini et al., 2015b). The disruption of immune homeostasis leading to parturition is expedited by both (Chen & Chen, 2013; Hinz & Scheidereit, 2014) endocrine and paracrine mediators generated when fetal growth is complete (Buhimschi et al., 2008; Dudley, 1999; Golightly, Jabbour, & Norman, 2011; lliodromiti et al., 2012; Keelan et al., 2003; Mendelson, 2009; Shynlova, Tsui, Jaffer, & Lye, 2009; Trivedi et al., 2012). Premature disruption of immune homeostasis and an overwhelming host inflammatory response due to infectious or other noninfectious risks can lead to PTB and pPROM (Romero et al., 2014b; Romero et al., 2006; Xu et al., 2018). Histologic chorioamnionitis (HCA), the infiltration of neutrophils into fetal membranes, is often associated with PTB and pPROM and contributes to neonatal morbidity and mortality (Chaiworapongsa et al., 2002; Gomez-Lopez, Romero, Xu, et al., 2017; Menon, Taylor, & Fortunato, 2010). Similarly, decidual cells and immune cells residing in decidua are in an immune harmonious state(Mclntire, Ganacias, & Hunt, 2008). Endogenous activation of inflammation due to decidual senescence(Cha, Hirota, & Dey, 2012) or immune activation in response to signals from fetus can disrupt immune homeostasis of this tissue. (Bartmann et al., 2014; Erlebacher, 2013; Gomez-Lopez et al., 2014; J. Liu, Dong, Wang, & Li, 2019; S. Liu et al., 2017; Mor, 2008; Presicce et al., 2015; Rinaldi et al., 2017; Schumacher et al., 2018). In summary, uterine tissue immune balance, a harmonious situation between both maternal and fetal tissues and immune cells harbored by them, is a key factor that maintains pregnancy (Alijotas-Reig et al., 2014; Arenas-Hernandez et al., 2016; Challis et al., 2009; Erlebacher, 2013; Figueiredo & Schumacher, 2016; Gomez-Lopez et al., 2014;

King, Kelly, Sallenave, Bocking, & Challis, 2007; S. Liu et al., 2017; Mor, 2008; Negishi, Takahashi, Kuwabara, & Takeshita, 2018a; Peltier, 2003; Rinaldi et al., 2017; Song & Shi, 2014; Southcombe, Tannetta, Redman, & Sargent, 2011; Szekeres-Bartho, 2002).

Pro-inflammatory activity is one of the key triggers of parturition at term and preterm. One is a physiologic and natural response, while the other is a pathologic and untimely event. Increased pro-inflammation imbalances and collapses all other homeostatic states of various pregnancy-associated tissues to ensure parturition. It is hypothesized that each tissue has its own inflammatory contributions, and we have been investigating how fetal membranes either endogenously, and/or in response to exogenous mediators, lead to an inflammatory state that can cause cellular and fetal membrane ECM degradation and a dysfunctional membrane status. A dysfunctional membrane loses the following capacities: 1) mechanical and structural disruption to support intrauterine cavity; and 2) controlling structural damage that contributes to functional disruptions such as a loss of antimicrobial resistance, immunologic compromises, and a loss of permeability functions resulting in fetal membrane collapse. In the next two segments, we will review how the endogenous activation of inflammatory mediators, primarily inflammatory cytokines, chemokines, and metalloproteinases, causes localized inflammation in the fetal membranes. We will review whether these are capable of disrupting membranes to predispose them to either rupture or generate inflammation to cause labor.

2.1.   Fetal membrane stretches and strain and inflammation

The amnion membrane component of the fetal membranes, which is constantly hydrated by the amniotic fluid, is highly elastic and can withstand pressure without undergoing any rupture during normal pregnancy. Pressure on and stretching of the amnion membrane *in utero* are caused by the growing fetus and an increase in amniotic fluid volume as gestation progresses (Adams Waldorf et al., 2015; C. E. Kendal-Wright, 2007). The amnion ECM is comprised of tropoelastins, elastin cross-linking enzymes, lysyl oxidase, and lysyl oxidase-like (LOXL) enzymes that contribute to the amnion's mechanical function (Bryant-Greenwood, 1998; Malak et al., 1993; Polettini et al., 2016; Strauss, 2013). Polettini et al. showed that changes to these enzymes can predispose the membrane to premature rupture, resulting in preterm delivery (Polettini et al., 2016). The membrane undergoes mechanical stretching as it grows and develops alongside the fetus, distends further at term and detaches from the uterine wall (Joyce et al., 2016; Millar, Stollberg, DeBuque, & Bryant-Greenwood, 2000). This has been validated in classical studies utilizing an *in vitro* model of amnion tissue stretch (Buerzle et al., 2013), mimicking fetal descent (Burzle, Mazza, & Moore, 2014; Mauri, Ehret, et al., 2015; Mauri, Perrini, Ehret, De Focatiis, & Mazza, 2015). and a balloon inflation approach to mimic a situation like polyhydramnios in non-human primates. Both studies showed stretching-induced inflammation (Adams Waldorf et al., 2015). Over-distension of the membranes at term or preterm is also mimicked *in vitro* by cyclic stretching of the amnion (Joyce et al., 2016; C. E. Kendal-Wright, 2007; Kendal-Wright, Hubbard, & Bryant-Greenwood, 2008; Kendal-Wright, Hubbard, Gowin-Brown, & Bryant-Greenwood, 2010). Cyclic stretch has been shown to increase cellular stress and pro-inflammatory cytokine production, indicative of a laboring phenotype (C. E. Kendal-Wright, 2007; Kendal-Wright et al., 2010). However, persistent stretch, as reported by

Kendal-Wright et al., demonstrated physical, but not static, strain placed on the amnion cells induces pro-labor chemokines like Interlukin-8 (IL-8) (Kendal-Wright et al., 2008). In support of this finding, we have seen that physiologic stretch experienced by the human amnion membrane does cause stress signals such as p38 mitogen activated protein kinase (MAPK) activation, but this activation does not result in the downstream activation of inflammatory mediators such as NF-kB (manuscript under review). Therefore, it is likely that stretch-associated inflammation is not sufficient to cause membrane damage but helps to remodel them to withstand sustained stretching throughout pregnancy.

2.2. Fetal organ maturation signals are pro-inflammatory in amniochorion cells

Fetal organ maturation is a key developmental signal from the fetus. Biochemicals such as endothelins (indicating kidney maturation) (Gryglewski, Chlopicki, Uracz, & Marcinkiewicz, 2001; Lockwood, 1994), platelet activation factor (Hoffman, Truong, & Johnston, 1986), surfactant proteins from the fetal lung (Condon, Jeyasuria, Faust, & Mendelson, 2004; Mendelson et al., 2017; Montalbano et al., 2013), epidermal growth factor (Cai, Huang, Leung, & Burd, 2014), and brain-derived neurotrophic factor (Antonakopoulos et al., 2018) are a few of the signals that are considered pro-inflammatory in the amniotic fluid; their presence can increase the overall inflammation. Although these signals are present in the amniotic fluid during fetal growth at low levels, their increased bioavailability may exceed the threshold of a balanced immune status and induce inflammation from fetal membrane cells, specifically amnion epithelial cells that are the innermost lining of the amniotic cavity and constantly bathed in the amniotic fluid and associated biochemicals. Reinl & England summarized a mechanistic process of such signaling that can lead to myometrial smooth muscle cell activation, resulting in parturition (Reinl & England, 2015). Similarly, we further documented in animal model studies that fetal exosomes can travel from the fetal to the maternal side, reaching the uterus and cervix to trigger parturition (Reinl & England, 2015). In summary, fetal biochemical signals of organ maturation have the capacity to cause inflammation in fetal membrane cells that can disrupt membrane functions at term.

One of the above-described mechanisms may contribute to tissue remodeling during pregnancy, whereas others can be a signal of parturition. However, our recent discoveries suggest that there are two other key mechanisms by which fetal membranes generate inflammation that can be detrimental to pregnancy and lead to parturition mechanisms. We will focus the next segments on explaining some of the novel mechanisms involved in fetal membrane maintenance during gestation and how two unique events (senescence (mechanism of aging) and cellular transitions such as epithelial-to-mesenchymal transition (EMT) and mesenchymal-to-epithelial transition (MET)) generate inflammation within the fetal membranes that can promote parturition. These studies are currently focused on amnion membrane cells (amnion epithelial cells [AEC] and amnion mesenchymal cells [AMC]).

3. Fetal membrane senescence, a mechanism of aging, and aging associated inflammation

Fetal membranes start their development at embryogenesis, grow with the fetus, and undergo multiple cycles of developmental changes during gestation. Amnio-chorion fusion happens during the early second trimester, resulting in a single unit structure (Menon & Moore, 2020; Menon et al., 2018). As with any other type of cells, replication of the fetal membrane cells leads to senescence (Campisi, 1997a; von, 2003), a pathway to aging and inflammation (Campisi, 1997b; Chambers & Akbar, 2020). The "Hayflick Phenomenon" explains this process, where cell division is halted after a certain number of divisions (Hayflick, 1961; Hayflick & Moorhead, 1961). Two key function-based definitions proposed by Masoro et al. (Masoro, 1995) and Finch et al. (Finch, 1992) may aid in the understanding of the biologic aging of fetal membranes: 1) fetal membranes are expected to deteriorate during gestation once their maturation is completed during gestation once its maturation is completed around the 12th week of pregnancy, and will be vulnerable to subtle changes in the intrauterine environment, decreasing survival ability; and 2) senescence is a mechanism associated with the deterioration of the membranes, which alters its function and decreases vitality (Menon, 2016). A recent report by Behnia et al. showed an association between amniochorionic membrane senescence and term pregnancies. Additional work determined the mechanism of the development of senescence through various *in vitro* and *in vivo* models (Behnia, Taylor, et al., 2015c). The following features were observed in the development of fetal membrane senescence.

3.1. Amniochorionic membrane senescence shows gestational age dependent telomere attrition

Telomeres are specialized DNA-protein structures located at the ends of eukaryotic chromosomes. Cellular replication leads to their reduction in size and this is considered as one of the biomarkers for aging (Rodier, Kim, Nijjar, Yaswen, & Campisi, 2005; Sanders & Newman, 2013; von & Martin-Ruiz, 2005). The examination of amniochorionic membranes from various gestational ages (starting from 22 weeks) until term (40 weeks) showed a progressive decline in telomere length in both amniochorionic membranes and fetal DNA samples from cord blood (Menon et al., 2012b). We were also able to demonstrate that cell-free fragments of telomeres are shed from the cells and increased in the amniotic fluid of women at term labor compared to term not in labor (Menon, Behnia, et al., 2016). Telomere reduction in fetal membranes is correlated with fetal growth; therefore, the peak of membrane aging is paralleled with the completion of fetal growth and organ maturation, suggesting that senescence is likely a physiologic process preparing membranes for delivery.

3.2. Amniochorion membrane senescence is aided by oxidative stress (OS) build up in the amniotic cavity and activation of p38MAPK

The intrauterine environment undergoes redox changes during pregnancy, as detailed in many reports (G.J. Burton, 2009; Burton & Jaunaiux, 2001; Myatt, 2010; Myatt & Cui, 2004). Term pregnancy is characterized by redox imbalance and the accumulation of reactive oxygen species (ROS) in the amniotic fluid and in feto-placental tissues (Longini et

al., 2007; Menon, Boldogh, et al., 2014; Polettini, Richardson, & Menon, 2018). Multiple reasons can be linked to the build-up of ROS at the feto-maternal interface that can be seen in a previous reference (Menon, 2014) [Figure 1]. The examination of OS-mediated signaling revealed that fetal membrane cells respond with the increased activation of p38MAPK, a responder of stress signals that can determine cell fate (apoptosis, senescence and or other forms of cell deaths) (Choi, Lee, Ha, & Kim, 2011; Jin et al., 2018; Menon et al., 2013; Menon & Papaconstantinou, 2016). p38MAPKs are a family of four stress response signaling isoforms (p38MAPKα, β, γ, δ) that are evolutionarily conserved serine/threonine kinases whose functions differ significantly. We observed activation of of p38MAPKα, a form that is reportedly more common in cells (Ruiz-Bonilla et al., 2008). The activation of p38MAPK by phosphorylation results in cell cycle inhibition in amnion cells via the down-regulation of glycogen synthase kinase 3 beta (GSK3β) in fetal membranes (Lavu et al., 2019; Lavu et al., 2020).

3.3. p38MAPK activation results in cellular senescence

Using multiple models, both *in vitro* and *in situ*, we were able to demonstrate that p38MAPK activation is also a progressive event in fetal membrane cells and maximal activation seen when OS is high (Bonney et al., 2016). As shown in mouse models, p38MAPK activation correlates with gestational age along with the deactivation of GSK3β (Bonney et al., 2016; Lavu et al., 2020). Senescent cells are characterized by multitudes of changes and produce biochemicals that are specific indicators of senescence and cause injury to cells(Dimri & Campisi, 1994). Senescence-associated galactosidase (SA-β–Gal) is widely used as a biomarker of replicative senescence. As detailed by Kurz et al, SA-β–Gal is a manifestation of residual lysosomal activity at a suboptimal pH, which becomes detectable due to the increased lysosomal content in senescent cells (Kurz, Decary, Hong, & Erusalimsky, 2000). we were able to demonstrate that fetal membrane cells develop senescence-associated β-Galactosidase activity, a key marker of senescence (Behnia, Taylor, et al., 2015c; Coppe et al., 2008; Menon, Behnia, et al., 2016; Polettini et al., 2018). Senescent cells often show injury to organelles and nuclear membranes. Damage to the nuclear lamina leads to nuclear morphology in senescent cells and nuclear membrane Lamin B1 loss is detrimental to cell survival Freund, Laberge, Demaria, & Campisi, 2012). As a morphological marker of senescent associated nuclear injury, we tested Lamin B1 in fetal membrane cells. In addition to development of SA-β–Gal, we were able to confirm a loss of Lamin B, (Menon, Behnia, et al., 2016), as electron micrographs revealed morphologic changes (enlargement) to organelles such as the mitochondria, ER, and damage to the nuclear membrane (Menon, Boldogh, et al., 2014), DNA fragmentation (Menon et al., 2013; Menon, Polettini, Syed, Saade, & Boldogh, 2014), and the development of unique forms of inflammation (Menon, Behnia, et al., 2016). All of these effects were inhibited by p38MAPK inhibitors, confirming the role of this signal in inducing senescence in fetal membranes.

3.4. Senescent cells release senescence associated secretory phenotype (SASP) and damage associated molecular pattern markers (DAMPs)

Senescence is characterized by inflammation known as SASP as SASP is represented by a unique congregation of various biochemical markers. Although SASP is represented by well

reported cytokines, chemokines, growth factors, metalloproteinases (MMPs), inhibitors and receptors, and vascular growth factors, collective presence of specific markers are unique in senescent cells. Transcriptome analysis of senescent amniochorion cell showed the increased expression of SASP transcripts in term labor and in response to OS *in vitro* compared to their respective controls (Behnia, Taylor, et al., 2015c; Menon, Behnia, et al., 2016). Of note, SASP markers have been reported to be higher in various biological compartments during term labor (although not termed SASP markers) compared to term not in labor conditions. This further associates senescence and SASP with parturition (Behnia, Taylor, et al., 2015c). Most of these SASP markers are known pro-labor factors in feto-maternal tissues (Behnia, Taylor, et al., 2015c). Based on our data, a bioinformatics analysis of SASP markers was performed which revealed that pathological pathways and cellular signaling represented by SASP were derived due to cellular damage resulting from senescence. In our *in vitro* and *in vivo* models, administration of N-acetyl cystine (an anti-oxidant) and p38MAPK inhibitor SB 203580 reversed senescence and SASP, supporting the hypothesis that fetal membrane senescence and inflammation accompanying it are an OS-induced p38MAPK mediated phenomenon. Moreover, cells were further analyzed for degenerative tissue markers. Our studies revealed that OS-induced senescent amnion cells release the high mobility group box (HMGB)1 protein as well as cell free fetal telomere fragments (Bredeson et al., 2014; Menon, Behnia, et al., 2016). These markers belong to a class of Alarmins or damage associated molecular pattern markers (DAMPs) representing cellular injury and or tissue damage . DAMPs are intracellularly sequestered molecules and are hidden from recognition by the immune system under normal physiological conditions. However, under conditions of cellular stress/tissue injury, these molecules can either be actively secreted by stressed immune cells and considered as endogenous danger signals, because they induce potent inflammatory responses by activating the innate immune system during non-infectious inflammation (Roh & Sohn, 2018) (Land, 2015a, 2015b). DAMPs can arise from intracellular proteins, such as HMGB1, histones, IL-33, IL-1α, S100 proteins, heat-shock proteins (HSPs), DNA and RNA as well as extracellular matrix proteins such as decorin, biglycan, low molecular weight hyaluronan etc.. Besides these, cellular organelles and They can function through a variety of receptors like toll like receptor (TLR) 2, 4, 6 and 9, receptor for advanced glycation endproducts (RAGE) among other on various cell types to cause inflammatory activation in recipient cell(Roh & Sohn, 2018). TLRs expressions are well reported in the fetal membranes.(Abrahams et al., 2013; Agrawal & Hirsch, 2012) and their differential role during infection, parturition at term and preterm are well reported (Hoang et al., 2014; Ilievski, Lu, & Hirsch, 2007; Lim, Barker, & Lappas, 2014; Moco et al., 2013; Sato et al., 2016).

