UREA AND CREATININE IN VAGINAL WASHING FLUID IN DIAGNOSIS OF PRELABOUR PREMATURE RUPTURE OF MEMBRANES

Thesis

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INTRODUCTION

Premature rupture of membranes (PROM) is the rupture of the amniotic membranes prior to the completion of 37 weeks of gestation ***(Kale et al., 2007).*** It occurs in about 2-4% of preterm pregnancies ***(Mulhair et al., 2009).*** It is associated with a series of complications including fetal and maternal infection, and cord complications and preterm delivery ***(Wiberg-Itzel et al., 2005).***

The approach to the diagnosis of membrane rupture is clinical, with over 90% of cases being confirmed based on the presence of a suspicious history and ultr-asonographic finding followed by documentation of fluid passing from the cervix or the presence of a nitrazine/ ferning positive vaginal pool of fluid, but the nitrazine and ferning tests can be falsely positive ***(Kim et al., 2005).***

So it is therefore important to achieve accurate diagnosis by identifying the presence of specific amniotic fluid markers in vaginal environment. Among the markers evaluated were prolactin ***(Shahin and Raslan, 2007),*** α-fetoprotein, di-amine insulin-like growth factor binding protein-1 ***(Gaucherand et al., 1997),*** human chorionic gonadotropin ***(Kim et al., 2005)*** and fetal fibronectin ***(Chen and Dudenhousen, 2008).***

Some consider α-fetoprotein as an unuseful marker for PPROM because of overlap in concentrations between women with and without rupture of membrane ***(Kale et al., 2007).***

Some consider fetal fibronectin testing for the diagnosis of PPROM is not recommended because a positive test may reflect disruption of the deciduas rather than membrane rupture ***(Esim et al., 2003).***

Vaginal fluid creatinine determination for the diagnosis of premature rupture of membranes (PROM) is less expensive and easier to measure than human chorionic gonadotropin and α-fetoprotein, and appears to be more accurate than human chorionic gonadotropin ***(Li and Chang, 2000).***

Vaginal fluid urea and creatinine may be helpful in diagnosing PROM because fetal urine is the most important source of amniotic fluid in the second half of pregnancy ***(Kafali and Oksuzler, 2007).***

It has been reported that pregnant women in the early gestational age has a mean creatinine concentration of 0.6mg/dl in the amniotic fluid, similar to that found in maternal serum ***(Waters and Mercer, 2009).***

Creatinine concentration in amniotic fluid increases gradually between 20 and 32 weeks of gestation and more rapidly thereafter, when they were two to four times higher than maternal serum. Creatinine values in the amniotic fluid that best represent fetal maturity are 1.5-2.0mg/dl. A creatinine concentration of 1.75mg/dl or more correlates significantly with a gestational age of 37 weeks or more ***(Oliveira et al., 2002).***

AIM OF THE STUDY

The aim of this study is to evaluate the reliability of vaginal fluid urea and creatinine for the diagnosis of premature rupture of membranes (PROM).

Chapter 1

THE FETAL MEMBRANES: STRUCTURE AND FUNCTION

The placenta (Fig. 1-1) is a fetal organ situated between mother and fetus. It is essential for fetal growth and development. In addition to serving as a conduit for maternal fuels destined to nourish the growing fetus it fulfils a wide spectrum of other functions including the synthesis of various hormones and growth factors, detoxification of maternal xenobiotics, immunologic barrier and dissipation of thermic energy resulting from foetal metabolism ***(Hiden and Desoye, 2010).***

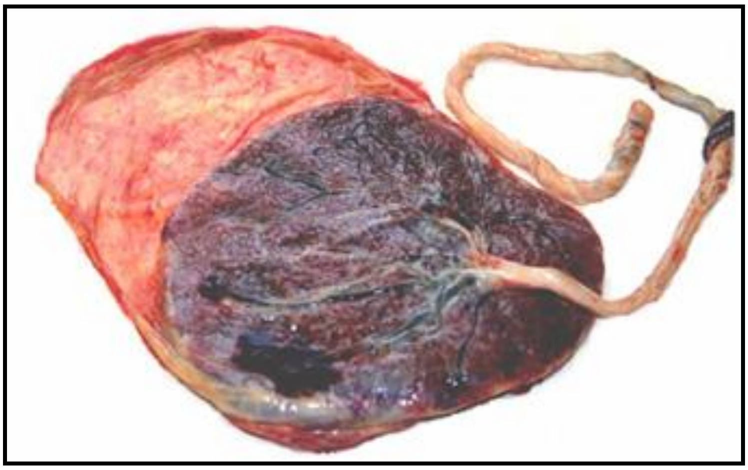

Fig. (1): Picture of the placenta, the fetal membranes and the umbilical cord ***(Dominguez 2009)***

