The Physiology of Cervical Ripening and the Induction of Labour: A Potential Role for the Nitric Oxide Donor Isosorbide Mononitrate

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Declaration:

I certify that this thesis is an original piece of work. Chapters 1 and 2 are a review of the physiology of cervical ripening and the induction of labour. Before writing these chapters I conducted a literature search. In chapter 3 the results of a study examining the maternal and fetal haemodynamic effects following the intra-vaginal administration of isosorbide mononitrate are presented. In this study I recruited and consented all patients, collected all data and performed analysis of the data. The role of others in this study is referred to in the text.

Antony Edward Nicoll.

Abstract:

The ideal agents for the induction of labour should be clinically effective, easy to administer, cost-effective and safe for both mother and fetus. Mechanical devices, oestrogens, progesterone antagonists, relaxin, and prostaglandins have all been used to induce cervical ripening, with varying rates of efficacy and adverse effects. The intra-vaginal administration of the nitric oxide donor isosorbide mononitrate (IMN) is effective at inducing cervical ripening in the first trimester of pregnancy. When used to induce cervical ripening in the first trimester, intra-vaginal IMN also has fewer adverse effects than intra-vaginal prostaglandins. Nitric oxide is a potent vasodilator. The effects of intra-vaginal IMN on maternal and fetal haemodynamics in pregnant women at term are not known.

The first two chapters of this thesis provide a review of the physiology of parturition and of the agents that can be used to artificially induce cervical ripening. The final part of this thesis will present the results of a study of the maternal and fetal haemodynamic effects of intra-vaginal IMN administered to pregnant women at term.

Study Design:

A double blind randomised controlled trial. 36 women were randomised to receive 20 milligrams intra-vaginal IMN (n=13), 40 milligrams intra-vaginal IMN (n=11), or no treatment (a vaginal examination only) (n=12). Maternal pulse, blood pressure and fetal heart rate were recorded before the administration of any treatment and subsequently at 30-minute intervals until 360 minutes. Umbilical artery resistance index and pulsatility index measurements were made at 0, 180 and 330 minutes. Participating women were asked to complete a symptom questionnaire immediately before the administration of the treatment and again at 360 minutes. On completion of the study cervical score was determined, and if required further cervical ripening was achieved with prostaglandins in accordance with the local protocol. Other outcome measures included mode of delivery, length of labour for those women who progressed to a vaginal delivery, volume of blood loss at delivery, umbilical cord pH, Apgar scores at 1 and 5 minutes, analgesic requirements in labour and whether or not infants required admission to the neonatal unit.

Results:

Mean maternal pulse rate was greater following the intra-vaginal administration of 20 and 40 milligrams IMN compared with the control group ($p = 0.003$ and $p = 0.01$ respectively). Mean maternal systolic and diastolic blood pressures were lower following the intra-vaginal administration of 40 milligrams IMN compared with the control group ($p = 0.006$ and $p = 0.001$). Mean maternal diastolic blood pressure was lower following the intra-vaginal administration of 20 milligrams IMN compared to the control group ($p = 0.01$). Neither dose of IMN had a significant effect on mean fetal heart rate and umbilical artery resistance or pulsatility index. 69% and 91% of participants reported a headache following the administration of 20 mg and 40 mg IMN respectively, compared to 8% of the control group ($p = 0.001$). None of the study participants required treatment for maternal hypotension, or maternal or fetal tachycardia. The numbers were too small to demonstrate any statistically significant change in cervical score and there were no statistically significant differences in other outcome measures.

Conclusion:

The intra-vaginal administration of 20 mg and 40mg IMN to pregnant women at term has an effect on maternal haemodynamics, but this effect does not appear to be of clinical significance. In this study no effect on fetal haemodynamics was demonstrated following the intra-vaginal administration of 20 mg and 40mg IMN to pregnant women at term. This thesis lays the foundation for further studies using IMN as a cervical ripening agent during the induction of labour in pregnant women at term.

Introduction:

Despite advances in our understanding of the cellular and biochemical events that occur during parturition, the search for the factors that initiate and regulate this complex process continues. There are many reasons why a greater knowledge of this process would be beneficial. In circumstances where the continuation of pregnancy presents a risk to the mother or the fetus, for example in severe preeclampsia or intra-uterine growth restriction, an ability to fully comprehend the events of parturition could lead to the development of more physiological, and therefore safer, methods for the artificial induction of labour. A greater awareness of the events of parturition could provide us with the means to help those women who experience dysfunctional labour.

In addition, pre-term labour affects up to 10% of all pregnancies and is the major cause of neonatal mortality and morbidity. In recent years neonatal survival following pre-term delivery has greatly improved, although not through advances in obstetric intervention [1]. A better understanding of the physiology of parturition could also help us to overcome this continuing problem.

Human parturition consists of two distinct phases [2]. The first phase occurs during the final weeks of pregnancy and involves re-modelling of cervical connective tissue. This preparatory phase of parturition is known as cervical ripening. The second phase of parturition is much shorter than the first and lasts for hours. This phase is known as active labour and consists of regular uterine activity with progressive dilation of the cervix and leads to delivery of the infant. By considering human parturition in this way, strategies have developed for the artificial induction of labour. A frequently used method involves the induction of the first phase of parturition with a cervical ripening agent, followed by amniotomy and infusion of oxytocin to initiate the second phase.

The ideal agents for the induction of labour should be clinically effective, easy to administer, cost-effective and be safe for both the mother and fetus. Many agents have been used to induce cervical ripening including mechanical devices, oestrogens, progesterone antagonists, relaxin, and prostaglandins. These agents all vary in effectiveness and have a wide spectrum of adverse features.

It is now apparent that nitric oxide (NO) is a crucial factor in the process of cervical connective tissue remodelling during parturition in both animals and humans [3, 4]. Consequently, NO donors such as isosorbide mononitrate have been proposed as suitable agents for the pharmacological induction of cervical ripening in pregnant women [3, 5, 6, 7, 8]. Our experience of using NO donors in pregnant women at term is limited. There have been no studies examining the maternal and fetal haemodynamic effects of NO donors administered intra-vaginally to pregnant women at term for the induction of cervical ripening.

This thesis will first attempt to summarise the histological and biochemical changes of cervical ripening and the factors that regulate this process. There will subsequently be a review of the methods that have been used in clinical practice to artificially induce cervical ripening. Finally the results of a study will be presented, in which the NO donor isosorbide mononitrate was administered to pregnant women at term, in order to examine the effects on maternal and fetal haemodynamics. This study will hopefully lay the foundation for a future randomised controlled trial of NO donors, administered intra-vaginally to pregnant women at term, for the induction of cervical ripening.

I. The Physiology of Cervical Ripening:

The cervix is a unique structure in the female reproductive system and has many important functions. Outside pregnancy the cervix is a two-way channel enabling the passage of sperm into the upper part of the female reproductive tract in one direction, and the flow of menses in the other. The cervix has important immunological functions; the mucus produced by the glandular elements within the cervix is a barrier to pathogens and also sperm. Throughout pregnancy the cervix continues to act as a barrier to pathogens, but must also keep the fetus inside the uterus. During parturition the cervix undertakes a new role and must progressively soften, efface and dilate, to facilitate the passage of the fetus through to the outside world.

Anatomy of the Cervix:

The cervix is the inferior part of the uterus that protrudes into the vagina. It is situated between the bladder, which is located anteriorly, and the rectum, which lies posteriorly. The pubo-cervical fascia, the utero-sacral ligaments and the cardinal ligaments support the cervix. The cervix is cylindrical in shape and is approximately 2 - 3 centimetres long and 0.5 - 1 centimetre wide. In pre-pubescent females the cervix is twice as long as the uterine corpus, whilst in nulliparous females the cervix and the uterine body are approximately equal in length. In multiparous women the uterine corpus is approximately twice as long as the cervix [9].

The endocervical canal runs through the entire length of the cervix. The superior opening of the canal is located at the level of the peritoneal reflection of the bladder and is known as the internal os. The canal ends at the inferior opening, the external os. The endocervical canal is lined by tall columnar epithelial cells, which are interspersed with glands. These glandular epithelial cells end abruptly at the external os. The glandular epithelium is replaced by non-keratinised stratified squamous epithelium that is continuous with the epithelium of the vagina. The epithelial junction is referred to as the transformation zone.

In contrast to the uterine corpus, the cervix predominantly consists of connective tissue and only 15% of the cervix consists of smooth muscle [10]. While some of the deeper muscle fibres within the cervix may function as contractile elements [11], the significance of endocervical submucosal smooth muscle found in some women remains unclear and may represent a variation of normal [12].

The extra-cellular matrix of the cervix is rich in type I (~ 80%) and type III (~ 20%) collagen [13]. There is also a small amount of type IV collagen present in the basement membranes of the epithelial elements. Collagen fibres are synthesised from tropocollagens, which are bound together by covalent cross-links. These cross-links are important for collagen stability; mature collagen has more stable cross-links and is thus more resistant to degradation. In the cervix of non-pregnant females collagen fibres have a cable-like structure and are organised into dense bundles. The collagen fibres are held together by a relatively small amount of ground substance.

The ground substance mainly consists of large proteoglycan complexes that contain one or more glycosaminoglycans (GAGs) connected to a protein core. GAGs, formerly known as acid mucopolysaccharides, are large unbranched polysaccharide chains. They consist of disaccharide repeating units that contain a hexosamine (glucosamine or galactosamine) residue and mostly an uronic acid (glucuronic acid or iduronic acid) residue [13]. GAGs frequently contain a high amount of sulphate groups, making them extremely hydrophilic. Examples of GAGs include keratan sulphate, heparan sulphate and hyaluronic acid. In the cervix the main GAGs are condroitin sulphate and its epimer dermatan sulphate [13, 14, 15, 16].

There are at least three types of proteoglycan [14]. PG-S1 (biglycan) is a small proteoglycan with two dermatan sulphate chains, while PG-S2 (decorin) is also small but has only one dermatan sulphate chain. PG-L (chondroitin) is a large proteoglcan with both chondroitin and dermatan sulphate side chains. In the non-pregnant cervix PG-S2 is dominant [14].

The close association of the proteoglycan complexes and collagen fibres is crucial for the alignment of the collagen fibres. It is this relationship that provides the cervix with its tensile strength. The various GAGs vary in chain length and charge density and hence, in their ability to bind collagen.

For example, dermatan sulphate has a strong affinity for collagen and will therefore increase tissue rigidity. Hyaluronic acid has a high affinity for water molecules and binds least strongly to collagen and acts to destabilise the collagen fibres. Changes in proteoglycan and GAG composition will therefore have profound effects on the stability of collagen binding.

A small amount of elastin is also found within the cervical stroma [17]. Elastin fibres maintain the shape of the cervix and play a major role in keeping the cervix closed [9]. The function of elastin fibres assumes greater importance during pregnancy and indeed the "incompetent" cervix, which is associated with pre-term labour, has reduced amounts of elastin [18]. Elastin may also assist the cervix in returning to its pre-pregnant shape after delivery. Fibronectin and water are also found within the cervical extracellular matrix [9].

The composition of the cervical stroma is determined by fibroblasts, which synthesise and degrade the major extracellular matrix elements.

The Cervix During Pregnancy and Cervical Ripening:

During pregnancy there are profound changes in the composition of the cervix, which are most marked immediately before and during labour. There is an increase in cervical water content from 80 to 86% [19], which appears to be the result of an increase in hydrophilic GAGs [20]. In comparison to the non-pregnant cervix, there is a 30% reduction in cervical collagen concentration at 10 weeks gestation, and a 70% reduction at term [13]. There are also qualitative changes in cervical collagen. At term the collagen fibres appear thinner and are more widely spread out [20]. Cervical collagen fibres also decrease in fibre length [21]. Collagen synthesis continues throughout pregnancy, but the immature collagen does not develop strong cross-links with other collagen fibres. Consequently this newly formed collagen is more susceptible to degradation [9].

Matrix metalloproteinases (MMPs) are enzymes that are essential for connective tissue remodelling. At least 17 MMPs have been identified in humans. Collagen is resistant to most extracellular proteinases except collagenase (MMP-1) and leukocyte elastase [13]. MMP-1 is specific for collagen

and is secreted by a number of cells including fibroblasts and cleaves collagen at a specific site. MMP-1 is secreted as a pro-enzyme and is activated extra-cellularly by stromelysin (MMP-3). MMP-3 is activated by a number of enzymes including elastase [9]. MMP-3 is therefore essential for full collagenase activity. MMP-9 (type IV collagenase-gelatinase B) is also important in cervical collagen degradation, and activation of MMP-9 is dependent upon activating protein-1 (AP-1) [22]. There are 4 inhibitors of MMP activity: tissue inhibitor of metalloproteinase 1 - 4 (TIMP-1, TIMP-2, TIMP-3 and TIMP-4).

In the cervix there is active breakdown of collagen fibres during pregnancy. Cervical MMP-1 activity is increased during pregnancy and MMP-1 activity is maximal immediately before the onset of parturition [13, 23]. During parturition neutrophilic polymorphonuclear leukocytess are the main source of MMP-1 [24]. In the rat cervix, MMP-9 expression is increased in response to agents that have been shown to promote cervical ripening [25]. Leukocyte elastase degrades many proteins, including collagen, elastin and proteoglycans. Leukocyte elastase activity also increases throughout pregnancy [13].

At term the total proteoglycan concentration is halved and there is an increase in the amounts of PG-S1 and PG-L in comparison to PG-S2 [14, 26]. PG-S1 and PG-L bind less strongly to collagen fibres than PG-S2. In rats cervical ripening is associated with an increase in the ratio of PG-S2 to collagen [27]. The interaction of PG-S2 and collagen in the cervix at term appears to be an important factor in destabilising collagen fibres, resulting in a loss of tensile strength[28].

There also appears to be a qualitative change in the cervical GAG composition, with an increase in keratan sulphate during pregnancy. Chondroitin and dermatan sulphate concentrations decrease [29]. During pregnancy cervical hyaluronic acid content may remain unchanged [30, 31], or may decrease [13]. However, it is now apparent that cervical hyaluronic acid levels remain low during pregnancy and increase with the onset of parturition [32]. Hyaluronic acid stimulates MMP production in the rabbit cervix and stimulates neutrophil chemotaxis [32]. Hyaluronic acid also stimulates collagenase and gelatinase activity in the human lower uterine segment and may also do so in the cervix [32]. Changes

in proteoglycan and GAG composition of the cervix during pregnancy and labour alter the ground substance to which the collagen fibres are embedded and serve to destabilise the collagen.

During pregnancy there is also an eight-fold increase in the concentration of cervical elastin, which decreases rapidly after parturition [33]. The elastin fibres in the cervix of pregnancy are arranged in a characteristic fishnet appearance [34].

Throughout pregnancy the cervix is infiltrated by leukocytes [35]. In early pregnancy these are mainly T lymphocytes, with a small number of neutrophilic polymorphonuclear leukocytes present, along with a very small number of macrophages. At term there is a marked increase in the number of neutrophilic and eosinophilic polymorphonuclear leukocytes within the cervix and these cells are often surrounded by a halo of tissue that has reduced collagen content [20]. At term there is also a 10-fold increase in the number of macrophages compared to early pregnancy [35].

There also appears to be an increase in programmed cell death (apoptosis) of cervical smooth muscle cells at term and this may be an important factor in the initiation of cervical ripening [36].

In early pregnancy collagen and elastin fibres, along with fibroblasts and smooth muscle cells, align parallel to one another [9]. This arrangement assists in the maintenance of pregnancy. By term the changes in the composition of the cervical stroma lead to a structural derangement of the extracellular matrix elements, and hence a reduction in cervical rigidity. The cervical connective tissue changes are manifest clinically as progressive softening, shortening and dilatation of the cervix, with an associated change in cervical position. The "ripeness" of the cervix can be measured using the modified Bishop Score and this score is a good indicator of the preparedness of the cervix for parturition [37].

Cervical Ripening as an Inflammatory Reaction:

The physiological changes in the cervix during cervical ripening resemble those of the inflammatory reaction [38]. Following any tissue insult there is local vasodilatation that is associated with an increase

in blood flow. This is followed by an increase in vascular permeability and the exudation of proteins and fluid into the interstitial tissues. Oedema and fibrin deposits are seen in the interstitial spaces. The next stage in the inflammatory process is the adhesion of leukocytes to the vessel walls and their subsequent migration in response to chemotactic agents. The migrating cells initially consist of neutrophil polymorphs and are followed by macrophages and, later, eosinophils.

After the destructive phase of inflammation a rebuilding and re-modelling phase occurs. Fibroblasts proliferate and migrate into the tissues and synthesise collagen, proteoglycans and GAGs. The main GAG secreted during the inflammatory process is hyaluronic acid. Fibroblasts are also a source of prostaglandins and proteolytic enzymes. The synthesis of new connective tissue is associated with increased tissue degradation. The whole inflammatory reaction is controlled by a number of factors including cytokines, histamine and prostaglandins.

