Term Human Fetal Membranes Have a Weak Zone Overlying the Lower Uterine Pole and Cervix Before Onset of Labor

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ABSTRACT

The etiology of fetal membrane (FM) rupture is unknown. A hypothesis that the FM weakens by a process of collagen remodeling and apoptosis to facilitate rupture has been proposed. Human FMs reportedly exhibit a zone of altered histology, postulated to be the FM rupture site, but concomitant FM weakness has not been demonstrated. We hypothesized that a discrete zone of FM with marked weakness, histological change, and evidence of remodeling and apoptosis, develops in late gestation in the FM overlying the cervix. FM tissue from women undergoing prelabor cesarean delivery were peripherally marked to identify the FM overlying the cervix, cut with a procedure that facilitates remapping the rupture strength of FM pieces to their former location and orientation on a three-dimensional model, and tested for strength. A 10-cm FM zone centered at the cervical mark was compared with the remaining FM. Mean rupture strength within the cervical zone was 55% of the remaining FM. The cervical zone also exhibited increased MMP-9 protein, decreased tissue inhibitor of metalloproteinases-3 (TIMP-3) protein, and increased PARP cleavage coincident with the previously reported zone of altered histology. A discrete zone of weakness is present in term prelabor FMs overlying the cervix and has biochemical characteristics consistent with tissue remodeling and apoptosis.

INTRODUCTION

Rupture of the fetal membranes (ROM) is an event integral to the onset and development of labor. Rupture of membranes usually follows uterine contractions. In at least 10% of term labor and nearly 40% of premature labor, however, ROM precedes the initiation of contractions [1]. Because ROM is a major initiator of premature delivery, a clinical problem resulting in significant perinatal mortality and morbidity, it is of interest both physiologically and clinically.

ROM has, until recently, been assumed to result entirely from physical tearing of the membrane tissues under the stress of contractions. This is clearly not the case in circumstances where the rupture precedes contractions. Recent data in the rat model suggest that the amnion, the component of the fetal membrane (FM) with the major tissue strength, undergoes collagen remodeling as gestation progresses [2, 3]. Apoptosis has also been observed in rat amnion following end-gestational remodeling [3, 4]. As a result of remodeling and apoptosis, the FM is postulated to weaken and be more susceptible to tearing near the end of gestation [3, 4]. Apoptosis has also been suggested to occur near term in human amnion and chorion [5]. A zone of FM along the membrane tear line has been identified by Malak, McLaren, and others that exhibits altered morphology in comparison with remaining FM [6, 7]. This area of altered morphology has been further characterized by the same group of researchers to have increased matrix metalloproteinase (MMP) activity and apoptosis [5, 8]. They have further demonstrated that although there is significant variation in the degree and size of this zone between different patients, the zone is present in all patient membranes they examined [9]. Although these biochemical and histological changes in the FM have been the focus of significant investigation, a direct demonstration that such modifications actually cause or are associated with physical weakening of the membranes is lacking.

Detailed analysis of FM physical properties has not been carried out in recent years. Extensive reports were filed by Artal et al. in the 1970s [10, 11], Lavery and Miller in the early 1980s [12–14], and by two groups that include Ould and Helmig et al. [15, 16] and Schober et al. in the early 1990s [17]. All of these studies predated the initial work by Malak and Bell. None of these groups suggested the existence of a focal area of FM weakness overlying the cervix. Furthermore, none of these reports attempted to correlate measured physical properties with histological or biochemical characteristics of the membranes. These reports also predated current understanding of the process of apoptosis.

The purpose of this study was to determine whether an area of focal weakness is present in prelabor fetal membranes, at term, overlying the lower uterine pole and cervix, and whether this region has the biochemical and histological characteristics consistent with the region of high morphological change described by Malak and Bell.