3.5. DAMPs cause feedback activation of senescence

In support of our *in vitro* cell-based models, we conducted an analysis of inflammatory marker concentrations in three distinct biological fluids from the same pregnancy. Our report showed higher concentrations of HMGB1 term labor amniotic fluid and cord blood samples than maternal plasma, supporting the hypothesis that these DAMPs may function as fetal signals of parturition (Menon & Taylor, 2019). DAMPs, Further testing was conducted to determine their functional relevance. Data from our own and other studies using *in vitro* and *in vivo* models revealed the following: 1) both HMGB1 and cell-free fetal telomere DNA

fragments accumulate in the amniotic fluid and they are also powerful stimulants of OS, induce AEC p38MAPK activation and enhance senescence of fetal membranes (Bredeson et al., 2014; Menon, Behnia, et al., 2016; Polettini et al., 2015a); 2) the *in vivo* injection of telomere fragments leads to p38MAPK activation, senescence and PTB in animals (Polettini et al., 2015a); and 3) the intra-amniotic administration of HMGB1 can cause PTB in mouse models (Gomez-Lopez et al., 2016). In addition, our own work has shown senescence in AMCs and chorion trophoblast cells (Feng et al., 2019), which aligns with other labs which have also reported telomere reduction (Phillippe, Sawyer, & Edelson, 2019), senescence of the amniochorion (Gomez-Lopez, Romero, Plazyo, et al., 2017), placenta (Burstein, Frankel, Soule, & Blumenthal, 1973; Cox & Redman, 2017; Ferrari, Facchinetti, Saade, & Menon, 2016; Parmley, 1984), and decidua (Hirota et al., 2010b), along with their association with parturition. In summary, SASP and DAMPs are unique forms of inflammation that are released from senescent fetal membrane cells that can cause parturition.

The above sections summarize that fetal membrane senescence and senescence-associated inflammation can be mechanistically linked to normal term birth. Senescence and SASP/DAMP-mediated inflammation are normal physiologic responses of the fetal membrane as they reach longevity at term, correlating with the completion of fetal growth.

4. Can premature senescence cause preterm birth?

The primary purpose of studying senescence and its association with normal term parturition is to expand our knowledge and understand similar pathways that can contribute to PTB and pPROM. The hypothesis is that PTB and pPROM risk factors accelerate senescence, causing SASP/DAMP-associated inflammation, which induces membrane dysfunction and collapse of the intrauterine structure required to support fetal growth [Figure 2]. All of the above markers of OS and senescence were tested in tissue samples from PTB and pPROM. Also, *in vitro* and *in vivo* models were developed by exposing either membrane explants, primary fetal membrane cells, or animal models to various PTB-inducing stimuli. Clinical sample analysis showed that compared to PTB with intact membranes, pPROM had: 1) shorter fetal membrane and cord blood DNA telomere lengths at delivery (Menon et al., 2012b), 2) reduced levels of antioxidant enzymes (Dutta et al., 2016), 3) the increased evidence of OS, protein peroxidation, lipid peroxidation and DNA damage (Menon, Boldogh, et al., 2014; Menon et al., 2011), and 4) the increased activation of p38 MAPK and tissue senescence (Menon, Boldogh, et al., 2014).

In vitro models showed increased OS, p38MAPK activation, senescence and the generation of SASP in response to various risk factors such as cigarette smoke (Menon et al., 2013; Polettini et al., 2018), environmental pollutants (Behnia, Peltier, et al., 2016; Behnia, Peltier, Saade, & Menon, 2015), noninfectious (sterile) inflammation and infection (Dixon, Richardson, Sheller-Miller, Saade, & Menon, 2018) [Figure 2]. The incidence of senescence in response to infectious stimuli such as lipopolysaccharides (LPS) was much milder than any other risk factors tested in our system (Dixon et al., 2018). This could be due to the fact that all other risk factors than LPS are dominant OS inducers. We do not rule out the possibility that live bacteria or even heat-inactivated bacteria may produce a much more rigorous OS response and hence senescence. *In vitro* models also lack immune cells that are

recruited to control the invasion of pathogens by generating ROS radicals(Menon, Behnia, et al., 2016; Menon, Boldogh, et al., 2014; Menon et al., 2013).

Of note, we did not see a difference in inflammatory markers (mostly cytokines and chemokines) in the amniotic fluid, cord blood, or maternal plasma between infection and OS, suggesting that inflammation is a key trigger in both conditions, regardless of the mechanism or pathway (Menon & Taylor, 2019). Interestingly, molecular markers, cellular level changes in senescence and inflammatory mediators exhibited tremendous similarities between normal term birth and pPROM (specifically early pPROM <34 weeks), whereas they were different in PTB. These include increased reactive oxygen species, telomere length reduction, increase in markers of lipid and protein peroxidation, imbalance in MMPs and TIMPs, p38MAPK, senescence associated cellular organelle morphology, localized MMP9 activation, and loss of Lamin B1(Dutta et al., 2016; Menon, Boldogh, et al., 2014; Menon, McIntyre, Matrisian, & Fortunato, 2006; Menon et al., 2012a). This suggests that fetal membrane aging and aging-induced inflammation are a (patho)physiologic requirement for term labor and pPROM, causing membrane weakening and rupture prior to labor. Although inflammatory marker concentrations and their profiles are similar between PTB and pPROM, it is likely that mechanisms leading to the increase in fetal membrane inflammation is independent of senescence and SASP in PTB (Dutta et al., 2016). Conversely, we would like to emphasize that pPROM is a disease associated with fetal membrane senescence. OS induced by various pPROM risk factors prematurely ages fetal membranes, making them dysfunctional leading to mechanical and structural weakening and rupture (Behnia, Peltier, et al., 2015; Behnia, Sheller, & Menon, 2016b; Menon et al., 2013).

To note, DAMPs can be generated independently of senescence of cells and they are primarily produced in response to infection, specifically in response to Pathogen-associated molecular patterns (PAMPs)(Santoni et al., 2015). Therefore, infection associated adverse pregnancy events can produce DAMPS via PAMPs as well as by inducing senescence of cells(Elovitz, Wang, Chien, Rychlik, & Phillippe, 2003; Hoang et al., 2014; Jaiswal et al., 2013; Padron, Saito Reis, & Kendal-Wright, 2020).

5. Cellular transitions and generation of inflammation

The above section detailed how inflammation is generated in response to senescence. In this section we describe yet another mechanism by which fetal membrane cells generate local inflammation that can be beneficial during gestation for tissue remodeling or the overwhelming presence of which can lead to membrane degeneration. Fetal membrane cells have stem cell-like properties, as they can proliferate, migrate, express stem cell markers and are capable of transitioning into other cell types (L. Richardson & Menon, 2018). These properties are essential for fetal membrane remodeling and to maintain its integrity, as membranes are constantly under shear stress and stretch from the fluid and fetus, respectively, during pregnancy. Studies conducted using fetal membrane cells to examine cellular transition mechanisms and generation of inflammation are listed below.

5.1.　Fetal membrane microfractures

The remodeling of membrane cells includes the shedding of cells, leading to the development of microfractures in the membrane structure (L. S. Richardson et al., 2017). Microfractures are not just gaps created by cellular shedding or puckering, they also show degradation of the basement membrane and matrix collagen as well as function as a passage for shed cells (L. S. Richardson et al., 2017). This process generates localized inflammation, which is required for membrane matrix remodeling. Although intrauterine OS levels fluctuates during gestation (G. J. Burton, 2009; Jauniaux et al., 2006; Myatt, 2010), redox balance sustains the remodeling process (Agarwal, Gupta, & Sharma, 2005). However, the process is stalled as the membrane reaches longevity at term and demonstrates structural, functional, and biomolecular changes that are characteristic of aging (Behnia, Taylor, et al., 2015a). The number of microfractures and their morphometry (width and length) were higher in term laboring membranes than term not in labor membranes (L. S. Richardson et al., 2017). Similarly, pPROM had a higher number of microfractures than PTB with intact membranes (L. Richardson & Menon, 2018).

5.2.　Microfracture healing involves cellular transitions and inflammation

In a recent study, Richardson et al. created artificial scratches (representing microfractures) using AECs. The following observations were made during microfracture healing (L. Richardson & Menon, 2018): 1) epithelial cells proliferate and transition into mesenchymal cells (EMT) in the early stages of healing with the expression of cytoskeletal and cell adhesion markers; 2) the transition to mesenchymal cells increases the migratory capacity of AECs; 3) healing of wounds/microfractures is accompanied by cell transitioning back to the epithelium (MET); 4) amniotic fluid accelerated the healing process, whereas OS stopped healing; 5) antioxidants reversed OS effect and augmented healing, suggesting that OS can cause a terminal or static state of EMT and prevent membrane microfracture healing; and 6) migration and healing was associated with localized inflammation.

5.3.　EMT is associated with term parturition

In addition to wound healing, as described above, the transdifferentiation of EMT is seen in embryogenesis and required for embryonic stem cell differentiation (DaSilva-Arnold, James, Al-Khan, Zamudio, & Illsley, 2015). Furthermore, EMT is also pathologically associated with fibrosis and cancer metastasis (Hay, 1995; Lamouille, Xu, & Derynck, 2014). In reproductive tissues, EMT has been linked to various stages of placental development including the differentiation of cytotrophoblasts to extravillous trophoblast cells (DaSilva-Arnold et al., 2015), as well as trophoblast differentiation (Vicovac & Aplin, 1996). EMT is characterized by the repression of epithelial cell-associated genes and the concomitant activation of genes that transition them into a mesenchymal phenotype (Lamouille et al., 2014). The reverse of EMT is seen with MET, which generates epithelial cells during various developmental stages. During the early stages of development, MET facilitates the embryo's engagement in gastrulation and organogenesis (Pei, Shu, Gassama-Diagne, & Thiery, 2019). Based on our observations during microfracture healing, we hypothesized that the amnion layer fetal membrane may undergo EMT to generate inflammation that will weaken this layer. This hypothesis was aided by a couple of supportive reports suggesting

EMT associated with membrane rupture and healing (Janzen et al., 2017; Mogami, Hari Kishore, Akgul, & Word, 2017). We examined human and mouse amnion membranes and noted that membranes from term parturition have a substantially higher number of AMCs than epithelial AECs (L. S. Richardson, Taylor, & Menon, 2020). This helped to expand our hypothesis that term labor may be associated with a terminal state of EMT due to the accumulation of highly inflammatory AMC in the ECM, as previously described (Sato et al., 2016). Descriptive data determined the following: 1) compared to term not in labor, term labor is associated with amnion membrane EMT in both mice and humans (L. S. Richardson et al., 2020); 2) AECs during normal gestation exhibit a 'metastate' expressing both epithelial (cytokeratin) and mesenchymal marker (vimentin) (L. S. Richardson et al., 2020); 3) classic markers (cytoskeletal, adhesion, and transcription factors) associated with mesenchymal transitions were expressed in amnion layer; and 4) EMT is associated with MMP9 induction and collagen degradation that can cause basement membrane degradation to structurally and functionally weaken the membrane, cause dysfunction and predispose them to rupture. In summary, term labor is associated with EMT and localized inflammation [Figure 3].

5.4. Mechanisms of EMT mediated by transforming growth factor (TGF)-β

Using molecular and cell biological approaches, Richardson et al. reported that EMT in amnion cells are mediated by TGF-β. TGF-β is seen in the amniotic fluid during gestation, but its concentrations are higher at term labor compared to term not in labor samples (L. S. Richardson et al., 2020). The exposure of cells to OS experienced at term labor increased TGF-β release from AECs, an increase that was reduced by antioxidant NAC treatment (L. S. Richardson et al., 2020). Blocking OS induced TGF-β-mediated signaling through gene silencing of TGF-β-activated kinase 1 binding protein 1 (TAB1) reduced EMT transcription factors and mesenchymal junction markers, maintaining epithelial characteristics. The silencing of TGF-β reduced p38MAPK activation. The inhibition of EMT in AECs by treatment with p38MAPK inhibitors further supports the role of p38MAPK in TGF-β-TAB1-mediated EMT (L. Richardson, Dixon, Aguilera-Aguirre, & Menon, 2018). EMT accumulates AMCs in the matrix and prompt inflammatory activation in the membranes (Sato et al., 2016; Whittle, Gibb, & Challis, 2000). Therefore, OS-induced p38MAPK activation can cause both senescence and EMT in cells. It is still unclear whether these two processes are interdependent or independent; however, both of these processes co-exist at term labor membranes in both humans and mouse models of normal gestation and parturition [Figure 3].

5.5. Mechanism of MET mediated by progesterone

Mesenchymal cells perform endocrine functions during gestation; however, these are tightly regulated and require a limited number of cells. As mesenchymal cells are highly susceptible to inflammation and ROS(Sato et al., 2016), their numbers need to be tightly regulated, which is achieved by reprograming them back to epithelial cells through MET to maintain membrane integrity. MET will reestablish epithelium cell-to-cell contact and increase nascent collagen production to remodel the degraded matrix. The pregnancy maintenance hormone progesterone, an anti-inflammatory hormone, was tested to determine whether it mediated the reversal of EMT by promoting MET(L. S. Richardson et al.,

2020). This mechanism is also thought to regulate local inflammation. Progesterone, through the progesterone receptor membrane component 2 (PGRMC2), induces MET via the proto-oncogene c-MYC. The silencing of PGRMC2 using siRNA and/or reducing c-MYC using its pharmacologic inhibitor increased mesenchymal transcription factors and cellular junction markers indicative of the persistence of the mesenchymal fibroblastoid phenotype(L. S. Richardson et al., 2020) [Figure 3].

5.6. Cyclic EMT-MET maintains membrane integrity during gestation and a terminal state of EMT at term increases inflammation

Based on the mechanisms described above, fetal membranes maintain their integrity through a cyclic EMT-MET process. This helps to maintain the 10:1 epithelial to mesenchymal ratio during gestation (Myatt & Sun, 2010). TGF-β in the local cellular and amniotic fluid environment can promote EMT and progesterone-mediated MET can revert them back to AEC. This cyclic process balances the number of AMCs in the ECM and allows microfractures to reseal and the membrane to remodel. At term, increased OS promotes two key events that lead to a static state of EMT and the accumulation of AMC: 1) OS causes TGF-β levels to increase and promote EMT; and 2) the OS-mediated reduction in progesterone receptor expression leads to membrane functional progesterone withdrawal, resulting in a lack of MET. This will result in localized inflammation and matrix degradation, predisposing membrane weakening and preparing them for labor [Figure 3].