There are several similarities between the inflammatory reaction and the process of cervical ripening. As previously discussed, during cervical ripening there is an increase in tissue hydration and an alteration of the cervical extra-cellular matrix, with both qualitative and quantitative changes in collagen and proteoglycan composition. During cervical ripening there is also an influx of leukocytic cells. Many of the mediators and enzymes involved in the regulation of the acute inflammatory reaction are also involved in the regulation of cervical ripening.

The Regulation of Cervical Ripening:

Inflammation may be triggered by trauma, chemical agents, infective agents, or be part of a hypersensitivity reaction. The process of cervical ripening also appears to have many possible triggers. While it is recognised that some cases of pre-term labour are the result of an infective trigger [39], one must assume that in the healthy woman at term, other factors must be involved in the initiation of parturition.

As previously discussed, human parturition has two phases that are closely related, cervical ripening and active labour. In normal parturition cervical ripening must occur before active labour. Parturition

begins when the forces that favour cervical ripening and active labour overcome those that prevent these processes. Important factors in the regulation of parturition include cytokines, mechanical stress, apoptosis of cervical cells, endocrine factors (placental corticotrophin releasing hormone, progesterone, oestrogen, and oxytocin), relaxin, prostaglandins and nitric oxide.

Cytokines:

The inflammatory process is regulated by a diverse group of soluble proteins and polypeptides known as cytokines. Cytokines regulate cellular function in both normal and pathophysiological conditions. Cytokines usually contain between 100 and 200 amino acid residues and have a molecular weight of 10-20 kilodaltons. Cytokine function is mediated by their binding to specific membrane associated receptors [40]. Since cervical ripening is similar to the inflammatory process, this would suggest that cytokines play a role in the regulation of cervical connective tissue re-modelling during parturition.

The principal groups of cytokines are: interleukins (1-18); the interferons α, β and γ; colony stimulating factors (G-CSF, M-CSF and GM-CSF); tumour necrosis factors α and β; the transforming growth factor β family; and additional growth factors such as erythropoietin, nerve growth factor, epidermal growth factor, fibroblast growth factors, insulin-like growth factors and platelet derived growth factor [41]. The cytokines involved in the regulation of inflammation are the interleukins, TNF-α and -β, platelet activating factor (PAF) and the interferons.

Interleukin-1α and interleukin-1β are 17kD peptides that are synthesised by many cells, including fibroblasts, endothelial cells, monocytes and macrophages. IL-1α and IL-1β have similar biological effects and are recognised by the same receptors. They are pro-inflammatory and stimulate the production of IL-6, IL-8 and TNF [42]. IL-1α and IL-1β are important mediators of parturition. Amniotic fluid IL-1α concentrations increase in term labour [43].

IL-1α and IL-1β regulate cervical ripening by altering the activities of MMPs and by altering cervical GAG composition. IL-1α induces the expression of MMP-1 and MMP-3, down-regulates the expression of TIMPs and inhibits matrix synthesis [44]. IL-1β also induces MMP-1, -3, and -9 mRNA

expression in cultured human cervical smooth muscle cells in vitro, along with the elastinolytic enzyme cathepsin S [45]. The effects of IL-1 on MMP production are mediated by protein kinase C [46]. IL-1α stimulates fibroblast hyaluronic acid production [47].

Interleukin-8 is a small (6-8kD) peptide produced by monocytes, fibroblasts and chorio-decidual cells [48]. IL-1, TNF and aminopeptidase inhibitor (bestatin) regulate the production of IL-8 [49, 50].

IL-8 is a crucial factor in the regulation of cervical ripening [48]. Cervical fibroblasts have the capacity to produce IL-8 in large amounts during pregnancy [51]. Pre-term labour is associated with increased concentrations of amniotic fluid IL-8 [52, 53], and it has been suggested that the measurement of IL-8 in cervical secretions may be a useful marker for pre-term labour [54]. During parturition the production of IL-8 within the cervix is dependent on IL-1α [55].

IL-8 is a powerful neutrophil chemotactic agent, promotes neutrophil activation and also increases vascular permeability [42]. Thus IL-8 could account for the influx of neutrophilic polymorphonuclear leukocytes that is seen during cervical ripening. There is also evidence to suggest that IL-8 can influence cervical MMP activity during parturition and also alter GAG composition. IL-8 stimulates MMP-8 and MMP-9 release from neutrophils [56] and stimulates fibroblast hyaluronic acid production in vitro [47].

Tumour Necrosis Factor (TNF) is a 17kD peptide and is released from monocytes and macrophages. TNF appears to be another factor in the regulation of cervical ripening. TNF regulates the activities of other inflammatory cytokines including IL-1, IL-6, IL-8 and interferon, as well as phospholipase A_2, PAF and protein kinase C [42]. TNF-α also alters cervical connective tissue composition by stimulating the production of MMPs [57]. TNF-α induces MMP-1, -3, and -9 mRNA expression in cultured human cervical smooth muscle cells in vitro, along with the elastinolytic enzyme cathepsin S [45].

Transforming growth factor β can also increase MMP production [58] and this cytokine may also be an important factor in the regulation of cervical ripening, although further investigation is required.

Mechanical Factors:

Cervical ripening is an active process. However the pressure effects exerted passively on the cervix by the fetus undoubtedly play a role in stimulating the connective tissue changes of cervical ripening. Treatment of rats with the progesterone antagonist mifepristone induces qualitative changes in cervical collagen content but not full cervical ripening [59]. In this study Glassman et al concluded that the mechanical distension of the cervix caused by the fetus was essential for complete ripening to occur [59].

El Maradny et al demonstrated that stretching of fetal membranes and also myometrial cells from the lower uterine segment results in an increase in IL-8 concentration and collagenase activity in vitro [60]. It appears logical to suggest that passive mechanical stretching of the fetal membranes, the lower uterine segment and the cervix, by the fetal presenting part could promote the release of IL-8 and hence other pro-inflammatory cytokines, and promote the cervical connective tissue remodelling at term in vivo. It would be of interest to repeat the study of El Maradny et al using cervical tissue in order to determine whether or not stretching of cervical tissue also induces IL-8 and collagenase production.

The mechanical stress caused by myometrial activity may also be involved in the initiation of cervical ripening [9]. Myometrial contractions will cause sheer stress on the cervical tissues, promoting inflammatory changes within the cervix. Inflammatory mediators such as IL-1, IL-6 and TNF increase prostaglandin production in a variety of animal and human cells in vitro [40]. Prostaglandins will further promote myometrial contractility. It therefore appears that a positive loop is set up to drive forward the process of parturition once cervical ripening has been initiated.

Apoptosis:

The length of pregnancy is relatively constant within a species group and the idea of genetically determined cell death triggering parturition is appealing. In rats there is an increase in apoptosis of

cervical smooth muscle cells at term [36]. It has been suggested that apoptosis of cervical smooth muscle cells could be a factor in the initiation of human cervical ripening [36].

Apoptosis is regulated by cytokine and endocrine factors, including TNF-α and glucocorticoids [61, 62]. The administration of the progesterone antagonist onapristone inhibits apoptosis of smooth muscle cells and fibroblasts in the cervices of pregnant rats [63]. An increase in smooth muscle cell apoptosis in the cervix at term may simply be a response to other events in the parturition process, rather than an initiating factor. Further work is required to determine the exact role of apoptosis in the regulation of human cervical ripening.

Placental Corticotrophin Releasing Hormone:

In humans corticotrophin releasing hormone (CRH) is released from the hypothalamus and is a major factor in the hypothalamic-pituitary-adrenal axis. CRH is released in response to stress and stimulates adrenocorticotrophin (ACTH) release from the anterior pituitary gland. ACTH stimulates glucocorticoid release from the adrenal glands. A negative feedback loop exists, whereby glucocorticoids inhibit both CRH and ACTH release at the hypothalamus and pituitary gland.

CRH has been identified in the human placenta [64, 65]. Placental CRH is a peptide with identical size and biological activity to hypothalamic CRH, and is synthesised in syncytiotrophoblast cells [66]. CRH receptors have been found in the myometrium and placental CRH can stimulate myometrial activity [67]. Placental CRH synthesis is increased in the last 6-8 weeks of human pregnancy and high levels of placental CRH are associated with pre-term labour, pregnancy induced hypertension and intra-uterine growth restriction [68].

Hypothalamic CRH release is mediated by IL-1 and TNFα and is dependent upon nitric oxide production [69, 70, 71]. The link between inflammatory cytokines and CRH production probably also exists in the female reproductive tract [72]. In vitro experiments have shown that IL-1 and prostaglandins promote the production of placental CRH [73]. Progesterone may inhibit placental CRH

synthesis via its action on trophoblast glucocorticoid receptors [68]. In contrast to their inhibitory effects on hypothalamic CRH, glucocoticoids promote placental CRH gene expression [74].

In humans there is a rise in fetal cortisol production at term [75]. Fetal cortisol promotes fetal lung maturation, an important step in the preparation for delivery. Placental CRH stimulates the release of fetal ACTH from the fetal pituitary gland, which in turn stimulates further production of fetal cortisol from the fetal adrenal cortex. A positive "feed-forward" loop is established ensuring a rise in placental CRH.

Fetal ACTH also stimulates the production of dihydroepiandrosterone (DHEA) in the fetal adrenal gland. Increases in plasma DHEA concentrations are associated with cervical ripening [9]. DHEA appears to act synergistically with IL-8 in the process of cervical ripening, and acts by increasing MMP and elastase activities [76, 77]. In the placenta DHEA is converted to oestradiol. Oestradiol increases myometrial gap junction formation, increases the number of myometrial oxytocin receptors, and stimulates prostaglandin formation [75]. These changes will promote myometrial contractility, which further stimulates the process of cervical ripening.

The trigger for the rise in fetal cortisol production is unknown. The hypothalamic-pituitary-adrenal axis is activated in response to stress. An increase in fetal cortisol production could be a physiological reaction to a stressful event, such as infection or hypoxia. The placenta may not be meeting the demands of the fetus either through disease or because the fetus has out-grown its supplier. There may be a biological clock functioning within the fetal hypothalamus or the placenta (or both), which upon activation increases CRH production and thus triggers parturition.

Oestrogen:

In animals, including the sheep and the rat, plasma levels of oestrogen rise and progesterone levels fall immediately before the onset of parturition [78, 79]. In humans there does not appear to be a fall in plasma levels of progesterone, but an alteration in the ratio of plasma oestradiol to progesterone during the later stages of pregnancy and during parturition has been reported [80, 81, 82]. Women who

progress to spontaneous labour have a higher ratio of salivary oestrogen to salivary progesterone concentration than those women who do not labour spontaneously [83]. Furthermore, a raised salivary oestriol concentration may be a marker for pre-term labour [83]. Cervical ripening is associated with raised plasma 17β-oestradiol [9].

As discussed previously, in humans the shift from progesterone to oestrogen dominance at term is dependent on the activation of the fetal hypothalamic-pituitary-adrenal axis, and the placental conversion of DHEA to oestradiol [75]. An increase in oestrogen activity will have effects at the myometrial and cervical levels. In the myometrium oestrogens increase myometrial gap junction formation, oxytocin receptor synthesis, and prostaglandin synthesis [75]. These changes promote myometrial contractility.

In human cervix at term there is a reduction in cervical oestrogen and progesterone receptor protein, compared to non-pregnant controls, that is the result of an increase in receptor turnover [79]. Insulin-like growth factor-1 is a mediator for growth hormone that has also been proposed as a mediator for oestrogen action in the uterus [84]. The changes demonstrated by Stjernholm et al in the human cervix at term were associated with a four-fold rise in cervical IGF-1 mRNA which suggests that oestrogen activity is present in the cervix at the time of parturition. A subsequent study showed a further decrease in oestrogen and progesterone receptor protein and IGF-1mRNA concentrations occurred during parturition [82]. This implies that oestrogen activity is less important for cervical connective tissue remodelling once parturition is initiated.

Animal and human studies demonstrate that the oestrogens have a variety of effects on cervical connective tissue. In pregnant rats administration of oestradiol at term leads to a reduction in both the total and the soluble collagen content [85]. In the same study the administration of tamoxifen, an oestrogen antagonist with partial agonist activity, did not affect total cervical collagen content, but did reduce the amount of soluble cervical collagen [85]. In humans the administration of oestradiol reduces the total cervical collagen content and increases the amount of soluble collagen [20].

Oestrogens may alter cervical composition and facilitate cervical ripening by stimulating MMP activity. In guinea pigs 17β-oestradiol stimulates cervical MMP activity in vitro [86]. Oestrone sulphate increases MMP activity in cervical tissue taken from women at term in vitro [76]. However Sato et al demonstrated that 17β-oestradiol reduces MMP activity and increases TIMP production in the rat cervix in vitro [87]. Oestrogen replacement alone in pregnant rats at term following oophorectomy produces a compact and inextensible cervix, whereas the administration of oestrogen and relaxin produced changes that were similar to those of cervical ripening [78]. It may be that at physiological concentrations oestrogens do not directly exert much influence on MMP production, and hence MMP activity, and that other agents in the ripening cascade are more important in the regulation of cervical collagen content.

It appears likely that oestrogens influence cervical ripening through processes other than the direct alteration of cervical collagen composition. Oestrogens can induce apoptosis [9] and also promote cervical eosinophilic infiltration in pregnant rats at term [88]. Oestrogens also regulate the binding of relaxin, another mediator of cervical ripening, to porcine cervical cells [89]. It may be that the effects of oestrogen on the cervix are indirect and are mediated by prostaglandins, myometrial activity, or via relaxin. Further work is required to determine the exact mechanisms by which oestrogens regulate cervical ripening.

Progesterone:

In animals there is a shift from progesterone to oestrogen dominance at term and this change is believed to be a crucial factor in determining the timing of the onset of parturition [78, 82]. In sheep fetal cortisol stimulates the production of 17α-hydroxyprogesterone lyase, an enzyme that is responsible for progesterone breakdown. The human placenta does not contain 17 α-hydroxyprogesterone lyase, and so a rise in fetal cortisol will not lead to a reduction in progesterone. However fetal cortisol may displace progesterone from glucocorticoid receptors in the myometrium and cervix, thereby simulating progesterone "withdrawal" [67].

It has long been recognised that progesterone inhibits cervical ripening [19]. Antagonists to progesterone such as mifepristone (RU 486), induce cervical ripening in both animals and humans [27, 90, 91, 92]. In rat cervices the administration of a progesterone antagonist is associated with a decrease in the amount of mature cross-linked collagen, an increase in the amount of soluble cervical collagen, but a decrease in cervical hydration [59, 93]. The administration of the progesterone antagonist onapristone also increases the PG-S2 (decorin) / collagen ratio and alters cervical extensibility of the pregnant rat cervix [94].

In humans mifepristone induces cervical softening and dilatation in vivo, and inhibits cervical collagen synthesis in vitro but does not alter the total amount of cervical collagen [92]. In the human cervix there is a reduction in progesterone receptor at term and during parturition that is associated with cervical connective tissue remodelling [79, 82].

Progesterone appears to regulate cervical ripening by inhibiting cervical collagen breakdown [79]. Progesterone inhibits the IL-1 induced production of MMP-1, MMP-3, and MMP-9, and augments IL-1 induced production of TIMP-1 and TIMP-2 from rabbit cervical fibroblasts in vitro [9, 22, 87]. It is likely that mifepristone promotes cervical ripening by inducing collagen fragmentation and breakdown [92] and this effect may be mediated by prostaglandins [95].

Endothelial adhesion molecules are crucial for the adhesion of leukocytes to the vascular endothelium prior to chemotaxis, a crucial part of the process of cervical ripening. Whilst progesterone alone does not appear to affect the expression of these endothelial adhesion molecules in umbilical vein endothelial cells in vitro [96], the progesterone antagonist onapristone increases the expression of the endothelial adhesion molecule endothelial leukocyte adhesion molecule-1 (ELAM-1) in umbilical vein endothelial cells in vitro [97]. The combination of onapristone and TNFα led to increased expression of ELAM-1, along with expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). These effects are also seen when 17β oestradiol is used rather than onapristone [96].

Progesterone antagonists also increase the production of IL-8 in the human placenta resulting in neutrophil polymorph chemotaxis and activation [98]. Hypothetically the same events could occur in the cervix during cervical ripening, in response to progesterone "withdrawal". There is also evidence to suggest that progesterone is an inhibitor of apoptosis. Progesterone inhibits the aminoglutethamide-induced apoptosis of bovine corpus luteal cells in vitro, whilst the progesterone antagonists, mifepristone and onapristone, increased apoptotic cell death [99]. Conversely Leppert et al demonstrated that the administration of the progesterone antagonist onapristone inhibited apoptosis of smooth muscle cells and fibroblasts in the cervices of pregnant rats [63].

Therefore, during pregnancy progesterone inhibits the degradation of the cervical collagen and hence conserves the tensile strength of the cervix. At term there appears to be a "withdrawal" of progesterone activity likely to be the result of progesterone receptor antagonism by fetal cortisol [67]. The suppression of MMPs and induction of TIMPs by progesterone is lost and there is an influx of inflammatory cells into the cervix. It is again likely that the "withdrawal" of progesterone activity only partially influences cervical ripening, and that it is one of many factors that regulate cervical ripening.