MATERIALS AND METHODS

Biological Samples

Fetal membranes were collected from 12 patients who had elective cesarean delivery at term (37–39 wk) before the initiation of labor. Patients were recruited for this study with informed consent, using a protocol approved by the institutional review board. Tissues were brought to the laboratory within 10 min of delivery of the placenta. Each FM was systematically and completely sectioned into pieces using the procedure described below. The full-thickness FM fragments were washed briefly in Hanks
balanced salt solution (HBSS; pH 7.4) and then kept moist throughout strength testing. Samples from all specimens were submitted for pathological review. Selected FM fragments were tested for biochemical evidence of remodeling and apoptosis. Membranes were excluded if infection was suspected from clinical history or if chorioamnionitis was detected on pathological review.

Marking Procedure

After delivery of the infant and before expulsion of the placenta with membranes, the FM overlying the internal cervical os was identified by direct visualization and marked with Gentian Violet. This was performed per operatively by the delivering obstetrician using a cotton-tipped applicator dipped in gentian violet dye.

Topographical Mapping

Membrane cutting procedure. We used measured distances from the placental disk rim and from the tear-line (in the case of vaginal studies) to determine from where to take samples for strength testing. Using this methodology, we noted significant intraplacental and interpatient strength variability, as has been reported by others [10–17]. We were not able to identify a zone of weakness as we report in this present study. For the studies reported here we adopted a much more meticulous methodology.

The entire FMs were cut for testing and the precise location and orientation of each cut piece, relative to both the placental disc and the region that formerly overlay the cervix, were determined (Fig. 1).

The placenta and its membranes were placed on a specially designed cutting board (1 m × 1 m) covered with white paper. After identifying the area overlying the cervix (i.e., the marked area), it was used as a reference point to lay out the FM. The first cut was made perpendicular to the surgical incision line just lateral to the marked area. This cut was continued until the placental rim was reached. The second cut was made perpendicular to the other side of the incision line and continued to the placental rim in the same way. Cuts were then made perpendicular to these cut surfaces at intervals sufficient to allow the membranes to lay flat (Fig. 1). Once the FM was flat, secondary cuts were made parallel to the placental rim such that each resulting piece was large enough for two to three strength measurements. Generally, such pieces were 3–4 cm wide and 6–10 cm long (parallel to the rim). After each such piece was cut, the perimeter of the cut was outlined on the paper. Both the paper and piece were marked with tissue dye at one end to record the orientation, and the piece was removed from the cutting board and placed in a small numbered beaker with HBSS. The piece number was also recorded on the paper (Fig. 1). Following cutting and removal of all pieces, the position of the placental rim and umbilical cord were outlined on the paper and the placental disk was removed from the cutting board. The placenta was removed, a glass pane was placed over the cutting board and marked paper. Tracing paper was used to record the position and orientation of all cut pieces, the placental rim, and the marked area indicating the membranes overlying the cervix. After strength testing was completed, the rupture strength values were also recorded in the proper positions on the outline of each piece on the tracing (Fig. 1). The outer borders of the FM were cut from the tracing. This two-dimensional (2-D) map was then folded to show the original 3-D, physical configuration, of the amniotic sac, with superimposed rupture strength results.

Designation of the area overlying the cervix versus remaining FM. An area of 10 cm in diameter (about 78.5 cm²) centered on the cervical mark on the placenta was arbitrarily designated as the area overlying the cervix. All physical, biochemical, and histological testing performed on FM pieces or parts of a piece within this zone were then compared with those of the remaining FM. This diameter was chosen because 1) it represents the maximal cervical dilatation, 2) it is large enough to allow a number of puncture tests to be performed within it (we averaged 5, range 4–7), and 3) it is well within the boundaries that McParland et al. described as having altered morphology (which they reported as 119.4 cm² [9]).

Physical Testing

FM physical properties were determined by American Society for Testing and Materials standards using modified industrial rupture testing equipment (Com-Ten Industries, St. Petersburg, FL; Fig. 2). Membranes were supported within a 2.5-cm diameter fixture (Fig. 2B). A motor driven, 1-cm-thickness serrated plancher was forced perpendicular to the membrane surface at a speed of 8.4 cm/min. Displacement of the membrane and the resultant force were collected continuously and analyzed by data reduction software. Rupture forces and maximum displacements were determined from the generated force/displacement curves.

