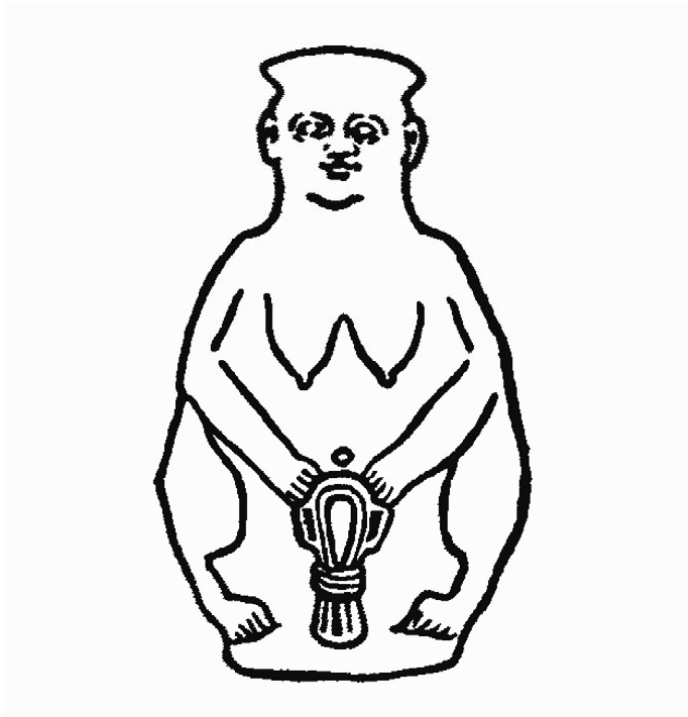


Misoprostol's preoperative cervical ripening effect

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Illustration page 1: Figure 1. Hieroglyphic form of the human uterus from the Third Egyptian Dynasty about 4500 BC. From Kranz and Philips (1962).

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“Felix qui potuit rerum cognoscere causas”

(Happy is the man who understands the causes of things)

- Vergilius, *Georgics* 29 BC, book 2, line 490

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What has surprised and touched me most during these seven years is the way Norwegian women so selflessly, readily and graciously agree to participate in scientific

trials. They do so without any remuneration, possibly with inconvenience to themselves and only because they want to contribute to 'the greater good'. The trials have only been possible because of such people; any potential benefit to medicine and society is thanks to them.

Lastly, but not least, none of this would have been possible without my mother. She ensured that my brothers and I had the option for an education and a future in Norway. Her unfailing unconditional support and encouragement have provided the foundation for my life.

Hammerfest, July 2009

Kevin Sunde Oppegaard

1. ABSTRACT

Background Misoprostol is used for cervical ripening before first trimester termination of pregnancy, as well as in non-pregnant women before intrauterine procedures. The optimal dose, dosage routes and outcomes were not well documented.

Aims The aims of this Ph.D. project were to study the effect of different doses and dosage routes of misoprostol on cervical ripening prior to first trimester termination of pregnancy, and on non-pregnant pre- and postmenopausal women prior to operative hysteroscopy.

Patients and methods Four prospective randomised trials designed and conducted in accordance with the recommendations in the CONSORT statement:

Trial I Five hundred and fifty-one women were randomised in a double-blind placebo-controlled trial either to 200 or 400 micrograms of oral misoprostol 10-16 hours before vacuum aspiration. The trial was conducted from January to June 2002.

Trial II Three hundred and thirty-eight women were randomised in a trial to 400 micrograms of oral misoprostol the evening before or 400 micrograms of self-administered vaginal misoprostol at home the same day as vacuum aspiration. The trial was conducted from April to August 2004.

Trial III Eighty-six women referred to day-care operative hysteroscopy were randomised to 1000 micrograms of misoprostol or placebo in one of two randomised double-blind and placebo-controlled sequential trials, based on their menopausal status. Two separate but identical parallel trials were conducted between August 2006 and April 2007, one for premenopausal women and one for postmenopausal women.

Trial IV Seventy-two postmenopausal women, not currently using hormone therapy, referred to day-care operative hysteroscopy, were randomised to 1000 micrograms of misoprostol or placebo in a double-blind placebo-controlled sequential trial. The women had administered a 25-microgram vaginal estradiol tablet daily for 14 days before the operation. In addition, they had self-administered either 1000 micrograms vaginal misoprostol or self-administered a vaginal placebo the evening before the operation. The trial was conducted from January 2008 to May 2009.

Results and conclusions

Trial I Two hundred micrograms of oral misoprostol compared with 400 micrograms oral misoprostol taken 10-16 hours before first trimester termination of pregnancy results in less preoperative vaginal bleeding and in a statistically, although not clinically, significant reduction in cervical dilatation. Neither dose was considered optimal for achieving cervical ripening. Our study supports smaller studies that suggested that oral misoprostol is inferior to vaginal misoprostol as regards efficacy, as well as causing a higher frequency of side effects.

Trial II Self-administered vaginal misoprostol prior to first trimester termination of pregnancy is more effective for cervical ripening compared with oral misoprostol, has a high acceptability rate and results in fewer side effects.

Trial III One thousand micrograms of self-administered vaginal misoprostol 12 hours before operative hysteroscopy is effective for cervical ripening in premenopausal but not postmenopausal women, compared with placebo.

Trial IV One thousand micrograms of self-administered vaginal misoprostol 12 hours prior to day-care hysteroscopy, after 14 days pre-treatment with vaginal estradiol, has a significant cervical ripening effect compared with placebo in postmenopausal women.