Oxytocin:

The presence of oxytocin is critical for the initiation and maintenance of parturition. Oxytocin is a short polypeptide with a molecular weight of 1000 [100]. Oxytocin, along with vasopressin, is released from the posterior lobe of the pituitary gland. Oxytocin is synthesised in the paraventricular and supraoptic nuclei by the formation of large precursor molecules that are cleaved and stored in the neurohypophysis. Oxytocin and vasopressin have similar structures and differ only by the substitution of 2 amino acid residues.

Oxytocin is released in a pulsatile manner from the neurohypophysis in response to a number of stimuli including breast stimulation, sensory stimuli from the lower genital tract, and cervical stretching [100]. The release of oxytocin following breast stimulation results in oxytocin-induced contraction of mammary myoepithelial cells, the milk-ejection reflex [101]. Vaginal and cervical

stretching leads to oxytocin-induced myometrial contractions through Ferguson's reflex [102]. Both oestrogen and progesterone regulate the function of oxytocin at the receptor level and facilitate the uterine response. During parturition oxytocin stimulates the production and release of arachidonic acid from decidual cells, potentiating its effects on the myometrium [103, 104]. Oxytocin also stimulates vascular smooth muscle relaxation and has an antidiuretic effect [100].

While it is clear that oxytocin has direct effects on the myometrium, it is recognised that oxytocin has no direct effect on cervical tissue composition [105]. Any changes in cervical composition associated with pharmacological oxytocin administration are likely to be the result of sheer stress caused by myometrial contractions, and the actions of other mediators of cervical ripening that are induced by oxytocin such as prostaglandins.

Relaxin:

Relaxin is a 6000-dalton dimeric polypeptide hormone that is structurally related to insulin and was first isolated in its pure form in 1926 [106]. The hormone consists of two chains, an A chain of 24 amino acids and a B chain of 29 amino acids, that are linked by 2 disulfide bridges, with additional disulfide linkage in the A chain [107]. Relaxin promotes cervical connective tissue changes in both animals and humans.

In pregnant rats the administration of relaxin decreases cervical collagen concentration, increases cervical collagen solubility and increases cervical concentrations of hyaluronic acid[108]. In rats cervical ripening is inhibited by the administration of an antibody specific to relaxin [109]. In humans serum concentrations of relaxin remain constant throughout pregnancy but cervical tissue concentrations of relaxin rise towards the end of pregnancy [110]. In the human cervix relaxin increases gelatinase and collagenase activities, and also increases GAG synthesis [111].

The action of relaxin during cervical ripening appears to be dependent on the presence of oestrogen. The administration of relaxin and oestrogen to pregnant rats at term following oophorectomy, results in changes similar to those of cervical ripening [78, 88]. Oestrogen and relaxin appear to have a

synergistic effect on increasing porcine cervical collagen synthesis and secretion, and oestrogens regulate the binding of relaxin to cervical cells [89]. Relaxin therefore maintains the cervical ripening process in the presence of oestrogen. Relaxin regulates the binding of the oestromedin, IGF-1, to human decidudal cells [107] and it is possible that this is the mechanism by which relaxin regulates the effects of oestrogen in the cervix.

Relaxin also has effects in the myometrium and other tissues. Purified porcine relaxin inhibits spontaneous contractility of myometrium from pregnant rats and pigs in vitro [107]. Relaxin also inhibits the spontaneous contractility of non-pregnant human myometrium in vitro [112]. However the same effects could not be demonstrated on pregnant human myometrium in vitro [113], and it appears that human relaxin only has a minor effect on myometrial activity in late pregnancy [114].

Relaxin stimulates the release of prorenin from cultured human decidual cells and also regulates the synthesis and release of decidual prolactin. Relaxin increases insulin binding to adipocytes and may have a regulatory role in lipid metabolism during pregnancy. Growth hormone secretion may also be regulated by relaxin, and relaxin may be involved in the expansion of plasma volume that is seen in pregnancy, which is caused by re-setting of the plasma osmolality-arginine vasopressin system [107].

Prostaglandins:

The administration of exogenous prostaglandins produces cervical ripening at any stage in pregnancy [115]. Prostaglandins are a closely related family of C20 carboxylic acids. Prostaglandins consist of a pentane ring that has two fatty acyl chains attached to adjacent carbons. The structure of the pentane ring differs between the different sub-classes of prostaglandins (A, B, C, D, E. F or J). Prostacyclin (PGI_2) and thromboxane A_2 have a different ring structure to the other prostaglandins. Each prostaglandin also has a numeric designation (1, 2, 3) that indicates the number of double bonds in the fatty acyl backbone [116].

The biological effects of prostaglandins are mediated via receptors that are attached to the cell membrane or intracellular organelles. These receptors are specific to the type of prostaglandin as

determined by the pentane ring. However prostaglandin receptors do not distinguish between the molecules in the fatty acyl backbone.

The most common precursor for the synthesis of prostaglandins is arachidonic acid (*all-cis*-5, 8, 11, 14 eicosatetraenoic acid). Arachidonic acid and other precursors are liberated from the cell membrane by phospholipases, principally phospholipase A_2 (PLA_2). Following their release arachidonic acids are metabolised to a number of biologically active compounds, which are known as eicosanoids. Prostaglandins are derived from these eicosanoids via the cyclo-oxygenase (COX) pathway. Leukotrienes and lipoxins are also derived from arachidonic acids [117].

Cyclo-oxygenase exists in two forms. COX-1 is present in a wide variety of tissues and is known as non-inducible COX. COX-2 is found in only a small number of tissues and is referred to as inducible COX. COX-2 is expressed in very low quantities under basal conditions. However the induction of COX-2 is of greater significance in the regulation of COX activity. Non-steroidal anti-inflammatory drugs prevent cervical ripening by inhibiting prostaglandin synthesis [118]. The local administration of the COX-2 antagonist nimesulide, to pregnant rats also inhibits cervical ripening [119].

Prostaglandin E_2, $F_{2\alpha}$ and I_2 are important regulators of cervical ripening [120]. PGE_2 appears to be a more important mediator of cervical ripening than $PGF_{2\alpha}$, while the role of PGI_2 in the process of cervical ripening remains unclear [121].

Prostaglandins appear to promote cervical ripening by inducing qualitative and quantitative changes in cervical collagen and by altering the GAG/proteoglycan composition. Animal studies have shown a variety of effects of prostaglandins on cervical composition. PGE_2 administered to pregnant rats at term appears to have no significant effect on cervical hydration, total collagen content, and appears to decrease the quantity of soluble collagen [93]. In the same study $PGF_{2\alpha}$ decreased cervical tissue hydration, had no effect on total cervical collagen content or quantity of soluble collagen. The inhibition of cyclo-oxygenase produced no significant effects on total collagen content but did decrease the amount of soluble collagen. The author concluded that prostaglandins do not act alone in the regulation of cervical ripening [93].

El Maradny et al demonstrated that the administration of PGE_2 to pregnant rabbits, had no effect on total cervical water content, but an increase in cervical hydration was seen following the administration of repeated doses of prostaglandin [122]. In the same study the administration of PGE_2 increased the number of neutrophil polymorphs, significantly decreased the total collagen concentration, but had no effect on MMP activity.

In humans the administration of the prostaglandin analogue gemeprost in the first trimester of pregnancy significantly reduces total collagen content [121]. PGE_2 administration was associated with an influx of neutrophil polymorphs. Conversely Greer et al demonstrated that the administration of gemeprost had no statistically significant effect on cervical GAG concentrations [121].

Norman et al showed that cervical biopsies taken following delivery, from pregnant women who had received intra-cervical PGE_2 at term, had reduced collagen content compared to non-pregnant controls [123]. In women who had received PGE_2 there were no differences in collagen content between women who delivered by caesarean section and those who delivered vaginally, suggesting that cervical remodelling had occurred early in labour. PGE_2 stimulates the synthesis of GAG in cervical fibroblasts in vitro [124]. Norman et al demonstrated that women who had been treated with PGE_2 in vivo had significantly increased cervical proteoglycan synthesis, particularly larger proteoglycans such as PGL and biglycan, compared to pregnant and non-pregnant controls [123].

The mechanisms by which prostaglandins regulate cervical ripening are not fully understood. The effects of prostaglandins on the cervix may not be the result of direct activation of MMPs that degrade collagen fibres [121]. Prostaglandin analogues applied to the human cervix decrease collagen cross-linking [125], and it has been suggested that prostaglandins activate poteases that cleave cross-links between cervical collagen fibrils [126].

In cervical ripening prostaglandins may also act as vasodilatory agents and increase vascular permeability, thereby facilitating inflammatory cell infiltration [124]. During cervical ripening prostaglandins may also promote the adhesion of granulocytes to the vascular endothelium prior to

chemotaxis. PGE_2 increases the expression of the endothelial adhesion molecule ICAM-1 in umbilical vein endothelial cells in vitro [96]. Denison et al demonstrated that PGE_2 release from the cervix inhibited the release of secretory leukocyte protease inhibitor (SLPI) [127]. SLPI is a strong inhibitor of neutrophil function and elastase release. A fall in SLPI activity would result in neutrophil recruitment and activation, and thus facilitate the process of cervical ripening.

There is evidence to support that prostaglandins are one of the links between the endocrine changes that occur at the end of pregnancy and the cervical changes that are seen during parturition. Oestradiol stimulates prostaglandin production within the uterus [19]. The induction of cervical ripening in sheep using oestradiol is associated with an increased production of prostaglandins from the cervix [128]. It is likely that progesterone inhibits prostaglandin synthesis and enhances prostaglandin catabolism [121]. Antiprogestins have the opposite effects [129, 130]. However in vitro studies have shown that progesterone and dexamethasone do not appear to effect cervical PGE_2 release [131].

Prostaglandin synthesis and bioavailability may therefore be regulated by other non-endocrine factors. Both PGE_2 and $PGF_{2\alpha}$ are released from the fetal and maternal sides of intact fetal membranes in response to bacterial endotoxin (lipopolysaccharide) in vitro [132]. Bacterial endotoxin is a component of the cell wall of gram-negative bacteria. Infection has been implicated in the pathogenesis of pre-term labour. However bacterial endotoxin does not appear to cross the intact fetal membrane [133] and this prompted Rajasingham et al to suggest that another factor, perhaps a cytokine, was important in stimulating prostaglandin synthesis in response to infection.

It is now apparent that prostaglandins regulate cervical ripening by altering cytokine activity [40]. Prostaglandins inhibit the production of IL-1 from macrophages and peripheral blood monocytes [134, 135]. PGE_2 stimulates the release of IL-8 from human cervical tissue in vitro [127]. In extra-uterine sites PGE_2 acts synergistically with IL-8 to promote neutrophil chemotaxis [136]. The prostaglandin analogue misoprostol decreases IL-1β in the pregnant rat cervix in vitro [137].

During cervical ripening prostaglandin activity appears to be mediated by cytokines. However there is also evidence to suggest that cytokines regulate prostaglandin activity. Prostaglandin production is regulated by the availability of arachidonic acid, via an increase in phopsholipase A_2 (PLA_2) activity, and also cyclo-oxygenase (COX) activity. IL-1, IL-6 and TNF increase PLA_2 activity in a variety of animal and human cells in vitro [40]. In the rat glomerular mesangial cells TNF induced PLA_2 release in vitro is blocked by dexamethasone [138]. IL-1 and TNF also stimulate COX [40].

IL-1 stimulates the production of prostaglandins by fetal membrane cells [139]. TNF-α stimulates the production of PGE_2 from amnion cells in vitro [140]. Anti-IL-1β inhibits lipopolysaccharide induced PGE_2 production from human decidual cells in vitro [141]. Klim et al also demonstrated that TGF-β2 also inhibits lipopolysaccharide induced PGE_2 production from human decidual cells in vitro. PGE_2 release from the cervix is stimulated by nitric oxide in vitro, while conversely IL-8 has no effect on PGE_2 release from the cervix in vitro [131].

Therefore prostaglandin activity is essential for successful cervical ripening to occur. Prostaglandins appear to be one of the links between the endocrine changes that are seen in the later stages of human and animal pregnancy, and the inflammatory changes that are visualised in the cervix during parturition.

Nitric Oxide:

It is now clear that nitric oxide (NO) is a crucial factor in the process of cervical connective tissue remodelling during parturition in both animals and humans [3, 4]. NO, previously described as endothelial dependent relaxing factor, is a small, uncharged gas molecule that is an important biological mediator in humans. It is a free radical with a half-life of a few seconds [142]. NO is synthesised from the amino acid l-arginine by the enzyme nitric oxide synthase. There are three isoforms of the enzyme; neuronal, inducible, and endothelial nitric oxide synthase (NOS I, II and III).

Haemodynamic Effects of NO:

In the vascular system NO induces vasodilatation, inhibits platelet aggregation, prevents neutrophils and platelet aggregation to endothelial cells, inhibits smooth muscle cell proliferation and migration, and maintains endothelial cell barrier function [143]. In the non-pregnant state NO is thought to play an important role in the control of systemic blood pressure, by maintaining resistance vessels in a constant state of relaxation. NO relaxes smooth muscle by activating soluble guanylate cyclase [142]. There are several mechanisms controlling the concentration of NO at the vascular site; an increase in the basal release of NO will reduce resistance and increase flow; NO may be released in response to specific receptor activation; NO may be released via non-receptor mechanisms such as neuronal stimuli; NO may also be released in response to sheer stress [144].

In normal human pregnancy there is a marked increase in intravascular volume, heart rate and cardiac output. Endogenous NO, in conjunction with other agents such as prostacyclin, has been implicated in the mechanism of pregnancy-induced vasodilatation [145]. In the rat NO is an important regulator of maternal blood pressure in pregnancy [146]. This appears to be oestrogen dependent [147].

NO and the Utero-Placental Circulation:

Pregnancy is associated with an increase in flow in the utero-placental circulation along with the development of the feto-placental circulation [148]. There is continuous forward flow velocity in the umbilical artery, which suggests that there is low impedance to flow in the placental circulation. In the pregnant guinea pig NO is important for uterine artery relaxation [144]. The increase in NOS activity in pregnant guinea pigs is also oestrogen dependent [144]. Chang et al showed that the infusion of a NOS inhibitor to sheep increased feto-placental resistance and reduced umbilical artery flow [149]. In humans endogenous NO appears to contribute to the maintenance of vasodilatation in the utero-placental circulation in order to preserve low resistance to flow [145]. In humans NO relaxes both placental and umbilical vessels in vitro [150, 151]. In the human utero-placental circulation it appears that NO is produced locally in response to shear stress caused by blood flow [152].

NO and the Myometrium:

In animals both NOS II expression and NO production in the uterus are increased during pregnancy to maintain uterine quiescence [153, 154, 155, 156]. In animals uterine NOS II expression and NO production decrease during term and pre-term labour [157]. However these changes do not appear to occur in humans [158]. NO donors relax the myometrium [159] and these agents have been proposed as tocolytic agents in the management of pre-term labour [160, 161]. NO donors have also successfully been used to treat intrapartum fetal distress caused by uterine hypertonus [162].

NO and Inflammation:

NO has pro-inflammatory properties and has been implicated in tissue damage and apoptosis. The NOS II system is important in tissue remodelling and inflammation. The induction of NOS II can lead to sustained high concentrations of NO, promoting inflammatory effects such as vasodilatation, oedema, cytotoxicity, tissue remodelling and the activation of cytokines [163]. Therefore if NO plays a prominent role in the inflammatory process, it would seem likely that it plays a vital role in the process of cervical ripening.

NO and Cervical Ripening:

Animal models suggest that NO is another important factor in the process of cervical ripening. In the rat cervix there is a low production of NO during pregnancy and a high production of NO during term and pre-term labour [164]. Treatment of pregnant guinea pigs with an inhibitor of NOS (L-NAME) induces pre-term parturition but delays cervical ripening [165]. L-NAME treatment of pregnant rats also causes delayed cervical ripening [164]. Lipopolysaccharide (LPS), a product of bacterial infection, can induce cervical ripening in pregnant rats that can be inhibited by L-NAME [166]. This suggests that NO is important in the pathogenesis of infection-induced pre-term labour. In guinea pigs the direct application of an NO donor, sodium nitroprusside (SNP), to the cervix can induce macroscopic changes similar to cervical ripening, along with histological evidence of cervical

connective tissue remodelling [167]. The intra-cervical application of SNP to pregnant guinea pigs induces cervical ripening by decreasing the amount of collagen cross-links [168].