6. Can premature EMT activation and lack of MET cause preterm birth?

Like senescence, EMT at term is a normal and physiologic process to ensure inflammation and membrane dysfunction and prepare the fetal tissues for parturition. The goal of understanding these mechanisms is to see whether the premature activation of EMT can cause pathways leading to PTB and pPROM. Unpublished reports from our lab have shown that EMT markers and inflammation are evident in PTB, but not in pPROM when tissues are gestationally age-matched. These markers include decreased E-cadherin (epithelial marker) and increased vimentin and N-cadherin (both mesenchymal markers). This contrasts with senescence, which was dominant in pPROM, but not in PTB when membranes were intact. We are not elaborating this section as it is still under investigation. In summary, PTB and pPROM may have similar risk factors but the cellular biologic mechanisms that generate inflammation in fetal membranes are different. pPROM involves an accelerated senescence, generating inflammation that collapses the structural and functional integrity of the fetal membrane, whereas PTB has a terminal state of EMT, forcing inflammation.

7. How do membrane senescence and EMT associated inflammation promote parturition?

Multiple mechanisms have been proposed to destabilize membranes. Collagenolytic processes by endogenously generated MMPs and an imbalance in MMP/inhibitor (tissue inhibitor of matrix metalloproteinase -TIMP) have been well reported. It was unclear, however, how these MMPs are activated. In this review, we introduced two new mechanistic events that can generate localized inflammation. Senescence and EMT-

associated Inflammation of the membranes generate an array of cytokine mediators that belong to different classes with distinct functions that imbalance membrane function and cause them to collapse. These inflammatory mediators are disseminated to the maternal side via senescent and EMT impacted cell-derived extracellular vesicles (EV; exosomes of 50-150 nm). Our studies have shown that EV carrying this inflammatory load at term can reach the myometrium, decidua, and cervix and cause parturition-associated inflammatory activation (Hadley et al., 2018; Jin & Menon, 2017; Menon, 2019; Menon et al., 2017; Sheller-Miller, Trivedi, Yellon, & Menon, 2019). These EV-mediated mechanisms can also be considered as "fetal signals of parturition", indicating fetal readiness for delivery either at term or preterm. In preterm birth, maternal plasma contains fetal exosomes that are different to those in normal term birth. Fetal exosomes carrying inflammatory pathway mediators can be identified in maternal plasma as early as the late first trimester, indicating underlying pathophysiological conditions (Menon et al., 2020; Menon et al., 2019). Sheller-Miller et al. showed that exosomes carrying inflammatory mediators can cause preterm parturition in animal models (Sheller-Miller et al., 2019). As shown in Figure 4, senescent cells generate inflammatory mediator-enriched exosomes that can reach maternal uterine tissues and cause inflammatory activation (Hadley et al., 2018), and transition them into an active state of labor. Inflammatory mediators are not just cytokines, chemokines or immune cells, but also include SASP and DAMP-enriched exosomes (Sheller-Miller, Urrabaz-Garza, Saade, & Menon, 2017). In summary, we demonstrated two unique forms of inflammatory activation in fetal membranes and how they can mechanistically mediate parturition.

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REFERENCES

Abrahams VM, Potter JA, Bhat G, Peltier MR, Saade G, & Menon R (2013). Bacterial modulation of human fetal membrane Toll-like receptor expression. Am J Reprod Immunol, 69(1), 33–40. doi:10.1111/aji.12016 [PubMed: 22967004]

Adams Waldorf KM, Singh N, Mohan AR, Young RC, Ngo L, Das A, … Johnson MR (2015). Uterine overdistention induces preterm labor mediated by inflammation: observations in pregnant women and nonhuman primates. Am J Obstet Gynecol, 213(6), 830.e831–830.e819. doi:10.1016/j.ajog.2015.08.028 [PubMed: 26284599]

Agarwal A, Gupta S, & Sharma RK (2005). Role of oxidative stress in female reproduction. Reprod Biol Endocrinol, 3, 28. doi:10.1186/1477-7827-3-28 [PubMed: 16018814]

Agrawal V, & Hirsch E (2012). Intrauterine infection and preterm labor. Semin Fetal Neonatal Med, 17(1), 12–19. doi:10.1016/j.siny.2011.09.001 [PubMed: 21944863]

Alijotas-Reig J, Llurba E, & Gris JM (2014). Potentiating maternal immune tolerance in pregnancy: a new challenging role for regulatory T cells. Placenta, 35(4), 241–248. doi:10.1016/j.placenta.2014.02.004 [PubMed: 24581729]

Ananth CV, & Vintzileos AM (2006). Epidemiology of preterm birth and its clinical subtypes. J Matern. Fetal Neonatal Med, 19(12), 773–782. doi:TQ734253495047V7 [pii];10.1080/14767050600965882 [doi] [PubMed: 17190687]

Andrews WW, Goldenberg RL, Faye-Petersen O, Cliver S, Goepfert AR, & Hauth JC (2006). The Alabama Preterm Birth study: polymorphonuclear and mononuclear cell placental infiltrations, other markers of inflammation, and outcomes in 23- to 32-week preterm newborn infants. Am.

J. Obstet. Gynecol, 195(3), 803–808. doi:S0002-9378(06)00866-0 [pii];10.1016/j.ajog.2006.06.083 [doi] [PubMed: 16949415]

Antonakopoulos N, Iliodromiti Z, Mastorakos G, Iavazzo C, Valsamakis G, Salakos N, … Vrachnis N (2018). Association between Brain-Derived Neurotrophic Factor (BDNF) Levels in 2(nd) Trimester Amniotic Fluid and Fetal Development. Mediators Inflamm, 2018, 8476217. doi:10.1155/2018/8476217 [PubMed: 30622436]

Arechavaleta-Velasco F, Ogando D, Parry S, & Vadillo-Ortega F (2002). Production of matrix metalloproteinase-9 in lipopolysaccharide-stimulated human amnion occurs through an autocrine and paracrine proinflammatory cytokine-dependent system. Biol. Reprod, 67(6), 1952–1958. [PubMed: 12444074]

Arenas-Hernandez M, Romero R, St Louis D, Hassan SS, Kaye EB, & Gomez-Lopez N (2016). An imbalance between innate and adaptive immune cells at the maternal-fetal interface occurs prior to endotoxin-induced preterm birth. Cell Mol Immunol, 13(4), 462–473. doi:10.1038/cmi.2015.22 [PubMed: 25849119]

Barker DJ, Gelow J, Thornburg K, Osmond C, Kajantie E, & Eriksson JG (2010). The early origins of chronic heart failure: impaired placental growth and initiation of insulin resistance in childhood. Eur. J. Heart Fail, 12(8), 819–825. doi:hfq069 [pii];10.1093/eurjhf/hfq069 [doi] [PubMed: 20504866]

Barker DJ, Osmond C, Forsen TJ, Thornburg KL, Kajantie E, & Eriksson JG (2013). Foetal and childhood growth and asthma in adult life. Acta Paediatr, 102(7), 732–738. doi:10.1111/apa.12257 [PubMed: 23560734]

Barker DJ, Thornburg KL, Osmond C, Kajantie E, & Eriksson JG (2010). The prenatal origins of lung cancer. II. The placenta. Am J Hum Biol, 22(4), 512–516. doi:10.1002/ajhb.21041 [PubMed: 20309992]

Bartmann C, Segerer SE, Rieger L, Kapp M, Sutterlin M, & Kammerer U (2014). Quantification of the predominant immune cell populations in decidua throughout human pregnancy. Am J Reprod Immunol, 71(2), 109–119. doi:10.1111/aji.12185 [PubMed: 24330065]

Beck S, Wojdyla D, Say L, Betran AP, Merialdi M, Requejo JH, … Van Look PF (2010). The worldwide incidence of preterm birth: a systematic review of maternal mortality and morbidity. Bull World Health Organ, 88(1), 31–38. doi:10.2471/BLT.08.062554 [PubMed: 20428351]

Behnia F, Peltier M, Getahun D, Watson C, Saade G, & Menon R (2016). High bisphenol A (BPA) concentration in the maternal, but not fetal, compartment increases the risk of spontaneous preterm delivery. J Matern. Fetal Neonatal Med, 1–7. doi:10.3109/14767058.2016.1139570 [doi]

Behnia F, Peltier MR, Saade GR, & Menon R (2015). Environmental Pollutant Polybrominated Diphenyl Ether, a Flame Retardant, Induces Primary Amnion Cell Senescence 1. Am. J. Reprod. Immunol doi:10.1111/aji.12414 [doi]

Behnia F, Sheller S, & Menon R (2016a). Mechanistic Differences Leading to Infectious and Sterile Inflammation. Am J Reprod Immunol, 75(5), 505–518. doi:10.1111/aji.12496 [PubMed: 26840942]

Behnia F, Sheller S, & Menon R (2016b). Mechanistic Differences Leading to Infectious and Sterile Inflammation 3. Am. J Reprod Immunol doi:10.1111/aji.12496 [doi]

Behnia F, Taylor BD, Woodson M, Kacerovsky M, Hawkins H, Fortunato SJ, … Menon R (2015a). Chorioamniotic Membrane Senescence: A Signal for Parturition? Am. J. Obstet. Gynecol doi:S0002-9378(15)00517-7 [pii];10.1016/j.ajog.2015.05.041 [doi]

Behnia F, Taylor BD, Woodson M, Kacerovsky M, Hawkins H, Fortunato SJ, … Menon R (2015b). Chorioamniotic membrane senescence: a signal for parturition? Am J Obstet Gynecol, 213(3), 359 e351–316. doi:10.1016/j.ajog.2015.05.041 [PubMed: 26025293]

Behnia F, Taylor BD, Woodson M, Kacerovsky M, Hawkins H, Fortunato SJ, … Menon R (2015c). Chorioamniotic Membrane Senescence: A Signal for Parturition? 1. Am. J. Obstet. Gynecol doi:S0002-9378(15)00517-7 [pii];10.1016/j.ajog.2015.05.041 [doi]

Berghella V, & Saccone G (2019). Cervical assessment by ultrasound for preventing preterm delivery. Cochrane Database Syst Rev, 9, CD007235. doi:10.1002/14651858.CD007235.pub4 [PubMed: 31553800]

Bonney EA, Krebs K, Saade G, Kechichian T, Trivedi J, Huaizhi Y, & Menon R (2016). Differential senescence in feto-maternal tissues during mouse pregnancy. Placenta, 43, 26–34. doi:10.1016/j.placenta.2016.04.018 [PubMed: 27324096]

Boonkasidecha S, Kannan PS, Kallapur SG, Jobe AH, & Kemp MW (2017). Fetal skin as a pro-inflammatory organ: Evidence from a primate model of chorioamnionitis. PLoS One, 12(9), e0184938. doi:10.1371/journal.pone.0184938 [PubMed: 28957335]

Bredeson S, Papaconstantinou J, Deford JH, Kechichian T, Syed TA, Saade GR, & Menon R (2014). HMGB1 promotes a p38MAPK associated non-infectious inflammatory response pathway in human fetal membranes. PLoS One, 9(12), e113799. doi:10.1371/journal.pone.0113799 [PubMed: 25469638]

Brosens I, Pijnenborg R, Vercruysse L, & Romero R (2011). The "Great Obstetrical Syndromes" are associated with disorders of deep placentation. Am J Obstet Gynecol, 204(3), 193–201. doi:10.1016/j.ajog.2010.08.009 [PubMed: 21094932]

Bryant-Greenwood GD (1998). The extracellular matrix of the human fetal membranes: structure and function. Placenta, 19(1), 1–11. [PubMed: 9481779]

Buerzle W, Haller CM, Jabareen M, Egger J, Mallik AS, Ochsenbein-Koelble N, … Mazza E (2013). Multiaxial mechanical behavior of human fetal membranes and its relationship to microstructure. Biomech Model Mechanobiol, 12(4), 747–762. doi:10.1007/s10237-012-0438-z [PubMed: 22972367]

Buhimschi CS, Baumbusch MA, Dulay AT, Oliver EA, Lee S, Zhao G, … Buhimschi IA (2009). Characterization of RAGE, HMGB1, and S100beta in inflammation-induced preterm birth and fetal tissue injury. Am J Pathol, 175(3), 958–975. doi:10.2353/ajpath.2009.090156 [PubMed: 19679874]

Buhimschi CS, Baumbusch MA, Dulay AT, Oliver EA, Lee S, Zhao G, … Buhimschi IA (2009). Characterization of RAGE, HMGB1, and S100beta in inflammation-induced preterm birth and fetal tissue injury. Am. J. Pathol, 175(3), 958–975. doi:S0002-9440(10)60607-4 [pii];10.2353/ajpath.2009.090156 [doi] [PubMed: 19679874]

Buhimschi CS, Turan OM, Funai EF, Azpurua H, Bahtiyar MO, Turan S, … Buhimschi IA (2008). Fetal adrenal gland volume and cortisol/dehydroepiandrosterone sulfate ratio in inflammation-associated preterm birth. Obstet Gynecol, 111(3), 715–722. doi:10.1097/AOG.0b013e3181610294 [PubMed: 18310376]

Bukowski R, Sadovsky Y, Goodarzi H, Zhang H, Biggio JR, Varner M, … Baldwin DA (2017). Onset of human preterm and term birth is related to unique inflammatory transcriptome profiles at the maternal fetal interface. PeerJ, 5, e3685. doi:10.7717/peerj.3685 [PubMed: 28879060]

Burstein R, Frankel S, Soule SD, & Blumenthal HT (1973). Aging of the placenta: autoimmune theory of senescence. Am. J. Obstet. Gynecol, 116(2), 271–276. doi:0002–9378(73)91062–4 [pii]

Burton GJ (2009). Oxygen, the Janus gas; its effects on human placental development and function. J Anat, 215(1), 27–35. doi:10.1111/j.1469-7580.2008.00978.x [PubMed: 19175804]

Burton GJ (2009). Oxygen, the Janus gas; its effects on human placental development and function. J. Anat, 215(1), 27–35. doi:JOA978 [pii];10.1111/j.1469-7580.2008.00978.x [doi] [PubMed: 19175804]

Burton GJ, & Jaunaiux E (2001). Maternal vascularisation of the human placenta: does the embryo develop in a hypoxic environment? Gynecol Obstet Fertil, 29(7-8), 503–508. [PubMed: 11575145]

Burzle W, Mazza E, & Moore JJ (2014). About puncture testing applied for mechanical characterization of fetal membranes. J Biomech Eng, 136(11). doi:10.1115/1.4028446

Cai YJ, Huang L, Leung TY, & Burd A (2014). A study of the immune properties of human umbilical cord lining epithelial cells. Cytotherapy, 16(5), 631–639. doi:10.1016/j.jcyt.2013.10.008 [PubMed: 24364910]

Campisi J (1997a). Aging and cancer: the double-edged sword of replicative senescence. J. Am. Geriatr. Soc, 45(4), 482–488. [PubMed: 9100719]

Campisi J (1997b). The biology of replicative senescence. Eur. J. Cancer, 33(5), 703–709. doi:S0959-8049(96)00058-5 [pii];10.1016/S0959-8049(96)00058-5 [doi] [PubMed: 9282108]

Canzoneri BJ, Feng L, Grotegut CA, Bentley RC, Heine RP, & Murtha AP (2013). The Chorion Layer of Fetal Membranes Is Prematurely Destroyed in Women With Preterm Premature Rupture of the Membranes. Reprod. Sci doi:1933719113483009 [pii];10.1177/1933719113483009 [doi]

Cao B, Stout MJ, Lee I, & Mysorekar IU (2014). Placental Microbiome and Its Role in Preterm Birth. Neoreviews, 15(12), e537–e545. doi:10.1542/neo.15-12-e537 [PubMed: 25635174]

Cappelletti M, Presicce P, Lawson MJ, Chaturvedi V, Stankiewicz TE, Vanoni S, … Divanovic S (2017). Type I interferons regulate susceptibility to inflammation-induced preterm birth. JCI Insight, 2(5), e91288. doi:10.1172/jci.insight.91288 [PubMed: 28289719]

