INTRODUCTION

Preterm labor (PTL) refers to effective uterine contractions leading to cervical changes occurring before 37 weeks of gestation. It is classified according to gestational age into mild preterm (32 to <37 weeks), very preterm (28 to <32 weeks), extremely preterm (<28 weeks); and according to birth weight into low birth weight (LBW <2500 grams), very low birth weight (VLBW <1500 grams), extremely low birth weight (ELBW <1000 grams) ***(WHO et al., 2012).***

Preterm birth is the leading direct cause of neonatal death. It is responsible for 27% of neonatal deaths worldwide, comprising over one million deaths annually. The risk of neonatal mortality decreases as gestational age at birth increases, but the relationship is nonlinear ***(Lawn et al., 2010).*** The burden of preterm birth includes neonatal morbidity and long-term sequelae, including neurodevelopmental deficits and an increased risk of chronic disease in adulthood ***(Mwaniki et al., 2012).***

Worldwide, the preterm birth average incidence is estimated to be about 11% (range 5% [parts of Europe] to 18% [parts of Africa]), and about 15 millions children are born preterm each year (range 12 to 18 millions) ***(WHO, 2008).*** Of these preterm births, 84% occur at 32 to 36 weeks, 10% occur at 28 to <32 weeks, and 5% occur at <28 weeks ***(Blencowe et al., 2012).***

Approximately 70 to 80% of preterm births occur spontaneously. 40-50% of preterm births are related to premature uterine contractions, 20-30% of preterm births are related to preterm premature rupture of membranes (PPROM). The remaining 20 to 30% of preterm births are medically indicated because of maternal or fetal issues, such as preeclampsia, placenta previa, abruptio placenta, fetal growth restriction or multiple gestations ***(Muglia and Katz, 2010).***

Progesterone appears be important in maintaining uterine quiescence in the second half of pregnancy, possibly by limiting the production of stimulatory prostaglandins and inhibiting the expression of contraction-associated protein genes within the myometrium, including ion channels, oxytocin and prostaglandin receptors, and gap junctions ***(Norwitz and Lye, 2009).***

Although levels of progesterone in the maternal circulation do not change significantly in the weeks preceding labor, the onset of labor both at term and preterm is associated with a functional withdrawal of progesterone activity at the level of the uterus ***(Lockwood et al., 2010).***

The new proposed phenotypic classification of preterm birth aims to take into consideration pathophysiologic events occurring with the mother, placental or fetal compartment. Therefore, the pathophysiologic mechanisms of preterm birth

are likely to expand to include factors such as poor progesterone effect ***(Zakar and Mesiano, 2011)***.

Progesterone, IM injections and vaginal pessaries were found to be effective for the prevention of PTL in women with a previous history of PTL and in those with a mid-trimester short cervix ***(Dodd et al., 2013).***

The efficacy of progesterone supplementation for prevention of preterm birth depends primarily on appropriate patient selection. In addition, there is evidence from in vitro and animal research that the type of progesterone, formulation, dose, and route of delivery have a significant impact on efficacy ***(Kuon et al., 2010).***

The review of 36 randomized controlled trials (RCT), involving a total of 8523 women considered to be at increased risk of preterm birth, and 12,515 infants, found that where progesterone was given (intramuscularly in most studies and transvaginally in others), it had beneficial effects ***(Dodd et al., 2013).***

Support for the use of progesterone for preventing preterm birth is provided by RCT and meta-analyses. Intramuscular administration of progesterone is painful and patient compliance may be reduced because of the need for medical assistance. Vaginal administration can be associated with unpleasant vaginal discharge. Only a few studies have investigated the oral route using micronized progesterone,

although it is cheaper, easier to use and more acceptable ***(Rai et al., 2009).*** For these reasons, we plan to compare the effect of receiving oral micronized progesterone, versus placebo, on incidence of the preterm birth ***(Utrocare®, October Pharma, Sixth of October city, Egypt)***.

AIM OF THE WORK

The aim of this study is to evaluate the role of oral micronized progesterone (OMP) versus placebo, in the prevention of preterm birth in patients with a past history of preterm delivery.

Research Hypothesis:

Progesterone has a role in prevention of preterm delivery among women with history of preterm birth.

Null Hypothesis:

The rate of preterm delivery in patients receiving oral micronized progesterone is the same as that in patients receiving placebo.

Research Question:

Is oral micronized progesterone more effective than placebo in preventing preterm birth?

Clinical application:

The risk of neonatal mortality decreases as gestational age at birth increases. Preterm birth is the leading direct cause of neonatal morbidity and mortality, in addition to its effect on maternal morbidity rates. So in this study, we will compare the incidence of the preterm birth among patients receiving oral micronized progesterone versus placebo.

