INTRODUCTION

Surgical termination of pregnancy is associated with complications such as uterine perforation, cervical laceration and incomplete evacuation of the uterus. Cervical ripening prior to surgical termination of pregnancy has been shown to reduce the associated operative morbidity ***(Ledingham et al., 2001).***

A previous study has confirmed that preoperative cervical ripening with a prostaglandin analogue can reduce the incidence of complications, including the duration of postoperative vaginal bleeding, readmission for abnormal vaginal bleeding, pelvic infection, and the incidence of recurettage ***(Li et al., 2003a).***

Nitric oxide is a free radical with a very short half-life. The nitric oxide-generating system has been shown to exist in human female genital tract, and there is an up-regulation of the system with advanced gestation. The nitric oxide donors have been tested in various clinical applications, including cervical ripening during first-trimester pregnancy and third-trimester pregnancy, treatment of preeclampsia and preterm labor. No information is available concerning the application of nitric oxide donor for second trimester cervical ripening ***(Li et al., 2003b).***

Studies in animals have shown that nitric oxide (NO) is the final common pathway in cervical ripening. NO donors have been shown to have similar efficacy and fewer side effects than prostaglandins for cervical ripening before surgical termination of pregnancy in the first trimester. In contrast to prostaglandins, these drugs are uterine relaxant and thus have the theoretical advantage of achieving cervical ripening without uterine contractions ***(Sharma et al., 2005).***

Human studies using nitric oxide donors for cervical ripening have been conducted. It has been shown that nitric oxide donors are effective in priming the cervix before suction evacuation of the uterus in terminating first-trimester pregnancies, though it is less effective than prostaglandin analogues ***(Thomson et al., 1997; Thomson et al., 1998; Facchinetti et al., 2000; Ledingham et al., 2001).***

NO donors have been shown to stimulate prostaglandin production in the human cervix after vaginal administration ***(Ledingham et al., 1999; Ekerhovd et al., 2002).*** Even if NO does not stimulate prostaglandin production, the cervical softening effects of NO donors and prostaglandins would still be additive ***(Garfield et al., 1998).***

If this hypothesis is correct, this would allow the use of a lower dose of prostaglandin needed to affect cervical

softening if a NO donor is given in combination with a prostaglandin compared with a prostaglandin given alone. Such a strategy might reduce the side effects associated with larger doses of either agents used alone. Combination therapy might therefore produce an optimal therapeutic strategy for first trimester preoperative cervical ripening ***(Ledingham et al., 2001).***

AIM OF THE WORK

Primary Objective

To compare the efficacy of misoprostol combined with isosorbide mononitrate in cervical ripening prior surgical evacuation of first trimester missed abortion, as demonstrated by larger baseline cervical dilatation in mm and decreased force required to dilate the cervix.

Secondary Objectives

- To compare safety and evaluate adverse events recorded during the study.
- To correlate the effectiveness of the medication with clinical parameters, including duration of the operation and occurrence of cervical trauma.

Chapter One

EMBRYOLOGY, ANATOMY AND HISTOLOGY OF THE UTERUS AND CERVIX

Embryology:

In the absence of the anti-müllerian hormone (AMH), which is secreted from the developing testes, the squamous epithelium of the portio vaginalis and the mucous membrane of the cervical canal are developed from the Müllerian ducts, along with regression of the mesonephric duct system ***(O'Rahilly 1977; Byskov and Hoyer 1994).***

The cervical stroma is derived from a layer of mesenchyme that surrounds these structures early in fetal life ***(Davies and Kusania, 1962).***

By the 10th week of the intrauterine life, differentiation of the cervix from the uterine body begins, and it is clearly recognized as a separate entity by the 20th week of life. In a 22-week fetus, the smooth muscle is well differentiated in the corpus uteri but absent from the wall of the cervix ***(Moore and Persaud, 1993).***

Within 2 weeks of birth the reduction of hormonal stimulation results in a reduction in length of the uterus to about 25mm; two thirds of this length is still the cervix. The infantile stage remains until about 2 years before the

menarche when the uterus doubles its length and its weight increases 10 folds. Maturation to the adult stage occurs over the next 2 or 3 years ***(Hughesdon, 1952).***