Cha J, Hirota Y, & Dey SK (2012). Sensing senescence in preterm birth. Cell Cycle, 11(2), 205–206. doi:18781 [pii];10.4161/cc.11.2.18781 [doi] [PubMed: 22189716]

Chaiworapongsa T, Romero R, Kim JC, Kim YM, Blackwell SC, Yoon BH, & Gomez R (2002). Evidence for fetal involvement in the pathologic process of clinical chorioamnionitis. Am J Obstet Gynecol, 186(6), 1178–1182. doi:S0002937802000157 [pii] [PubMed: 12066094]

Challis JR, Lockwood CJ, Myatt L, Norman JE, Strauss JF 3rd, & Petraglia F (2009). Inflammation and pregnancy. Reprod Sci, 16(2), 206–215. doi:10.1177/1933719108329095 [PubMed: 19208789]

Chambers ES, & Akbar AN (2020). Can blocking inflammation enhance immunity during aging? J Allergy Clin Immunol, 145(5), 1323–1331. doi:10.1016/j.jaci.2020.03.016 [PubMed: 32386656]

Chavan AR, Griffith OW, & Wagner GP (2017). The inflammation paradox in the evolution of mammalian pregnancy: turning a foe into a friend. Curr Opin Genet Dev, 47, 24–32. doi:10.1016/j.gde.2017.08.004 [PubMed: 28850905]

Chen J, & Chen ZJ (2013). Regulation of NF-kappaB by ubiquitination. Curr Opin Immunol, 25(1), 4–12. doi:10.1016/j.coi.2012.12.005 [PubMed: 23312890]

Choi TG, Lee J, Ha J, & Kim SS (2011). Apoptosis signal-regulating kinase 1 is an intracellular inducer of p38 MAPK-mediated myogenic signalling in cardiac myoblasts. Biochim Biophys Acta, 1813(8), 1412–1421. doi:10.1016/j.bbamcr.2011.04.001 [PubMed: 21530592]

Conde-Agudelo A, Romero R, & Nicolaides KH (2020). Cervical pessary to prevent preterm birth in asymptomatic high-risk women: a systematic review and meta-analysis. Am J Obstet Gynecol. doi:10.1016/j.ajog.2019.12.266

Condon JC, Jeyasuria P, Faust JM, & Mendelson CR (2004). Surfactant protein secreted by the maturing mouse fetal lung acts as a hormone that signals the initiation of parturition. Proc Natl AcadSci U S A, 101(14), 4978–4983. doi:10.1073/pnas.0401124101

Coppe JP, Patil CK, Rodier F, Sun Y, Munoz DP, Goldstein J, … Campisi J (2008). Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS. Biol, 6(12), 2853–2868. doi:08-PLBI-RA-2566 [pii];10.1371/journal.pbio.0060301 [doi] [PubMed: 19053174]

Cox LS, & Redman C (2017). The role of cellular senescence in ageing of the placenta. Placenta, 52, 139–145. doi:10.1016/j.placenta.2017.01.116 [PubMed: 28131318]

DaSilva-Arnold S, James JL, Al-Khan A, Zamudio S, & Illsley NP (2015). Differentiation of first trimester cytotrophoblast to extravillous trophoblast involves an epithelial-mesenchymal transition. Placenta, 36(12), 1412–1418. doi:10.1016/j.placenta.2015.10.013 [PubMed: 26545962]

Devaskar SU, & Thamotharan M (2007). Metabolic programming in the pathogenesis of insulin resistance. Rev. Endocr. Metab Disord, 8(2), 105–113. doi:10.1007/s11154-007-9050-4 [doi] [PubMed: 17657604]

Di Renzo GC, & Roura LC (2006). Guidelines for the management of spontaneous preterm labor. J. Perinat. Med, 34(5), 359–366. doi:10.1515/JPM.2006.073 [doi] [PubMed: 16965221]

Di Sarno R, Raffone A, & Saccone G (2019). Effects of progestogens in women with preterm premature rupture of membranes. Minerva Ginecol, 71(2), 121–124. doi:10.23736/S0026-4784.18.04335-6 [PubMed: 30318880]

Dimri GP, & Campisi J (1994). Molecular and cell biology of replicative senescence. Cold Spring Harb. Symp. Quant. Biol, 59, 67–73. [PubMed: 7587128]

Dixon CL, Richardson L, Sheller-Miller S, Saade G, & Menon R (2018). A distinct mechanism of senescence activation in amnion epithelial cells by infection, inflammation, and oxidative stress. Am J Reprod Immunol, 79(3). doi:10.1111/aji.12790

Dodd JM, Flenady VJ, Cincotta R, & Crowther CA (2008). Progesterone for the prevention of preterm birth: a systematic review. Obstet. Gynecol, 112(1), 127–134. doi:10.1097/AOG.0b013e31817d0262 [doi];112/1/127 [pii] [PubMed: 18591318]

Dudley DJ (1999). Immunoendocrinology of preterm labor: the link between corticotropin-releasing hormone and inflammation. Am J Obstet Gynecol, 180(1 Pt 3), S251–256. [PubMed: 9914628]

Dunand C, Hoffmann P, Sapin V, Blanchon L, Salomon A, Sergent F, … Alfaidy N (2014). Endocrine gland-derived endothelial growth factor (EG-VEGF) is a potential novel regulator of human parturition. Biol Reprod, 91(3), 73. doi:10.1095/biolreprod.114.119990 [PubMed: 25122063]

Dutta EH, Behnia F, Boldogh I, Saade GR, Taylor BD, Kacerovsky M, & Menon R (2016). Oxidative stress damage-associated molecular signaling pathways differentiate spontaneous preterm birth and preterm premature rupture of the membranes 8. Mol Hum Reprod, 22(2), 143–157. doi:gav074 [pii];10.1093/molehr/gav074 [doi] [PubMed: 26690900]

Edey LF, O'Dea KP, Herbert BR, Hua R, Waddington SN, MacIntyre DA, … Johnson MR (2016). The Local and Systemic Immune Response to Intrauterine LPS in the Prepartum Mouse. Biol Reprod, 95(6), 125. doi:10.1095/biolreprod.116.143289 [PubMed: 27760748]

El KM, Pandey V, Stetzer B, Mercer BM, Kumar D, Moore RM, … Moore JJ (2006). Fetal membranes from term vaginal deliveries have a zone of weakness exhibiting characteristics of apoptosis and remodeling. J. Soc. Gynecol. Investig, 13(3), 191–195. doi:S1071–5576(05)00417-X [pii];10.1016/j.jsgi.2005.12.010 [doi]

Elci E, Gunes Elci G, & Sayan S (2020). Comparison of the accuracy and reliability of the AmniSure, AMNIOQUICK, and AL-SENSE tests for early diagnosis of premature rupture of membranes. Int J Gynaecol Obstet, 149(1), 93–97. doi:10.1002/ijgo.13097 [PubMed: 31925795]

Elovitz MA, Wang Z, Chien EK, Rychlik DF, & Phillippe M (2003). A new model for inflammation-induced preterm birth: the role of platelet-activating factor and Toll-like receptor-4. Am J Pathol, 163(5), 2103–2111. doi:10.1016/S0002-9440(10)63567-5 [PubMed: 14578208]

Erlebacher A (2013). Immunology of the maternal-fetal interface. Annu Rev Immunol, 31, 387–411. doi:10.1146/annurev-immunol-032712-100003 [PubMed: 23298207]

Faye-Petersen OM (2008). The placenta in preterm birth. J Clin Pathol, 61(12), 1261–1275. doi:10.1136/jcp.2008.055244 [PubMed: 19074631]

Feng L, Allen TK, Marinello WP, & Murtha AP (2019). Roles of Progesterone Receptor Membrane Component 1 in Oxidative Stress-Induced Aging in Chorion Cells. Reprod Sci, 26(3), 394–403. doi:10.1177/1933719118776790 [PubMed: 29783884]

Fernandez-Macias R, Martinez-Portilla RJ, Cerrillos L, Figueras F, & Palacio M (2019). A systematic review and meta-analysis of randomized controlled trials comparing 17-alpha-hydroxyprogesterone caproate versus placebo for the prevention of recurrent preterm birth. Int J Gynaecol Obstet, 147(2), 156–164. doi:10.1002/ijgo.12940 [PubMed: 31402445]

Ferrari F, Facchinetti F, Saade G, & Menon R (2016). Placental telomere shortening in stillbirth: a sign of premature senescence? J Matern Fetal Neonatal Med, 29(8), 1283–1288. doi:10.3109/14767058.2015.1046045 [PubMed: 26004986]

Figueiredo AS, & Schumacher A (2016). The T helper type 17/regulatory T cell paradigm in pregnancy. Immunology, 148(1), 13–21. doi:10.1111/imm.12595 [PubMed: 26855005]

Finch CE (1992). Mechanisms in senescence: some thoughts in April 1990 3. Exp. Gerontol, 27(1), 7–16. [PubMed: 1499686]

Fortunato SJ, Menon R, Swan KF, & Lyden TW (1994). Organ culture of amniochorionic membrane in vitro. Am. J. Reprod. Immunol, 32(3), 184–187. [PubMed: 7880402]

Fox H (1981). Invited review. A contemporary approach to placental pathology. Pathology, 13(2), 207–223. [PubMed: 7254904]

Freund A, Laberge RM, Demaria M, & Campisi J (2012). Lamin B1 loss is a senescence-associated biomarker. Mol. Biol. Cell, 23(11), 2066–2075. doi:mbc.E11-10-0884 [pii];10.1091/mbc.E11-10-0884 [doi] [PubMed: 22496421]

Gao L, Rabbitt EH, Condon JC, Renthal NE, Johnston JM, Mitsche MA, … Mendelson CR (2015). Steroid receptor coactivators 1 and 2 mediate fetal-to-maternal signaling that initiates parturition. J. Clin. Invest, 125(7), 2808–2824. doi:78544 [pii];10.1172/JCI78544 [doi] [PubMed: 26098214]

Garg M, Thamotharan M, Dai Y, Thamotharan S, Shin BC, Stout D, & Devaskar SU (2012). Early postnatal caloric restriction protects adult male intrauterine growth-restricted offspring from obesity. Diabetes, 61(6), 1391–1398. doi:db11-1347 [pii];10.2337/db11-1347 [doi] [PubMed: 22461568]

Gnecco JS, Anders AP, Cliffel D, Pensabene V, Rogers LM, Osteen K, & Aronoff DM (2017). Instrumenting a Fetal Membrane on a Chip as Emerging Technology for Preterm Birth Research. Curr Pharm Des. doi:10.2174/1381612823666170825142649

Goldenberg RL, Culhane JF, Iams JD, & Romero R (2008). Epidemiology and causes of preterm birth. Lancet, 371(9606), 75–84. doi:10.1016/S0140-6736(08)60074-4 [PubMed: 18177778]

Golightly E, Jabbour HN, & Norman JE (2011). Endocrine immune interactions in human parturition. Mol Cell Endocrinol, 335(1), 52–59. doi:10.1016/j.mce.2010.08.005 [PubMed: 20708653]

Gomez-Lopez N, Hernandez-Santiago S, Lobb AP, Olson DM, & Vadillo-Ortega F (2013). Normal and premature rupture of fetal membranes at term delivery differ in regional chemotactic activity and related chemokine/cytokine production. Reprod. Sci, 20(3), 276–284. doi:1933719112452473 [pii];10.1177/1933719112452473 [doi] [PubMed: 22836164]

Gomez-Lopez N, Romero R, Plazyo O, Panaitescu B, Furcron AE, Miller D, … Hassan SS (2016). Intra-Amniotic Administration of HMGB1 Induces Spontaneous Preterm Labor and Birth. Am. J. Reprod. Immunol, 75(1), 3–7. doi:10.1111/aji.12443 [doi] [PubMed: 26781934]

Gomez-Lopez N, Romero R, Plazyo O, Schwenkel G, Garcia-Flores V, Unkel R, … Dey SK (2017). Preterm labor in the absence of acute histologic chorioamnionitis is characterized by cellular senescence of the chorioamniotic membranes. Am J Obstet Gynecol. doi:10.1016/j.ajog.2017.08.008

Gomez-Lopez N, Romero R, Xu Y, Plazyo O, Unkel R, Than NG, … Hassan SS (2017). A Role for the Inflammasome in Spontaneous Labor at Term with Acute Histologic Chorioamnionitis. Reprod Sci, 24(6), 934–953. doi:10.1177/1933719116675058 [PubMed: 27852921]

Gomez-Lopez N, StLouis D, Lehr MA, Sanchez-Rodriguez EN, & Arenas-Hernandez M (2014). Immune cells in term and preterm labor. Cell Mol Immunol, 11(6), 571–581. doi:10.1038/cmi.2014.46 [PubMed: 24954221]

Gomez-Lopez N, Vadillo-Perez L, Hernandez-Carbajal A, Godines-Enriquez M, Olson DM, & Vadillo-Ortega F (2011). Specific inflammatory microenvironments in the zones of the fetal membranes at term delivery. Am. J. Obstet. Gynecol, 205(3), 235–224. doi:S0002-9378(11)00466-2 [pii];10.1016/j.ajog.2011.04.019 [doi]

Gonzalez JM, Dong Z, Romero R, & Girardi G (2011). Cervical remodeling/ripening at term and preterm delivery: the same mechanism initiated by different mediators and different effector cells. PLoS One, 6(11), e26877. doi:10.1371/journal.pone.0026877 [PubMed: 22073213]

Gryglewski RJ, Chlopicki S, Uracz W, & Marcinkiewicz E (2001). Significance of endothelial prostacyclin and nitric oxide in peripheral and pulmonary circulation. Med Sci Monit, 7(1), 1–16. [PubMed: 11208485]

Hadley EE, Sheller-Miller S, Saade G, Salomon C, Mesiano S, Taylor RN, … Menon R (2018). Amnion Epithelial Cell Derived Exosomes Induce Inflammatory Changes in Uterine Cells. Am J Obstet Gynecol. doi:10.1016/j.ajog.2018.08.021

Hamilton S, Oomomian Y, Stephen G, Shynlova O, Tower CL, Garrod A, … Jones RL (2012). Macrophages infiltrate the human and rat decidua during term and preterm labor: evidence that decidual inflammation precedes labor. Biol Reprod, 86(2), 39. doi:10.1095/biolreprod.111.095505 [PubMed: 22011391]

Hay ED (1995). An overview of epithelio-mesenchymal transformation. Acta Anat (Basel), 154(1), 8–20. [PubMed: 8714286]

Hayflick L (1961). The establishment of a line (WISH) of human amnion cells in continuous cultivation. Exp Cell Res, 23, 14–20. [PubMed: 13712490]

Hayflick L, & Moorhead PS (1961). The serial cultivation of human diploid cell strains. Exp Cell Res, 25, 585–621. [PubMed: 13905658]

Hinz M, & Scheidereit C (2014). The IkappaB kinase complex in NF-kappaB regulation and beyond. EMBO Rep, 15(1), 46–61. doi:10.1002/embr.201337983 [PubMed: 24375677]

Hirota Y, Daikoku T, Tranguch S, Xie H, Bradshaw HB, & Dey SK (2010a). Uterine-specific p53 deficiency confers premature uterine senescence and promotes preterm birth in mice. J Clin Invest, 120(3), 803–815. doi:10.1172/JCI40051 [PubMed: 20124728]

Hirota Y, Daikoku T, Tranguch S, Xie H, Bradshaw HB, & Dey SK (2010b). Uterine-specific p53 deficiency confers premature uterine senescence and promotes preterm birth in mice. J. Clin. Invest, 120(3), 803–815. doi:40051 [pii];10.1172/JCI40051 [doi] [PubMed: 20124728]

Hoang M, Potter JA, Gysler SM, Han CS, Guller S, Norwitz ER, & Abrahams VM (2014). Human fetal membranes generate distinct cytokine profiles in response to bacterial Toll-like receptor and nod-like receptor agonists. Biol Reprod, 90(2), 39. doi:10.1095/biolreprod.113.115428 [PubMed: 24429216]