Chapter I

PRETERM BIRTH

Preterm labor (PTL) refers to effective uterine contractions leading to cervical changes occurring before 37weeks of gestation. It is classified according to gestational age into mild preterm (32 to <37weeks), very preterm (28 to <32 weeks), extremely preterm (<28 weeks); and according to birth weight into low birth weight (LBW <2500 grams), very low birth weight (VLBW <1500 grams), extremely low birth weight (ELBW <1000 grams) ***(WHO et al., 2012).***

Significant progress has been made in the care of premature infants, but not in reducing the prevalence of preterm birth. In the United States, there has been a 21% rise in the rate of preterm births since 1990, which peaked in 2006 with 12.8% of all 4 million annual live births born at less than 37 weeks of gestation. The incidence in Europe and other developed countries lies between 5-9%. East Asian and Hispanic women typically have a low preterm birth rate. However, the incidence of preterm birth continues to rise. Part of this escalation is due to the increased indicated preterm delivery of artificially conceived multiple pregnancies, which account for 15-20% of all preterm births ***(Offiah et al., 2012).***

Preterm birth is the principal cause of infant mortality in developed countries. One in 8 births in the United States in 2005 were preterm, compared to 1 in 18 births in Ireland and Finland.

The infant mortality rate in Ireland in 2010 was 3.89 per 1000 live births and in the United States 6.8 per 1000 live births. The main cause of the United States' high infant mortality rate when compared with Europe is the very high percentage of preterm births in the United States, the period when infant mortality is greatest ***(Mac Dorman & Mathews, 2010).***

Pathophysiology of preterm labor

Cervical Ripening

The collagen content of the cervix, both type I and type III, undergoes marked changes in pregnancy. The spaces between the collagen bundles become dilated as early as 8 to 14 weeks gestation. Although there is an increase in the total collagen content of the cervix at term, the collagen concentration is reduced by 30 to 50 percent compared with the non- pregnant cervix. This arises because other components of the cervix, the water, and non-collagen proteins are increasing in relatively greater amounts. In addition, the collagen fibrils are reduced in size ***(Mahmoud et al., 2013)***.

During pregnancy, hyaluronic acid concentration in the cervix is very low, but increases rapidly at the onset of labor. Hyaluronic acid has a high affinity for water molecules and hence can maintain tissue hydration. Hyaluronic acid can stimulate collagenase production ***(Akgul et al., 2014).***

Collagenase is a lytic enzyme, now called matrix metalloproteinase which is produced by fibroblasts and leukocyte is secreted in a latent form, procollagenase, which is activated by cleavage of the pro-enzyme by plasmin or stromelysin to the active form ***(Becher et al., 2010).***

Ripening refers to the increased softening, dispensability, effacement, and early dilatation of the cervix that can be detected by pelvic examination ***(Ducarme et al., 2016).***

Cervical ripening is an active biochemical process, which occurs independent of uterine contractions, and is similar to an inflammatory reaction. During this process the inflammatory cascade is activated, including the release of proinflammatorycytokines, the infiltration of white blood cells, the release and activation of degradative enzymes (matrix metalloproteinases), a changing synthesis of extracellular matrix proteins and glycoproteins, an increase in collagen turnover, a disruption of tightly aligned collagen fibrils, changes in the decorin/collagen ratio, and increased extracellular fluid due to hyaluronic acid ***(Ducarme et al., 2016).***

Various human agents are involved in cervical ripening, including progesterone, relaxin, prostaglandins, and local mediators such as proinflammatory cytokines and nitrous oxide (NO). However, the exact biochemical mechanisms responsible for the rearrangement of extracellular matrix during cervical ripening are still poorly understood. Nevertheless, the dissolution

of collagen fibers via enzymatic degradation and/or 'dilution' by increasing proteoglycan concentrations is the pivotal event during cervical ripening, resulting in the subsequent decrease in cervical resistance ***(Gonzalez et al., 2011).***

Progesterone seems to exert an overall control on cervical ripening. Antiporgestins are effective agents in inducing cervical ripening in all species investigated including humans. In guinea pigs, the progesterone agonist (promegestone) completely blocked onapristone-induced cervical ripening, indicating that this effect was mediated by the progesterone receptor ***(Stricker et al., 2015).***

Progesterone has an inhibitory effect on cervical ripening and parturition in those animals in which a decrease in progesterone at term results in ripening and labor. Such a decrease does not occur in the human, but progesterone is a potent anti-inflammatory agent, and could still be an important physiologic inhibitor of the ripening process in vivo by inhibiting neutrophil influx and activation ***(House et al., 2014).***