A characteristic plot of force versus deflection (Fig. 3) illustrates mechanical properties that are routinely determined for FM using this system. Initially, the intact chorioamniotic membrane resists the force required for displacement. Typically, the force displacement plot is initially curvilinear, followed by an approximately linear segment leading to the point of maximum force where the choric component of the FM breaks. After the rupture of the chorion, the rest of the curve represents the amniotic component of strength. Strength required to puncture the chorionic component of the chorioamniotic membrane. Strength-2 represents the force required to puncture the remaining amniotic component. Although the chorionic component of the fetal membranes ruptures first at a higher break force than the amnion, studies we have performed with separated amnion and chorion membranes clearly indicate that the amnion is the stronger membrane. The sharing of the load between the adherent individual membrane components results in the chorion breaking at a higher strength when the membranes are intact (data to be separately reported). Ductility is defined as the distance that the tip of the probe moves from the first contact with the membrane to the development of peak force (peak deflection). Work-to-rupture is the amount of work required to puncture the membrane (area under the force-displacement curve). Stiffness represents the ratio of an increment in force to an increment of displacement; the slope of the force displacement diagram. Secant stiffness, which is illustrated in Figure 3 and hereafter referred to as Stiffness, is the slope of the chord connecting the point of contact (no deflection) to the point of initial rupture. This represents an average stiffness over the test interval. Note that these mechanical characteristics are not strictly equivalent to the engineering properties of stress, modulus of elasticity, toughness, and strain.

Physical measurements were performed on all pieces cut from FM of each of the 12 patients. Most pieces were cut in a manner that allowed multiple physical testing procedures (most frequently three) to be performed on a single piece. Knowledge of both the position and orientation of each piece from the mapping procedure described above allowed complete characterization of the physical properties over the entire fetal membrane.

Western Blotting

After strength testing, some membrane fragments were washed in ice-cold PBS, weighed, and homogenized for 30 sec on ice (Tekmar Tissuemizer, Cincinnati, OH) in 5 ml/100 mg ice-cold RIPA buffer (PBS containing 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM sodium orthovanadate, 0.1 mg/ml phenylmethylsulfonyl fluoride, and 1X Protease Inhibitor Cocktail Set III [Calbiochem, San Diego, CA]). Homogenates were centrifuged on ice, and supernatant was collected. Fifty μg of sterile gauze, and clarified by centrifugation at 12 000 × g for 15 min at 10°C. Proteins were quantitated by Bio-Rad (Hercules, CA) DC DC protein assay. Forty micrograms of protein was loaded into each lane and separated on 4%–15% Tris HCl gels by SDS-PAGE. Separated proteins were semi-dry electroblotted to polyvinylidenefluoride (Amersham, Buckinghamshire, UK.), washed in TBST (20 mM Tris, 137 mM NaCl pH 7.6 containing 0.1% Tween-20), blocked in IB (5% nonfat milk/TBST), and incubated overnight at 10°C with antibodies to proteins of interest in IB. Multiple antibodies were used to definitively identify each of the proteins in this report; all antibodies identified the same bands and produced the same results. The antibodies tested were as follows: PARP cleavage fragment (Chemicon, Temecula, CA, 1:100; Oncogene Research Products, San Di- ego, CA, 1:50; or CalBiochem, 1:50), TIMP-3 (CalBiochem, 1:25; or San- ta Cruz Biotechnology, Santa Cruz, CA, 1:1000) or MPP-9 (Oncogene Research Products, 1:100; Chemicon, 1:100; or Santa Cruz Biotechnology, 1:100). The antibodies actually used for all work in this report were as follows: PARP cleavage fragment (Chemicon, 1:100), TIMP-3 (Cal- Biochem, 1:25), and MPP-9 (Oncogene Research Products, 1:100). Standards for MPP-9 and TIMP-3 were from CalBiochem; no SDS-PAGE standard was available for the PARP. Postwashing the blots were incubated for 30 min with the appropriate immunoglobulin G horseradish peroxidase conjugate (Santa Cruz Biotechnology) diluted 1:3000 in IB, washed extensively in TBST, developed using ECL-Plus (Amersham) according to the manufacturer’s protocol, and exposed against Fuji Super RX film. Quantitative values for Western blots were obtained by densitometry using Image J version 1.29k software (W. Rasband, Na- tionation Institutes of Health, MD). A wide range of proteins, commonly used to ensure even loading of wells, were not used in these studies because of known differences in the cell/tissue composition
FIG. 1. Fetal membrane cutting procedure. A) Marked membranes showing location of primary cuts required to lay the membranes flat. B) Membranes with secondary cuts. C) Paper tracing reproduction showing cuts, orientation and location of all pieces, and strength test results.