Hoffman DR, Truong CT, & Johnston JM (1986). The role of platelet-activating factor in human fetal lung maturation. Am J Obstet Gynecol, 155(1), 70–75. doi:10.1016/0002-9378(86)90081-5 [PubMed: 3728606]

Huszar G, & Naftolin F (1984). The myometrium and uterine cervix in normal and preterm labor. N Engl J Med, 311(9), 571–581. doi:10.1056/NEJM198408303110905 [PubMed: 6379460]

Ilievski V, Lu SJ, & Hirsch E (2007). Activation of toll-like receptors 2 or 3 and preterm delivery in the mouse. Reprod. Sci, 14(4), 315–320. doi:14/4/315 [pii];10.1177/1933719107302959 [doi] [PubMed: 17644803]

Iliodromiti Z, Antonakopoulos N, Sifakis S, Tsikouras P, Daniilidis A, Dafopoulos K, … Vrachnis N (2012). Endocrine, paracrine, and autocrine placental mediators in labor. Hormones (Athens), 11(4), 397–409. doi:10.14310/horm.2002.1371 [PubMed: 23422762]

Jaiswal MK, Agrawal V, Mallers T, Gilman-Sachs A, Hirsch E, & Beaman KD (2013). Regulation of apoptosis and innate immune stimuli in inflammation-induced preterm labor. J Immunol, 191(11), 5702–5713. doi:10.4049/jimmunol.1301604 [PubMed: 24163412]

Janzen C, Sen S, Lei MY, Gagliardi de Assumpcao M, Challis J, & Chaudhuri G (2017). The Role of Epithelial to Mesenchymal Transition in Human Amniotic Membrane Rupture. J Clin Endocrinol Metab, 102(4), 1261–1269. doi:10.1210/jc.2016-3150 [PubMed: 28388726]

Jauniaux E, Poston L, & Burton GJ (2006). Placental-related diseases of pregnancy: Involvement of oxidative stress and implications in human evolution. Hum Reprod Update, 12(6), 747–755. doi:10.1093/humupd/dml016 [PubMed: 16682385]

Jin J, & Menon R (2017). Placental exosomes: A proxy to understand pregnancy complications. Am J Reprod Immunol. doi:10.1111/aji.12788

Jin J, Richardson L, Sheller-Miller S, Zhong N, & Menon R (2018). Oxidative stress induces p38MAPK-dependent senescence in the feto-maternal interface cells. Placenta, 67, 15–23. doi:10.1016/j.placenta.2018.05.008 [PubMed: 29941169]

Joyce EM, Diaz P, Tamarkin S, Moore R, Strohl A, Stetzer B, … Moore JJ (2016). In-vivo stretch of term human fetal membranes. Placenta, 38, 57–66. doi:10.1016/j.placenta.2015.12.011 [PubMed: 26907383]

Keelan JA (2018). Intrauterine inflammatory activation, functional progesterone withdrawal, and the timing of term and preterm birth. J Reprod Immunol, 125, 89–99. doi:10.1016/j.jri.2017.12.004 [PubMed: 29329080]

Keelan JA, Blumenstein M, Helliwell RJ, Sato TA, Marvin KW, & Mitchell MD (2003). Cytokines, prostaglandins and parturition--a review. Placenta, 24 Suppl A, S33–46. [PubMed: 12842412]

Kendal-Wright CE (2007). Stretching, mechanotransduction, and proinflammatory cytokines in the fetal membranes. Reprod. Sci, 14(8 Suppl), 35–41. doi:14/8_suppl/35 [pii];10.1177/1933719107310763 [doi] [PubMed: 18089608]

Kendal-Wright CE (2007). Stretching, mechanotransduction, and proinflammatory cytokines in the fetal membranes. Reprod Sci, 14(8 Suppl), 35–41. doi:10.1177/1933719107310763 [PubMed: 18089608]

Kendal-Wright CE, Hubbard D, & Bryant-Greenwood GD (2008). Chronic stretching of amniotic epithelial cells increases pre-B cell colony-enhancing factor (PBEF/visfatin) expression and protects them from apoptosis. Placenta, 29(3), 255–265. doi:10.1016/j.placenta.2007.12.008 [PubMed: 18272217]

Kendal-Wright CE, Hubbard D, Gowin-Brown J, & Bryant-Greenwood GD (2010). Stretch and inflammation-induced Pre-B cell colony-enhancing factor (PBEF/Visfatin) and Interleukin-8 in amniotic epithelial cells. Placenta, 31(8), 665–674. doi:10.1016/j.placenta.2010.06.007 [PubMed: 20598369]

Kim KW, Romero R, Park HS, Park CW, Shim SS, Jun JK, & Yoon BH (2007). A rapid matrix metalloproteinase-8 bedside test for the detection of intraamniotic inflammation in women with preterm premature rupture of membranes. Am. J. Obstet. Gynecol, 197(3), 292–295. doi:S0002-9378(07)00816-2 [pii];10.1016/j.ajog.2007.06.040 [doi] [PubMed: 17826425]

King AE, Kelly RW, Sallenave JM, Booking AD, & Challis JR (2007). Innate immune defences in the human uterus during pregnancy 6. Placenta, 28(11-12), 1099–1106. doi:S0143-4004(07)00145-2 [pii];10.1016/j.placenta.2007.06.002 [doi] [PubMed: 17664005]

Kramer MS, Papageorghiou A, Culhane J, Bhutta Z, Goldenberg RL, Gravett M, … Villar J (2012). Challenges in defining and classifying the preterm birth syndrome. Am. J. Obstet. Gynecol, 206(2), 108–112. doi:S0002-9378(11)02169-7 [pii];10.1016/j.ajog.2011.10.864 [doi] [PubMed: 22118964]

Kshirsagar SK, Alam SM, Jasti S, Hodes H, Nauser T, Gilliam M, … Petroff MG (2012). Immunomodulatory molecules are released from the first trimester and term placenta via exosomes. Placenta, 33(12), 982–990. doi:10.1016/j.placenta.2012.10.005 [PubMed: 23107341]

Kumar D, Novince R, Strohl A, Mercer BM, Mansour JM, Moore RM, & Moore JJ (2009). A new methodology to measure strength of adherence of the fetal membrane components, amnion and the choriodecidua. Placenta, 30(6), 560–563. doi:S0143-4004(09)00109-X [pii];10.1016/j.placenta.2009.03.014 [doi] [PubMed: 19410292]

Kumar D, Schatz F, Moore RM, Mercer BM, Rangaswamy N, Mansour JM, … Moore JJ (2011). The effects of thrombin and cytokines upon the biomechanics and remodeling of isolated amnion membrane, in vitro. Placenta, 32(3), 206–213. [PubMed: 21300402]

Kurz DJ, Decary S, Hong Y, & Erusalimsky JD (2000). Senescence-associated (beta)-galactosidase reflects an increase in lysosomal mass during replicative ageing of human endothelial cells. J Cell Sci, 113 (Pt 20), 3613–3622. [PubMed: 11017877]

Lamouille S, Xu J, & Derynck R (2014). Molecular mechanisms of epithelial-mesenchymal transition. Nat Rev Mol Cell Biol, 15(3), 178–196. doi:10.1038/nrm3758 [PubMed: 24556840]

Land WG (2015a). The Role of Damage-Associated Molecular Patterns (DAMPs) in Human Diseases: Part II: DAMPs as diagnostics, prognostics and therapeutics in clinical medicine. Sultan Qaboos Univ Med J, 15(2), e157–170. [PubMed: 26052447]

Land WG (2015b). The Role of Damage-Associated Molecular Patterns in Human Diseases: Part I - Promoting inflammation and immunity. Sultan Qaboos Univ Med J, 15(1), e9–e21. [PubMed: 25685392]

Langen ES, Sit A, Sherwin K, Lyell DJ, Blumenfeld YJ, & El-Sayed YY (2018). A Double-Blind, Randomized, Placebo-Controlled Trial of 17 Alpha-hydroxyprogesterone Caproate in the Management of Preterm Premature Rupture of Membranes. Am J Perinatol, 35(8), 779–784. doi:10.1055/s-0037-1617428 [PubMed: 29298456]

Lappas M (2013). NOD1 and NOD2 regulate proinflammatory and prolabor mediators in human fetal membranes and myometrium via nuclear factor-kappa B. Biol. Reprod, 89(1), 14. doi:biolreprod.113.110056 [pii];10.1095/biolreprod.113.110056 [doi] [PubMed: 23740944]

Lash GE (2015). Molecular Cross-Talk at the Feto-Maternal Interface. Cold Spring Harb Perspect Med, 5(12). doi:10.1101/cshperspect.a023010

Lavu N, Richardson L, Radnaa E, Kechichian T, Urrabaz-Garza R, Sheller-Miller S, … Menon R (2019). Oxidative stress-induced downregulation of glycogen synthase kinase 3 beta in fetal membranes promotes cellular senescencedagger. Biol Reprod, 101(5), 1018–1030. doi:10.1093/biolre/ioz119 [PubMed: 31292604]

Lavu N, Sheller-Miller S, Kechichian T, Cayenne S, Bonney EA, & Menon R (2020). Changes in mediators of pro-cell growth, senescence, and inflammation during murine gestation. Am J Reprod Immunol, 83(3), e13214. doi:10.1111/aji.13214 [PubMed: 31814178]

Lim R, Barker G, & Lappas M (2014). The TLR2 ligand FSL-1 and the TLR5 ligand Flagellin mediate pro-inflammatory and pro-labour response via MyD88/TRAF6/NF-kappaB-dependent signalling. Am J Reprod Immunol, 71(5), 401–417. doi:10.1111/aji.12229 [PubMed: 24635133]

Liu J, Dong P, Wang S, & Li J (2019). Natural killer, natural killer T, helper and cytotoxic T cells in the decidua from recurrent spontaneous abortion with normal and abnormal chromosome karyotypes. Biochem Biophys Res Commun, 508(2), 354–360. doi:10.1016/j.bbrc.2018.11.156 [PubMed: 30503343]

Liu S, Diao L, Huang C, Li Y, Zeng Y, & Kwak-Kim JYH (2017). The role of decidual immune cells on human pregnancy. J Reprod Immunol, 124, 44–53. doi:10.1016/j.jri.2017.10.045 [PubMed: 29055791]

Lockwood CJ (1994). Recent advances in elucidating the pathogenesis of preterm delivery, the detection of patients at risk, and preventative therapies. Curr. Opin. Obstet. Gynecol, 6(1), 7–18. [PubMed: 8180354]

Longini M, Perrone S, Vezzosi P, Marzocchi B, Kenanidis A, Centini G, … Buonocore G (2007). Association between oxidative stress in pregnancy and preterm premature rupture of membranes. Clin. Biochem, 40(11), 793–797. doi:S0009-9120(07)00129-4 [pii];10.1016/j.clinbiochem.2007.03.004 [doi] [PubMed: 17442295]

Malak TM, Ockleford CD, Bell SC, Dalgleish R, Bright N, & Macvicar J (1993). Confocal immunofluorescence localization of collagen types I, III, IV, V and VI and their ultrastructural organization in term human fetal membranes. Placenta, 14(4), 385–406. [PubMed: 8248033]

Manuck TA, Esplin MS, Biggio J, Bukowski R, Parry S, Zhang H, … Proteomics Network for Preterm Birth, R. (2015). The phenotype of spontaneous preterm birth: application of a clinical phenotyping tool. Am J Obstet Gynecol, 212(4), 487 e481–487 e411. doi:10.1016/j.ajog.2015.02.010 [PubMed: 25687564]

Martin LF, Richardson LS, da Silva MG, Sheller-Miller S, & Menon R (2019). Dexamethasone induces primary amnion epithelial cell senescence through telomere-P21 associated pathwaydagger. Biol Reprod, 100(6), 1605–1616. doi:10.1093/biolre/ioz048 [PubMed: 30927408]

Masoro EJ (1995). Glucocorticoids and aging 1. Aging (Milano.), 7(6), 407–413. [PubMed: 8835077]

Mauri A, Ehret AE, Perrini M, Maake C, Ochsenbein-Kolble N, Ehrbar M, … Mazza E (2015). Deformation mechanisms of human amnion: Quantitative studies based on second harmonic generation microscopy. J BIomech, 48(9), 1606–1613. doi:10.1016/j.jbiomech.2015.01.045 [PubMed: 25805698]

Mauri A, Perrini M, Ehret AE, De Focatiis DS, & Mazza E (2015). Time-dependent mechanical behavior of human amnion: macroscopic and microscopic characterization. Acta Biomater, 11, 314–323. doi:10.1016/j.actbio.2014.09.012 [PubMed: 25240983]

McCabe ER, Carrino GE, Russell RB, & Howse JL (2014). Fighting for the next generation: US Prematurity in 2030. Pediatrics, 134(6), 1193–1199. doi:10.1542/peds.2014-2541 [PubMed: 25367536]

McIntire RH, Ganacias KG, & Hunt JS (2008). Programming of human monocytes by the uteroplacental environment. Reprod Sci, 15(5), 437–447. doi:10.1177/1933719107314065 [PubMed: 18579853]

Mendelson CR (2009). Minireview: fetal-maternal hormonal signaling in pregnancy and labor. Mol Endocrinol, 23(7), 947–954. doi:10.1210/me.2009-0016 [PubMed: 19282364]

Mendelson CR, Montalbano AP, & Gao L (2017). Fetal-to-maternal signaling in the timing of birth. J Steroid Biochem Mol Biol, 170, 19–27. doi:10.1016/j.jsbmb.2016.09.006 [PubMed: 27629593]

Menon R (2008). Spontaneous preterm birth, a clinical dilemma: etiologic, pathophysiologic and genetic heterogeneities and racial disparity. Acta Obstet. Gynecol. Scand, 87(6), 590–600. doi:791847679 [pii];10.1080/00016340802005126 [doi] [PubMed: 18568457]

Menon R (2014). Oxidative stress damage as a detrimental factor in preterm birth pathology 14. Front Immunol, 5, 567. doi:10.3389/fimmu.2014.00567 [doi] [PubMed: 25429290]

Menon R (2016). Human fetal membranes at term: Dead tissue or signalers of parturition? Placenta, 44, 1–5. doi:10.1016/j.placenta.2016.05.013 [PubMed: 27452431]

Menon R (2019). Initiation of human parturition: signaling from senescent fetal tissues via extracellular vesicle mediated paracrine mechanism. Obstet Gynecol Sci, 62(4), 199–211. doi:10.5468/ogs.2019.62.4.199 [PubMed: 31338337]

Menon R, Behnia F, Polettini J, Saade GR, Campisi J, & Velarde M (2016). Placental membrane aging and HMGB1 signaling associated with human parturition. Aging (Albany NY), 8(2), 216–230. doi:10.18632/aging.100891 [PubMed: 26851389]

Menon R, Boldogh I, Hawkins HK, Woodson M, Polettini J, Syed TA, … Taylor RN (2014). Histological evidence of oxidative stress and premature senescence in preterm premature rupture of the human fetal membranes recapitulated in vitro. Am J Pathol, 184(6), 1740–1751. doi:10.1016/j.ajpath.2014.02.011 [PubMed: 24832021]

Menon R, Boldogh I, Urrabaz-Garza R, Polettini J, Syed TA, Saade GR, … Taylor RN (2013). Senescence of primary amniotic cells via oxidative DNA damage. PLoS One, 8(12), e83416. doi:10.1371/journal.pone.0083416 [PubMed: 24386195]

Menon R, Bonney EA, Condon J, Mesiano S, & Taylor RN (2016). Novel concepts on pregnancy clocks and alarms: redundancy and synergy in human parturition. Hum Reprod Update, 22(5), 535–560. doi:10.1093/humupd/dmw022 [PubMed: 27363410]

Menon R, Debnath C, Lai A, Guanzon D, Bhatnagar S, Kshetrapal P, … Salomon C (2020). Protein Profile Changes in Circulating Placental Extracellular Vesicles in Term and Preterm Births: A Longitudinal Study. Endocrinology, 161(4). doi:10.1210/endocr/bqaa009

Menon R, Debnath C, Lai A, Guanzon D, Bhatnagar S, Kshetrapal PK, … Garbhini Study, T. (2019). Circulating Exosomal miRNA Profile During Term and Preterm Birth Pregnancies: A Longitudinal Study. Endocrinology, 160(2), 249–275. doi:10.1210/en.2018-00836 [PubMed: 30358826]

Menon R, & Fortunato SJ (2004). Fetal membrane inflammatory cytokines: a switching mechanism between the preterm premature rupture of the membranes and preterm labor pathways. J. Perinat. Med, 32(5), 391–399. doi:10.1515/JPM.2004.134 [doi] [PubMed: 15493713]

Menon R, Fortunato SJ, Milne GL, Brou L, Carnevale C, Sanchez SC, … Taylor RN (2011). Amniotic fluid eicosanoids in preterm and term births: effects of risk factors for spontaneous preterm labor. Obstet. Gynecol, 118(1), 121–134. doi:10.1097/AOG.0b013e3182204eaa [doi];00006250-201107000-00017 [pii] [PubMed: 21691170]

Menon R, McIntyre JO, Matrisian LM, & Fortunato SJ (2006). Salivary proteinase activity: a potential biomarker for preterm premature rupture of the membranes. Am. J. Obstet. Gynecol, 194(6), 1609–1615. doi:S0002-9378(06)00296-1 [pii];10.1016/j.ajog.2006.02.052 [doi] [PubMed: 16731078]

Menon R, Mesiano S, & Taylor RN (2017). Programmed Fetal Membrane Senescence and Exosome-Mediated Signaling: A Mechanism Associated With Timing of Human Parturition. Front Endocrinol (Lausanne), 8, 196. doi:10.3389/fendo.2017.00196 [PubMed: 28861041]

Menon R, & 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(1), 147–162. doi:10.1016/j.ogc.2019.10.004 [PubMed: 32008665]

Menon R, N. N, Bredeson S Polettini J (1907). Fetal Membranes: Potential Source of Preterm Birth Biomarkers. In P. V Preedy VR (Ed.), General Methods in Biomarker Research and Their Applications (pp. 483–529): Springer Science Publisher. (Reprinted from: Not in File).

