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The physiology of fetal membrane weakening and rupture: Insights gained from the determination of physical properties *revisited*

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ABSTRACT

Rupture of the fetal membranes (FM) is precipitated by stretch forces acting upon biochemically mediated, pre-weakened tissue. Term FM develop a para-cervical weak zone, characterized by collagen remodeling and apoptosis, within which FM rupture is thought to initiate. Preterm FM also have a weak region but are stronger overall than term FM. Inflammation/infection and decidual bleeding/abruption are strongly associated with preterm premature FM rupture (pPROM), but the specific mechanisms causing FM weakening-rupture in pPROM are unknown. There are no animal models for study of FM weakening and rupture. Over a decade ago we developed equipment and methodology to test human FM strength and incorporated it into a FM explant system to create an *in-vitro* human FM weakening model system. Within this model TNF (modeling inflammation) and Thrombin (modeling bleeding) both weaken human FM with concomitant up regulation of MMP9 and cellular apoptosis, mimicking the characteristics of the spontaneous FM rupture site. The model has been enhanced so that test agents can be applied directionally to the choriodecidual side of the FM explant consistent with the *in-vivo* situation. With this enhanced system we have demonstrated that the pathways involving inflammation/TNF and bleeding/Thrombin induced FM weakening overlap. Furthermore GM-CSF production was demonstrated to be a critical common intermediate step in both the TNF and the Thrombin induced FM weakening pathways. This model system has also been used to test potential inhibitors of FM weakening and therefore pPROM. The dietary supplement α-lipoic acid and progestogens (P4, MPA and 17α-hydroxyprogesterone) have been shown to inhibit both TNF and Thrombin induced FM weakening. The progestogens act at multiple points by inhibiting both GM-CSF production and GM-CSF action. The use of a combined biomechanical/biochemical *in-vitro* human FM weakening model system has allowed the pathways of fetal membrane weakening to be delineated, and agents that may be of clinical use in inhibiting these pathways to be tested.

Contents

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1. Background

1.1. Introduction

Normal pregnancy requires that the physical integrity of the fetal membranes be maintained until term delivery. However, premature failure of the FM-aka pPROM is responsible for nearly 40% of all preterm births [1]. Preterm birth, in turn, is a major global public health problem with the estimated 14.9 million preterm infants born each year being responsible for 35% of the world's 3.1 million annual neonatal deaths [2,3]. This is the second largest direct cause of death in children less than 5 years [2,3]. The preterm birth rate in the US was particularly high at 12% in 2010, making the US one of the ten countries globally with the highest number of preterm births [2]. The most recent estimates show some progress with the latest estimates at 9.57% [4].

The study of the rupture of fetal membranes (FM) has generally been pursued largely from a cell biology and tissue histology perspective. Although this approach has provided certain insight, the practical bottom line is how very macroscopic changes to FM physical properties develop and result in a large defect in what had previously been intact tissue. Any theory of the etiology of FM rupture must, therefore, ultimately be tested with a model in which changes to tissue physical properties can be demonstrated. We have developed a line of work integrating biochemical and histological studies with biomechanical testing of FM strength. This has resulted in unique insights into the FM rupture process and is the subject of this review.

1.2. Rupture of the FM and the onset of labor

Rupture of membranes, although part of and necessary for the delivery process, is likely controlled separately from uterine contractions [5–7]. In the majority of women who deliver at term, the labor process initiates with contractions. This is followed by spontaneous FM rupture (SROM), or commonly by artificial FM rupture (AROM) prior to delivery. In at least 10% of women, however, contractions follow the rupture of membranes. More significantly, in pregnancies delivered preterm, approximately 40% have ruptured membranes prior to the onset of contractions [1,8,9]. Acute inflammation associated increased proteolytic enzymes and activation of cytokines likely play an important role in preterm births following pPROM; 26–50% placentas following pPROM show acute chorioamnionitis [10]. Rates of bacterial colonization of second trimester placental parenchyma may be up to 79% in deliveries at 23 weeks gestation decreasing to 43% at 27 weeks [11]. Until recently it was believed that inflammation leading to preterm birth was always maternal in origin. Recent data suggest fetal origin of inflammation is also possible, especially fetal gut microbe-derived, contributing to preterm birth [12,13]. There is a significant body of literature highlighting differences between patients who have early onset contractions and those with pPROM, supporting the idea that developmental events leading to early contractions are different from those leading to early rupture of the membranes [5–7,14].

1.3. Evidence for a programmed weakening process leading to ROM

Stretch forces alone are not entirely responsible for FM weakening as the force generated by contractions are not adequate to rupture FM without pre-weakening (15). More likely, the rupture of membranes is the result of a remodeling-maturation process analogous to that seen in the cervix. In both cervix and amnion changes in collagen type and matrix cause initial structural weakening, which is then followed by cellular apoptosis. These changes in FM have been documented in animal models [16–18]. Rat FM demonstrate globalized amnion cellular apoptosis and collagen remodeling [17]. However, human FM from term deliveries do not have these global changes, but rather develop a focal area overlying the cervix that exhibits morphological changes with collagen remodeling and apoptosis [19–26]. These findings have been replicated and extended in our studies [27,28].

1.4. Changes in tissue biochemistry and weakness

Numerous apoptotic activators present in amniotic fluid increase with gestation, with infection, and even with rupture of membranes, and induce apoptosis in FM derived cells and in intact membranes [29–34]. Most also increase transcription or activation of MMPs, especially MMP9 [32,35,36]. Synergy between MMP activation and apoptotic change leading to rupture of the FM has been proposed [17,25,37,38]. Promoter polymorphisms of some cytokines (TNF and IL-1) and MMPs (-1,-8,-9) have also been associated with patients with pPROM [39–42].

Although it had been generally assumed that collagen