Structural characteristics of term human fetal membranes prior to labour: identification of an area of altered morphology overlying the cervix

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Premature rupture of fetal membranes can have serious clinical implications, especially for the initiation of preterm labour and its consequences. To account for this phenomenon many studies have attempted to identify membrane features that may be uniquely associated with the site of rupture. Our previous work has identified an area of the fetal membrane, following spontaneous term birth which exhibits alterations consistent with structural weakness. The aim of this study was to determine if these changes existed prior to labour. In formalin-fixed paraffin-embedded tissue sections an area of the fetal membrane overlying the cervix, termed the ‘cervical membranes’, was characterized by an increased thickness of the connective tissue layer (215% increase, P < 0.01) and decreased thickness of both the cytotrophoblast (36% reduction, P < 0.01) and decidual layers (64% reduction, P < 0.01) compared to the rest of the membrane. This resulted in the cervical membranes being significantly thinner (P < 0.05) than the rest of the membrane. Similar changes were also detected in frozen sections of fetal membranes. These regional differences have two important implications in that: (i) the cervical membrane may represent a region of structural weakness susceptible to rupture during labour, and (ii) the paracrine relationships between fetal membranes and the myometrium may be qualitatively affected within different regions of the uterus.

Key words: amnion/chorion/fetal membranes/preterm birth/ PROM

Introduction

In most pregnancies labour begins at term in the presence of intact fetal membranes (Alger and Pupkin, 1986). Without intervention they usually remain intact until they spontaneously rupture near the end of the first stage of labour. However, ~10% of term pregnancies (Mead, 1980), and up to 60% of preterm deliveries are preceded by pre-labour rupture of the membranes (PROM) (Keirse et al., 1989). Although the mechanisms of PROM are not well understood, infection has been implicated in the aetiopathology of a proportion of cases (Gibbs et al., 1992). Given the serious implications of PROM in both term and preterm pregnancies, the elucidation of the mechanisms responsible for PROM and its prevention is of primary concern.

To account for the phenomenon of fetal membrane rupture, many studies have attempted to identify features of the fetal membranes that may be uniquely associated with the site of rupture after their spontaneous rupture; unfortunately these studies have been contradictory (Bou-Resli et al., 1981; Halburt et al., 1989). However, the comprehensive mapping of fetal membranes, obtained as frozen sections after their spontaneous rupture at term, detected an area of the fetal membranes that exhibited unique morphological features which were only found within a restricted area along the line of rupture (Malak and Bell, 1994). This restricted area has been termed the ‘zone of altered morphology’ (ZAM) and the features described were consistent with its potential structural weakness (Malak and Bell, 1994, 1996). These included marked disruption of the connective tissue layers and marked reduction of the thickness, and hence cellularity, of both the cytotrophoblast and decidual layers. Given the structural features of the ZAM and its restricted localization to an area within the rupture line, it has been proposed that the ZAM may have represented the site of initial fetal membrane rupture in response to the increased intra-amniotic pressures experienced during labour (Malak and Bell, 1993; Bell and Malak, 1997).

The aim of the present study was to determine if the structural changes characteristic of the ZAM were present prior to labour. A biopsy procedure was designed with reference to the fetal membranes overlying the cervix since preliminary studies employing frozen sections indicated that this area exhibited altered structural characteristics (Abdel-Malak et al., 1993). It had also been demonstrated that the area of the fetal membranes over the cervix was always detected within the final rupture line after their spontaneous rupture after term (Bourne, 1962). Confirmation of these regional structural differences in the fetal membranes before labour and rupture may indicate that an area of the fetal membranes is generated prior to labour providing a region of structural weakness and hence a programmed susceptibility to rupture.

Materials and methods

Patient details

Fetal membranes were obtained from women (n = 12, formalin fixed tissue; n = 6 frozen tissue) undergoing elective Caesarean sections between 38 and 39 weeks of pregnancy for repeat Caesarean section, breech birth or cephalopelvic disproportion. Infected membranes were
Well forceps and an area of 4–5 cm² around the clip was removed.

Following the delivery of the baby, the fetal membranes overlying the cervix were located and marked by the application of Spencer–Well forceps and an area of 4–5 cm² was removed from the following areas of the fetal membrane (Figure 1).