Menon R, & Papaconstantinou J (2016). p38 Mitogen activated protein kinase (MAPK): a new therapeutic target for reducing the risk of adverse pregnancy outcomes. Expert Opin Ther Targets, 1–16. doi:10.1080/14728222.2016.1216980

Menon R, Polettini J, Syed TA, Saade GR, & Boldogh I (2014). Expression of 8-oxoguanine Glycosylase in Human Fetal Membranes. Am. J. Reprod. Immunol doi:10.1111/aji.12220 [doi]

Menon R, Richardson LS, & Lappas M (2018). Fetal membrane architecture, aging and inflammation in pregnancy and parturition. Placenta. doi:10.1016/j.placenta.2018.11.003

Menon R, & Taylor BD (2019). Exploring Inflammatory Mediators in Fetal and Maternal Compartments During Human Parturition. Obstet Gynecol, 134(4), 765–773. doi:10.1097/AOG.0000000000003470 [PubMed: 31503157]

Menon R, Taylor RN, & Fortunato SJ (2010). Chorioamnionitis--a complex pathophysiologic syndrome. Placenta, 31(2), 113–120. doi:10.1016/j.placenta.2009.11.012 [PubMed: 20031205]

Menon R, Yu J, Basanta-Henry P, Brou L, Berga SL, Fortunato SJ, & Taylor RN (2012a). Short fetal leukocyte telomere length and preterm prelabor rupture of the membranes. PLoS One,7(2), e31136. doi:10.1371/journal.pone.0031136 [PubMed: 22348044]

Menon R, Yu J, Basanta-Henry P, Brou L, Berga SL, Fortunato SJ, & Taylor RN (2012b). Short fetal leukocyte telomere length and preterm prelabor rupture of the membranes. PLoS. One, 7(2), e31136. doi:10.1371/journal.pone.0031136 [doi];PONE-D-11-21450 [pii] [PubMed: 22348044]

Mercer BM, Goldenberg RL, Meis PJ, Moawad AH, Shellhaas C, Das A, … McNellis D (2000). The Preterm Prediction Study: prediction of preterm premature rupture of membranes through clinical findings and ancillary testing. The National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. Am. J. Obstet. Gynecol, 183(3), 738–745. doi:S0002-9378(00)64569-6 [pii] [PubMed: 10992202]

Mercer BM, & Lewis R (1997). Preterm labor and preterm premature rupture of the membranes. Diagnosis and management. Infect. Dis. Clin. North Am, 11(1), 177–201. [PubMed: 9067791]

Mesiano S, Chan EC, Fitter JT, Kwek K, Yeo G, & Smith R (2002). Progesterone withdrawal and estrogen activation in human parturition are coordinated by progesterone receptor A expression in the myometrium. J. Clin. Endocrinol. Metab, 87(6), 2924–2930. doi:10.1210/jcem.87.6.8609 [doi] [PubMed: 12050275]

Mesiano S, Wang Y, & Norwitz ER (2011). Progesterone receptors in the human pregnancy uterus: do they hold the key to birth timing? Reprod. Sci, 18(1), 6–19. doi:1933719110382922 [pii];10.1177/1933719110382922 [doi] [PubMed: 20889955]

Millar LK, Stollberg J, DeBuque L, & Bryant-Greenwood G (2000). Fetal membrane distention: determination of the intrauterine surface area and distention of the fetal membranes preterm and at term. Am J Obstet Gynecol, 182(1 Pt 1), 128–134. [PubMed: 10649167]

Miyazaki C, Moreno Garcia R, Ota E, Swa T, Oladapo OT, & Mori R (2016). Tocolysis for inhibiting preterm birth in extremely preterm birth, multiple gestations and in growth-restricted fetuses: a systematic review and meta-analysis. Reprod Health, 13, 4. doi:10.1186/s12978-015-0115-7 [PubMed: 26762152]

Moco NP, Martin LF, Pereira AC, Polettini J, Peracoli JC, Coelho KI, & da Silva MG (2013). Gene expression and protein localization of TLR-1, −2, −4 and −6 in amniochorion membranes of pregnancies complicated by histologic chorioamnionitis. Eur J Obstet Gynecol Reprod Biol, 171(1), 12–17. doi:10.1016/j.ejogrb.2013.07.036 [PubMed: 24125907]

Mogami H, Hari Kishore A, Akgul Y, & Word RA (2017). Healing of Preterm Ruptured Fetal Membranes. Sci Rep, 7(1), 13139. doi:10.1038/s41598-017-13296-1 [PubMed: 29030612]

Mohan AR, Sooranna SR, Lindstrom TM, Johnson MR, & Bennett PR (2007). The effect of mechanical stretch on cyclooxygenase type 2 expression and activator protein-1 and nuclear factor-kappaB activity in human amnion cells. Endocrinology, 148(4), 1850–1857. doi:en.2006-1289 [pii];10.1210/en.2006-1289 [doi] [PubMed: 17218407]

Montalbano AP, Hawgood S, & Mendelson CR (2013). Mice deficient in surfactant protein A (SP-A) and SP-D or in TLR2 manifest delayed parturition and decreased expression of inflammatory and contractile genes. Endocrinology, 154(1), 483–498. doi:10.1210/en.2012-1797 [PubMed: 23183169]

Mor G (2008). Inflammation and pregnancy: the role of toll-like receptors in trophoblast-immune interaction. Ann N Y Acad Sci, 1127, 121–128. doi:10.1196/annals.1434.006 [PubMed: 18443339]

Mor G, Cardenas I, Abrahams V, & Guller S (2011). Inflammation and pregnancy: the role of the immune system at the implantation site. Ann N Y Acad Sci, 1221, 80–87. doi:10.1111/j.1749-6632.2010.05938.x [PubMed: 21401634]

Mor G, & Kwon JY (2015). Trophoblast-microbiome interaction: a new paradigm on immune regulation. Am J Obstet Gynecol, 213(4 Suppl), S131–137. doi:10.1016/j.ajog.2015.06.039 [PubMed: 26428492]

Muglia LJ, & Katz M (2010). The enigma of spontaneous preterm birth. N Engl J Med, 362(6), 529–535. doi:10.1056/NEJMra0904308 [PubMed: 20147718]

Murtha AP, & Menon R (2015). Regulation of fetal membrane inflammation: a critical step in reducing adverse pregnancy outcome. Am J Obstet Gynecol, 213(4), 447–448. doi:10.1016/j.ajog.2015.07.008 [PubMed: 26410204]

Myatt L (2010). Review: Reactive oxygen and nitrogen species and functional adaptation of the placenta. Placenta, 31 Suppl, S66–S69. doi:S0143-4004(09)00411-1 [pii];10.1016/j.placenta.2009.12.021 [doi] [PubMed: 20110125]

Myatt L, & Cui X (2004). Oxidative stress in the placenta. Histochem. Cell Biol, 122(4), 369–382. doi:10.1007/s00418-004-0677-x [doi] [PubMed: 15248072]

Myatt L, & Sun K (2010). Role of fetal membranes in signaling of fetal maturation and parturition. Int J Dev Biol, 54(2-3), 545–553. doi:10.1387/ijdb.082771lm [PubMed: 19924634]

Nancy P, & Erlebacher A (2012). [Epigenetic repression of chemokine expression at the maternal-fetal interface as a mechanism of feto-maternal tolerance]. Med Sci (Paris), 28(12), 1037–1039. doi:10.1051/medsci/20122812005 [PubMed: 23290395]

Negishi Y, Takahashi H, Kuwabara Y, & Takeshita T (2018a). Innate immune cells in reproduction. J Obstet Gynaecol Res. doi:10.1111/jog.13759

Negishi Y, Takahashi H, Kuwabara Y, & Takeshita T (2018b). Innate immune cells in reproduction. J Obstet Gynaecol Res, 44(11), 2025–2036. doi:10.1111/jog.13759 [PubMed: 30058156]

Nogami M, Kimura T, Seki S, Matsui Y, Yoshida T, Koike-Soko C, … Nikaido T (2016). A Human Amnion-Derived Extracellular Matrix-Coated Cell-Free Scaffold for Cartilage Repair: In Vitro and In Vivo Studies. Tissue Eng Part A, 22(7-8), 680–688. doi:10.1089/ten.TEA.2015.0285 [PubMed: 27019057]

Norwitz ER, Bonney EA, Snegovskikh VV, Williams MA, Phillippe M, Park JS, & Abrahams VM (2015). Molecular Regulation of Parturition: The Role of the Decidual Clock. Cold Spring Harb Perspect Med, 5(11). doi:10.1101/cshperspect.a023143

Olson DM, Zaragoza DB, Shallow MC, Cook JL, Mitchell BF, Grigsby P, & Hirst J (2003). Myometrial activation and preterm labour: evidence supporting a role for the prostaglandin F receptor--a review. Placenta, 24 Suppl A, S47–S54. doi:10.1053/plac.2002.0938 [PubMed: 12842413]

Osman I, Young A, Jordan F, Greer IA, & Norman JE (2006). Leukocyte density and proinflammatory mediator expression in regional human fetal membranes and decidua before and during labor at term. J Soc Gynecol Investig, 13(2), 97–103. doi:10.1016/j.jsgi.2005.12.002

Padron JG, Saito Reis CA, & Kendal-Wright CE (2020). The Role of Danger Associated Molecular Patterns in Human Fetal Membrane Weakening. Front Physiol, 11, 602. doi:10.3389/fphys.2020.00602 [PubMed: 32625109]

Park JS, Park CW, Lockwood CJ, & Norwitz ER (2005). Role of cytokines in preterm labor and birth. Minerva Ginecol, 57(4), 349–366. [PubMed: 16170281]

Parmley T (1984). Placental senescence. Adv. Exp. Med. Biol, 176, 127–132. [PubMed: 6496213]

Pavlicev M, & Norwitz ER (2018). Human Parturition: Nothing More Than a Delayed Menstruation. Reprod Sci, 25(2), 166–173. doi:10.1177/1933719117725830 [PubMed: 28826363]

Pei D, Shu X, Gassama-Diagne A, & Thiery JP (2019). Mesenchymal-epithelial transition in development and reprogramming. Nat Cell Biol, 21(1), 44–53. doi:10.1038/s41556-018-0195-z [PubMed: 30602762]

Peltier MR (2003). Immunology of term and preterm labor. Reprod Biol Endocrinol, 1, 122. doi:10.1186/1477-7827-1-122 [PubMed: 14651749]

Petraglia F, Arcuri F, de Ziegler D, & Chapron C (2012). Inflammation: a link between endometriosis and preterm birth. Fertil Steril, 98(1), 36–40. doi:10.1016/j.fertnstert.2012.04.051 [PubMed: 22658345]

Phillippe M, Sawyer MR, & Edelson PK (2019). The telomere gestational clock: increasing short telomeres at term in the mouse. Am J Obstet Gynecol, 220(5), 496 e491–496 e498. doi:10.1016/j.ajog.2019.01.218 [PubMed: 30690015]

Polettini J, Behnia F, Taylor BD, Saade GR, Taylor RN, & Menon R (2015a). Telomere Fragment Induced Amnion Cell Senescence: A Contributor to Parturition? PLoS. One, 10(9), e0137188. doi:10.1371/journal.pone.0137188 [doi];PONE-D-15-22883 [pii] [PubMed: 26397719]

Polettini J, Behnia F, Taylor BD, Saade GR, Taylor RN, & Menon R (2015b). Telomere Fragment Induced Amnion Cell Senescence: A Contributor to Parturition? PLoS One, 10(9), e0137188. doi:10.1371/journal.pone.0137188 [PubMed: 26397719]

Polettini J, Richardson LS, & Menon R (2018). Oxidative stress induces senescence and sterile inflammation in murine amniotic cavity. Placenta, 63, 26–31. doi:10.1016/j.placenta.2018.01.009 [PubMed: 29486853]

Polettini J, Silva MG, Kacerovsky M, Syed TA, Saade GR, & Menon R (2016). Screening of lysyl oxidase (LOX) and lysyl oxidase like (LOXL) enzyme expression and activity in preterm prelabor rupture of fetal membranes. J Perinat Med, 44(1), 99–109. doi:10.1515/jpm-2014-0337 [PubMed: 26011922]

Presicce P, Senthamaraikannan P, Alvarez M, Rueda CM, Cappelletti M, Miller LA, … Kallapur SG (2015). Neutrophil recruitment and activation in decidua with intra-amniotic IL-1beta in the preterm rhesus macaque. Biol Reprod, 92(2), 56. doi:10.1095/biolreprod.114.124420 [PubMed: 25537373]

Reinl EL, & England SK (2015). Fetal-to-maternal signaling to initiate parturition 1. J Clin Invest, 125(7), 2569–2571. doi:82576 [pii];10.1172/JCI82576 [doi] [PubMed: 26098207]

Richardson L, Dixon CL, Aguilera-Aguirre L, & Menon R (2018). Oxidative Stress-Induced TGF-beta/TAB1-mediated p38MAPK activation in human amnion epithelial cells. Biol Reprod. doi:10.1093/biolre/ioy135

Richardson L, Gnecco J, Ding T, Osteen K, Rogers LM, Aronoff DM, & Menon R (2019). Fetal Membrane Organ-On-Chip: An Innovative Approach to Study Cellular Interactions. Reprod Sci, 1933719119828084. doi:10.1177/1933719119828084 [PubMed: 30791822]

Richardson L, & Menon R (2018). Proliferative, Migratory, and Transition Properties Reveal Metastate of Human Amnion Cells. Am J Pathol, 188(9), 2004–2015. doi:10.1016/j.ajpath.2018.05.019 [PubMed: 29981743]