NO is also important factor in human cervical ripening. There is an increase in NO production in the human cervix during the ripening process. Human cervical biopsies obtained from women within 10 minutes of delivery show an increase in NOS II expression compared with non-pregnant controls [4]. The NO donors, isosorbide mononitrate and glyceryl trinitrate are effective at reducing the force required to dilate the cervix prior to surgical termination, in women in the first trimester of pregnancy, when compared to no treatment [5]. Although these NO donors were not as effective as the PGE_2 analogue gemeprost at inducing cervical changes, this study still demonstrates that NO is a factor in human cervical remodelling. Pregnant women who deliver prematurely have higher concentrations of the metabolites of NO in their vaginal secretions between 22 and 32 weeks gestation, compared to those women who deliver at term [168]. This provides further evidence that NO is another regulator of the process of parturition.

The mechanisms by which NO regulates cervical ripening are not yet fully understood, but are again likely to link with the endocrine and biochemical changes that are associated with parturition. There is evidence from animal studies to suggest that NO influences MMP production in tissues. NO stimulates gelatinase activity in rat mesangial cells [169]. Incubation of rabbit articular chondrocytes with IL-1 stimulated NO release and also MMP-2 (gelatinase) and MMP-9 release [170]. In cultured chondrocytes NO can also influence GAG synthesis [170]. The full effect of NO on collagen and proteoglycan metabolism in both the animal and human cervix has yet to be determined. In humans the in vivo and in vitro administration of NO donors to cervical tissue obtained from women in the first trimester of pregnancy does not have any significant effect on cervical MMP-2, MMP-9 and TIMP secretion [171].

Synthesis of NO can be altered by endocrine factors and also by cytokines [157]. In rats uterine NO production is gestationally regulated and progesterone dependent [164]. IL-1β induces iNOS expression in rat ovarian tissue in vitro [172]. In humans, the pro-inflammatory cytokines IL-6, IL-8 and TNF-α are associated with the induction of NO production during the acute phase of sepsis [173]. Inducible

NOS is activated by the pro-inflammatory cytokines IL-1, IFN-γ and TNF-α, and inhibited by the anti-inflammatory cytokines IL-4, IL-10 and TGF-β [174]. IL-1 appears to be the most effective cytokine in stimulating NO release from iNOS containing cells [167]. Neutrophils and macrophages cells are likely to be the source of NO during cervical ripening as they contain iNOS and are capable of producing large quantities of NO [175]. The placenta is another potential source of NO [176].

NO may also influence other mediators of cervical ripening. NO is a powerful inducer of COX-2 and elevates local PGE_2 concentrations in inflamed tissues [177]. Local application of an NO donor to the human cervix increases $PGF_{2\alpha}$ and decreases thromboxane A_2 synthesis [178]. NO also stimulates IL-8 and PGE_2 release from the human cervix in vitro [131]. Further work is required to assess what effects NO has on other cytokines involved in cervical ripening, in order to determine the exact role of NO in the ripening cascade.

Therefore NO represents another metabolic mediator of the cervical ripening, acting at the end of the process [2]. During cervical ripening it is likely that NO stimulates prostaglandin synthesis by inducing COX-2 and may act along with PGE_2 to increasing vascular permeability, cytotoxicity and by promoting tissue damage. NO may activate other MMPs and alter glycosaminoglycan composition by mechanisms that have as yet not been determined. NO may also regulate apoptosis again by mechanisms that are still to be determined.

Conclusion:

To conclude this section I will attempt to summarise the sequence of events that occur in the female reproductive tract in the later stages of pregnancy, in order to provide an overview of the physiology of parturition. Some of what follows is speculative and may of course be disproved by future research.

During pregnancy there are profound changes in the composition of the cervix that facilitate the process of cervical ripening. There is both a reduction in total cervical collagen concentration with an associated increase in immature collagen content. There is a marked increase in cervical tissue hydration. The total cervical proteoglycan concentration is halved and there are also qualitative

changes in the cervical GAG composition. These changes act to destabilise the cervical extra-cellular matrix.

The histological and biochemical changes of cervical ripening are similar to an inflammatory reaction. During cervical ripening there is vasodilatation and an increase in vascular permeability that is followed by an influx of neutrophilic polymorphonuclear leukocytes and macrophages into the cervical stroma. Cervical ripening is associated with apoptosis of cervical smooth muscle cells and fibroblasts, the significance of which is uncertain. Neutrophil polymorphs are the main source of matrix metalloproteinases (MMPs) during cervical ripening. MMPs are enzymes that are essential for collagen degradation. Leukocyte elastase is also essential for successful cervical connective tissue remodelling. The activation of these enzymes promotes the further connective tissue changes that are required for successful parturition.

Human parturition may be thought of as consisting of two phases; a preparatory phase which is cervical ripening, and a phase of myometrial activity that is associated with progressive cervical dilatation, known as active labour. Human parturition appears to be regulated by a number of factors. These factors, whilst independent of one another, can regulate other factors in the parturition chain. It appears that once the cascade of events in human parturition has been initiated, these factors interact with one another to ensure that successful parturition occurs.

The activation of the fetal hypothalamic-pituitary-adrenal-placental axis appears to be the trigger for human parturition. This axis may be triggered by maternal or fetal stress, or by a "biological clock" within the mother, fetus or placenta. This activation of this axis will produce a rise in fetal cortisol, DHEA and placental CRH. Fetal cortisol may displace progesterone from glucocorticoid receptors in the placenta, myometrium and cervix, thereby simulating progesterone "withdrawal". In the placenta the "withdrawal" of progesterone in conjunction with an increase in fetal cortisol production will promote placental CRH synthesis. The mediators of cervical ripening will also promote the synthesis of placental CRH. IL-1 and prostaglandins promote the production of placental CRH. Placental CRH stimulates the release of fetal ACTH from the fetal pituitary gland, which in turn

stimulates further production of fetal cortisol from the fetal adrenal cortex. A positive "feed-forward" loop is therefore established ensuring a rise in fetal cortisol, DHEA and placental CRH.

In the placenta DHEA is converted to oestradiol. Oestradiol increases myometrial gap junction formation, increases the number of myometrial oxytocin receptors, and stimulates prostaglandin formation. These changes are essential to promote myometrial contractions. Oxytocin release from the pituitary will further promote myometrial activity. Myometrial contractions, along with pressure from a descending fetal presenting part, cause mechanical stress on the cervix. Mechanical stress, along with the hormonal changes of parturition, is important in initiating the inflammatory cascade of cervical ripening.

Prostaglandins, NO, relaxin and cytokines regulate the connective tissue re-modelling that occurs in the cervix during cervical ripening, in response to the endocrine changes of parturition and mechanical stress. Stretching of the cervix may result in an increase in IL-8 and prostaglandin activity, most likely from cervical fibroblasts in response to IL-1. Progesterone "withdrawal" leads to increased expression of endothelial adhesion molecules allowing the adhesion of leukocytes to the vascular endothelium prior to chemotaxis. IL-8 is a powerful neutrophil chemotactic agent, and promotes neutrophil activation and increases vascular permeability. PGE_2 stimulates the release of IL-8 and may act synergistically with IL-8 to promote neutrophil chemotaxis.

Prostaglandins may also act as vasodilator agents and increase vascular permeability, further facilitating inflammatory changes. The local endocrine changes, namely a rise in oestradiol and a "withdrawal" of progesterone also stimulate prostaglandin production and reduces prostaglandin catabolism. The "withdrawal" of progesterone will also stimulate NO production from neutrophil polymorphs possibly mediated by IL-6, IL-8 and TNF-α. NO may promote further PGE_2 release from the cervix via COX-2 induction and NO may act along with PGE_2 in increasing vascular permeability, cytotoxicity and by promoting tissue damage. IL-1, IL-6 and TNF also increase the production of prostaglandins via increased PLA_2 activity.

In the cervix oestrogens may initiate apoptosis of cervical smooth muscle cells and fibroblasts and also promote cervical eosinophilic infiltration. Fetal cortisol, NO and TNF-α may also regulate apoptosis of cervical cells. Oestrogens also regulate the binding of relaxin to cervical cells. These effects of oestrogen during cervical ripening may be mediated by insulin-like growth factor.

The final stages of cervical ripening result in the activation of MMPs, and hence connective tissue remodelling. The "withdrawal" of progesterone allows IL-1 to induce the expression of MMP and down-regulate the expression of TIMP. The effects of IL-1 on MMP production are mediated by protein kinase C. IL-1 and IL-8 stimulate MMP release from neutrophils and fibroblasts and also stimulate fibroblast hyaluronic acid production, altering the cervical glycosaminoglycan structure. DHEA appears to act synergistically with IL-8 by increasing MMP activity. Relaxin increases MMP activity, and also increases cervical GAG synthesis. TNF-α and TGF-β may also be involved in cervical MMP production. NO may also stimulate MMP activity either directly, or via other mediators such as IL-1. NO may also influence cervical GAG synthesis.

The overall effect of the connective tissue changes that occur in the cervix during parturition facilitate further cervical remodelling during active labour and, along with myometrial contractions, should lead to successful delivery of the infant.

II. The Induction of Cervical Ripening:

Introduction:

The decision to attempt to artificially induce labour is one that the obstetrician does not take lightly. The objective of artificially inducing cervical ripening and uterine contractions is to provide greater benefit to the health of the mother and/or the fetus than if the pregnancy continued. The risks of the induction of labour are dependent upon parity, the individual's past obstetric history and the ripeness of the cervix [179]. The risks of induction of labour include increased rates of caesarean section and operative vaginal delivery compared to women who labour spontaneously, increased rates of fetal distress requiring medical intervention, wound dehiscence, epidural anaesthesia, intra-partum pyrexia, chorioamnionitis and post-partum haemorrhage [180]. The induction of labour is associated with a caesarean section rate of 25 - 40%, with some series reporting a caesarean rate as high as 47% [181].

There are many reasons as to why one would wish to initiate the potentially hazardous process of the induction of labour. There may be concerns regarding the well-being of the fetus; for example a pregnancy compromised by intra-uterine growth restriction or severe rhesus disease. Labour may be induced because of concerns regarding the health of the mother, for example worsening preeclampsia. Labour induction is occasionally performed for the convenience of the mother in the absence of any clear medical indication or health benefit. The risks of "social induction" must be clearly explained to women before the decision to induce labour is made.

In the United Kingdom the most common indication for the induction of labour is prolonged pregnancy. Pregnancies that continue beyond 42 weeks gestation are associated with an increased risk of stillbirth, neonatal and infant mortality [182]. Induction of labour should be offered routinely to all women whose pregnancies continue beyond 41 weeks gestation [183]. Induction of labour during this time is associated with reduced caesarean section rate, reduced rate of operative vaginal delivery, reduced frequency of fetal distress, meconium staining, macrosomia, and decreased risk of fetal and neonatal death [184].

The aim of the induction of labour is to successfully mimic the physiological process of parturition leading to a vaginal delivery, whilst creating minimal discomfort and risk to the mother and fetus. The ideal agent used for the induction of cervical ripening and the induction of labour should be effective, easy to administer, acceptable to the patient, safe for both mother and fetus and cost-effective.

Before the induction of labour begins, the cervix should be examined to determine its "ripeness". The ripeness of the cervix can be determined using the modified Bishop Score [37]. The cervical length, consistency, position and dilatation should be determined, along with the station of the fetal presenting part in relation to the ischial spines. Cervical dilatation appears to be the most important factor in predicting the length of the latent phase of labour and the likelihood of success of induction of labour [185, 186].

If the cervix is unfavourable (Bishop Score <7), then cervical ripening is required. When the cervix is favourable (Bishop Score $\geq$ 7), amniotomy should be performed. In some patients, most commonly multiparous women, amniotomy is often enough to provoke regular myometrial activity. 88% of women with a favourable cervix will labour within 24 hours by amniotomy alone [187]. Nevertheless following amniotomy synthetic oxytocin is usually used to stimulate active labour. If oxytocin is commenced at the time of amniotomy rather than delayed, there are reduced rates of operative delivery, post-partum haemorrhage and a shorter time interval from induction to delivery [105].

The first chapter of this thesis described the physiology of cervical ripening and the factors that initiate and regulate this process. A number of strategies have developed in an attempt to artificially induce cervical ripening, based on our understanding of the physiology of parturition. Once sufficient cervical ripening has occurred the second phase of parturition, known as active labour, may be induced with amniotomy and intravenous oxytocin infusion.

Membrane Sweeping:

Membrane sweeping has been used since 1810 to promote cervical ripening prior to the induction of labour. During routine cervical examination, the inferior pole of chorioamniotic membranes is separated from the lower uterine segment using a circular "sweeping" motion of the examining finger that has been inserted through the cervix. This procedure is relatively non-invasive, safe, easy to perform and inexpensive [188, 189].

It is believed that membrane sweeping stimulates cervical ripening by promoting endogenous prostaglandin release [190]. Membrane sweeping is associated with increased phospholipase A_2 activity and increased serum $PGF_{2\alpha}$ metabolites [191].

There are a number of studies that have demonstrated that membrane sweeping is effective at inducing parturition. Sweeping of the membranes once at term reduces the need for induction of labour in multiparous women with a favourable Bishop Score [192]. In women with an unfavourable cervix and a negative fetal fibronectin test at 39 weeks gestation, membrane sweeping every 3 days from 39 weeks gestation is associated with a lower gestational age at the time of delivery, a higher Bishop score on admission for delivery, a higher rate of spontaneous labour, a lower number of women requiring induction of labour, and a lower number of women who are undelivered at 42 weeks gestation [193]. Cammu and Haitsma demonstrated that weekly membrane sweeping is associated with a reduced number of primigravid women whose pregnancies progress beyond 287 days and hence a decreased rate of induction of labour in these women [194]. In women with unfavourable cervices at 41 weeks gestation daily membrane sweeping is associated with increased cervical ripening, shorter intervals to delivery, higher rates of spontaneous labour and reduced numbers of women requiring induction of labour at 42 weeks gestation compared to daily administration of prostaglandin [195].

However, membrane sweeping once in primigravid and multigravid women at term with unfavourable cervices (Bishop Score <6) has little effect on the rates of induction of labour [192]. Sweeping the membranes once between 38 and 40 weeks gestation is not associated with an increase in women who progress into spontaneous labour within seven days of the procedure [194, 196]. Multiple

sweeping therefore appears to be more effective than single membrane sweeping, especially in primigravid women.

Membrane sweeping is not entirely free of risk. The potential risks of membrane sweeping are infection, membrane rupture and ante-partum haemorrhage from an undiagnosed placenta praevia [193]. Boulvain reported that membrane sweeping was not associated with any significant adverse maternal or fetal outcome. However membrane sweeping appears to be associated with significant maternal discomfort during the procedure, and the vaginal examinations are perceived to take longer [192]. Membrane sweeping is also associated with an increase in vaginal bleeding and uterine contractions prior to the onset of labour that can cause considerable maternal anxiety [192].

Balloon Catheter Devices, and Mechanical Dilators:

Mechanical devices can be used to ripen the cervix prior to the induction of labour and are designed to ripen the cervix without causing uterine contractions. These techniques are useful in situations where induction of labour is necessary and uterine hyperstimulation is particularly undesirable, such as in multiparous women who have previously delivered by caesarean section, where there is a risk of uterine scar dehiscence, and also in pregnancies complicated by intra uterine growth restriction.

Like membrane sweeping, mechanical devices also appear to stimulate the release of endogenous prostaglandins from the decidua [197]. Histological examination of the cervix after such treatments shows an inflammatory response, with increased tissue hydration and a reduction in tissue collagen concentration [198].

Although the aim of mechanical techniques used to stimulate cervical ripening is to avoid uterine hyperstimulation, these methods are not entirely free of risk. Fetal distress can occur and there are risks of chorioamnionitis, membrane rupture and ante-partum haemorrhage. Mechanical procedures used to induce cervical ripening can also cause unwanted maternal discomfort.

The Foley single balloon catheter can induce cervical ripening, shorten the length of time between induction and delivery, reduce the rate of caesarean section and increase the rate of spontaneous vaginal delivery [199]. The use of an extra-amniotic balloon catheter device is inexpensive, safe, easy to use and reversible [200]. It appears to be well tolerated when administered to outpatients [201].

The Foley catheter is inserted into the cervical canal under aseptic conditions in the lithotomy position, the catheter balloon is filled with saline. In addition, traction may be applied to further encourage cervical ripening. The balloon either falls out spontaneously when cervical ripening has occurred, or is gently extracted at 12 hours.

The Foley catheter is as least as effective as PGE_2 when used for the induction of cervical ripening, is less expensive, and has a similar rate of intra-partum complications and delivery outcomes as PGE_2 gel [202, 203]. The Foley catheter also appears to have a shorter time interval between induction and delivery when compared to PGE_2, but in one study in which this was demonstrated, different methods of inducing uterine activity were used once cervical ripening had been achieved, making the interpretation of this finding difficult [202]. Adverse effects that may result from the use of the Foley catheter for the induction of cervical ripening include rupture of membranes, displacement of the fetal presenting part, vaginal bleeding, and infection [200]. However these adverse outcomes are rarely seen.

The instillation of normal saline into the extra-amniotic space via the Foley catheter further promotes cervical ripening and is more effective than intra-vaginal and intra-cervical PGE_2 in ripening the unfavourable cervix [200, 204]. However the installation of fluid through the Foley catheter has never been shown to be more effective than insertion of the Foley catheter alone.