Histology

After strength testing, areas of membrane fragments immediately adjacent to the tested regions were sampled for histological analysis. Tissue samples (approximate thickness 0.5 ± 0.3 cm) were pinned to corkboard and placed in buffered formalin. Strips of 3–4 mm were cut from the center of each region, embedded in paraffin, and sectioned at 3–4 μm onto electrostatically charged slides for routine hematoxylin-and-eosin (H&E) and other special stains. H&E-stained sections of each submitted sample were viewed initially for evidence of infection. Infected samples were not further analyzed. The orthogonal thickness of the following membrane layers was directly measured in five separate 200× microscopic fields: specimen using a 10 × 10 mm eyepiece reticle: subamniotic compact connective tissue, chorionic connective tissue, chorionic cellular zone, compact decidua, and loose decidua. The fetal membrane morphometric index (FMMI), a ratio of the noncellular to cellular sublayers (i.e., ratio of the thickness of the amniotic compact + amniotic fibroblast + amniotic spongy + chorionic reticular layers to the thickness of the chorionic cytotrophoblastic + decidual layers), was determined in the manner described by McParland et al. [9].

Statistical Analysis

Physical property measurements within the cervical zone (the area of the FM within a diameter of 10 cm centered at the cervix) for each placenta’s membrane were averaged and reported as the mean (strength, stiffness, work-to-rupture, and ductility). Measurements outside the cervical zone for each placenta were averaged and reported similarly. Differences in physical properties between the cervical zone and remaining areas were analyzed using the Student t-test, two tail, with P < 0.05 considered statistically significant. Differences in biochemical and histological properties between the cervical zone and remaining areas were analyzed in the same manner as the physical properties. For Western blots of MMP-9, TIMP-3, and PARP, densitometer results for values within the cervical zone were compared with those in the remaining areas. For histological studies, measurements of thickness of the tissue layers (amnion epithelial layer, amnion fibroblast layer, amnion spongy layer, chorion connective tissue layer, chorion trophoblast layer, and decidua) were taken at five different locations on each specimen, averaged, and compared using a paired Student t-test. To determine the association of biochemical characteristics with changes in physical properties, a linear regression analysis was also performed.

RESULTS

Twenty-five patients undergoing Cesarean delivery without labor at term consented to the membrane marking procedure (identification of the cervical overlying FM) and detailed examination of their FM. Of the 25 specimens, 13 were discarded because the mark was not visible, the membranes were excessively torn during the delivery process, or there was clinical or histological evidence of infection. A 10-cm-diameter area was circumscribed around the cervical mark on the 3-D reconstructed tracing of the FM to delineate the cervical zone. The physical, biochemical, and histological characteristics of the membranes within this cervical zone were then compared with those of the remaining areas of the FM.

On the 3-D reconstructions, a discrete weak zone was apparent in each FM. In all 12 cases, this weak area was within the 10-cm circumscribed area around the cervical mark. The mean strength within the cervical zone (4.98 ± 1.38 N) was 55% of that of the mean in the remaining area (9.07 ± 2.61 N; P < 0.001; Fig. 4A). The cervical zone had an average of 5 puncture tests (range 4–7) of the total average of 25 puncture tests (range 19–36). The cervical zones of all 12 membranes tested showed a consistent pattern of weakness relative to the remaining areas (Fig. 4B). Also, most of the individual weak spots within each membrane were contained in the circumscribed FM area around the cervix (Fig. 4C). Strength-2 was also decreased in the
Fig. 3. Typical force vs. displacement curve. Strength indicates the force at which the chorionic component of the chorionamnion ruptures. Strength-2 indicates the force at which the amnionic component ruptures. (See Materials and Methods for additional details.)