Gross Anatomy:

The uterus consist of two basic parts the body and its endometrium and the uterine cervix. The uterus is primarily muscular organ, is located in the pelvic cavity of non-pregnant women and also during first trimester of pregnancy. It is situated between the bladder on its anterior surface and the rectum on its posterior surface. The hole of the non-pregnant uterus protrudes into the vagina and is approximately 2cm long, 0.5 - 1cm wide and cylindrical in shape. It is called the cervix ***(Leppert 1992; McMinn 1999; Cunningham et al. 2005).***

The uterus measures about 7.5 cm. in length, 5 cm. in breadth, at its upper part, and nearly 2.5 cm. in thickness; it weighs from 30 to 40 gm. It is divided into two portions. On the surface, about midway between the apex and base, is a slight constriction, known as the isthmus, and corresponding to this in the interior is a narrowing of the uterine cavity, the internal orifice of the uterus (internal os). The portion above the isthmus is termed the body and that below, the cervix. The part of the body which lies above a plane passing through the points of entrance of the uterine tubes is known as the fundus ***(Bannister et al., 2005).***

The cervix is the lower constricted segment of the uterus. It is somewhat conical in shape, with its truncated apex directed downward and backward, but is slightly wider in the middle than either above or below. Owing to its relationships, it is less freely movable than the body, so that the latter may bend on it. The long axis of the cervix is therefore seldom in the same straight line as the long axis of the body. The long axis of the uterus as a whole presents the form of a curved line with its concavity forward, or in extreme cases may present an angular bend at the region of the isthmus. The cervix projects through the anterior wall of the vagina, which divides it into an upper, supravaginal portion, and a lower, vaginal portion ***(Bannister et al., 2005).*** The supravaginal segment on its posterior surface is covered by peritoneum. Laterally, it is attached to the cardinal ligaments, and anteriorly, it is separated from the overlying bladder by loose connective tissue. The external os is located at the lower extremity of the vaginal portion of the cervix, the portio vaginalis ***(Cunningham et al., 2005).***

The cervix is bounded at its cephalic end by the internal os and at its caudal end by the external os. The internal os is located at the peritoneal reflection of the bladder. The whole cervical length is about 2.5-3 cm and its wall is about 1 cm thick throughout its length. The cervix is divided into two portions, the portio vaginalis and the portio supra vaginalis, according to the segments that

lie respectively below and above vaginal reflection. The vaginal reflection is located at about the junction of the lower and middle thirds of the cervix ***(Cunningham et al., 2005).***

The uterine supports (the pubocervical fascia anteriorly, the uterosacral ligaments posteriorly and the transverse cervical or cardinal ligament laterally) are attached to the cervix immediately superior to the vaginal reflection in the non-pregnant woman. They stabilize the cervix in the center of the pelvis and during pregnancy they stabilize the cervix helping the uterus to expel the baby in the second stage of labor ***(Danforth, 1983).***

Microscopic anatomy (Histology)

In the cervix the mucous membrane is sharply differentiated from that of the uterine cavity. It is thrown into numerous oblique ridges, which diverge from an anterior and posterior longitudinal raphe. In the upper two-thirds of the canal, the mucous membrane is provided with numerous deep glandular follicles, which secrete clear viscid alkaline mucus; and, in addition, extending through the whole length of the canal is a variable number of little cysts, presumably follicles which have become occluded and distended with retained secretion. They are called the ovula Nabothi. The mucous membrane covering the lower half of the cervical canal presents numerous papillae. The epithelium of the upper two-thirds is cylindrical and

ciliated, but below this it loses its cilia, and gradually changes to stratified squamous epithelium close to the external orifice. On the vaginal surface of the cervix the epithelium is similar to that lining the vagina; stratified squamous ***(Gartner and Hiatt, 2002).***

Two distinct systems of the cervix are of importance:

1. The mucous membrane.

The cervix is lined with a pale staining simple columnar mucous secreting epithelium and contains branched tubular mucous glands lined with the same type of epithelium extend to the lamina propria. A short distance above the level of the uterine ostium, the external os, the epithelium changes abruptly from the simple columnar to stratified squamous non keratinized epithelium ***(Gartner and Hiatt, 2002).***

2. The wall of substance of the cervix

The underlying stroma of the cervix is predominately extracellular connective tissue matrix, namely type I (70 %) and III (30%) collagen ***(Gartner and Hiatt, 2002)*** and a small amount of type IV collagen seen in the basement membranes.