Cervical membranes

Following the delivery of the baby, the fetal membranes overlying the cervix were located and marked by the application of Spencer–Well forceps and an area of 4–5 cm² around the clip was removed. The area contained within the jaws of the forceps was not used since light microscopy revealed that this area was too damaged. Therefore only areas adjacent to the clip were sampled.

Mid-zone area

This was half-way between the cervical area and the placental edge, more than 10–12 cm from the cervical area. At least three specimens were taken from each area per patient. Specimens were then washed briefly in phosphate-buffered saline (pH 7.4). Fetal membrane strips were rolled with the amnion innermost and then fixed.

Tissue fixation

Formalin-fixed tissue sections

Fetal membrane rolls were placed in buffered (pH 7.6) formalin and fixed for 24 h before processing and mounting in paraffin wax.

Cryostat tissue sections

Rolls of fetal membranes were placed in plastic cups filled with tissue embedding compound; Tissue-Tek, OCT (BDH, Poole, UK) tissue blocks were snap-frozen in liquid hexane and dry-ice mixture (−70°C), then stored at −80°C until used. Samples for formalin fixation and frozen tissue were not taken from the same patient, since correct specific localized sampling would only allow for the assessment of one tissue treatment per patient. Thin tissue sections were cut and then stained with haematoxylin and eosin. Stained tissue sections were examined under light microscopy connected to an image capture system. This system comprised a Apple Macintosh Centris Running Image (TM) version 1.49. Each section of roll was divided into quadrants and areas of thickness were measured along the intersections of 12 to 6 o’clock and 3 to 9 o’clock. This was done for each roll with 8–10 intersections counted per roll. Each captured image was measured following calibration of the image with an internal scale slide. The thicknesses (µm) of the fetal membrane connective tissue, trophoblast and decidual layers were determined. Measurements were taken only from sections cut vertically, showing a single layer of amniotic epithelium. Separation of the amnion and chorion layers occasionally occurred in the formalin-fixed paraffin-embedded tissue; the spaces were subtracted from the final measurement in order to obtain the final connective tissue layer. Thickness measurements were represented as mean ± SD.

Statistics

Data values from formalin-fixed paraffin-embedded tissue were normally distributed and comparisons were made between the two different membrane zones using Student’s t-tests. Frozen tissue samples, which were normally distributed, had originally included additional membrane areas to those fixed in formalin, therefore two-way analysis of variance was carried out. Scheffe’s test was used for subsequent comparisons between different membrane zones.

Results

The constituent layers of both frozen and formalin-fixed fetal membranes were arranged as previously described by Malak and Bell (1993) and the major layers are illustrated in Figure 2A. For clarity only pictures of formalin-fixed tissue are included. The mid-zone area was characterized as follows. The amniotic layer consisted of a single layer of non-ciliated cuboidal cells resting on a basement membrane. The underlying connective tissue layers (CTL) included an acellular layer (compact layer) which overlays a layer consisting of connective tissue and fibroblast-like stellate-shaped cells (fibroblast layer). These layers comprised the amniotic part of the fetal membrane. Interfacing between the amnion and the chorion was the spongy layer which was formed of a fine, loose, wavy fibrillar network and a few cells. The chorion was composed of three layers: the reticular, the chorionic basement membrane and the cytotrophoblast layer. The reticular layer was composed of a network of fibres in which fusiform and stellate-shaped cells were embedded. The chorionic basement membrane underlay the trophoblast layer which comprised a thick layer of polygonal cytotrophoblastic cells. This layer contained two morphologically distinct cell types (Malak and Bell, 1993). The cells nearest the reticular layer, the basal cells, were vacuolated and tightly adherent to each other with narrow intracellular spaces (basal cytotrophoblasts). Nearer to the decidua, superficial trophoblastic cells with wider intercellular spaces were located. The decidua comprised of a thick layer of well-defined nucleated cells.