Richardson LS, Taylor RN, & Menon R (2020). Reversible EMT and MET mediate amnion remodeling during pregnancy and labor. Sci Signal, 13(618). doi:10.1126/scisignal.aay1486

Richardson LS, Vargas G, Brown T, Ochoa L, Sheller-Miller S, Saade GR, … Menon R (2017). Discovery and Characterization of Human Amniochorionic Membrane Microfractures. Am J Pathol. doi:10.1016/j.ajpath.2017.08.019

Rinaldi SF, Makieva S, Saunders PT, Rossi AG, & Norman JE (2017). Immune cell and transcriptomic analysis of the human decidua in term and preterm parturition. Mol Hum Reprod, 23(10), 708–724. doi:10.1093/molehr/gax038 [PubMed: 28962035]

Robins JC, Marsit CJ, Padbury JF, & Sharma SS (2011). Endocrine disruptors, environmental oxygen, epigenetics and pregnancy. Front Biosci (Elite Ed), 3, 690–700. [PubMed: 21196344]

Rodier F, Kim SH, Nijjar T, Yaswen P, & Campisi J (2005). Cancer and aging: the importance of telomeres in genome maintenance. Int. J. Biochem. Cell Biol, 37(5), 977–990. doi:S1357-2725(04)00380-2 [pii];10.1016/j.biocel.2004.10.012 [doi] [PubMed: 15743672]

Roh JS, & Sohn DH (2018). Damage-Associated Molecular Patterns in Inflammatory Diseases. Immune Netw, 18(4), e27. doi:10.4110/in.2018.18.e27 [PubMed: 30181915]

Romero R, Dey SK, & Fisher SJ (2014a). Preterm labor: one syndrome, many causes. Science, 345(6198), 760–765. doi:345/6198/760 [pii];10.1126/science.1251816 [doi] [PubMed: 25124429]

Romero R, Dey SK, & Fisher SJ (2014b). Preterm labor: one syndrome, many causes. Science, 345(6198), 760–765. doi:10.1126/science.1251816 [PubMed: 25124429]

Romero R, Espinoza J, Kusanovic JP, Gotsch F, Hassan S, Erez O, … Mazor M (2006). The preterm parturition syndrome. BJOG, 113 Suppl 3, 17–42. doi:10.1111/j.1471-0528.2006.01120.x

Romero R, Gotsch F, Pineles B, & Kusanovic JP (2007). Inflammation in pregnancy: its roles in reproductive physiology, obstetrical complications, and fetal injury. Nutr. Rev, 65(12 Pt 2), S194–S202. [PubMed: 18240548]

Rosen T, Kuczynski E, O'Neill LM, Funai EF, & Lockwood CJ (2001). Plasma levels of thrombin-antithrombin complexes predict preterm premature rupture of the fetal membranes. J. Matern. Fetal Med, 10(5), 297–300. [PubMed: 11730490]

Rubens CE, Sadovsky Y, Muglia L, Gravett MG, Lackritz E, & Gravett C (2014). Prevention of preterm birth: harnessing science to address the global epidemic. Sci Trans! Med, 6(262), 262sr265. doi:10.1126/scitranslmed.3009871

Ruiz-Bonilla V, Perdiguero E, Gresh L, Serrano AL, Zamora M, Sousa-Victor P, … Munoz-Canoves P (2008). Efficient adult skeletal muscle regeneration in mice deficient in p38beta, p38gamma and p38delta MAP kinases. Cell Cycle, 7(14), 2208–2214. doi:10.4161/cc.7.14.6273 [PubMed: 18641461]

Saito S, Lin YC, Murayama Y, Hashimoto K, & Yokoyama KK (2012). Human amnion-derived cells as a reliable source of stem cells 19. Curr. Mol. Med, 12(10), 1340–1349. doi:CMM-EPUB-20120924-2 [pii] [PubMed: 23016591]

Sanders JL, & Newman AB (2013). Telomere Length in Epidemiology: A Biomarker of Aging, Age-Related Disease, Both, or Neither? Epidemiol. Rev doi:mxs008 [pii];10.1093/epirev/mxs008 [doi]

Santoni G, Cardinali C, Morelli MB, Santoni M, Nabissi M, & Amantini C (2015). Danger- and pathogen-associated molecular patterns recognition by pattern-recognition receptors and ion channels of the transient receptor potential family triggers the inflammasome activation in immune cells and sensory neurons. J. Neuroinflammation, 12, 21. doi:10.1186/s12974-015-0239-2 [doi];s12974-015-0239-2 [pii] [PubMed: 25644504]

Sato BL, Collier ES, Vermudez SA, Junker AD, & Kendal-Wright CE (2016). Human amnion mesenchymal cells are pro-inflammatory when activated by the Toll-like receptor 2/6 ligand, macrophage-activating lipoprotein-2. Placenta, 44, 69–79. doi:10.1016/j.placenta.2016.06.005 [PubMed: 27452440]

Schliefsteiner C, Peinhaupt M, Kopp S, Logl J, Lang-Olip I, Hiden U, … Wadsack C (2017). Human Placental Hofbauer Cells Maintain an Anti-inflammatory M2 Phenotype despite the Presence of Gestational Diabetes Mellitus. Front Immunol, 8, 888. doi:10.3389/fimmu.2017.00888 [PubMed: 28824621]

Schmitz T, Sentilhes L, Lorthe E, Gallot D, Madar H, Doret-Dion M, … Kayem G (2019). Preterm premature rupture of the membranes: Guidelines for clinical practice from the French College of Gynaecologists and Obstetricians (CNGOF). Eur J Obstet Gynecol Reprod Biol, 236, 1–6. doi:10.1016/j.ejogrb.2019.02.021 [PubMed: 30870741]

Schumacher A, Sharkey DJ, Robertson SA, & Zenclussen AC (2018). Immune Cells at the Fetomaternal Interface: How the Microenvironment Modulates Immune Cells To Foster Fetal Development. J Immunol, 201(2), 325–334. doi:10.4049/jimmunol.1800058 [PubMed: 29987001]

Seferovic MD, Pace RM, Carroll M, Belfort B, Major AM, Chu DM, … Aagaard KM (2019). Visualization of microbes by 16S in situ hybridization in term and preterm placentas without intraamniotic infection. Am J Obstet Gynecol, 221(2), 146 e141–146 e123. doi:10.1016/j.ajog.2019.04.036 [PubMed: 31055031]

Sharp AN, Heazell AE, Crocker IP, & Mor G (2010). Placental apoptosis in health and disease. Am J Reprod Immunol, 64(3), 159–169. doi:10.1111/j.1600-0897.2010.00837.x [PubMed: 20367628]

Sheller-Miller S, Trivedi J, Yellon SM, & Menon R (2019). Exosomes Cause Preterm Birth in Mice: Evidence for Paracrine Signaling in Pregnancy. Sci Rep, 9(1), 608. doi:10.1038/s41598-018-37002-x [PubMed: 30679631]

Sheller-Miller S, Urrabaz-Garza R, Saade G, & Menon R (2017). Damage-Associated molecular pattern markers HMGB1 and cell-Free fetal telomere fragments in oxidative-Stressed amnion epithelial cell-Derived exosomes. J Reprod Immunol, 123, 3–11. doi:10.1016/j.jri.2017.08.003 [PubMed: 28858636]

Shynlova O, Tsui P, Jaffer S, & Lye SJ (2009). Integration of endocrine and mechanical signals in the regulation of myometrial functions during pregnancy and labour. Eur J Obstet Gynecol Reprod Biol, 144 Suppl 1, S2–10. doi:10.1016/j.ejogrb.2009.02.044 [PubMed: 19299064]

Simhan HN, & Canavan TP (2005). Preterm premature rupture of membranes: diagnosis, evaluation and management strategies. BJOG, 112 Suppl 1, 32–37. doi:BJO00582 [pii]; 10.1111/j.1471-0528.2005.00582.x [doi] [PubMed: 15715592]

Sinkey RG, Guzeloglu-Kayisli O, Arlier S, Guo X, Semerci N, Moore R, … Lockwood CJ (2020). Thrombin-Induced Decidual Colony-Stimulating Factor-2 Promotes Abruption-Related

Preterm Birth by Weakening Fetal Membranes. Am J Pathol, 190(2), 388–399. doi:10.1016/j.ajpath.2019.10.020 [PubMed: 31955792]

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(1-2), 215–220. [PubMed: 9786463]

Smith R, Mesiano S, & McGrath S (2002). Hormone trajectories leading to human birth. Regul Pept, 108(2-3), 159–164. [PubMed: 12220740]

Smith R, & Nicholson RC (2007). Corticotrophin releasing hormone and the timing of birth. Front Biosci, 12, 912–918. doi:2113 [pii] [PubMed: 17127348]

Snegovskikh VV, Schatz F, Arcuri F, Toti P, Kayisli UA, Murk W, … Norwitz ER (2009). Intra- amniotic infection upregulates decidual cell vascular endothelial growth factor (VEGF) and neuropilin-1 and −2 expression: implications for infection-related preterm birth. Reprod. Sci, 16(8), 767–780. doi:1933719109336623 [pii];10.1177/1933719109336623 [doi] [PubMed: 19474288]

Song D, & Shi Y (2014). Immune system modifications and feto-maternal immune tolerance. Chin Med J (Engl), 127(17), 3171–3180. [PubMed: 25189965]

Southcombe J, Tannetta D, Redman C, & Sargent I (2011). The immunomodulatory role of syncytiotrophoblast microvesicles 40. PLoS. One, 6(5), e20245. doi:10.1371/journal.pone.0020245 [doi];PONE-D-11-01938 [pii] [PubMed: 21633494]

Strauss JF 3rd. (2013). Extracellular matrix dynamics and fetal membrane rupture. Reprod Sci, 20(2), 140–153. doi:10.1177/1933719111424454 [PubMed: 22267536]

Sun K, He P, & Yang K (2002). Intracrine induction of 11beta-hydroxysteroid dehydrogenase type 1 expression by glucocorticoid potentiates prostaglandin production in the human chorionic trophoblast. Biol Reprod, 67(5), 1450–1455. doi:10.1095/biolreprod.102.005892 [PubMed: 12390875]

Svensson-Arvelund J, Mehta RB, Lindau R, Mirrasekhian E, Rodriguez-Martinez H, Berg G, … Ernerudh J (2015). The human fetal placenta promotes tolerance against the semiallogeneic fetus by inducing regulatory T cells and homeostatic M2 macrophages. J Immunol, 194(4), 1534–1544. doi:10.4049/jimmunol.1401536 [PubMed: 25560409]

Szekeres-Bartho J (2002). Immunological relationship between the mother and the fetus. Int Rev Immunol, 21(6), 471–495. [PubMed: 12650238]

Tattersall M, Engineer N, Khanjani S, Sooranna SR, Roberts VH, Grigsby PL, … Johnson MR (2008). Pro-labour myometrial gene expression: are preterm labour and term labour the same? Reproduction, 135(4), 569–579. doi:10.1530/REP-07-0461 [PubMed: 18367515]

Thornburg KL (2015). The programming of cardiovascular disease. J Dev Orig Health Dis, 6(5), 366–376. doi:10.1017/S2040174415001300 [PubMed: 26173733]

Trivedi S, Joachim M, McElrath T, Kliman HJ, Allred EN, Fichorova RN, … Extremely Low Gestational Age Newborns study, i. (2012). Fetal-placental inflammation, but not adrenal activation, is associated with extreme preterm delivery. Am J Obstet Gynecol, 206(3), 236 e231–238. doi:10.1016/j.ajog.2011.12.004 [PubMed: 22264652]

Vicovac L, & Aplin JD (1996). Epithelial-mesenchymal transition during trophoblast differentiation. Acta Anat (Basel), 156(3), 202–216. doi:10.1159/000147847 [PubMed: 9124037]

Villar J, Papageorghiou AT, Knight HE, Gravett MG, Iams J, Waller SA, … Goldenberg RL (2012). The preterm birth syndrome: a prototype phenotypic classification. Am. J. Obstet. Gynecol, 206(2), 119–123. doi:S0002-9378(11)02171-5 [pii];10.1016/j.ajog.2011.10.866 [doi] [PubMed: 22177191]

Vink JY, Qin S, Brock CO, Zork NM, Feltovich HM, Chen X, … Gallos G (2016). A new paradigm for the role of smooth muscle cells in the human cervix. Am J Obstet Gynecol, 215(4), 478 e471–478 e411. doi:10.1016/j.ajog.2016.04.053 [PubMed: 27166013]

Vogel JP, Nardin JM, Dowswell T, West HM, & Oladapo OT (2014). Combination of tocolytic agents for inhibiting preterm labour. Cochrane Database Syst Rev(7), CD006169. doi:10.1002/14651858.CD006169.pub2 [PubMed: 25010869]

von ZT (2003). Replicative senescence and the art of counting 20. Exp. Gerontol, 38(11-12), 1259–1264. doi:S0531556503002316 [pii] [PubMed: 14698805]

von ZT, & Martin-Ruiz CM (2005). Telomeres as biomarkers for ageing and age-related diseases. Curr. Mol. Med, 5(2), 197–203. [PubMed: 15974873]

Wadhwa PD, Culhane JF, Rauh V, & Barve SS (2001). Stress and preterm birth: neuroendocrine, immune/inflammatory, and vascular mechanisms. Matern Child Health J, 5(2), 119–125. [PubMed: 11573837]

Wallack L, & Thornburg K (2016). Developmental Origins, Epigenetics, and Equity: Moving Upstream. Matern Child Health J, 20(5), 935–940. doi:10.1007/s10995-016-1970-8 [PubMed: 27029539]

Whittle WL, Gibb W, & Challis JR (2000). The characterization of human amnion epithelial and mesenchymal cells: the cellular expression, activity and glucocorticoid regulation of prostaglandin output. Placenta, 21(4), 394–401. doi:10.1053/plac.1999.0482 [PubMed: 10833375]

Xu Y, Romero R, Miller D, Silva P, Panaitescu B, Theis KR, … Gomez-Lopez N (2018). Innate lymphoid cells at the human maternal-fetal interface in spontaneous preterm labor. Am J Reprod Immunol, 79(6), e12820. doi:10.1111/aji.12820 [PubMed: 29457302]

Yellon SM (2017). Contributions to the dynamics of cervix remodeling prior to term and preterm birth. Biol Reprod, 96(1), 13–23. doi:10.1095/biolreprod.116.142844 [PubMed: 28395330]

