INTRODUCTION

Premature Rupture Of Membranes (PROM) occurs in 10% of all gestations and about 2-4% of preterm pregnancies *(Modena et al., 2004)*.

Correct diagnosis of PROM has great importance because failure of diagnosis can lead to unwanted obstetric complications like chorioamnionitis, preterm birth, on the other hand over diagnosis can lead to unnecessary interventions like hospitalization. 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 or ultra-sonographic finding followed by documentation of fluid passing from the cervix *(Hasan and Cevdet, 2007)*.

The management of patients with PROM, regardless of gestational age, remains controversial, and it is therefore important to achieve accurate diagnosis by identifying the presence of specific amniotic fluid markers in vaginal environment. The methods used to diagnose PROM are variable and based as much on clinical evaluation as on biological

tests, which are useful in the cases of clinically asymptomatic patients and/or in the ones with unclear PROM. These tests include the measurement of vaginal pH, prolactin, Alpha Fetroprotein (AFP), diamine oxydase, Insulin Like Growth Factor Binding Protein-1 (IGFBP-1), human chorionic gonadotropin and fetal fibronectin. All these tests have advantages and drawbacks, up to now there is no gold standard diagnostic test for PROM *(Esim et al., 2003 and Darj et al., 1998)*.

Fetal urine is the most important source of amniotic fluid in the second half of pregnancy. So, vaginal fluid urea and creatinine may be helpful in diagnosis of PROM *(Hasan and Cevdet, 2007)*.

Vaginal fluid creatinine determination has been used in the clinical studies to diagnose PROM *(Li Hy and Chang, 2000; Gurbuz et al., 2004)*.

Li Hy and Chang, (2000) have found creatinine less expensive and easier to measure than hCG and AFP and appears to be more accurate than hCG. More recently *Gurbuz et al. (2004)* reported that the specificity, positive predictivity and negative

predictivity were all 100% in detecting PROM by evaluation of vaginal fluid creatinine concentration with a cut-off value of 0.12mg/dL and they speculated that vaginal creatinine measurement is cheaper and faster than other methods, and has higher sensitivity and specificity to establish accurate diagnosis.

AIM OF THE WORK

The aim of this study was to evaluate the reliability of vaginal fluid urea and creatinine for the diagnosis of Premature Rupture Of Membranes (PROM).

ANATOMY AND BIOCHEMISTRY OF THE FETAL MEMBRANES

The membranous structure that surrounds the developing fetus and forms the amniotic cavity is derived from fetal tissue and is composed of two layers: the amnion (inner layer) and the chorion (outer layer). The amnion is a translucent structure adjacent to the amniotic fluid, which provides necessary nutrients to the amnion cells. The chorion is a more opaque membrane that is attached to the decidua (i.e., maternal tissue that lines the uterus during pregnancy). The amnion and chorion are separated by the exocelomic cavity until approximately three months gestation, when they become fused. Intact, healthy fetal membranes are required for an optimal pregnancy outcome (*Benirschke and Kaufmann, 1995).*

Extracellular matrix (ECM) proteins synthesized by several cell types within the amnion and chorion confer both strength and elasticity to the fetal membranes. During or just prior to labor, the

breakdown of these proteins is regulated by matrix metalloproteinases (MMPs) and their inhibitors. These biochemical changes in the fetal membranes reduce their integrity and elasticity, make them more vulnerable to rupture, and may contribute to the initiation of parturition *(Bryant, 1998).*

Anatomy:

Inspection of the fetal membranes following delivery reveals amnion that is mildly adherent to the fetal side of the chorion. Small amounts of maternal decidual tissue can be observed attached to the outer, maternal side of the chorion *(Bryant, 1998).*

Cellular anatomy:

Fetal membranes, as the name implies, are genetically identical to the fetus. The membranes contain many cell types, but are avascular and without nerve cells. The cells appear columnar where the membranes are attached to the placenta, but become more flattened or cuboidal adjacent to the deciduas *(Parry and Strauss, 1998*).

Amnion:

The amnion is loosely composed of three layers of cells:

- The inner compact layer, which varies greatly in thickness, consists of epithelial cells attached to a basement membrane (Figs. 1 and 2) *(Parry and Strauss, 1998)*.
- The mesenchymal cell layer, the thickest of the amnion layers, is comprised of dispersedly distributed fibroblasts.
- The outer intermediate layer, also known as the spongy layer or zona spongiosa, is adjacent to the chorion and can swell to facilitate sliding of the amnion across the chorion. In this way, the intermediate layer provides a cushioning mechanism to reduce stress applied to the fetal membranes *(Behzad et al., 1994).*