Fig. 4. Fetal membrane strength. A) Mean strength of cervical zones for each patient is less than the mean strength of the remaining areas (P < 0.001). B) Individual patient data showing a weaker cervical zone (□) for each of the 12 patients relative to the remaining areas (■). C) Scattergram of the results of individual strength tests for each patient showing the cervical (●) and remaining areas (○).

Fig. 5. Comparison of means for cervical vs. remaining areas (n = 12) are shown for work to rupture (A, P = 0.002), stiffness (B, P = 0.002), and ductility (C, P = 0.014).

Remodeling and Apoptosis

To determine whether the cervical zone of the FM exhibited characteristics suggestive of collagen remodeling and apoptosis, processes believed to be central to the postulated biological program of membrane weakening, protein levels of representative enzymes were examined in the FM area overlying the cervix, and the remaining area more distant from the cervix. The zone of FM overlying the cervix exhibited increased MMP-9 protein (cervical vs. remaining P < 0.01; Fig. 6, A and B). MMP-9 protein also showed cervical zone (data not shown). In addition to strength, other physical parameters showed differences in the FM zone overlying the cervix. Work-to-rupture (0.012 ± 0.006 J vs. 0.025 ± 0.011 J, P = 0.002), Stiffness (9.14 ± 2.51 N/cm vs. 14.47 ± 3.38 N/cm, P = 0.002), and (displacement at rupture) ductility (0.56 ± 0.097 cm vs. 0.67 ± 0.101 cm, P = 0.014) were all less in this cervical zone (Fig. 5).
an inverse linear relationship with FM strength \(P < 0.001, r^2 = 0.92; \text{Fig. } 6\text{C})\). The cervical zone of the FM showed a decrease in TIMP-3 protein (cervical vs. remaining \(P < 0.01\; \text{Fig. } 6, \text{A and B}\)). TIMP-3 protein was predominant in areas distal to the cervix (\text{Fig. } 6, \text{A and B}) and increased in parallel with FM strength \((P < 0.05, r^2 = 0.41; \text{Fig. } 6\text{C})\). TIMP-1, TIMP-2, and TIMP-4 were also examined. TIMP-1 was barely detectable; TIMP-2 and TIMP-4 proteins were detectable in all specimens but did not change with strength (data not shown). The cervical FM areas also showed increased PARP cleavage compared with the remaining areas of the membranes, suggesting increased apoptosis (cervical vs. remaining \(P < 0.01\; \text{Fig. } 6, \text{A and B}\)). PARP cleavage showed an inverse linear relationship with strength \((P < 0.01, r^2 = 0.81; \text{Fig. } 6\text{C})\).

Histology

An area of high morphological change in the zone of FM overlying the cervix was identified by Malak and Bell in 1994 [6]. They followed this initial study with several reports suggesting that this histological change reflected a developmental process by which FM weakened. No correlating data documenting changes in physical properties have been presented, however. In the most recent report from this group, two histological findings were cited in patients undergoing cesarean delivery similar to those reported here: the decidual layer was thinner and the FMMI (i.e., ratio of the relatively acellular to cellular layers—see Materials and Methods) was higher in the cervical zone. We specifically examined the FM for these characteristics. In our specimens we also found that the decidual layer in the region overlying the cervix was significantly thinner \((56.6 \pm 44.4 \mu m)\) than the remaining areas \((121.7 \pm 29.8 \mu m, P = 0.0007; \text{Fig. } 7\text{A})\). The FMMI index was also greater in the cervical zones \((1.50 \pm 1.55)\) than the FMMI in the remaining areas \((0.47 \pm 0.27, P = 0.0175; \text{Fig. } 7\text{B})\). The total membrane thickness was also determined. When both fetal layers (amnion and chorion) and the maternal decidua were considered, the cervical zone was thinner \((191 \pm 54 \mu m \text{ vs. } 233 \pm 54 \mu m, P < 0.05)\). When the amniochorion alone was considered, the cervical zone was thicker than the remaining areas \((136 \pm 23 \mu m \text{ vs. } 112 \pm 31 \mu m, P < 0.02)\).