The Atad Ripener Device is a recently developed double balloon device that operates using the same principle as the Foley balloon catheter, and is effective at inducing cervical ripening in 92% of primigravid and multigravid women [205]. The Atad double balloon device appears to cause less maternal discomfort than the single balloon Foley catheter, and appears to be more efficacious than the single balloon device and have a lower failure rate (8% vs. 16%) [205]. Further randomised-

controlled trials are required in order to determine whether or not this device is a more acceptable agent than other methods of inducing cervical ripening.

Dilapan is a synthetic polyacrylnitrile hygroscopic cervical dilator that is safe and effective at inducing cervical ripening prior to the induction of labour [206]. Dilapan dilators are inserted into the cervical canal and removed 12 hours after insertion. However the use of Dilapan as a cervical ripening agent is not associated with any reduction in the total length of labour, nor does it reduce the rate of caesarean section in those women who are attending for the induction of labour who have received oxytocin without any ripening agent [206].

Dihydroepiandrosterone (DHEA) and Oestrogen:

During human parturition increases in plasma dihydroepiandrosterone (DHEA) concentrations are associated with cervical ripening [9]. DHEA is synthesised in the fetal adrenal gland in response to fetal ACTH. In the placenta DHEA is converted to oestradiol. The alteration of the ratio of oestrogen to progesterone prior to the onset of parturition is believed to stimulate prostaglandin production that will in turn promote cervical ripening.

The twice-weekly intra-venous administration of 100 milligrams DHEA from 37 weeks gestation is associated with significant increases in Bishop Score until 14 days after first injection, compared to controls [207]. There was also a significantly shorter induction to delivery interval associated with DHEA administration. However this study does not include information regarding maternal and neonatal outcomes, and it is therefore difficult to comment on the safety of DHEA when used for the induction of cervical ripening. The use of an agent for the induction of cervical ripening that must be injected repeatedly is also questionable.

The local application of oestradiol cream is effective at inducing cervical ripening and is associated with a reduction in the caesarean section rate [105]. Oestradiol is as effective as intra-vaginal PGE2 gel at inducing labour with less uterine activity [208]. The administration of 4 milligrams of oestradiol cream into the anterior fornix at 6 hourly intervals is as effective at inducing cervical ripening as the

intra-cervical administration of 0.5 milligrams of PGE2 gel at 6 hourly intervals, and also intravenous infusion of low dose oxytocin [209]. Magann et al noted that although the overall rate of caesarean section was high (59%), there were no differences in delivery outcomes between the three agents.

Despite there being some evidence to suggest that the local application of oestradiol cream is effective at inducing cervical ripening, the use of oestrogens to induce cervical ripening appears to have gone out of vogue. The studies that have investigated the effectiveness of oestrogens at inducing cervical ripening are small, and it seems that further work is required before oestrogens can be considered as alternatives to those agents currently used to induce cervical ripening.

Mifepristone:

Progesterone inhibits cervical ripening [19]. Mifepristone (RU 486) is a steroid compound that antagonises progesterone at the receptor level [198]. Mifepristone is effective at terminating pregnancy in early pregnancy and at term [210, 211]. Mifepristone increases myometrial contractility and responsiveness to endogenous and exogenous prostaglandins and also promotes cervical ripening [212]. It is likely that mifepristone promotes cervical ripening by inducing collagen fragmentation and breakdown [92].

The oral administration of 200 milligrams of mifepristone on 2 consecutive days is effective at inducing cervical ripening in pregnant women at term [213]. This regime appears to be safe, even for those women with a history of previous caesarean section [214]. However the oral administration of a single dose of 200 milligrams of mifepristone to pregnant women beyond 41 weeks gestation appears to have only modest effects on cervical ripening [215]. The oral administration of a single dose of 400 milligrams of mifepristone is also effective at inducing cervical ripening [216]. A dose of 400 milligrams of mifepristone appears to increase the number of women who progress into spontaneous labour and also shortens the induction to delivery interval when compared to the oral administration of 2 doses of 200 milligrams of mifepristone [216].

The use of mifepristone for the induction of cervical ripening may not be without adverse maternal, fetal and neonatal adverse effects. Mifepristone may increase the incidence of uterine hypertonus when oxytocin is used for augmentation of labour [216]. Mifepristone has anti-glucocorticoid properties that do not appear to be of any clinical significance in adults, even following the administration of a single high dose (2 grams), or following prolonged administration (200 milligrams daily for 3 months) [217]. Mifepristone crosses the placenta and could therefore adversely affect the fetus, in particular by causing neonatal hypoglycaemia [218].

The numbers involved in studies in which mifepristone has been used to induce cervical ripening in pregnant women at term are too small to draw conclusions about the fetal and neonatal safety of mifepristone when used as a cervical ripening agent, although no significantly increased adverse outcomes have been seen. Therefore mifepristone might be a suitable alternative to those agents currently in use for the induction of cervical ripening, although further studies are required to fully evaluate the risks and benefits of this agent.

Oxytocin:

It is recognised that oxytocin has no direct effect on cervical tissue composition [105]. Any changes in cervical composition associated with pharmacological oxytocin administration are likely to be the result of the indirect action of sheer stress caused by myometrial contractility. However, continuous low dose oxytocin has been used as an effective cervical ripening agent in doses of 1 – 4 milliUnits / minute [219, 220, 221].

The use of intra-venous oxytocin does not appear to be more effective at inducing cervical ripening as locally administered PGE_2 [209, 222, 223]. Jackson demonstrated no difference in the degree of cervical ripening, vaginal delivery rates and successful induction rates, when low dose oxytocin infusion was compared with repeated intra-cervical doses of 0.5 milligrams of PGE_2 gel administered at 6 hourly intervals [222]. In the induction of cervical ripening there appears to be a greater likelihood of vaginal delivery within 24 hours in those women that receive PGE_2 gel as opposed to oxytocin [222].

Pollnow et al also demonstrated that intravenous oxytocin administration for the induction of cervical ripening is not as effective as locally administered PGE_2 [223]. The administration of intra-venous oxytocin at 2 milliUnits / minute over 10 hours is not as effective at inducing cervical ripening as 2 intra-vaginal doses of 4 milligrams of PGE_2 gel [223]. In addition there were less successful inductions and the induction to delivery interval was greater in the group that received oxytocin. There were no differences between the two groups for caesarean section rates, meconium stained amniotic fluid, Apgar Scores and umbilical cord pH.

The use of intra-venous oxytocin may also have adverse effects on both mother and fetus. Prolonged oxytocin use is associated with water intoxication, and may cause fetal distress due to uterine contractions on an unripe cervix [198]. Because of the potential to cause hyper-stimulation there are situations in which one would wish to avoid the use of oxytocin for the induction of cervical ripening, for example in patients with a history of caesarean section and or pregnancies complicated by intra-uterine growth restriction. Oxytocin is administered as a continuous intra-venous infusion and will restrict patient mobility resulting in decreased patient satisfaction.

It appears that oxytocin should not be used for the induction of cervical ripening. Intravenous oxytocin should be reserved for the induction of myometrial contractions following amniotomy, after successful cervical ripening has been achieved by other methods.

Relaxin:

Relaxin, a large polypeptide hormone, appears to be an integral component of human cervical connective tissue remodelling. Relaxin has an inhibitory effect on myometrial activity in many species, but human relaxin appears to have only a minor effect on myometrial activity in late pregnancy [114]. The idea of inducing cervical ripening without stimulating myometrial activity is appealing.

Early studies using purified porcine relaxin demonstrated that the intra-vaginal administration of 2 and 4 milligrams of purified porcine relaxin resulted in an increase in cervical score and a shorter

duration of labour, when compared to placebo [224, 225]. There was not a statistically significant difference in cervical ripening effect between 2 and 4 milligrams of purified porcine relaxin [225]. The intra-vaginal administration of 2 milligrams of purified porcine relaxin to outpatients also stimulated cervical ripening and appeared safe [225]. No significant cervical ripening effect was demonstrated following the intra-vaginal administration of 4 milligrams of purified porcine relaxin to outpatients, suggesting that using a higher dose might promote down-regulation of cervical relaxin receptors [225].

More recently however, the intra-vaginal administration of 1, 1.5, 2, and 4 milligrams of recombinant human relaxin gel produced no significant increase in cervical score when compared to placebo at 15 hours following administration [226, 227]. There were also no statistically significant differences in lengths of labour, modes of delivery, and serum relaxin concentrations when comparing these doses of relaxin to placebo [226, 227].

Although intra-vaginal recombinant human relaxin, gel is not associated with adverse maternal or fetal outcomes, is does not appear suitable for use as a cervical ripening agent. Intra-venous relaxin might improve tissue availability and hence increase the likelihood of achieving cervical ripening [227]. Nevertheless it must be asked whether or not women attending for the induction of labour would find such a regime more acceptable than those agents currently used to induce cervical ripening?

Prostaglandins:

Prostaglandins are currently the most commonly used agents used to artificially induce cervical ripening. In the induction of cervical ripening prostaglandins increase the likelihood of vaginal delivery by 33% [181]. Prostaglandins used for the induction of cervical ripening are more likely than placebo to increase cervical score, shorten the average length of labour, promote myometrial contractility, and to decrease the rate of operative delivery [181, 228]. Prostaglandins of the E series are superior to those of the F series when used to induce cervical ripening, as the ripening effects are greater and the systemic adverse effects are less [181].

Oral, intra-cervical, intra-vaginal and intra-venous prostaglandins have all been used to induce cervical ripening. The local administration of prostaglandins is more effective and is associated with fewer systemic adverse effects than oral or intra-venous administration. A single dose of 0.5 milligrams intra-cervical PGE_2 gel is associated with a greater increase in Bishop Score compared to a single dose of 1 milligram intra-vaginal PGE_2 gel [229]. Nevertheless when comparing intra-cervical and intra-vaginal administration of PGE_2 gel at these doses, there is no difference in total duration of labour, mode of delivery, and maternal and fetal adverse outcomes [229].

Stempel et al showed that there was no difference in Bishop Score when comparing the administration of 0.5 milligrams of intra-cervical PGE_2 and 5 milligrams of intra-vaginal PGE_2 for the induction of pre-labour cervical ripening [230]. Again there was no difference in total duration of labour, mode of delivery, and maternal and fetal adverse outcomes between the groups that received intra-cervical and intra-vaginal PGE_2. Because the intra-vaginal method of PGE_2 administration is easier and the preparation is cheaper, it is more widely practised.

PGE_2 gel may be administered on an outpatient basis and this method of inducing cervical ripening appears safe and cost effective [228, 231]. The daily administration of 2 milligrams of intra-vaginal PGE_2 gel between 38 and 40 weeks gestation is safe for both mother and fetus and is effective in initiating labour [228]. However the out-patient administration of intra-vaginal PGE_2 gel does not appear to reduce the overall frequency of pregnancies that have exceeded their estimated date of delivery, nor significantly reduce the number of inductions of labour and the rates of caesarean section for these women [228]. There does not appear to be any increase in cervical ripening associated with repeated intra-cervical doses of 0.5 milligrams of PGE_2 gel when compared to placebo, in women attending for routine out-patient antenatal surveillance beyond their estimated date of delivery [231]. Furthermore in this study there was no difference in the numbers of women who required induction of labour, in the numbers who required additional prostaglandin gel when admitted for induction of labour, and in the total length of labour.

PGE_2 is usually administered as a gel but is also effective at inducing cervical ripening when administered intra-vaginally as a sustained release preparation [232, 233, 234]. It has been shown that 10 milligrams of PGE_2, administered intra-vaginally as a continuous release preparation over 12 hours, is at least as effective as intra-cervical doses of 0.5milligrams of PGE_2 administered 6 hourly [232, 233]. The continuous release preparation is associated with a shorter ripening period and also a decreased induction to delivery interval when compared to repeated doses of intra-cervical PGE_2 gel [232, 233]. There is no difference in the lengths of latent and active labour associated with continuous release PGE_2 when compared to intra-cervical PGE_2 and there were no significant differences in modes of delivery and side effect profile [232, 233]. Nonetheless sustained release PGE_2 preparations are more likely to be associated with uterine hyper-stimulation than PGE_2 gel, although the sustained release preparation is easily removed and the uterine effects usually disappear within 15 minutes of withdrawal [198].

In the United Kingdom primigravid women with an unfavourable cervix usually receive an initial dose of 2 milligrams of PGE_2 gel intra-vaginally. Further doses of 1 milligram may then be administered at 6 hourly intervals to a maximum of 4 milligrams. In multigravid women or primigravid women with a favourable cervix an initial dose of 1 milligram is used, followed by doses of 1 milligram at 6 hourly intervals to a maximum of 3 milligrams [183].

Mackenzie and Burns showed that a single dose of 2 milligrams of PGE_2 gel is as effective at inducing cervical ripening as 2 doses of 2 milligrams for both primigravid and multigravid women [235]. A single administration of 2 milligrams of PGE_2 gel is also more cost effective than repeated administration [235]. The use of prostaglandins administered in doses of 3 milligrams at 6 hourly intervals, followed by amniotomy and oxytocin infusion when cervical ripeness has been achieved, is associated with an 80% vaginal delivery rate [180].

The use of repeated intra-vaginal doses of PGE_2 gel in conjunction with the use of an intra-cervical single balloon Foley catheter appears to provide additional ripening effects compared to the use of PGE_2 alone [236]. The use of a single balloon Foley catheter in combination with 4 hourly intra-vaginal

administration of 4 milligrams of PGE_2 gel is associated with more effective cervical ripening, shorter induction to delivery interval and a higher rate of vaginal deliveries within 24 hours when compared to the intra-vaginal administration of 25 micrograms of misoprostol at 4 hourly intervals [237]. However the use of a Foley balloon catheter in combination with the intra-vaginal administration of 25 micrograms of misoprostol does not appear to have any significant ripening advantages when compared to misoprostol alone [238].

Prostaglandin receptors are widely distributed and therefore prostaglandins administered to induce cervical ripening will not only have effects on the cervix, but also on other tissues. Prostaglandins that are administered in order to induce cervical ripening will also provoke myometrial contractions. Uterine hyperstimulation occurs in 7% of cases [181]. The hyper-stimulation of uterine activity with an unripe cervix can provoke fetal distress and also placental abruption, requiring emergency delivery by caesarean section. There are also prostaglandin receptors within the gastro-intestinal tract and lungs and prostaglandins may induce nausea, vomiting, diarrhoea and asthmatic episodes.

The prostaglandin E_1 analogue misoprostol is also more effective than placebo at stimulating cervical ripening [239]. Misoprostol has advantages over PGE_2 gel as it is far less expensive, and is easier to administer [240]. Initial studies comparing the intra-vaginal administration of misoprostol with intra-cervical PGE_2 suggested that misoprostol was more effective than PGE_2 at inducing cervical ripening and associated with a shorter induction to delivery time interval [239, 240, 241, 242].

However in these studies there were some concerns about potential harmful effects of misoprostol. Wing et al reported that whilst there were no differences in the frequency of abnormal fetal heart rate patterns, the administration of 50 micrograms of misoprostol at 3 hourly intervals was associated with an increase in myometrial activity and with the passage of thick meconium, when compared to the intra-cervical administration of 0.5 milligrams of PGE_2 gel at 6 hourly intervals [240]. Buser et al also reported an increase in non-reassuring fetal heart rate patterns in conjunction with uterine hyper-stimulation, and an increase in the frequency of caesarean section associated with the administration of 50 micrograms of misoprostol at 4 hourly intervals [241].

Conversely Chuck and Huffaker concluded that the intra-vaginal administration of 50 micrograms of misoprostol for the induction of cervical ripening was more effective and as safe as the intra-cervical administration of PGE_2 at 4 hourly intervals [242]. The intra-vaginal administration of 50 micrograms of misoprostol at 4 hourly intervals is as safe and effective at inducing cervical ripening as extra-amniotic saline infusion via a Foley single balloon catheter [243]. Indeed in this study the rate of caesarean section was low (23%), demonstrating the effectiveness of both agents at inducing cervical ripening.

Gottschall et al demonstrated that the administration of a single dose of 100 micrograms of misoprostol intra-vaginally is effective at inducing cervical ripening and did not appear to be associated with greater rates of uterine hyper-stimulation, or abnormal fetal heart rate patterns when compared to the administration of a single dose of 5 milligrams of intra-vaginal PGE_2 [244]. The administration of 25 micrograms of misoprostol at 3 hourly intervals does not appear to be associated with an increase in myometrial activity or the occurrence of thick meconium, and is effective at inducing cervical ripening when compared to intra-cervical PGE_2 [239].