Water, glycosaminoglycans, and proteoglycans are important constituents of the uterine cervical matrix as well, especially dermatan sulfate, hyalouronic acid, and heparin sulfate ***(Gartner and Hiatt, 2002).***

Fibronectin, a different protein other than the fetal fibronectin, is present in the stroma also. Elastin, the functional protein of elastic fibers, is found in the cervix in physiologic amounts. The elastic fibers are 2-4 µm wide and run between the bundles of collagen fibers. They are located in a band that is 20-30 µm thick and parallel to a plane from the external os to the internal os under the epithelium of the portio vaginalis and under the epithelium of the endocervical canal at the external os ***(Leppert et al., 1986)***. These elastic fibers are very thin compared with elastic fibers of other tissue ***(Leppert and Yu, 1991)***.

Of importance is the fact that the ratio of elastin to collagen is highest at the area of the internal os, meaning that there is more elastic fiber compared with collagen at the internal os. The greatest amount of smooth muscles is just below the internal os and these smooth muscles tapper off toward the external os ***(Leppert et al., 1986).***

Functional Histobiochemistry of the Cervix:

The uterine cervix begins abruptly with a 2-3 mm transition from the myometrium ***(Uldbjerg and Ulmsten, 1990).*** The cervix is a unique organ composed predominately of the extracellular matrix: collagen and elastin fibers, glycosaminoglycans and proteoglycans as ground substance. Smooth muscle cells, which comprise 10-15% of cervical tissue, undergo programmed cell death and play a role in cervical softening ***(Leppert, 1995)***

The results of studies by ***Danforth et al. (1960)*** are suggestive that the physical properties of the cervix are determined, in large measure, by the state of the connective tissue, and that during pregnancy and labor the remarkable ability of the cervix to dilate is the result of dissociation of collagen. The concentrations of total collagen were 38.8 and 64.3-72.4% of dry-defatted tissues for uterine body and cervix ***(Leppert and Yu, 1991)***

Minamoto et al (1987) have studied collagen types in the uterine cervix in pregnant and non-pregnant states by immunoperoxidase staining with the use of type-specific anticollagen antibodies. The non-pregnant cervix, composed of dense fibrous tissues, was diffusely stained with antibodies to type I and type III collagens. Type IV collagen was located only in the basement membrane region. Type I collagen and type III collagen fibers constitute 80% and 20% respectively and are covalently cross linked with glycosaminoglycans***. (Uldbjerg and Ulmsten, 1990)***

Elastin is another important component of the extracellular matrix of the human uterine cervix. Elastic fibers are organized parallel to and between collagen fibers. They assemble in a band 20 to 30 Micrometers thick. These thin sheets are capable of being stretched in any direction. Elastin, in its closed state, allows the uterus to retain the fetus during gestation. With mechanical stress, the elastin component can distend to twice its length to allow the

cervix to dilate for parturition. There is evidence to suggest that elastin has an essential role in maintaining a pregnancy by keeping the cervix closed. ***(Leppert et al., 1986)***

Hughesdon (1952), noted that the outer third of the wall of the cervix, like corpus, is muscular and the inner third consists of moderately cellular connective tissue, rich in collagen and contain some bundles of immature muscle fibers. He referred to the outer muscle layer as the extrinsic muscle of cervix. Internally, forming the bulk of cervix, lies a broad mass of fibrous connective tissue. It contains a variable proportion of scattered muscle bundles, to which be referred as the intrinsic muscle of the cervix.