DISCUSSION

We show for the first time that fetal membranes from term, cesarean deliveries, with no preceding labor, have a circumscribed zone of weakness in the region overlying the cervix. This zone also shows increased MMP-9 protein, decreased TIMP-3 protein, and increased PARP cleavage, suggesting that it is undergoing greater collagen remodeling and apoptosis relative to the remaining areas of the FM. Histological examination of this zone reveals characteristics similar to the area of high morphological change described previously in a series of reports by other researchers [5–9]. As in the histological studies reported by that group, a meticulous methodology of cutting and examining the membranes was required to obtain meaningful physical property data.

Measurement of the appropriate physical characteristics of FM, including strength, is not straightforward. Three methodologies have been used: tensile testing, burst testing, and puncture testing. In tensile testing, strips of FM are cut, the ends of each strip of FM are placed in a vice grip, and the strips are pulled apart [10–11; 15–16]. This procedure utilizes methods common to tensile testing of metal rods and other materials. A major drawback is that stress is uniaxially applied rather than biaxially, as occurs in normal FM physiology. In burst testing, the membranes are clamped in a circular ring and a head of pressure (either fluid or air) is applied to the membrane. This testing procedure most closely mimics the in situ physiological stretching of FM over an open cervix. However, it is logistically difficult and requires relatively large pieces of FM for each test, allowing only one or two fragments per placenta to be examined [12–14, 18–21]. Puncture testing, which is described in Materials and Methods, was initiated because of the problems associated with other methodologies. It allows multiple small fragments of a single FM to be examined in a biaxial test procedure. A concern that
puncture testing is not reproducible because the quantitative results depend on the size of the probe and tissue holder used as dispelled by Schober et al. [22]. They demonstrated that puncture test results using any size probe and tissue holder could be quantitatively compared with results of puncture tests with different size equipment, and also with burst testing results, via a mathematical formula built on the ratio of the probe-to-tissue holder diameters [22]. Using this formula, our data are comparable to those predicted by Schober et al. [22], given the ratio of our probe-to-tissue holder diameters (1.0/2.5 = 0.4); for example, Schober’s data predicts FM strength of 8.4 N with our equipment, which compares with our data mean of 9.07 N. We adopted the puncture testing procedure because of these advantages.

Our studies confirm the variability in FM strength over its surface reported by previous investigators, but demonstrate that much of this is due to the physiological pattern related to the weak zone overlying the cervix. Artal et al. [10], using a modified Harvard pump to stretch FM pieces from areas around the placental rim and rupture site (28 patients), noted considerable variation among samples but no statistically significant difference in work or strain to rupture between the two sites. Similarly, Lavery and Miller [13] reported no difference in work or strain to rupture between FM areas adjoining the placenta and the rupture site, using fluid pressure, in 15 patients with ROM at the time of labor and 13 term deliveries with premature ROM (PROM). Pressman et al. [23] demonstrated that FM tensile strength increases during mid-trimester (18–20 wk) with a plateau from 20 to 39 wk, and a subsequent precipitous decrease thereafter. In addition, they reported that the tensile strength of FM adjacent to the chorionic plate did not differ from areas adjacent to the point of rupture. All previous groups failed to detect a weak zone over the cervix. The lack of FM homogeneity and technical problems associated with the strength testing methodologies and membrane cutting procedures may explain this. Specifically, previous investigators had the following problems: 1) fewer and larger membrane fragments were tested, 2) there was no method to determine the position of the cervix, and 3) no method was available to relate strength testing between adjacent fragments (their intra uterine topological relationship was lost). Our study demonstrates the importance of using a meticulous testing methodology in FM physical property studies: 1) marking and identifying the FM overlying the cervix, 2) cutting the membranes along specific grids with respect to the tear line and the placental disc, and 3) marking each cut piece to document its exact location and orientation. Only after adopting this level of detailed analysis was the underlying pattern of FM physical properties, in relation to position relative to placental disc and the cervix, apparent.