Cervical membranes

Significant differences in the fetal membrane structure were detected in specimens from this region, in both formalin-fixed and frozen tissue, as compared to the mid-zone (Figure 2B). In formalin-fixed tissue the total thickness of the fetal membranes in the cervical area (298 ± 60 µm) was significantly less (P < 0.05) as compared to the mid-zone area (327 ± 64 µm) (Table I). The most significant internal structural difference was the increase in the thickness of the
Figure 2. (A) The mid-zone area of fetal membranes. The single layer of amniotic epithelium (AE) rests on an amniotic basement membrane. The connective tissue layer (CTL) is densely packed. Trophoblast (T), decidual (D) cell layers. (B) The cervical area of the fetal membranes. Note the marked swelling of the connective tissue layer (CTL) and the pronounced thinning of the trophoblast (T) and decidual layers (D) compared to the mid-zone area. Haematoxylin and eosin staining. Scale bars = 100 µm.

### Table I.
The mean ± SD thickness (µm) of the pre-labour affected formalin-fixed fetal membrane samples

<table>
<thead>
<tr>
<th>Fetal membrane layers</th>
<th>Cervical area (n = 12)</th>
<th>Mid-zone area (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Connective tissue</td>
<td>183 ± 61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85 ± 27</td>
</tr>
<tr>
<td>Trophoblast</td>
<td>63 ± 25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98 ± 31</td>
</tr>
<tr>
<td>Decidual</td>
<td>52 ± 21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>144 ± 64</td>
</tr>
<tr>
<td>Total membrane thickness</td>
<td>298 ± 60&lt;sup&gt;b&lt;/sup&gt;</td>
<td>327 ± 64</td>
</tr>
</tbody>
</table>

<sup>a</sup> = number of patients, <sup>b</sup>P < 0.01, <sup>a</sup>P < 0.05.

The whole connective tissue layer of the cervical membranes (215% increase, P < 0.01) compared to the mid-zone (Figures 2B and 3A). However, the cervical membranes also exhibited a significant reduction in the thickness of the decidual (64%, P < 0.01) and cytotrophoblast layers (36%, P < 0.01) as compared to the mid-zone (Table I, Figure 3B and C). Frozen tissue sections showed similar changes to the fetal membrane structure, as seen in the formalin-fixed tissue, including the thickening of the connective tissue layer (123%, P < 0.01), and the thinning of both the trophoblastic (31% reduction, P < 0.01) and decidual layers (43% reduction, P < 0.01). The overall thickness of the fetal membrane was also reduced in the cervical area (22% reduction, P < 0.01) (Table II).
over the cervix is associated with the rupture site and the workers have alluded to the possibility that the area directly but less extreme than those observed in the ZAM. Previous describe in the present study in the cervical zone were similar, frequently located at one end of the tear. The alterations we area within the post-delivery rupture tear and was most obtained at term and after vaginal delivery identified an area of structurally altered morphology, termed the ZAM (Malak and Bell, 1994). The ZAM was always detected as a restricted of the cellular cytотrophoblastic and decidual layers and a significant thickening of the whole connective tissue layer. Differences in the thickness of the connective tissue and cytотrophoblast layers between the two fixation methods were evident; however, both formalin-fixed and frozen tissue showed the same overall structural changes described above, suggesting that these were real changes and not an artefact of the tissue fixation process. These changes indicate the presence of an area of significant structural alteration overlying the cervix prior to labour. The detection of regional differences in the structure of the fetal membrane demonstrates the need to regionally sample the fetal membrane when investigating fetal membrane biology.

Previous work on spontaneously ruptured fetal membranes obtained at term and after vaginal delivery identified an area of structurally altered morphology, termed the ZAM (Malak and Bell, 1994). The ZAM was always detected as a restricted area within the post-delivery rupture tear and was most frequently located at one end of the tear. The alterations we describe in the present study in the cervical zone were similar, but less extreme than those observed in the ZAM. Previous workers have alluded to the possibility that the area directly over the cervix is associated with the rupture site and the studies of Bourne et al. (1962) directly supports the hypothesis. These workers, by staining the area of the fetal membranes overlying the cervix with a trypan blue dye accessed through the cervix prior to rupture and delivery, demonstrated that this area was included in the post-delivery tear and was located at one end of the tear. Given the similarities, the ZAM most likely arises from the cervical area of the fetal membranes we have detected prior to labour, with the increased structural alterations resulting from the combined effects of the process of labour and delivery.