Misoprostol is also effective at inducing cervical ripening in pregnant women at term following oral administration [245]. Although not as effective as vaginal administration of misoprostol at inducing cervical ripening, there is a lower rate of caesarean section for non-reassuring fetal heart rate pattern associated with the oral administration of misoprostol [245]. The oral administration of 50 micrograms of misoprostol at 4 hourly intervals is as effective at inducing labour as the Foley balloon catheter used in combination with oxytocin infusion in multiparous women [246]. The rates of uterine hyperstimulation in this study were significantly lower in the group of women that received repeated doses of misoprostol compared to the Foley catheter-oxytocin group. There were no significant differences between the modes of delivery or adverse outcomes. The oral administration of misoprostol for the induction of labour appears to be less efficacious in nulliparous women [246]. The oral administration of 25 micrograms of misoprostol appears to be safe and effective at inducing labour [247].

A meta-analysis comparing PGE_2 and misoprostol used for the induction of cervical ripening, showed that overall there was a shorter induction to delivery interval associated with misoprostol. However, misoprostol administration was associated with a higher incidence of uterine hyper-stimulation, and higher rates of abnormal fetal heart rate patterns and higher frequency of the presence of meconium stained liquor [248]. There was insufficient power in the meta-analysis to comment on the safety of misoprostol with regard to perinatal outcomes. This suggests that for the moment PGE_2 should remain the prostaglandin of choice for the induction of cervical ripening.

The Future - Cytokines and Nitric Oxide Donors:

There are many factors that regulate the inflammatory-like process of cervical ripening. In recent years it has been recognised that cytokines and nitric oxide (NO) are important regulators of physiological cervical ripening in both animals and humans.

IL-1, IL-8 and TNF-α have been suggested as potential cervical ripening agents [51, 249, 250]. The maternal, fetal and neonatal effects of these cytokines following administration to pregnant women at term are unknown. Preliminary studies should involve in vitro analysis of the effects of these cytokines on human cervical tissue, followed by their administration to pregnant women in the first trimester prior to surgical termination of pregnancy, in order to gain some evidence of effectiveness at inducing cervical ripening and safety in humans. Subsequent studies should then focus on using these cytokines to induce cervical ripening at term, with particular attention on maternal, fetal and neonatal adverse outcomes.

NO donors have also been proposed as alternatives to other agents that are currently used to induce cervical ripening [3, 5, 6, 7, 8]. In animals NO production in the uterus is increased during pregnancy to maintain uterine quiescence [153, 154, 155, 156]. In animals uterine iNOS expression and NO production decrease during term and pre-term labour [157]. However these changes do not appear to occur in humans [158]. However, in humans NO donors relax the myometrium [159] and these agents have been proposed as tocolytic agents in the management of pre-term labour [160, 161]. NO donors have also successfully been used to treat intrapartum fetal distress caused by uterine hypertonus [162]. The idea of

stimulating cervical ripening without myometrial activity is appealing and this is one reason why NO donors could be more acceptable than those agents that are currently used for the induction of cervical ripening.

In the pregnant guinea pig NO is important for uterine artery relaxation [144]. Endogenous NO appears to contribute to the maintenance of vasodilatation and hence flow, in the utero-placental circulation [145]. The use of a cervical ripening agent that promotes utero-placental vasodilatation could be useful in situations where there is evidence of fetal compromise, for example in pregnancies complicated by intra-uterine growth restriction. This is another reason why NO donors could be more advantageous than agents that are currently used for the induction of cervical ripening.

The NO donor, sodium nitroprusside (SNP) is effective at inducing cervical ripening in guinea pigs [167]. In humans the NO donors, isosorbide mononitrate and glyceryl trinitrate (GTN), are effective at inducing cervical changes in the first trimester of pregnancy [5]. Although these NO donors were not as effective as the PGE_2 analogue gemeprost at inducing cervical ripening a subsequent study showed that NO donors induced cervical changes with less adverse effects than gemeprost [6].

However NO is an important regulator of the maternal vasculature and the administration of NO donors to pregnant women at term could have profound cardiovascular effects. It is likely that NO is also an important regulator of the fetal vascular system, and NO donors may also induce adverse fetal cardiovascular events.

There are currently no published studies examining the maternal and fetal haemodynamic effects of NO donors that are administered intra-vaginally to pregnant women at term for the induction of cervical ripening. NO donors would appear to be effective at inducing cervical ripening at term in animals and during the first trimester of pregnancy in humans. Whether or not NO donors are effective at inducing cervical ripening in pregnant women at term remains to be seen. Before this question is answered the effects of NO donors on maternal and fetal haemodynamics needs to be assessed. This will pave the way for further studies examining the effects of NO donors on cervical ripening in pregnant women at term.

III. The Vaginal Application of the Nitric Oxide Donor Isosorbide Mononitrate for the Induction of Cervical Ripening: A Randomised Controlled Trial to Determine Effects on Maternal and Fetal Haemodynamics:

Introduction:

The ideal agent for the pharmacological induction of cervical ripening should be easy to administer, cost effective, acceptable to both patient and clinician and produce adequate cervical change with minimal maternal and fetal adverse effects. In the United Kingdom the agent most widely used for the induction of cervical ripening is intra-vaginal prostaglandin E_2 gel. However prostaglandins are not without adverse effects. These include uterine hyper stimulation, and subsequent fetal compromise, as well as gastro-intestinal upset (see chapter II for review).

Nitric oxide (NO) is responsible for the activity of endothelial dependent relaxing factor and is an important biological mediator in humans [251, 252]. NO exerts its effects in part via the stimulation of guanylate cyclase, and acts as a generalised smooth muscle relaxant. NO mediated smooth muscle relaxation is seen not only in vascular smooth muscle, but also in gastric smooth muscle, and the myometrium. It is now apparent that NO is another crucial factor in the regulation of cervical ripening (see chapter I for review).

NO donors are a class of drugs that liberate NO *in vivo*. These drugs have been used for over one hundred years in the treatment of ischaemic heart disease and cardiac failure. Within obstetrics NO donors have been used to induce myometrial relaxation in the treatment of pre-term labour [160, 161,253, 254, 255], uterine hyperstimulation [162], to facilitate fetal extraction during caesarean section [256] and to assist with the manual removal of a retained placenta [257]. NO donors have also been used to improve fetal blood flow [258].

Several authors have suggested that NO donors could be considered as alternatives to prostaglandins for the induction of cervical ripening [3, 5, 6, 7, 8]. The idea of stimulating cervical ripening without stimulating myometrial activity is appealing. The use of a cervical ripening agent that also would not

adversely affect the utero-placental circulation is also beneficial to the fetus, especially in circumstances in which there is evidence of utero-placental insufficiency. However one area of concern regarding the use of NO donors to induce cervical ripening is the potential risk of maternal hypotension as a consequence of the systemic vasodilatory effects of NO. Any fall in maternal blood pressure could reduce utero-placental blood flow, and cause fetal compromise.

In humans the intra-vaginal administration of 40 milligrams of the NO donor isosorbide mononitrate (IMN) is effective at inducing cervical ripening in the first trimester of pregnancy[5]. Furthermore in the first trimester of pregnancy intra-vaginal IMN has fewer adverse effects than the prostaglandin analogue gemeprost when used to induce cervical ripening [6]. Before studies are performed to determine the efficacy of intra-vaginal IMN as a cervical ripening agent in pregnant women at term, it is important to determine the effects of this agent on maternal and fetal haemodynamics. The third chapter of this thesis will therefore present the results of a study that principally aims to determine the maternal and fetal haemodynamic effects following the intra-vaginal administration of IMN to pregnant women at term during the induction of labour.

Methods:

The study was performed at the Glasgow Royal Maternity Hospital between August 1998 and July 1999. Primigravid women who were attending for the induction of labour were asked to participate in the study. The local ethics committee granted approval. Written informed consent was obtained from each participant prior to recruitment (Appendix I).

Women who wished to participate in the study were assessed by vaginal examination. Assessment of the cervix included an assessment of consistency, length, dilation, position and station of the fetal presenting part as described by Bishop in 1964 and modified by Calder in 1977 [37]. A cardiotocograph was performed to ensure that there was no acute fetal compromise. An ultrasound scan was performed to measure fetal abdominal circumference and the amniotic fluid index (the sum of the maximum vertical cord-free pockets obtained from each of the four quadrants of the uterus). Women with the following characteristics were excluded: cervix with a bishop score >7, multiparity,

multiple pregnancy, history of unexplained antepartum haemorrhage, pregnancy induced hypertension/preeclampsia, breech presentation, fetal abdominal circumference $<5^{th}$ centile for gestational age, amniotic fluid index $<5^{th}$ centile for gestational age, history of cardio-respiratory illness, headache, or a non-reassuring fetal cardiotocograph.

Participating women were randomised to receive one of three treatments. The first group received 20 milligrams IMN (one tablet) administered into the posterior fornix of the vagina. The second group received 40 milligrams IMN (two tablets) administered into the posterior fornix of the vagina. The third group was the control group and received a vaginal examination only. The treatment options were randomised using random number tables in groups of twelve (block randomisation) by the academic supervisor, Dr Jane Norman.

Treatment allocations were placed into opaque envelopes numbered sequentially with a unique study number and sealed. Following recruitment of each patient to the study, the envelopes were opened in order by an independent observer (one of the medical staff of the Glasgow Royal Maternity Hospital), who then administered the treatment. The independent observer then discarded information on the treatment allocation, and the unique study number was used to code the patients' measurements. AE Nicoll subsequently performed all the measurements on both mother and fetus whilst blind to the treatment allocation. Participating women also remained blind to the treatment received. At the end of the study period, the treatment allocation used for each unique study number was revealed, and the results analysed.

Maternal pulse, maternal blood pressure and fetal heart rate were recorded, immediately prior to treatment and subsequently every 30 minutes using an electronic *Dynamap*^{TM} machine. The fetal heart rate was recorded continuously using a cardiotocograph monitor from 30 minutes before treatment to the end of the study period. The umbilical artery resistance index and pulsatility index were determined at baseline, at 180 minutes and 330 minutes using continuous wave Doppler ultrasound. The umbilical artery Doppler studies were performed with the patient in a 15^0left lateral tilted position. A freely floating loop of cord was identified using real time imaging (*Ultramark 4 Plus*, 3.5 MHz curvilinear probe). In the absence of fetal body or breathing movements, signals were

then collected from the umbilical artery using a hand-held 4 MHz continuous was probe (*Sonicaid vasoflo 3*). Resistance index (RI) and pulsatility index (PI) were measured from three consecutive uniform waveforms. These Doppler recordings were made by the same observer (AE Nicoll), who was blinded to the allocated treatment.

Participating women were asked to complete a symptom questionnaire immediately before the administration of the treatment and again at 360 minutes (Appendix II). This questionnaire asked whether or not the following symptoms were present; headache, drowsiness, nausea, indigestion, abdominal pain, pelvic pain, bloating, loss of appetite, diarrhoea, constipation, breast pain, faintness, tiredness, hot flushes, vomiting and dysuria.

Cervical score was assessed immediately prior to recruitment and again at 360 minutes in order to assess for change in cervical score. Both examinations were performed by the same observer (AE Nicoll), who remained blind to the treatment allocation. If the cervical score remained less than 7 at 360 minutes after treatment allocation, participating women received cervical "priming" with intra-vaginal PGE_2 gel in accordance with the local induction of labour protocol. Women with a cervical score greater than 7 underwent forewater amniotomy the following morning, again in accordance with the local induction of labour protocol.

The following parameters of the subsequent labour and delivery were recorded: prostaglandin and oxytocin requirements, mode of delivery, length of labour for women who had a vaginal delivery, analgesia used during labour, volume of blood loss at delivery, umbilical cord venous pH, Apgar scores at 1 and 5 minutes and whether or not the infant required admission to the neonatal unit. In those patients in whom an adverse outcome was seen, the patient notes were examined by an independent observer (the academic supervisor, Dr Jane Norman) to ensure that the adverse effect was not attributable to the intra-vaginal administration of IMN.

Comparisons were made between the three treatment groups. Statistical analysis of the numerical data was performed by analysis of variance using a general linear model of repeated measures (*SPSS for Windows*, Version 8). Significant differences in symptoms between the groups were determined by the

Chi squared test. A p value of less than 0.05 was regarded as statistically significant. It was calculated that a study of 36 patients would have an 80 % power to detect the following differences between each of the IMN groups and the control group at the 5% significance level; maternal pulse 16 beats per minute, systolic blood pressure 12 mmHg, diastolic blood pressure 8mmHg, fetal heart rate 13 beats per minute, umbilical artery RI 10%, umbilical artery PI 15% and cord pH 0.11.

Results:

38 women were recruited to the study. 2 women were excluded from the study after they had been allocated a treatment, but before its administration. One of these women was excluded because of an undiagnosed breech presentation and the other because treatment administration was delayed, and in the interval, spontaneous labour ensued. These women were therefore excluded from the analysis.

Patient characteristics, maternal and neonatal outcomes are shown in Table I. 13 women received 20 milligrams IMN. These women had a median age of 26 years (range 19-36 years) and a median gestation of term + 9 days (range term + 3 - term + 11 days). 11 women received 40 milligrams IMN. These women had a median age of 27 years (range 17 - 34 years) and a median gestation of term + 9 days (range term + 6 - term + 10 days). 12 women received a vaginal examination only. These women had a median age of 28 years (range 16 -38 years) and a median gestation of term + 9 days (range term + 7 - term + 11 days). The indication for induction of labour (as defined by the obstetrician caring for the woman) was post dates pregnancy in all women.

Mean maternal pulse was higher following the administration of 20 and 40 milligrams IMN compared to the control group ($p = 0.003$ and $p = 0.01$ respectively) (Figure I). The greatest difference in mean maternal pulse rate between either of the IMN groups and the control group was at 300 minutes, with a difference in mean maternal pulse of 21 beats per minute (95% CI 5 - 37 beats per minute) between the 20 milligrams IMN and the control group. There was no significant difference in maternal pulse rate between the IMN groups.

Both 20 and 40 milligrams IMN were associated with lower mean maternal blood pressures compared to the control group (Figure II). The intra-vaginal administration of 40 milligrams IMN was associated with a reduction in maternal systolic blood pressure compared to no treatment ($p = 0.006$). The difference in mean maternal systolic blood pressure between the 40 milligrams IMN and the control group was greatest at 300 minutes (greatest difference = 15 mmHg, 95% CI 2 - 26 mmHg). There was no significant difference in mean maternal systolic blood pressure between the 20 milligrams IMN group when compared with both the 40 milligrams IMN group and the control group.

Both 20 and 40 milligrams IMN were associated with a fall in maternal diastolic blood pressure compared to no treatment ($p = 0.01$ and $p = 0.001$ respectively). The greatest difference in mean maternal diastolic blood pressure occurred between the 40 milligrams IMN and the control group (greatest difference = 16 mmHg, 95% CI 7 - 24 mmHg). Mean maternal diastolic blood pressure was significantly lower following the administration of 40 milligrams IMN compared to the administration of 20 milligrams ($p = 0.01$). During the study none of the women experienced any symptoms of hypotension or required treatment for hypotension.

There was no statistically significant difference in fetal heart rate following the administration of 20 and 40 milligrams IMN compared to the group that received no treatment. There was also no statistically significant difference in fetal heart rate between the IMN groups (Figure III). There was no evidence of fetal compromise on cardiotocograph monitoring during the study. Neither dose of IMN had a statistically significant effect on umbilical artery Doppler indices (Table II).

The most frequent maternal side effect associated with IMN was headache, which occurred in 69% (9/13) of women who received 20 milligrams IMN and 91% (10/11) of women who received 40 milligrams IMN, compared with only 8% (1/12) women in the control group ($p=0.001$). There were no other statistically significant differences in reported symptoms (abdominal pain, pelvic pain, hot flushes, faintness, drowsiness, tiredness, heartburn, nausea and vomiting) (Figure IV). During the study none of the women reported any of the following symptoms; constipation, diarrhoea, loss of appetite, bloating, breast tenderness, dysuria or change in fetal movements.

The women that received 40 milligrams IMN had higher mean cervical scores at 360 minutes compared to those women that had received 20 milligrams IMN or no treatment, but these differences in cervical score did not reach statistical significance. More women in the control group required further cervical priming in the form of prostaglandin E_2 compared to both IMN groups. Again these differences were not statistically significant. There were also no statistically significant differences in the number of women in each group that required intravenous oxytocin during labour induction, although fewer women in the control group required oxytocin (Table I).

There were no statistically significant differences in the length of labour for those women who had a spontaneous vaginal delivery, mode of delivery, analgesia use during labour, volume of blood loss following delivery, umbilical venous cord pH, Apgar scores at 1 minute and 5 minutes or the rates of admission to the neonatal unit (Table I).