The human placenta consists of a fetal component (the chorionic plate) and a maternal component (the deciduas). The two parts are held together by chorionic villi that connect the cytotrophoblastic shell of the chorionic sac to the deciduas basalis. The fetal part of the placenta, which includes the amniotic and chorionic fetal membranes, separates the fetus from the endometrium (Fig. 2) ***(Parolini and Soncini, 2006).***

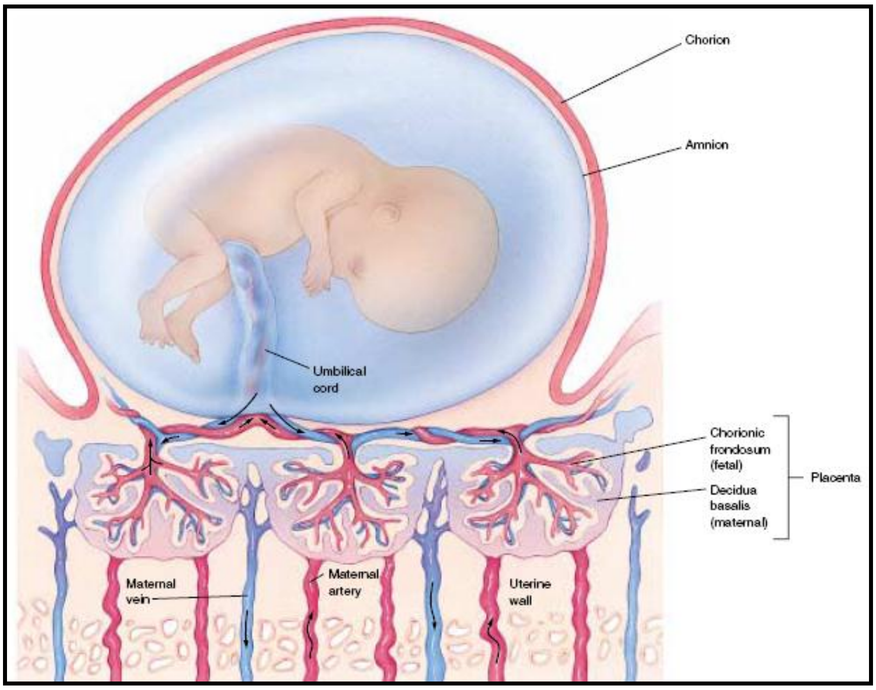

Fig. (2): Schematic representation of the placenta ***(Johnson et al., 2002).***

The Fetal Membranes

The fetal membranes (chorioamnion) form a highly specialized interface between mother and fetus, which is necessary for successful maintenance and termination of pregnancy in higher vertebrates ***(Lorenzi et al., 2010).***

The membranes normally insert at the edge of the placenta and contain the amniotic fluid and the fetus. The amnion covers embryo from the inner side, while the chorion does it from the outer side ***(Dominguez 2009).***

The chorioamnionic membrane forms the outer limits of the sac that encloses the fetus and the innermost layer of this sac is formed by the amnion (Fig. 3). The collagen-rich amnion confers tensile strength to the amniotic sac and results in the resistance to rupture typically observed in fetal membranes ***(Parolini and Soncini, 2006).***

The basal plate is covered by syncytiotrophoblast on the maternal side and consists of a basal decidua, which is the portion of the decidua where the implantation site is located ***(Parolini & Soncini, 2006).***