Zhang J, Shynlova O, Sabra S, Bang A, Briollais L, & Lye SJ (2017). Immunophenotyping and activation status of maternal peripheral blood leukocytes during pregnancy and labour, both term and preterm. J Cell Mol Med, 21(10), 2386–2402. doi:10.1111/jcmm.13160 [PubMed: 28429508]
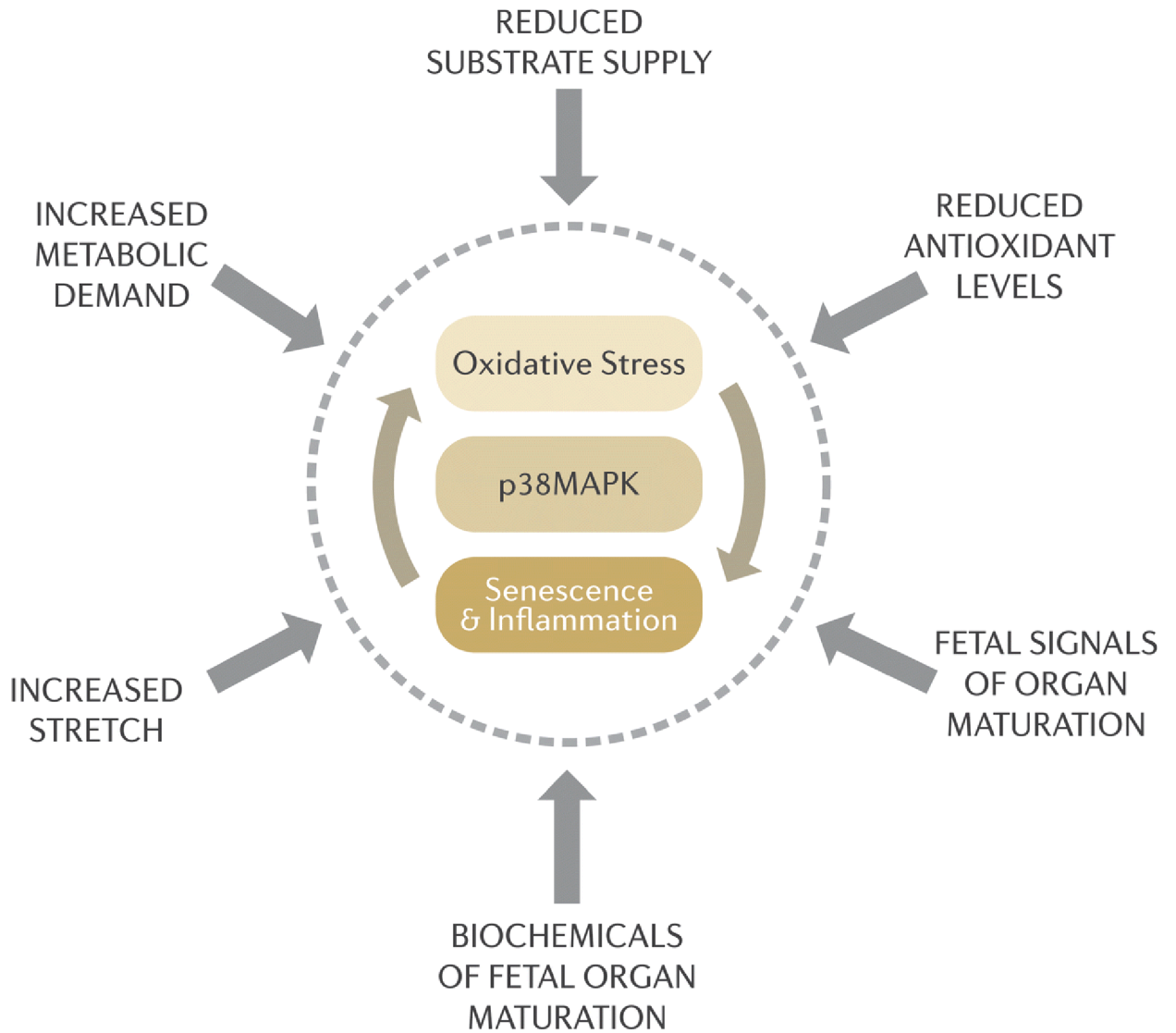
Figure 1: Oxidative stress induces p38MAPK mediated activation of senescence and inflammation in fetal membranes at term.

Various factors as shown in the figure can contribute to excessive reactive oxygen species (ROS) build-up in the intra-amniotic cavity. This ROS can accelerate fetal membrane senescence and senescence-associated secretory phenotype (SASP). Senescence and SASP factors, in a feedback loop, can cause further damage to non-senescent and neighboring tissues to cause further enhanced inflammation. This is a natural and physiological process during normal parturition.
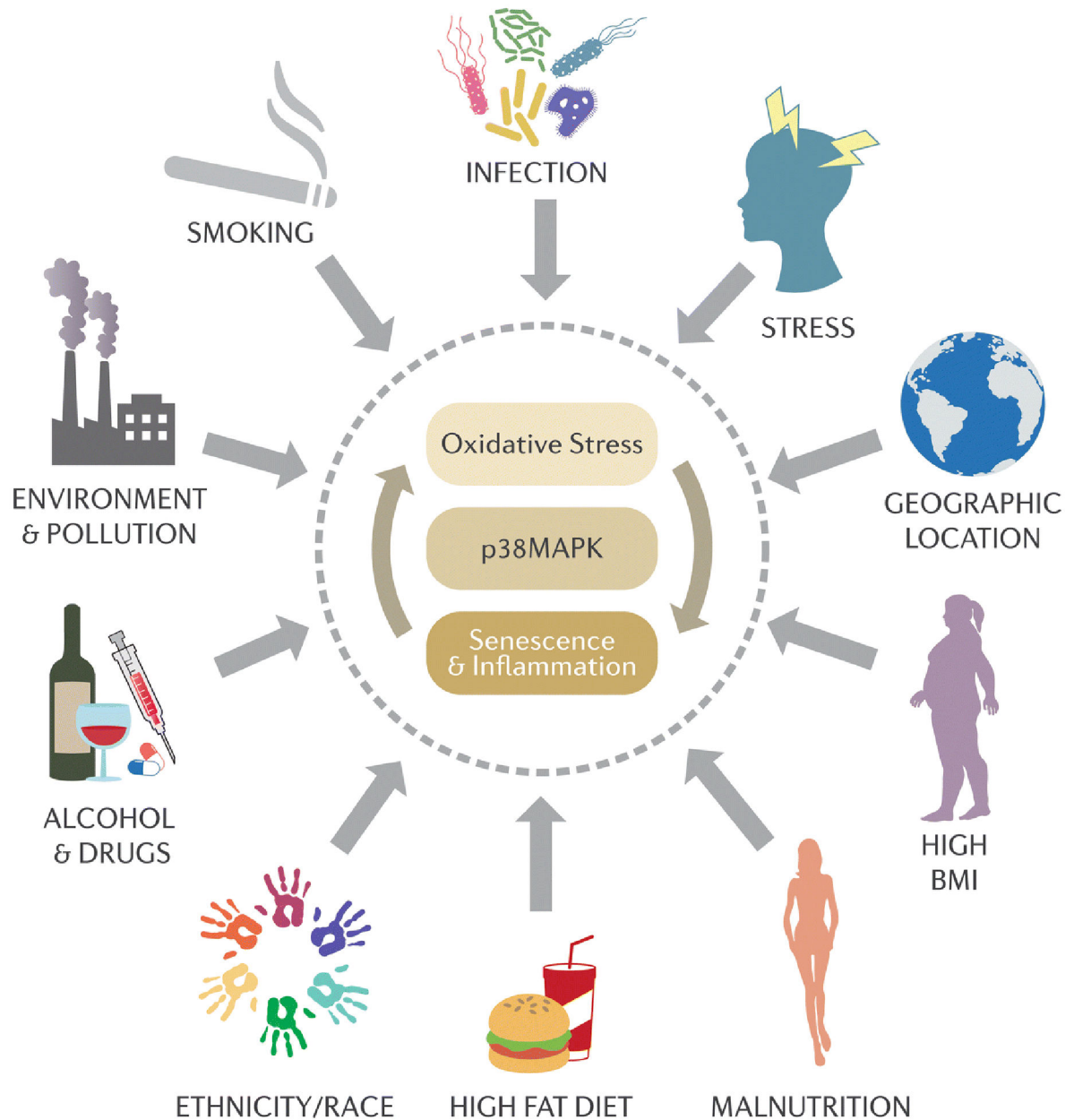
Figure 2: Pregnancy risk factors increase oxidative stress and cause increased activation of p38MAPK, senescence and inflammation in fetal membranes in preterm pregnancies.
Multiple risk factors can cause an increase in ROS in the intra-amniotic cavity. Pathways of ROS generation, characteristics of oxidative stress and p38MAPK activation may not be the same for all risk factors. Regardless, many of these factors can increase p38MAPK activation pathologically prior to term. Senescence and SASP factors can cause preterm labor and or pPROM. Premature activation of p38MAPK is the pathological activation of senescence leading to preterm parturition.
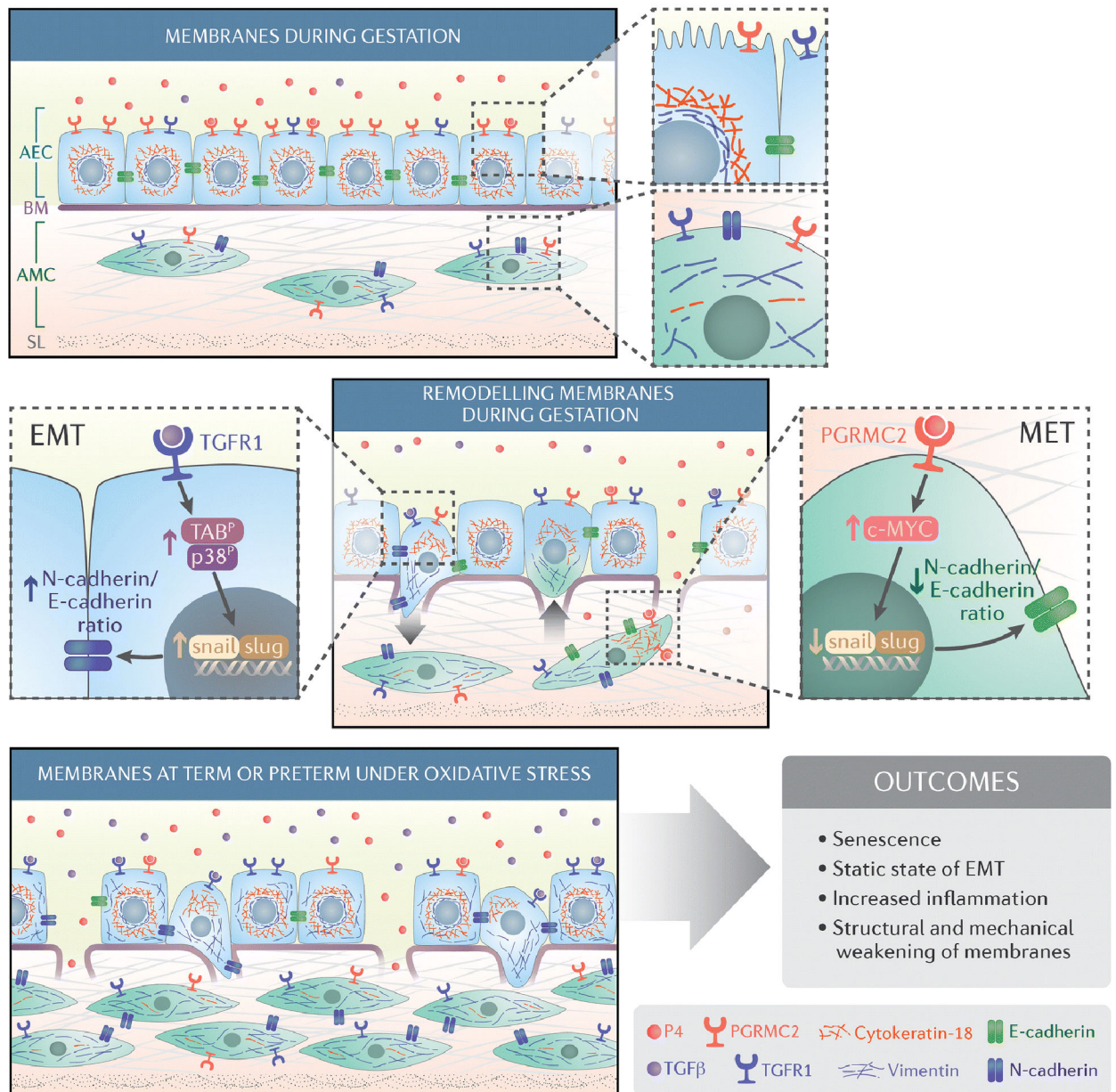
Figure 3: Schematics of changes to membrane structure during normal gestation and parturition.

Top panel: Membranes during gestation: Fetal membranes, specifically amnion layer undergoes a cyclic transition of EMT←→MET that maintains membrane homeostasis and a 10:1 ratio between AEC and AMC.

Middle panel: During gestation, the TGF-b/TAB/p38MAPK-mediated pathway forces the EMT to shed AECs which are transformed into AMC. Since the accumulation of AMC is an unstable state, these cells are transitioned back to AMC by the P4/PGRMC2/c-MYC pathway.

Bottom panel: At term, ROS buildup and p38MAPK activation (see Figure 1), can lead to a terminal state of EMT with the accumulation of AMC and no MET to balance the cell

ratio between AEC and AMC. This is an unstable state of inflammation and cause local inflammatory build-up, matrix degradation and membrane weakening.
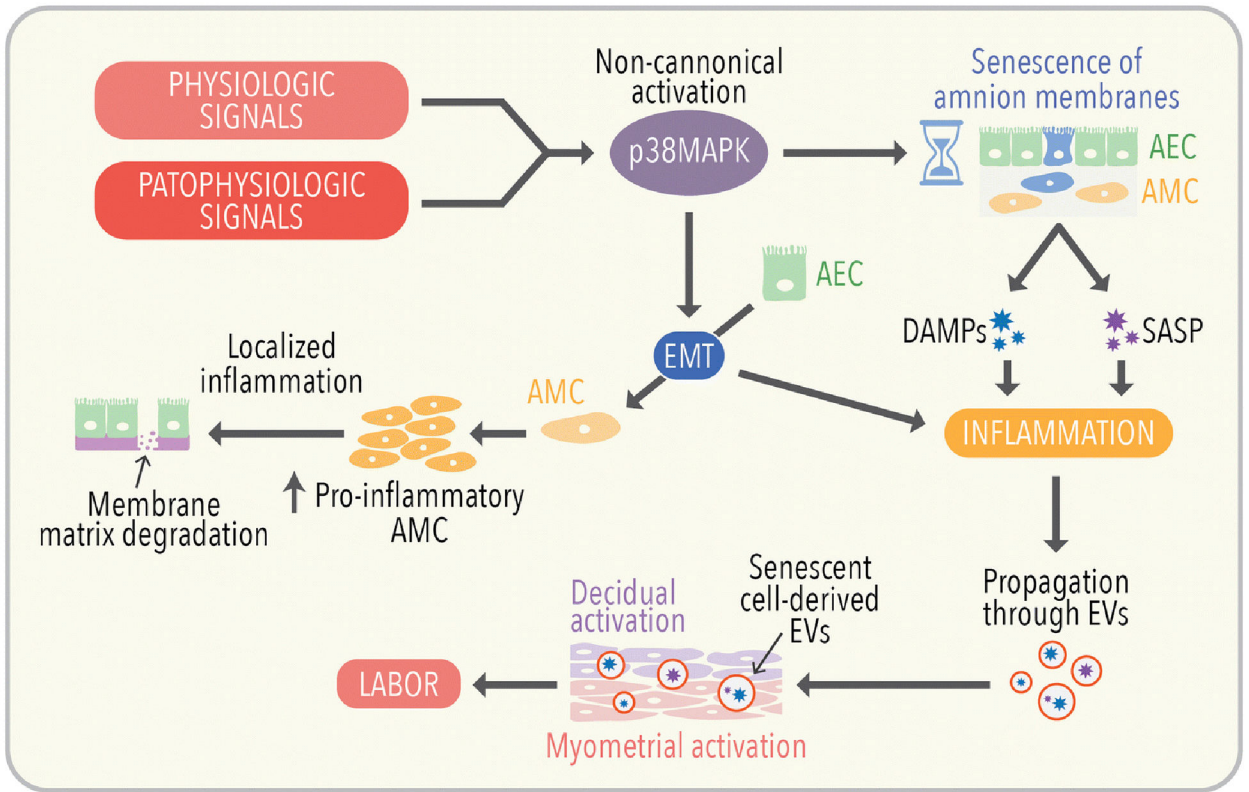
Figure 4: Two distinct mechanisms of inflammatory activation in fetal membranes and fetal inflammatory signaling for parturition.

Physiologic (Fig 1) or pathologic (Fig 2) signals increase ROS in the intra-amniotic cavity and cause non-canonical activation of p38MAPK in human fetal membrane cells. p38MAPK activation can lead to:

1. Senescence of the fetal membrane cells, the production of SASP and the generation of DAMPS.

2. p38MAPK forces a terminal state of EMT and the accumulation of AMCs, causing membrane matrix damage and weakening along with increased localized inflammation. Inflammatory mediators generated are packaged into extracellular vesicles released by fetal membrane cells – fetal signals – that can reach the myometrium and decidua and cause their activation (inflammation) which can transition these tissues from their quiescent state to an active state of labor.