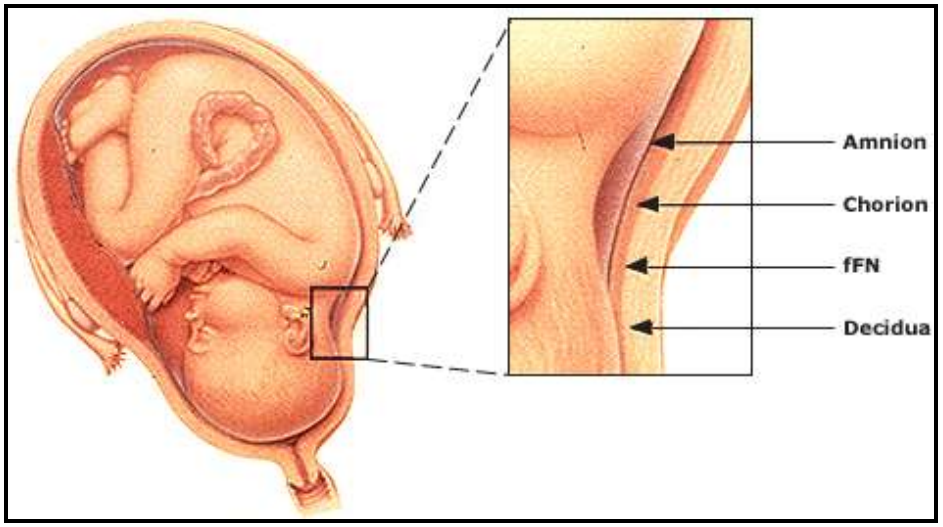

Fig. (1): Amnion chorion

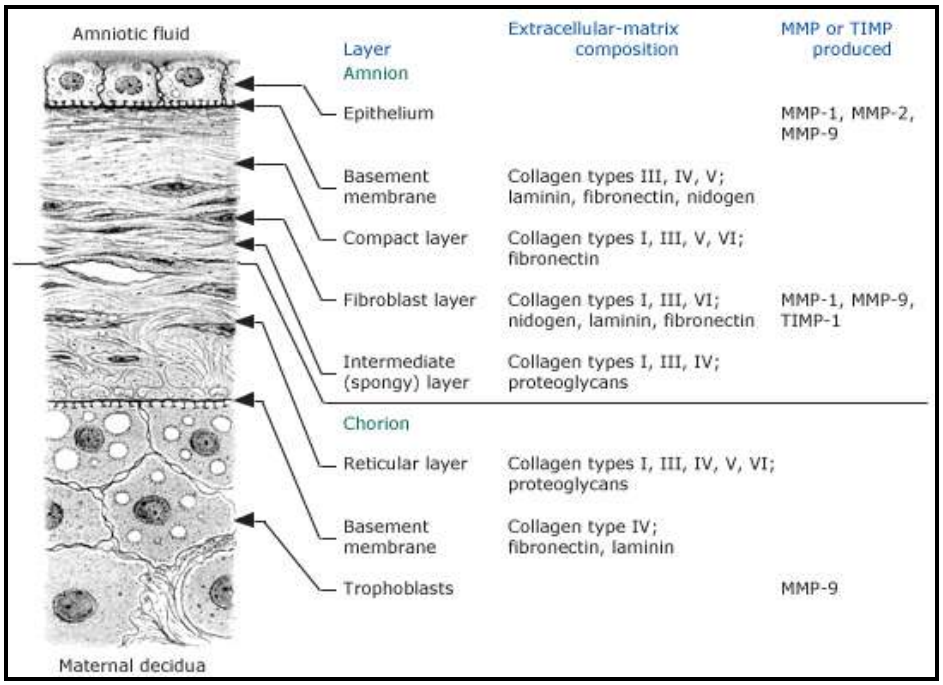

Fig. (2): Layers of amnion chorion

Chorion:

The chorion is composed of two layers: an outer reticular and an inner cytotrophoblast layer. The reticular layer is structurally similar to the amnionic mesenchyme, and is predominately made up of fibroblasts and macrophages. A basal lamina or pseudobasement membrane separates the reticular layer from the underlying cytotrophoblast layer *(Bryant-Greenwood, 1998).*

The cytotrophoblast cells of the chorion, similar to all other trophoblasts found outside of the placental villus, are known as extravillous trophoblasts. They vary in appearance from small, round, proliferating cells to large, polygonal, well-differentiated cells. Although thicker than the amnion, the chorion plays only a minor role in maintaining the tensile strength of the fetal membranes *(Bryant-Greenwood, 1998).*

Biochemistry of fetal membrane strength:

Fetal membrane strength is influenced by a number of proteins including extracellular membrane proteins, metalloproteinases, and tissue inhibitors of metalloproteinases.

Extracellular membrane proteins:

The strength and integrity of fetal membranes derive from extracellular membrane proteins including collagens, fibronectin, and laminins.

Collagens:

The collagens are a large family of tripple helical ECM proteins that interact with other components of the extracellular matrix. Interstitial collagens (types I and III) located primarily in the compact layer of amnion play a critical role in maintaining the integrity of the fetal membranes and are the primary regulators of their tensile strength *(Parry and Strauss, 1998).*

The cellular source of compact layer collagens is uncertain. In one report, mesenchymal cells expressed significantly higher levels of collagens I and III than did epithelial cells. However, another study using fetal membrane organ cultures found that both epithelial and mesenchymal cells produce interstitial collagens. The epithelial cell contribution to collagen synthesis is likely to be dependent upon gestational age, as the density of mesenchymal cells in the amnion decreases

in the third trimester. Thus, both epithelial and mesenchymal cells appear to contribute to the pool of interstitial collagens in the compact layer *(Casey and MacDonald, 1996).*

Type IV and VII collagens also make important contributions to the integrity of the fetal membranes.