Histological changes during pregnancy:

The collagen fibers are loosened and the loosening process consists of three components namely, (1) Separation of individual fibers into a number of very fine fibrils, (2) Actual fiber reabsorption, and (3) Replacement by fluid with increasing in the vascularity of the cervix as pregnancy advances ***(Hughsedon, 1952).*** The remodeling is achieved by changes in the quality and quantity of collagen fibers and ground substance and their interplay, which influences the biomechanical behavior of the cervix and contributes to pathologic condition such as cervical incompetence (CI) ***(Bauer et al., 1997)***.

Throughout pregnancy, collagen is actively synthesized. It is also continuously remodeled by collagenases, secreted from both cervical cells and neutrophils in an apparently slow and precise fashion. Collagen is degraded by collagenases both intracellular, to remove structurally defective procollagen to prevent the formation of weak structural collagen, and extracellular, to slowly weaken (so- called softening or ripening) the collagen matrix to allow delivery of the pregnancy ***(Junqueira et al., 1980).***

Even by the end of the first trimester, the collagen bundles become less tightly packed. This results in an overall decrease in the collagen concentration. However, with smooth muscle cells and elastic tissue, the collagen fibers align in a definite direction parallel to each other. Thus, the cervix will feel softer than the nonpregnant cervix, but is situated to maintain the pregnancy in utero ***(Ludmir and Sehdev, 2000).***

Cervical remodeling along with uterine contractile activation are the 2 key events that facilitate the birth of young. Cervical remodeling in preparation for parturition begins early in pregnancy; is a slow, progressive process; and can be loosely divided into 4 overlapping, phases: softening, ripening, dilation, and repair ***(Martin et al., 2006).*** The initial softening phase is a slow, progressive process of increased turnover of matrix components, resulting in reorganization of the collagen fibrillar

network. The second phase, termed cervical ripening, precedes uterine contractions of labor and involves increased synthesis of proteoglycans, glycosaminoglycans, and collagen. Despite an increase in collagen synthesis, collagen concentrations decrease and collagen solubility increases due to the dramatic increase in hydrophilic glycosaminoglycans during the ripening phase. The third phase is cervical dilation during labor. Cervical dilation involves leukocytes and release of proteases and collagenases into the extracellular matrix. Additional studies are indicated to determine the precise regulatory signaling pathways in these cells and the interactions between cervical epithelial cells and the underlying stromal fibroblasts ***(Brenda et al., 2007 and Word et al., 2007).***

The "Inflammatory Reaction" as a Mechanism for Cervical Ripening:

The normal process of cervical remodeling in multiple species has long been considered to be mediated by an inflammatory cascade whereby leukocytes invading the stromal matrix release proteases that degrade collagen and other extracellular matrix components ***(Timmons and Mahendroo, 2006).***

Inflammatory cells (neutrophils and macrophages) accumulate in the cervix during this process ***(Junqueira et al., 1980; Liggins, 1981 and Chwalisz et al., 1993)***. Pro-inflammatory cytokines, e.g. IL-1 B, tumor necrosis factor

(TNF), IL-8, have also been shown to be produced by the cervix and are thought to be involved in softening during pregnancy ***(Barclay et al., 1983 and Chwalisz et al., 1993).***

It is not clear whether infiltrating leukocytes are necessary for cervical ripening. In mice, experimentally induced neutrophils depletion has no effect on the timing or success of parturition, suggesting that neutrophils may not play a role in cervical ripening in the mouse. The role of monocytes, eosinophils, or other cell types in the ripening process is not clear ***(Timmons and Mahendroo, 2006).***

Cervical stromal cells and neutrophils metalloproteinases are inhibited by both tissue inhibitors of metalloproteinase and [alpha] 2-macroglobulin ***(Kitamura et al., 1980).*** These tissue inhibitors are found in the cervix throughout gestation and it appears that the balance between degrative enzymes and their inhibitors in part determines remodeling of collagen. At term, there is an increase in both the concentration of the proteases and their inhibitors ***(Rechberger and Woessner, 1993).***

The mechanisms responsible for the increase in the levels of collagenase inhibitors are unknown, but this increase appears to be able to suppress the action of degrative enzymes. During labor, the ratio between metalloproteinases and inhibitors increase to allow the net