Prostaglandins, particularly PGE2, have for a long time been thought the key mediators of cervical ripening ***(Sykes et al., 2014).***

Locally administered prostaglandins are effective in inducing cervical changes and the results of many experimental and clinical studies suggest that endogenous prostaglandins are involved in cervical ripening ***(Yount and Lassiter, 2013).***

Amniotic fluid concentration of PGE2 and PGF2 α correlate directly with the cervical score in women at term who are not in labor. In addition receptors for PGE2 and PGF2 α can be demonstrated in the cervix. Prostaglandins might affect cervical ripening by inducing the breakdown of collagen. PGE2 treatment will reduce collagen concentration similar to the changes seen in physiologic ripening, but it is uncertain whether this is the result of the breakdown of collagen ***(Karim, 2012).***

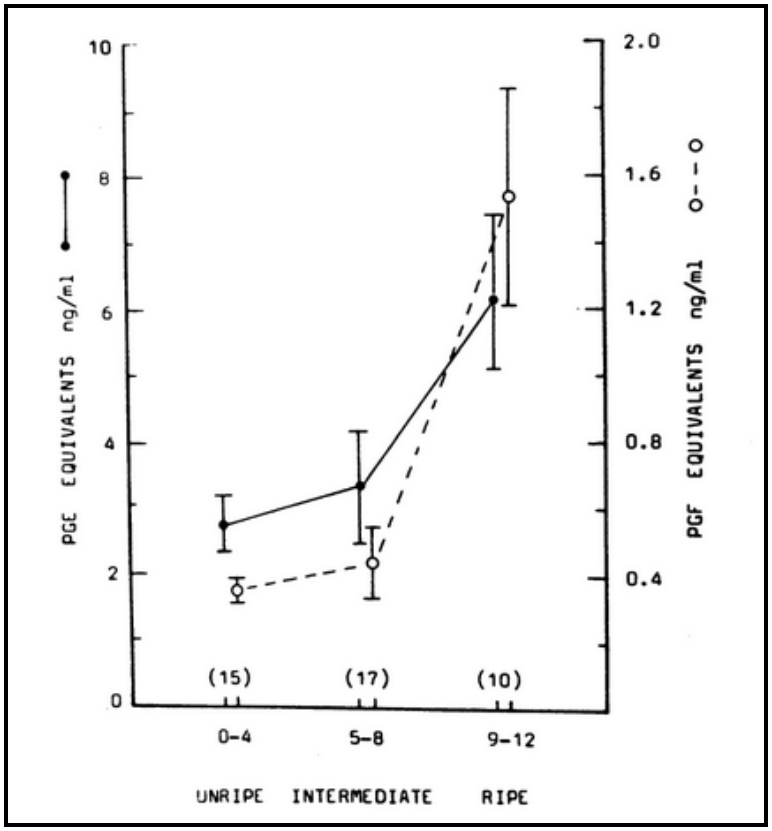

Fig. (1): Cervical score ***(Karim, 2012).***

Estrogens such as estradiol have been used to bring about cervical ripening in the clinical situation. The mechanism underlying these effects may be due in part to the induction of prostaglandin synthesis within the tissues. Estradiol might also be responsible for the influx of protease-producing leukocytes that could induce ripening ***(Andersson et al., 2008).***

There is good theoretical evidence that relaxin, a dimeric peptide hormone, plays a role in the process of cervical ripening in humans. It has been shown to increase collagenase activity perhaps via a mitogenic effect on fibroblasts, which are known to exhibit relaxin receptors. Pharmacologically, relaxin has been shown to cause cervical ripening in women ***(Gu et al., 2016).***

While the specific role of relaxin during human pregnancy is unknown, increased relaxin concentration in the maternal circulation might be associated with preterm labor perhaps by altering cervical connective tissue ***(Kota et al., 2013).***

Cervical ripening is considered to be a physiologic inflammatory process, characterized by an accumulation of neutrophils in the cervical stroma. Interleukin- 8 is an inflammatory cytokine that is capable of producing selective neutophilchemotaxis and activation. This cytokine can be produced by fibroblasts in non-pregnant and pregnant rabbits. Interleukin- 8 may have a synergistic interaction with PGE2 in promoting cervical ripening. Other cytokines, including interleukin-1 and tumor necrosis factor, have been shown to produce cervical ripening in animal studies ***(Timmons et al., 2010).***