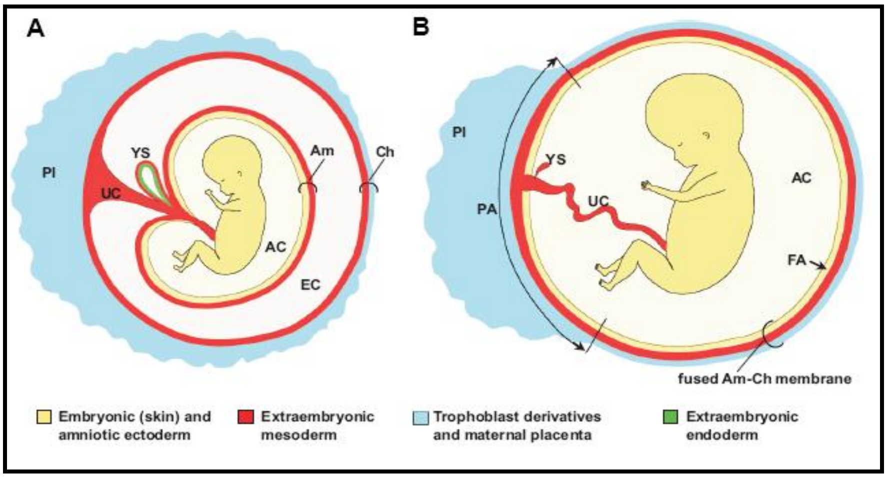

Fig. (3): Appearance of fetal membranes in primates. Schematic representation of a human fetus within its extraembryonic membranes around 10-12 weeks **(A)** and 20 weeks **(B)** of gestation. The umbilical cord consists of extraembryonic mesoderm and - endoderm. The endodermal component is not represented for simplicity ***(Dobreva et al., 2010).***

Decidua

The human decidua is a maternal organ, as opposed to the amnion and chorion, which are genetically derived from the fetus. The decidua is rich in extracellular matrix, decidual cells, macrophages and lymphocytes. While, on the one hand, an immunological barrier exists at the maternal-fetal interface, some hormones, such as prolactin, originating from the decidual cells, transverse the fetal membranes to the amniotic fluid ***(Weiss et al., 2007).***

The deeper layers of the parietal decidua, which remain in utero, are richly supplied with maternal blood vessels. *In the superficial parts of the decidua, maternal vessels are the exception.* Generally, only a few capillaries, smaller arterioles, and venules can be identified ***(Baergen, 2005)***

Amnion

The more or less excentrically inserted umbilical cord is covered with the amnion, which is the innermost layer of the human placenta. It is a tough, alymphatic, avascular, and aneural tissue with a shiny physical appearance ***(Tan et al., 2011).***

Although human amnion is often referred to as a single, continuous structure, it is anatomically divided into three regions: placental amnion (amnion on the chorionic plate), reflected amnion (amnion of the extraplacental fetal membranes), and umbilical amnion (amnion covering the surface of the umbilical cord) ***(Han et al., 2008).***

Cellular anatomy of the amnion (Fig. 4):

The avascular amnion, lying between the amniotic fluid and the chorion, has been described as being constructed from five layers: the amniotic epithelium, the basement membrane, the compact layer, the fibroblast layer and the spongy layer ***(Weiss et al., 2007).***

The amnion is comprised primarily of two cell types: the epithelial cuboid cells and the columnar cells. The amniotic epithelial cells (AE) on one side of the amnion create a continuous lining adjacent to the amniotic fluid, while on the other side of the amniotic epithelium is a thin layer of amniotic mesoderm (AM), throughout which a few fetal macrophages are sporadically distributed ***(Parolini and Soncini, 2006).***

Amniotic epithelial cells exhibit quite characteristic morphological features. They have a relatively small number of intracytoplasmic organelles, microvilli on the apical surface, abundant cytoplasmic processes to the lateral and basal sides, and loose intercellular connections between each other ***(Iwasaki et al., 2003).***

The amnion is loosely attached to the next layer, the chorionic plate, a tough fibrous tissue layer from the outer surface of which the villous trees originate ***(Lorenzi et al., 2010).***