Figure I - Mean maternal pulse rate at baseline and at 30 minute intervals following the intra-vaginal administration of 20 mg IMN, 40 mg IMN and no treatment (control group):

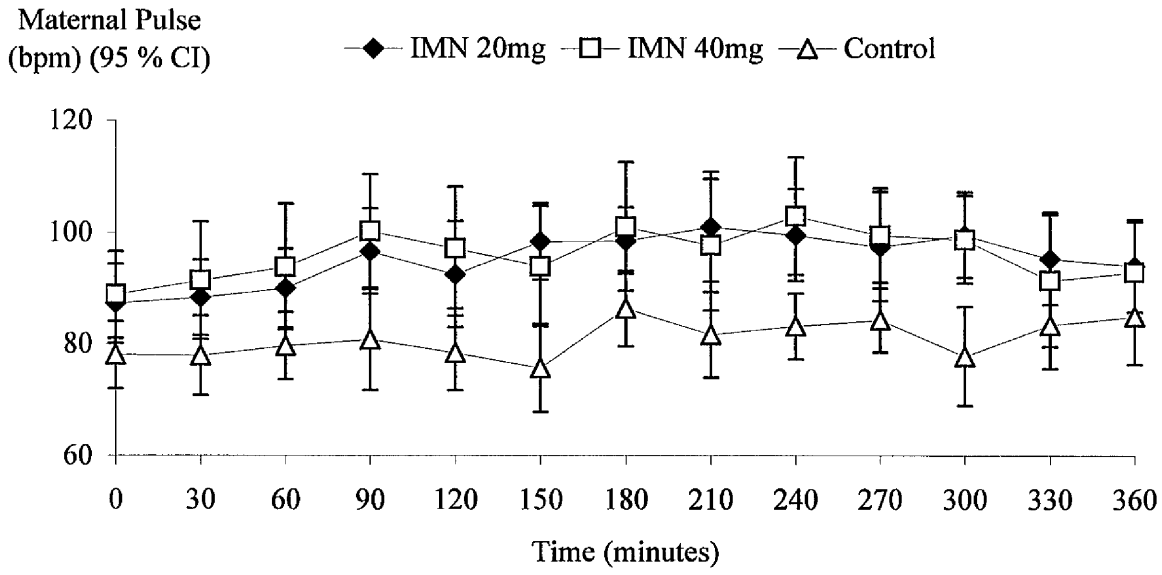

Figure II - Mean maternal systolic and diastolic blood pressure at baseline and at 30 minute intervals following the intra-vaginal administration of 20 mg IMN, 40 mg IMN and no treatment (control group):

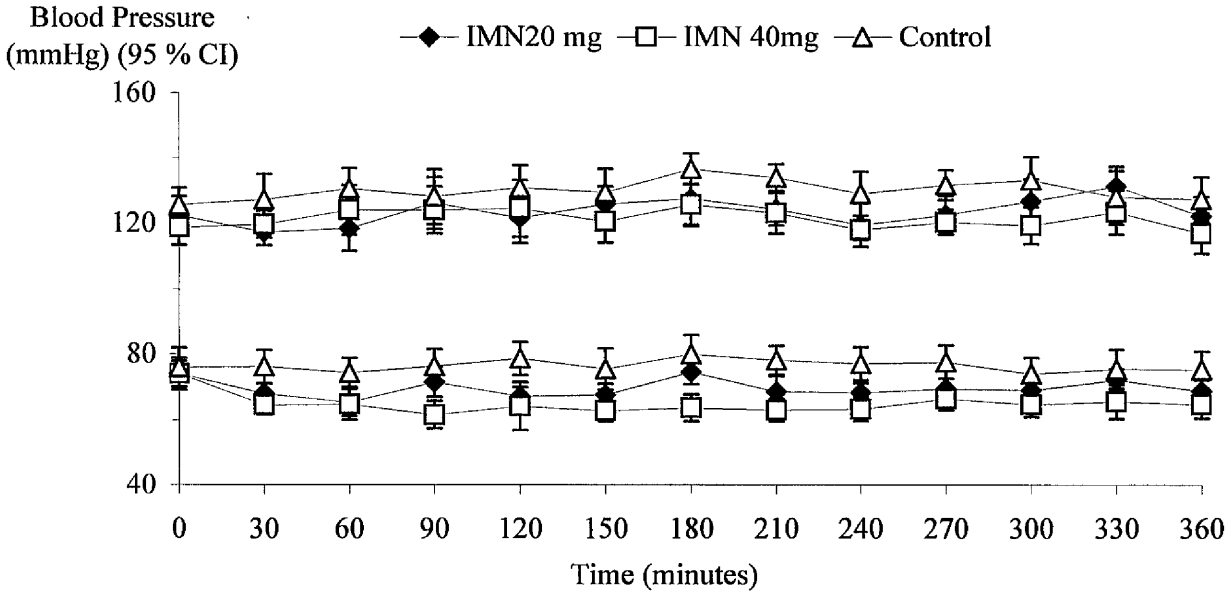

Figure III - Mean fetal heart rate per minute at baseline and at 30 minute intervals following the intra-vaginal administration of 20mg IMN, 40mg IMN and no treatment (control group):

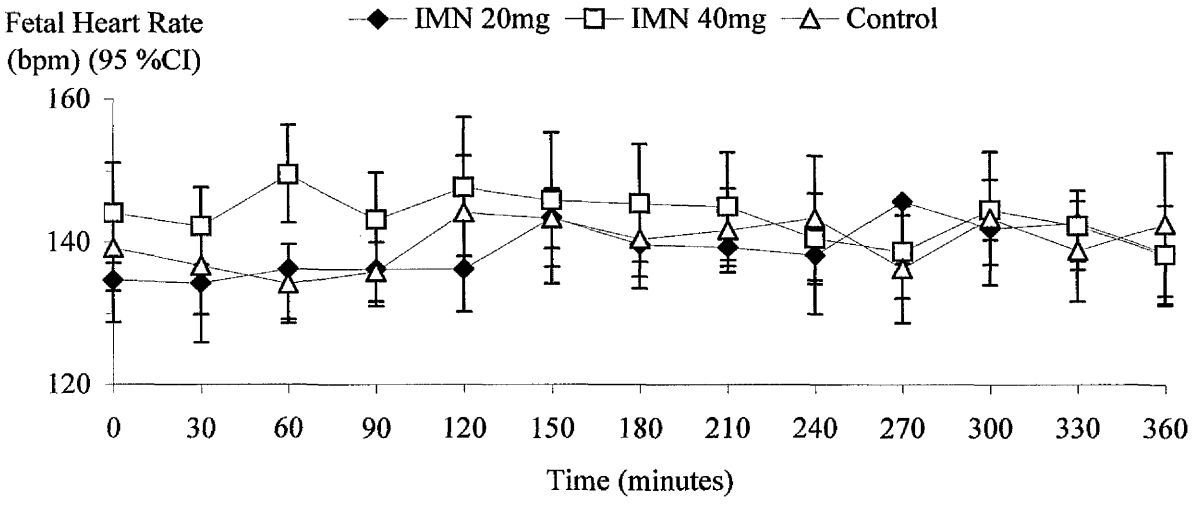

Figure IV - Maternal symptoms at 360 minutes following the intra-vaginal administration of 20 milligrams IMN, 40 milligrams IMN and no treatment (control group):

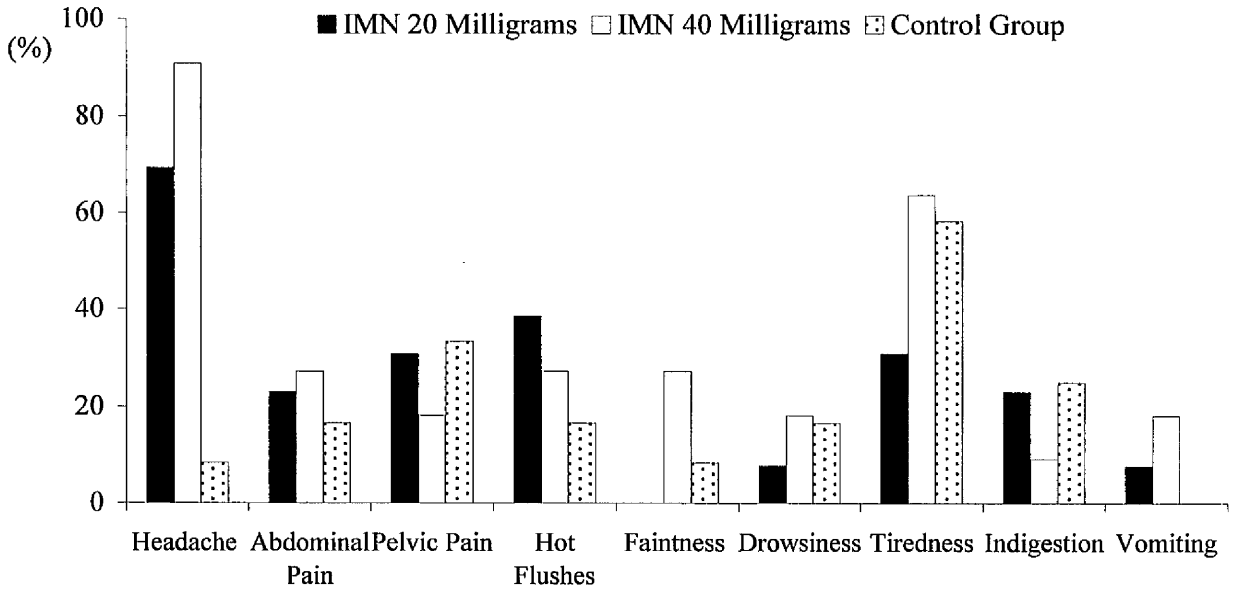

Table I - Description of Patients / Maternal and Neonatal Outcomes:

	IMN 20 (n=13)	IMN 40 (n=11)	Control (n=12)
Median Age (Years)	26 (19 - 36)	27 (17 - 34)	28 (16 - 38)
Median Gestation (Days)	289 (283 - 289)	289 (286 - 290)	289 (287 - 291)
Median Cervical Score 0 Min	4 (2 - 6)	4 (1 - 6)	4 (2 - 6)
Median Cervical score 360 Min	4 (2 - 8)	6 (3 - 9)	5 (2 - 9)
Number Requiring Prostaglandin	9 (69%)	7 (64%)	10 (84%)
Number Requiring Oxytocin	11 (85%)	11 (100%)	9 (75%)
Median Induction to Delivery Interval (Min)*	1628 (615 - 2142)	1760 (1062 - 2075)	1623 (1060 - 2394)
Median Length Stage I (Min)*	315 (30 - 865)	370 (115 - 550)	390 (250 - 895)
Median Length Stage II (Min)*	48 (5 - 172)	92 (32 - 173)	109 (4 – 139)
Median Length Stage III (Min)*	7 (2 - 19)	9 (2 - 25)	7 (3 - 16)

No statistically significant differences between the groups.

* Women delivered by LUSCS excluded.

Table I (continued) - Description of Patients / Maternal and Neonatal Outcomes:

	IMN 20 (n=13)	IMN 40 (n=11)	Control (n=12)
SVD	4 (31%)	5 (45%)	5 (42%)
Forceps / Ventouse	3 (23%)	4 (36%)	3 (25%)
LUSCS	6 (46%)	2 (18%)	4 (33%)
IM Opiate	11 (85%)	11 (100%)	6 (50%)
Epidural	10 (77%)	11 (100%)	11 (92%)
Median Estimated Blood Loss (ml)	400 (100 - 1000)	300 (100 - 2500)	300 (100 - 1250)
Median Umbilical cord pH	7.33 (7.03 - 7.39)	7.27 (7.19 - 7.36)	7.32 (7.16 - 7.37)
Median Apgar 1 Minute	9 (3 - 10)	9 (5 - 9)	9 (6 - 10)
Median Apgar 5 Minutes	9 (5 - 10)	9 (8 - 10)	10 (9 - 10)
SCBU Admissions	3 (23%)	2 (18%)	3 (25%)

No statistically significant differences between the groups.

Table II - Median (+ range) Umbilical Artery Resistance Index (RI) and Pulsatility Index (PI) at 0, 180 and 330 minutes following 20milligrams IMN, 40 milligrams IMN or no treatment (control):

	IMN 20 (n=13)	IMN 40 (n=11)	Control (n=12)
RI 0 min	0.58 (0.53 - 0.65)	0.55 (0.46 - 0.64)	0.55 (0.43 - 0.61)
RI 180 min	0.57 (0.48 - 0.65)	0.51 (0.45 - 0.57)	0.58 (0.5 - 0.62)
RI 330 min	0.53 (0.45 - 0.65)	0.54 (0.42 - 0.59)	0.56 (0.49 - 0.62)
PI 0 min	0.89 (0.75 - 1.1)	0.81 (0.64 - 0.99)	0.78 0.56 - 0.94)
PI 180 min	0.83 (0.66 - 1.07)	0.71 (0.61 - 0.84)	0.87 (0.68 - 0.98)
PI 330 min	0.76 (0.59 - 1.07)	0.79 (0.57 - 0.95)	0.85 (0.68 - 0.99)

No statistically significant differences between the groups.

Discussion:

NO donors are a class of drugs that act by liberating NO *in vivo*. They include isosorbide mononitrate (IMN) isosorbide dinitrate (IDN), sodium nitroprusside (SNP) and glyceryl trinitrate (GTN). The vasodilatory effects of NO donors are widely used in the treatment of ischaemic heart disease and cardiac failure. The adverse effects of these agents are mainly secondary to vascular smooth muscle relaxation and include hypotension, tachycardia and headache.

It has been suggested that the intra-vaginal administration of an NO donor might be considered as an alternative to agents that are currently used to induce cervical ripening during the induction of labour at term [3, 5, 6, 7, 8]. The relaxation effects of an NO donor in the myometrium and in the utero-placental circulation could provide advantages over other agents that are currently being used to induce cervical ripening. Conversely any hypotensive effects caused by an NO donor would be unwanted. Furthermore, profound maternal hypotension may compromise uterine blood flow and adversely affect fetal well-being.

When this study was designed there were no published studies regarding the use of intra-vaginal IMN administered to pregnant women at term to induce cervical ripening. In designing this study it was felt that systemic absorption would be minimal and that the maximal effects of the drug would be confined to the site of administration, i.e. the cervix. Our experience of using NO donors in pregnant women at term is limited, and it was therefore felt that a study of the potential adverse effects of such agents should be undertaken, prior to any larger study concerning the efficacy of IMN in inducing cervical ripening in pregnant women at term. As discussed the principal adverse effects of NO donors are related to changes in the vascular system. In theory NO donors such as IMN will cross the placenta and therefore the intra-vaginal administration of IMN could also have effects on the fetal haemodynamics.

In this study the administration of a single dose of 20 and 40 milligrams intra-vaginal IMN had a small, but statistically significant effect on maternal haemodynamics. Both 20 and 40 milligrams of IMN caused a rise in mean maternal heart rate and a fall in mean maternal diastolic blood pressure.

The intra-vaginal administration of 40 milligrams IMN was associated with a reduction in mean maternal systolic blood pressure and also produced a greater reduction in mean maternal diastolic blood pressure compared to the intra-vaginal administration of 20 milligrams IMN. There was a maximal increase in mean maternal pulse rate of 21 beats per minute and a maximal fall in mean maternal systolic and diastolic blood pressure of 15 mmHg and 16 mmHg respectively. During the study there were no episodes of symptomatic hypotension, nor did any woman require treatment because of these observed changes in blood pressure.

Although there are no published studies regarding the use of intra-vaginal IMN administered to pregnant women at term Thomson et al have examined mean maternal blood pressure following the intra-vaginal administration of 40 milligrams IMN to women in the first trimester undergoing surgical termination of pregnancy [6]. In contrast to the results observed in this study no significant effects were observed in women in the first trimester following the intra-vaginal administration of 40 milligrams IMN. The most likely reason for this discrepancy is that the frequent blood pressure measurements performed in the study reported here may reveal subtle fluctuations in mean maternal systolic and diastolic blood pressure that were not apparent when blood pressure was measured on only two occasions during the study of Thomson et al. Alternative explanations include differences in cervical composition between the first and third trimesters of pregnancy resulting in altered pharmacokinetics of vaginally administered IMN, and also the differences in the maternal circulation that exist between the first and third trimesters of pregnancy.

During pregnancy NO donors other than IMN have been administered for a number of indications via the intravenous, transdermal or sublingual route. The intravenous infusion of GTN to pregnant women in the third trimester of pregnancy with severe preeclampsia is associated with a small rise in mean maternal heart rate and a reduction in both mean maternal systolic and diastolic blood pressure [259]. The use of intravenous GTN in the treatment of intra-partum fetal distress caused by uterine hyperstimulation is associated with hypotension that requires treatment in 25% of cases [162].

Several authors have reported the use of transdermal GTN in the treatment of pre-term labour and in the prophylaxis of preeclampsia and have concluded that transdermal GTN does not appear to be

associated with maternal hypotension nor tachycardia [160, 161, 254, 260]. A rate of maternal tachycardia of only 2% is seen following the administration of 10 to 20 milligrams transdermal GTN to women in the treatment of pre-term labour [255].

Nevertheless there are studies that suggest that the administration of transdermal GTN is associated with maternal haemodynamic changes. Bisits et al reported that the administration of 10 milligrams GTN transdermally in the treatment of pre-term labour was associated with no changes in maternal heart rate, but was associated with a small but clinically insignificant fall in maternal systolic blood pressure [253]. The application of a 10 milligram GTN patch to women with pregnancies complicated by preeclampsia and intra-uterine growth restriction between 28 and 36 weeks gestation is not associated with any changes in mean maternal heart rate, but is associated with a reduction in mean maternal arterial pressure [261]. The fall in mean maternal arterial pressure caused by the administration of transdermal GTN is due to a reduction in systolic blood pressure [261].