There are several evidences suggesting that nitric oxide play a pivotal physiological role in cervical function. Treatment of pregnant guinea pigs with nitric oxide synthetase (NOS) inhibitor delayed physiological cervical ripening, resulting in prolonged deliveries ***(Chwalisz et al., 1994).*** Furthermore, (NOS) inhibitor treatment of pregnant rats significantly prolonged the duration of delivery, indicating indirectly a cervical dystocia ***(Kelly et al., 2011).***

More recent studies on humans support that concept. Vaginal administration of the nitric oxide donor isosorbidemononitrate induces cervical ripening in women at term ***(Kelly et al., 2011).***

Furthermore, cervical fluid nitric oxide metabolite level rises after cervical ripening, nitric oxide donor administration, or cervical manipulation, which supports a role for cervical nitric oxide in cervical ripening ***(Rahkola et al., 2011).***

In summary, NO represents the final metabolic pathway of cervical ripening acting in concert with prostaglandins (mainly PGE2) by inducing local vasodilatation, increasing vascular permeability and leukocyte infiltration, and other mechanisms responsible for the extracellular matrix remodeling such as modulation of proteoglycan synthesis ***(Ekman-Ordeberg et al., 2003).***

Pathogenesis of preterm labor:

Spontaneous preterm labor is a physiological heterogeneous syndrome. The cascade of events that culminate in spontaneous preterm labor has several possible underlying pathways. Four of these pathways are supported by a considerable body of clinical and experimental evidence:

1. Premature activation of the maternal or fetal hypothalamic-pituitary- adrenal axis.
2. Intrauterine infection or inflammation.
3. Decidual hemorrhage.
4. Excessive myometrial and fetal membrane over distention.

These pathways may be initiated weeks to months before clinically apparent preterm labor. The processes leading to preterm parturition may originate from one or more of these pathways ***(Hyagriv and Steve, 2007).***

Premature activation of the maternal or fetal hypothalamic- pituitary- adrenal (HPA) axis: Premature activation of the (HPA) axis can initiate preterm labor. Major maternal physical or psychological stress, which can activate the maternal HPA axis, has been associated with a slightly higher rate of preterm labor ***(Dole et al., 2003).***

Premature fetal HPA activation can result from the stress of uteroplacentalvasculopathy and has higher correlation with

subsequent preterm labor than maternal stress ***(Wadhwa et al., 2011).***

The mechanisms by which HPA activation are thought to cause preterm labor include, increase release of corticotropin-releasing hormone (CRH), which appears to program a "placental clock". Increased release of fetal pituitary adrenocorticotropic hormone (ACTH) secretion, which stimulates production of placental estrogenic compounds that may activate the myometrium and initiate labor ***(Gleicher, 2012).***

Corticotrophin-releasing hormone

It is a 41-amino acid peptide initially localized to the hypothalamus but also expressed by cells in the placenta, chorion, amnion, and uterine decidua ***(Kramer et al., 2010).***

The maternal plasma concentration of corticotropin-releasing hormone rises during the second half of pregnancy and peaks during labor ***(Farina et al., 2004).***

This hormone stimulates the production of prostaglandins by cells of the amnion, chorion, and decidua. Prostaglandins then directly stimulate uterine contractions and cause cervical ripening. Prostaglandins also stimulate the release of corticotropin-releasing hormone in the placenta, fetal membranes, and decidua, resulting in a positive paracrine feedback loop that drives preterm delivery. Whereas glucocorticoids inhibit the hypothalamic release of (CRH),

stress induced increases in fetal or maternal cortisol production enhance placental production of (CRH). The production stimulates the release of (ACTH) from the fetal pituitary gland which, in turn, stimulates fetal adrenal cortisol production by positive feedback loop. Cortisol also directly increases the production of corticotropin-releasing hormone by the placenta and fetal membranes and has local effects that stimulate the synthesis of prostaglandins in the fetal membranes ***(Hansen, 2013; Kota et al., 2013).***

Estrogen

Activation of the fetal HPA axis also leads to PTB through a pathway involving estrogens. Fetal pituitary ACTH secretion stimulates adrenal synthesis of dehydroepiandrosterone sulfate (DHEA-S), which is converted to 16- hydroxyl DHEA-S in the fetal liver. Placental CRH also can augment fetal adrenal DHEA production directly ***(Ding and He, 2007).***

The placenta converts these androgen precursors to estone (E1), estradiol (E2), and esteriol (E3), which in turn, activate the myometrium by increasing gap junction formation, oxytocin receptors, prostaglandin activity, and enzymes responsible for muscle contraction ***(Mungenast and Thalhammer, 2014).***