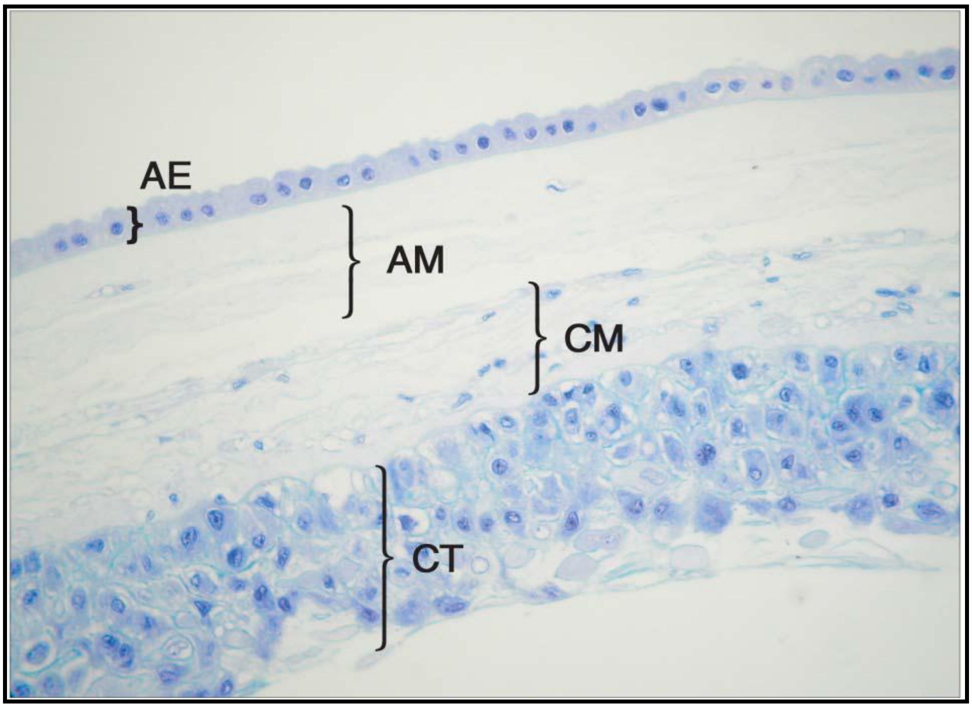

Fig. (4): Morphology of fetal membranes of human term placenta (Giemsa staining; AE = amniotic epithelium layer; AM = amniotic mesenchymal layer; CM = chorionic mesenchymal layer; CT = chorionic trophoblastic layer) ***(Parolini and Soncini, 2006).***

The amnion is formed from the epiblast from the inner cell mass at day eight after fertilization. That is prior to the start of organogenesis, which occurs approximately 3 weeks after fertilization. Therefore, amniotic cells have been hypothesized to retain pluripotent properties of early epiblast cells ***(Dominguez, 2009).***

As illustrated in Figure 5, the epiblast gives rise to the amnion as well as all three embryonic germ layers (ectoderm, endoderm and mesoderm) ***(Dominguez 2009).***

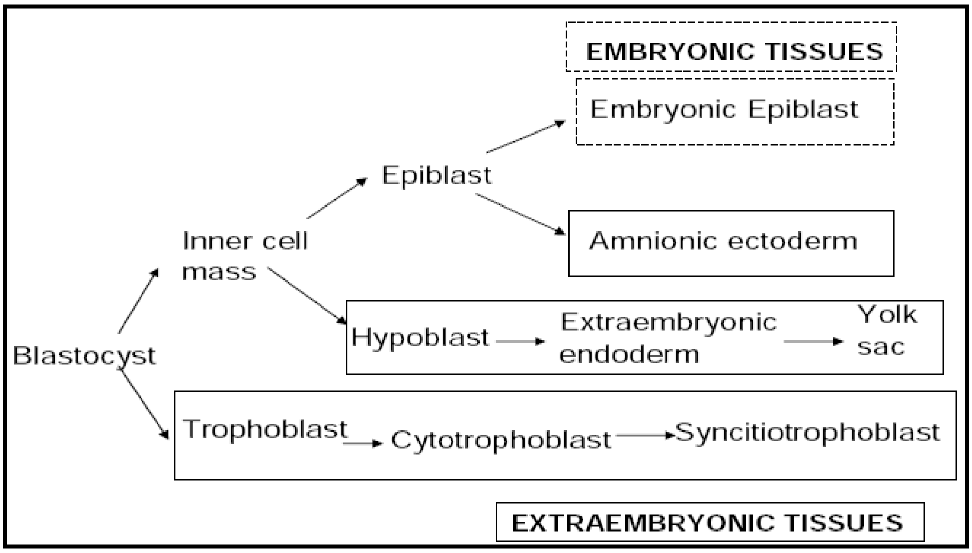

Fig. (5): Origin of the embryonic and extraembryonic tissues. Diagrammatic Illustration ***(Dominguez, 2009).***