- Type IV collagen, a basement membrane protein, is a product of both the epithelial cells of the amnion and the pseudobasement membrane of the chorion. This collagen provides a scaffolding on which to attach and assemble other components of the basement membrane, such as laminin and heparin sulfate proteoglycan *(Malak et al., 1993).*
- Type VII collagen is made by epithelial cells of the amnion. It stabilizes the fetal membranes by creating anchoring fibrils that link the basal lamina of the amnion to the extracellular matrix components *(Rousselle et al., 1997)*.

Other compact layer collagens (e.g., types V and VI) play a lesser role in maintaining fetal membrane strength *(Kanayama et al., 1985).*

Fibronectin:

The fibronectin that is present in basement membranes of the amnion and chorion is an oncofetal or fetal fibronectin (onfFN), a uniquely glycosylated molecule with a specific oncofetal epitope that is expressed by tumor cells and fetal tissue. onfFN is thought to be a "trophoblast glue" that promotes cellular adhesion at uterine-placental and decidual-fetal membrane interfaces. It is released when the extracellular matrix of the chorionic/decidual interface is disrupted prior to labor *(Lockwood et al., 1991).*

Clinically, the determination of oncofetal fibronectin levels in the cervical and vaginal secretions of pregnant women may be a biochemical marker for the diagnosis and prediction of preterm labor.

Laminins:

Laminins interact with type VII collagen to stabilize fetal membranes. They anchor cells to the basement membrane and the basement membrane to the underlying layers. Laminin 7, a novel laminin variant, has been found in fetal membranes associated with laminin 5 *(Champliaud et al., 1996)*.

Matrix metalloproteinases:

Matrix metalloproteinases are a family of enzymes with varied substrate specificities that decrease membrane strength by increasing collagen degradation. In situ hybridization and immuno-histochemical analyses have identified MMP-2 (gelatinase A) and MMP-9 (gelatinase B) mRNA, protein, and activity in amnion epithelial cells and chorionic trophoblasts in fetal membranes after delivery. MMP-1 (collagenase-1) and MMP-8 (colla-genase-2) hydrolyze triple helical regions of inter-stitial collagens (types I and III), which are sub-sequently degraded by MMP-9 and MMP-2. MMP-2 and MMP-9 also degrade type IV collagen, proteo-glycans, and fibronectin *(Fortunato et al., 2000).*

Tissue inhibitors of metalloproteinases:

Tissue inhibitors of MMPs (TIMPs) bind to matrix metalloproteinases and shut down proteolysis, thereby helping to maintain membrane integrity. In vivo and in vitro studies demonstrated TIMPs in amnion and chorion layers. Thus, cells above and below compact layer collagens secrete both MMPs

capable of hydrolyzing interstitial collagens as well as inhibitors that block this process *(Riley et al., 1999).*

Antimicrobial activity:

Infection/inflammation impacts survival of the cells comprising the fetal membranes. There is some evidence that the fetal membranes have antimicrobial activity, which may help protect them from rupture. Pattern recognition receptors (PRRs) in tissue are thought to note the presence of microorganisms and initiate a protective host response. The toll-like receptors (toll-like receptor-2 and -4) are a type of PRRs that are expressed on the amnion, particularly during labor and with histologic chorioamnionitis. These receptors may play a role in the antimicrobial activity of fetal membranes *(Stock et al., 2007).*

Biochemistry of fetal membrane elasticity:

Fetal membranes must have elastic properties to accommodate enlargement of the uterus resulting from increases in fetal size and amniotic fluid as pregnancy progresses. Elastic fibers consist of fibrillin-containing microfibrils that are cross-linked to elastin. These microfibrils are abundantly expressed in the

mesenchymal and compact layers of the amnion and the reticular and cytotrophoblast layers of the chorion. Elastin is also present and appears to contribute to the elasticity of fetal membranes *(Kim et al., 2004).*

Pathogenesis of rupture:

The fetal membranes normally remain undisturbed until late in gestation, most likely due to low MMP activity and high levels of TIMPs. When contractions begin or the membranes rupture, MMP activity in the amnion and chorion increases and levels of interstitial and basement membrane collagens decrease. The specific changes that occur are illustrated by the following observations *(Hampson et al., 1997)*:

- In humans and in rat models, MMP-9, MMP-2, MMP-3 (stromelysin), and interstitial collagenase activities in fetal membranes and amniotic fluid rise near delivery. In comparison, the level of TIMP-1 dramatically falls at these sites in association with labor *(Riley et al., 1999).*
- An increase in MMP-9 activity has been noted in extracts of fetal membranes that overlie the