The temporal development of these changes in the cervical membranes during normal pregnancy is not known, but indirect evidence may help to characterize its development during the latter weeks. Oncofetal fibronectin, which is present at high concentrations in fetal membranes and in amniotic fluid, is detected in cervico-vaginal secretions up to 3–4 weeks prior to labour (Lockwood et al., 1991, 1993). The structural features of the fetal membranes overlying the cervix suggests that this fibronectin could be released from such a disrupted extracellular matrix or could leak from the amniotic fluid through such a matrix; this may indicate that the kinetics of appearance in the cervico-vaginal secretion reflects the generation of changes in the cervical zone.

Given the degradative nature of the connective tissue layer disruption and that these morphological changes are consistent with the disruption of the major components of the connective tissue, the collagens, this would suggest the involvement of proteolytic enzymes, in particular matrix metalloproteinases. These are a family of zinc-dependent enzymes responsible for the degradation of most of the components of the extracellular matrix (for review see Birkedal-Hansen et al., 1993). There is accumulating evidence to suggest that labour and delivery induces the synthesis of these enzymes (Draper et al., 1995; Vadillo-Ortega et al., 1995; Qin et al., 1997); however, whether they are involved in the development of the structural alterations seen in the fetal membranes overlying the cervix needs to be determined.

The thinning of the cytотrophoblast layer and the absence or dramatic thinning of the decidua could be the result of a number of processes. First, in the region of the lower segment there is a limited blood supply to the decidua (MacDonald et al., 1991), which may result in the selective removal of cells due to the processes of either necrosis or apoptosis. Second, it could have occurred by progressive stretch. The significant reduction of the cytотrophoblast layer in the cervical membranes could have implications for the local action of uterotonins such as prostaglandins and endothelin. Uterotonins are produced in the amnion and are present in amniotic fluid, yet the cytотrophoblast layer possesses the capacity to degrade these factors and prevent their action upon the myometrium and cervix. It has been proposed that local involution of the cytотrophoblast layer could allow uterotonins to escape metabolism and act locally (Malak and Bell, 1996) and the demonstration of its decreased thickness prior to labour could be causally involved in uterotonin action.

The investigation of the mechanisms responsible for the development of this region of structural alteration may have important implications for our understanding of the mechanisms of pre-labour fetal membrane rupture and preterm birth. Whatever the causes of the preterm pre-labour rupture of the fetal membranes in the absence of infection, their common end effect must be the production of a structurally weakened fetal membrane that predisposes it to subsequent pre-labour rupture. Whether mediated by infection or not, this condition may be the result of the premature activation of the processes involved in the generation of an area of structural weakness equivalent to that in the cervical area in normal pregnancy (Malak and Bell, 1996). Indeed, studies have reported that pre-labour ruptured membranes in both term and preterm births exhibit the extensive structural changes of ZAM, and that membranes from preterm births exhibit more extensive structural changes compared to those in spontaneously ruptured fetal membranes of either term or preterm births (Malak et al., 1993, 1994). The appearance of ZAM in the fetal membranes from preterm births also raises the possibility that its premature

### Table II. The mean ± SD thickness (µm) of the pre-labour affected frozen sections fetal membrane samples

<table>
<thead>
<tr>
<th>Fetal membrane layers</th>
<th>Cervical area (n = 6)</th>
<th>Mid-zone area (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Connective tissue</td>
<td>120.5 ± 7.36*</td>
<td>97.4 ± 4.4</td>
</tr>
<tr>
<td>Trophoblast</td>
<td>81.8 ± 6.34*</td>
<td>119.1 ± 9.7</td>
</tr>
<tr>
<td>Decidual</td>
<td>90.4 ± 12.32*</td>
<td>159.8 ± 16.9</td>
</tr>
<tr>
<td>Total membrane thickness</td>
<td>292.8 ± 12.42*</td>
<td>376.5 ± 25.71</td>
</tr>
</tbody>
</table>

\( n = \) number of patients. \(^*\) \( P < 0.01 \).
appearance with the loss of the cytotrophoblast layer will allow premature action of uterotonins to induce idiopathic preterm labour.

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References


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