In their histological studies, Bell, Malak, McParland and others [5–9] used Babcock tissue forceps instead of a dye to mark the FM overlying the cervix before delivery of the placenta. They then used the mark as a reference point to orient their samples in relation to each other and to the uterine cavity. They identified a zone consisting of about 10% of total FM surface area that exhibited extreme altered morphology, characterized by an elevated connective tissue layer to cellular layer thickness ratio (FMMI). Within this zone, there was a gradual change to a more restricted area that exhibited extreme altered morphology but represented only 1.9% of the total FM surface area. This area of extreme altered morphology is centered in FM overlying the cervix. Using a very systematic membrane cutting methodology, they detected this zone of morphological change in every patient they examined [9]. Our initial attempts to identify a weak area in each FM we tested were unsuccessful. Only after adopting a meticulous and systematic approach, as in these reports, were we able to demonstrate a weak zone of FM overlying the cervix in every patient. We believe that this weak zone of FM overlying the cervix represents a previously unrecognized correlation of relative weakness of the “zone of high morphological change” identified by Malak and Bell [6].

Rupture of FM during labor, or PROM, has been associated with the expression and activation of MMPs and increased apoptosis. MMPs are a family of enzymes that hydrolyze specific components of the extracellular matrix. The regulation of MMP activity in extracellular matrix remodeling is a complex process that involves protease activation and interaction with specific TIMPs. A balance between MMPs and TIMPs is believed to exist during tissue remodeling to accommodate fetal growth, although an imbalance in favor of the MMPs leads to cervical ripening and FM rupture [24]. MMP-9 belongs to this protease family and shows increased expression in FM of several species during labor. Several authors have proposed that the induction of MMP-9 expression is one of the first biochemical events during labor [25–29]. TIMP-3 is a member of the TIMP family that has been extensively reported in human placental studies. Marvin et al. [30] recently reported that TIMP-3 expression is significantly decreased in FM of patients with preterm premature rupture of membranes. PARP is a conserved nuclear enzyme implicated in DNA repair.
in the apoptosis response. This protein is one of the main cleavage targets of caspase-3. None of the groups that conducted extensive investigation of FM physical properties reported correlations with tissue biochemical or histological characteristics. Two studies of collagen content and FM physical properties failed to show any association [19, 20]. Schoonmaker et al. [31] demonstrated that in vitro incubation of FM with high concentrations of bacterially derived collagenase or proteinase-producing bacteria weakened the membranes. In a more recent study, Uchide et al. [32] showed a very rough correlation between the ratio of MMP-9/TIMP-1 and membrane tensile strength. Our studies show a clear relationship of increased MMP-9, decreased TIMP-3, and increased PARP cleavage in the weakened FM area over the cervix. Furthermore, we show a linear correlation between the levels of these proteins and rupture strength.

A recent hypothesis suggests that the rupture of FM occurs as a result of a maturation process akin to that in cervix and involves collagen matrix remodeling and apoptosis. Such changes in amnion collagen have been well documented in both animal and human studies [2–4]. A zone of high morphological (histological) change identified by Bell, Malak, McLaren, and Taylor demonstrates collagen remodeling and apoptosis and has been suggested to contain the FM rupture site [5, 6]. They have also recently described increased MMP activation and chorion (cytotrophoblast) cell apoptosis in this zone of FM overlying the cervix [7, 8]. Most investigators have generally assumed that the biochemical changes associated with collagen remodeling and apoptosis directly cause weakening, leading to FM rupture. This has never been directly demonstrated. Our data, for the first time, correlate collagen remodeling and apoptosis with the physical weakness of the FM, providing strong support for the theory that, before birth, FM undergo a developmental process of remodeling that weakens them in preparation for rupture at the time of labor.

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REFERENCES