During the 4th week of gestation, small spaces appear between the inner cell embryonic mass and the trophoblast ring. By the 22nd day after the last menstruation, these spaces have coalesced to form the amniotic cavity. Around the 5th week of gestation, amniotic fluid begins to accumulate within the cavity. This fluid increases in quantity and causes the amnion to expand. Towards the end of the 12th week the amnion adheres with the mesenchymal inner surface of the chorionic plate, a "fusion" of amnion and chorion and the secondary yolk sac disappears ***(Dominguez, 2009).***

Chorion

The chorionic membrane is a tough fibrous layer carrying the fetal blood vessels. The amnion and chorion are easily separated and will readily slide along one another. This is due to the existence of the spongy layer, which is the result of incomplete fusion of the amnionic and chorionic mesoderm and is thus between the two layers ***(Baergen, 2005).***

The chorion has many villi on its outer surface which are more pronounced at the site of the placenta and attenuated outside this area resulting in formation of chorion frondosum (villous chorion) and chorion laeve (nonvillous chorion), respectively ***(Baradaran-Rafii et al., 2007).***

Cellular anatomy of the chorion (Fig. 4):

The chorionic membrane is derived from the trophoblast layer and is considered an extraembryonic tissue ***(Dominguez, 2009).***

The chorion consists of a reticular layer and a basement membrane on a bed of trophoblasts facing the decidua. The chorion is thicker than the amnion, but the tensile strength is attributed to the amnion ***(Weiss et al., 2007).***

While the amnion lacks trophoblasts (consisting of unique epithelial cells instead), the predominant cells of the chorion are trophoblasts (chorion laeve trophoblasts), similar to the placental villi, and the chorion therefore has a strong resemblance to the villous part of the placenta both in its morphology and its function ***(Iwasaki et al., 2003).***

A histological overview of the composition of the fetal membranes is shown in table (1).

Table (1): Composition of the Fetal Membranes: overview ***(Dominguez, 2009).***

Amniotic membrane	Bilayer	Amniotic epithelium + amniotic mesenchyma
Chorionic membrane	Trilayer	Chorionic mesenchyma + chorionic epithelium chorionic trophoblast

Biochemistry of Fetal Membrane Elasticity

While the chorion is thicker than the amnion, the tensile strength is attributed to the amnion. The thickness of the human term amnion varies between individuals and depends on the location of the sample (70 to 180 μm thick), but it is remarkably strong and elastic ***(Dobreva et al., 2010).***

The amnion has been found to be stronger than the chorion in controlled uniaxial mechanical tests. However, a uniaxial test does not replicate the *in vivo* loading conditions of the membranes, as membranes are loaded biaxially by the amniotic fluid pressure ***(Oyen et al., 2004).***

In vivo, the amnion withstands the progressive stretching of the growing embryo, internal and external traumas, and fast and slow pressure changes. Its strength is due to the epithelial layer and the amniotic mesodermal derived connective tissue in which 4 layers can be distinguished: the compact acelluler layer, the fibroblast layer, the spongy layer, and the reticular layer ***(Dobreva et al., 2010).***

The chorioamniotic extracellular matrix is composed of several different types of collagen arranged in a complex framework, maximizing its mechanical resistance. Major components are types I, III, IV, V, and VI collagens and abundant proteoglycans, which are embedded in the fibrous proteins. The principal tissue support is generated by fibers composed of types I and III collagens, which, in turn, are stabilized by a network of collagen types: IV, V, and VI ***(Zaga-Clavellina et al., 2011).***

The tractional resistance of the amniotic membrane is related mainly to the condensed layer of interstitial collagens type I and II. Collagen type I is the main