The sublingual administration of 5 milligrams isosorbide dinitrate (IDN) in the first and second trimesters of pregnancy is associated with a small increase in mean maternal heart rate and a small reduction in mean maternal arterial pressure [262, 263]. Despite these changes in maternal haemodynamics following the transdermal and sublingual administration of an NO donor to pregnant women, treatment for hypotension is rarely required.

This study is the first to determine maternal pulse and blood pressure changes following the intra-vaginal administration of IMN to pregnant women at term. It suggests that the intra-vaginal administration of IMN in the late third trimester of pregnancy has no clinically significant adverse effects on maternal haemodyamics. The small effect of IMN in stimulating maternal pulse rate and depressing maternal blood pressure is not sufficient to preclude the consideration of intra-vaginal IMN as a cervical ripening agent for healthy pregnant women at term. The stimulatory effect of IMN on maternal heart rate may however prevent the use of IMN in women with some cardiac conditions such as dysrhythmias and hypertrophic obstructive cardiomyopathy, although these conditions are rarely seen in pregnant women at term.

Despite evidence of effects on maternal pulse and blood pressure there were no statistically significant changes in fetal heart rate following the intra-vaginal administration of both 20 and 40 milligrams IMN. During this study there were also no cardiotocograph abnormalities that suggested fetal compromise after the intra-vaginal administration of IMN. These findings are consistent with previous studies in which an NO donor has been administered to women in the third trimester of pregnancy [160, 161, 253, 254, 255]. The intra-venous administration of 0.25-10.0 micrograms/kilogram GTN and the transdermal administration of 10 milligrams GTN to pregnant women in the third trimester of pregnancy have no effect on fetal heart rate [253, 259, 261, 264].

Doppler ultrasound is used to assess blood flow of the uterine, placental and fetal circulation. The resistance and pulsatility indexes (RI and PI) provide a measure of resistance to flow. In this study the intra-vaginal administration of 20 and 40 milligrams of IMN had no effect on umbilical artery RI and PI at 180 and 360 minutes after treatment. This finding is again consistent with previous observations following the administration of an NO donor to women in the third trimester of pregnancy. The administration of transdermal GTN for the treatment of pre-term labour has no effect on umbilical artery Doppler indices [253]. The administration of transdermal GTN to women in the third trimester of pregnancies complicated by preeclampsia and intrauterine growth restriction had no effect on umbilical artery RI and PI [261].

There is however a significant fall in maternal uterine artery RI and PI following the administration of transdermal GTN to pregnant women between 28 and 36 weeks gestation with pregnancies complicated by preeclampsia [261]. The intravenous infusion of GTN reduces maternal uterine artery RI and PI in women in the first trimester of pregnancy undergoing termination of pregnancy, and also in women between 24 and 26 weeks gestation with reduced placental blood flow[258]. However in women between 24 and 26 weeks gestation the intravenous infusion of GTN produced no statistically significant changes in umbilical and middle cerebral artery RI and PI [258]. These observations suggest that the administration of NO donors to pregnant women in the third trimester of pregnancy is less likely to affect fetal haemodynamics.

In humans the sublingual administration of the NO donor IDN is associated with a reduction in umbilical artery RI and PI in women in the first and second trimesters of pregnancy [262, 263]. The intravenous infusion of GTN in the third trimester of pregnancies complicated by severe preeclampsia is also associated with a reduction in umbilical artery PI during the time of infusion [259]. The increase in umbilical artery flow that has been demonstrated in these studies might be attributable to a direct effect of NO donors that have crossed the placenta. Alternatively, it may be that these umbilical artery responses are merely reactions to the increase in uterine perfusion caused by changes in the maternal systemic circulation in response to the administration of an NO donor.

It may be NO donors have some transient effects on fetal haemodynamics that are short lasting and this may explain why no changes in umbilical artery RI and PI were demonstrated in the study presented in this thesis and other studies. The changes in umbilical artery Doppler indices that were seen following the sublingual administration of IDN to pregnant women in the first and second trimesters occurred within 20 minutes [262, 263]. In the study presented in this thesis the decision to record Doppler indices at 180 and 330 minutes following treatment administration was based on the availability of the ultrasound facilities within the unit. It may be that by limiting the recordings to these times transient changes in fetal haemodynamics were missed.

However Bisits et al have demonstrated that the transdermal administration of GTN to pregnant women in the third trimester of pregnancy has no effect on umbilical and fetal arterial Doppler parameters at 30 minutes following the administration of the NO donor [253]. The differences in the effects of NO donors on umbilical Doppler indices may be the result of pharmacokinetic differences between the different preparations of NO donor and the different routes of administration.

In this study the main symptom reported by women following the intra-vaginal administration of 20 and 40 milligrams of IMN was headache, with frequencies (69 and 91%) that were significantly greater than in the control group (8%). Headache is a recognised side effect of NO donors. Following the intra-vaginal administration of 40 milligrams IMN in the first trimester of pregnancy headache was reported in 23% of cases [6]. There are a number of possible explanations for the differences in the frequency of headache in this study and that of Thomson et al[6]. Again the differences may be

explained by altered pharmacokinetics secondary to changes in cervical composition and maternal cardiovascular changes during the third trimester of pregnancy. However in the study performed by Thomson et al [6], the symptom questionnaire was completed at 180 minutes and it may be that if given more time, more women may have reported the symptom of headache.

In studies where the transdermal GTN has been administered to pregnant women for the treatment of pre-term labour, headache is one of the most frequently reported symptoms. In these studies the reported rates of headache are 20%[161], 30% [255], 31% [253], 33% [160], 64% [254], 100% [261]. Headache has also been reported following the intravenous infusion of GTN with reported rates varying from 12.5 to 65%[162, 264].

The intra-vaginal administration of 40 milligrams IMN caused a slight increase in cervical score compared to those groups that received 20 milligrams IMN and no treatment. Furthermore fewer women that received 20 and 40 milligrams IMN required prostaglandin E_2 for further cervical ripening compared to the group that received no treatment. These findings while not statistically significant, would suggest that intra-vaginal IMN might have a role to play in the pharmacological induction of cervical ripening.

The intra-vaginal administration of 20 and 40 milligrams IMN had no statistically significant effects on the subsequent course of labour and delivery, including length of labour, analgesic use during labour, operative delivery rates, blood loss at the time of delivery and neonatal outcomes. More women required oxytocin in the groups that received 20 and 40 milligrams IMN, and in these women the induction of labour to delivery interval was longer than in the group that received no treatment. These findings are perhaps a reflection of the relaxing effects that NO donors have on the myometrium.

There has only been one other study published in which an intra-vaginal NO donor has been administered to pregnant women at term for the induction of cervical ripening [266]. In this study pregnant women at term were randomised to receive 2 intra-vaginal doses of 500 micrograms GTN at 6 hourly intervals, or 2 intra-vaginal doses of 3 milligrams prostaglandin E_2. Following the intra-

vaginal administration of GTN there were fewer episodes of uterine hyperstimulation and the median Bishop Score at 12 hours was greater compared to the group that had received intra-vaginal prostaglandin E_2. Consistent with the results presented in this thesis, oxytocin requirements were less in the group that had received intra-vaginal PGE_2, and these women also had significantly shorter labours compared to the group that had received intra-vaginal GTN. There were no differences in operative delivery rates between the group that had received intra-vaginal GTN and the group that had received intra-vaginal PGE_2.

Chanrachakul et al also made some assessment of maternal and fetal haemodynamics following the intra-vaginal administration of GTN [265]. Following the intra-vaginal administration of GTN there were no significant changes in maternal heart rate, maternal blood pressure and fetal heart rate [265]. However maternal haemodynamic parameters were only assessed at 4 hourly intervals and fetal heart rate was not recorded continuously unless an abnormality was detected.

Headache was seen in only 9% of cases following the intra-vaginal administration of GTN[265]. There are a number of reasons to explain the differences in the rates of headache following the intra-vaginal administration of GTN and the study presented in this thesis. The differences may be due to differences in pharmacokinetics between intra-vaginal GTN and intra-vaginal IMN. On the other hand following the intra-vaginal administration of GTN patients were only asked to report symptoms when they were present, and no objective symptom questionnaire was completed. Therefore the true incidence of headache following the intra-vaginal administration of GTN may in fact be higher than that reported by Chanrachakul et al [265].

The intra-vaginal administration of GTN is also effective at inducing cervical ripening in the first trimester of pregnancy [5]. However in the first trimester of pregnancy GTN is not as effective as IMN at lowering cervical resistance [5]. It therefore follows that if intra-vaginal GTN is effective at inducing cervical ripening in pregnant women at term, IMN is also likely to be effective. A randomised trial of intra-vaginal IMN versus both placebo and the current agent of choice, prostaglandin E_2, is required to assess the efficacy of IMN as a cervical ripening agent and its adverse effects. Subsequently the intra-vaginal administration of IMN to pregnant women at term could be compared with other agents

that are used to induce cervical ripening such as mechanical devices, misoprostol and mifepristone. A power calculation indicates that a study with over 100 women in each arm will be required to determine whether or not IMN is an effective cervical ripening agent in the third trimester of human pregnancy.

Another area of future research is to determine which NO donor is the most appropriate for the induction of cervical ripening in pregnant women at term. A recently published study has shown that intra-cervical administration of 5 and 10 milligrams of the NO donor SNP is effective at inducing cervical ripening in the first trimester prior to termination of pregnancy [266]. In the first trimester of pregnancy the intra-cervical administration of 5 and 10 milligrams of SNP is not associated with any adverse effect on maternal blood pressure or maternal headache [266]. The efficacy SNP in the induction of cervical ripening in pregnant women at term has yet to be determined. With its low incidence of headache, intra-cervical SNP may be a more acceptable agent than intra-vaginal IMN in the induction of cervical ripening in pregnant women at term. Therefore a randomised controlled trial of intra-vaginal IMN versus other NO donors that are used to induce cervical ripening, such as intra-vaginal GTN and intra-cervical/vaginal SNP, should be conducted in pregnant women at term to determine which NO donor is the most effective with the lowest incidence of adverse effects.

One of the difficulties encountered in the study presented in this thesis was in the recruitment of patients. More than a hundred women were approached during the study period. Most women declined to participate stating that they were unwilling to participate in a study that involved the administration of a drug that had never before been given to pregnant women at term. Patients were also concerned that the intra-vaginal administration of IMN may in fact prolong the induction process, because of the relaxing effects that IMN has on the uterus. Now that this study has been completed there is more information that can be given to those pregnant women at term who are considering participating in a study in which intra-vaginal IMN is being administered, thereby facilitating recruitment. Recruitment to any future study on the efficacy of IMN could also be improved by conducting a multi-centre study, allowing more patients the opportunity to participate.

IV. Conclusion:

During pregnancy there are profound changes in the composition of the cervix that facilitate the process of cervical ripening. The mechanisms that regulate cervical ripening are incompletely understood. Our understanding of the physiological process of cervical ripening, and hence parturition, has allowed us to develop agents for the pharmacological induction of cervical ripening. Mechanical devices, oestrogen, relaxin, progesterone antagonists and prostaglandins have been used to induce cervical ripening in pregnant women at term. In the United Kingdom the current agent of choice is prostaglandin E_2 gel. Prostaglandin E_2 gel is not without adverse effects, principally uterine hyperstimulation and subsequent fetal compromise. In view of these adverse effects the search for an alternative agent for the induction of cervical ripening in pregnant women at term continues.

The intra-vaginal administration of an NO donor such as IMN has been proposed as an alternative to prostaglandin E_2 gel for the induction of cervical ripening in pregnant women at term. The study that is presented in this thesis is the first to report the intra-vaginal administration IMN to pregnant women at term. NO donors could theoretically induce both maternal and fetal haemodynamic changes. It was therefore felt that it was important to address this issue before any study was undertaken examining the efficacy of IMN as a cervical ripening agent in pregnant women at term.

The intra-vaginal administration of 20 and 40 milligrams IMN during the induction of labour in the third trimester of pregnancy causes a small but statistically significant rise in maternal heart rate and a small reduction in maternal blood pressure. Furthermore the intra-vaginal administration of 20 and 40 milligrams IMN had no significant effect on fetal heart rate or on umbilical artery Doppler indices, suggesting that intra-vaginal IMN has no effects on fetal haemodynamics. There were no clinically significant differences in maternal and fetal haemodynamics following the intra-vaginal administration of 20 and 40 milligrams IMN. The only statistically significant haemodynamic difference that was demonstrated was a lower mean maternal diastolic blood pressure and a higher incidence of headache following the intra-vaginal administration of 40 milligrams IMN. The only significant symptomatic maternal adverse effect was headache. It is perhaps a cause for concern that this was seen in over 90% of patients following the intra-vaginal administration of 40 milligrams

IMN. Nevertheless, the study presented in this thesis suggests that a dose of 40 milligrams is more effective than a dose of 20 milligrams IMN without any significant increase in adverse maternal or fetal adverse effects, other than maternal headache. Future studies on the efficacy of IMN as a cervical ripening agent in pregnant women at term should therefore concentrate on using a dose of 40 milligrams.

The ideal agent for the pharmacological induction of cervical ripening should be easy to administer, cost effective, acceptable to both patient and clinician and produce adequate cervical change with minimal maternal and fetal adverse effects. The vaginal administration of 40 milligrams IMN appears to fit these criteria. Intra-vaginal IMN is easy to administer, inexpensive, and has no clinically significant effects on maternal and fetal haemodynamics. I believe that the work presented in this thesis lays the foundation for future studies examining the efficacy of 40 milligrams IMN as a cervical ripening agent during the induction of labour.

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FOR OFFICE USE ONLY PROJECT NUMBER: [ ]

PATIENT INFORMATION SHEET AND CONSENT FORM

Are nitric oxide donors effective in inducing cervical ripening during the induction of labour at term?

You have been admitted to hospital to have your labour induced (started off). This is normally done using a drug called prostaglandin. Prostaglandins ripen (start to open) the cervix (neck of the womb), and also stimulate the womb to contract. Although prostaglandins are effective in inducing labour, they are sometimes associated with side effects such as painful contractions before labour starts, or with an abnormal fetal heart rate pattern.

We are keen to investigate another drug called isosorbide mononitrate. This drug has been used for many years in people with heart disease. We have found that it also ripens the cervix. In early pregnancy, isosorbide mononitrate ripens the cervix with fewer side effects than prostaglandin. We wish to test whether this also happens at the end of pregnancy.

We should like to invite you to take part in a study comparing isosorbide mononitrate with no treatment to find out whether isosorbide mononitrate causes cervical ripening at the end of pregnancy. If you agree to take part in the study, you will receive either isosorbide mononitrate (given vaginally), or no treatment (internal examination only). Over the next six hours, your blood pressure will be measured and we will record the baby's heart rate. We will also scan the baby to see if there is any change in blood flow to the baby (in early pregnancy, drugs like isosorbide mononitrate improve the blood flow to the baby). At the end of six hours, your labour will be induced in the usual manner. We hope that by using isosorbide mononitrate the induction of labour will be easier.

You should be aware that the side effects which can be caused by isosorbide mononitrate are a headache and a drop in blood pressure for a short while.

Please note that if you agree to take part, this Research Project may be of little benefit to you but the results may help other patients in the future. If you do not want to take part in the Research Project, or if at any time you wish to stop taking part you may do so. The care which you are presently receiving will not be affected in any way. If you do agree to take part in the Research Project, your own general practitioner will be told and will be given details of information about any care which you are to receive.

CONSENT

I (name).....

of (address).....

.....

agree to take part in the Research Project described above.

Dr..... has explained to me what I have to do, how it might affect me and the purpose of the Research Project.

Signed.....Date.....

Witness.....Date.....

Symptom questionnaire

The purpose of this questionnaire is to find out if the tablets we are going to give you make you feel unwell. We will ask you to complete this questionnaire immediately before the tablets are given, and then six hours later. If there is anything you do not understand, please ask.

Please tick the correct answer:

At this moment in time, are you suffering from any of the following:

headache:	yes☐	no☐
drowsiness	yes☐	no☐
nausea (sickness)	yes☐	no☐
indigestion	yes☐	no☐
abdominal pain (pain in the upper tummy)	yes☐	no☐
pelvic pain (pain in the lower tummy)	yes☐	no☐
bloating	yes☐	no☐
loss of appetite	yes☐	no☐
diarrhoea	yes☐	no☐
constipation	yes☐	no☐
breast pain	yes☐	no☐
weakness	yes☐	no☐
faintness	yes☐	no☐
tiredness	yes☐	no☐
hot flushes	yes☐	no☐

In the last six hours, have you had any of the following:

vomiting	yes☐	no☐
pain passing urine	yes☐	no☐