2. LIST OF PAPERS

This thesis is based on the following publications, referred to by their Roman numerals:

- I. Oppegaard KS, Abdelnoor M, Nesheim BI, Jerve F, Eskild A. The use of oral misoprostol for pre-abortion cervical priming: a randomised controlled trial of 400 mg versus 200 mg in first trimester pregnancies. *BJOG* 2004;111:154–159.
- II. Oppegaard KS, Qvigstad E, Nesheim BI. Oral versus self-administered vaginal misoprostol at home before surgical termination of pregnancy: a randomised controlled trial. *BJOG* 2006;113:58–64.

Correspondence: Nzewi C. Oral versus self-administered vaginal misoprostol at home before surgical termination of pregnancy by Oppegaard *et al.* *BJOG* 2006;113:979-980.

(*Trial protocol for III*) Oppegaard KS, Nesheim B-I, Istre O, Qvigstad E. Comparison of self-administered vaginal misoprostol versus placebo for cervical ripening prior to operative hysteroscopy using a sequential trial design (Trial protocol). *BJOG* 2007;114:769-e12.

- III. Oppegaard KS, Nesheim BI, Istre O, Qvigstad E. Comparison of self-administered vaginal misoprostol for cervical ripening prior to operative hysteroscopy using a sequential trial design. *BJOG* 2008;115:663, e1-9.

Correspondence: Lin F, Zheng F. Comparison of self-administered vaginal misoprostol for cervical ripening prior to operative hysteroscopy using a sequential trial design. *BJOG* 2008;115:1188.

Correspondence: Tsivos D, Lee CL. Comparison of self-administered vaginal

misoprostol for cervical ripening prior to operative hysteroscopy using a sequential trial design. *BJOG* 2008;115:1188-1189.

Correspondence: Burch D, Avasarala S. Comparison of self-administered vaginal misoprostol for cervical ripening prior to operative hysteroscopy using a sequential trial design. *BJOG* 2008;115:1720-1721.

(Trial protocol for IV) Oppegaard KS, Lieng M, Berg A, Istre O, Qvigstad E, Nesheim B-I. Does self-administered vaginal misoprostol result in cervical ripening in postmenopausal women after 14 days of pre-treatment with estradiol? Trial protocol for a randomized, placebo-controlled sequential trial. *BJOG* 2008;115:917-e10.

IV. Oppegaard KS, Lieng M, Berg A, Istre O, Qvigstad E, Nesheim B-I. A combination of misoprostol and estradiol for preoperative cervical ripening in postmenopausal women: a randomised controlled trial. Submitted to *BJOG* 2009.

The papers (I-III), manuscript (IV), protocols and correspondence listed above are reprinted in this thesis with kind permission from Wiley-Blackwell, Oxford, UK.

3. INTRODUCTION

3.1 THE UTERINE CERVIX

The cervix is the lower part of the uterus and is divided from the upper part, called the corpus, by a fibromuscular junction that includes a sphincter. The internal cervical ostium separates this fibrous cervix from the muscular corpus. The cervix projects through the anterior vaginal wall at the vaginal vault. The passage between the uterine cavity and the vagina is via the endocervical canal, which is fusiform in shape. The distance from the external to internal ostium is approximately three cm in an adult/fertile woman, flattened from front to back and measuring some seven mm at its widest point.¹

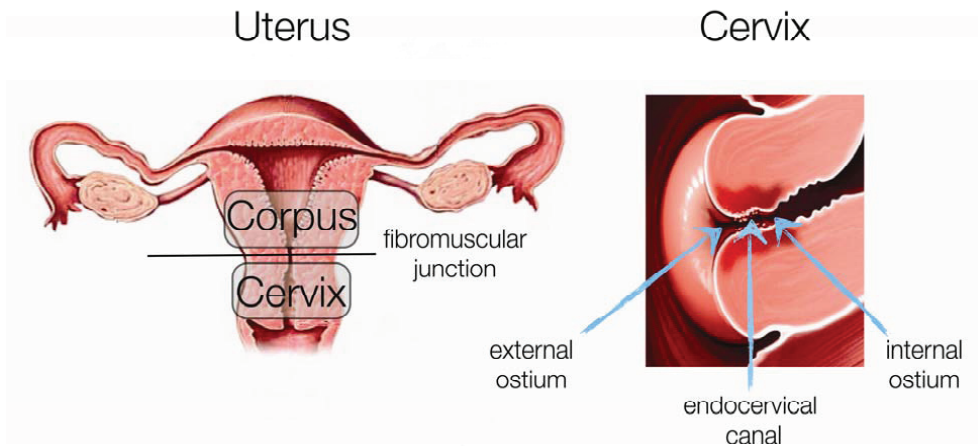
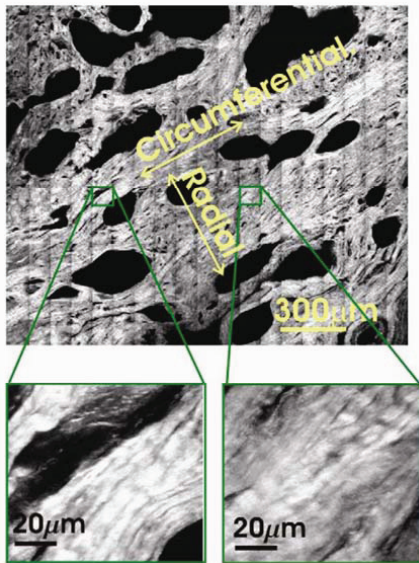


Figure 2: The human uterus and cervix

The basic structure of the human cervix is fibrous connective tissue, with collagen and its associated ground substance accounting for 80% of its dry weight. Light microscopic examinations and X-ray diffraction studies have shown three zones of collagen, estimated to represent between 64.3% and 72.4% of the fibrous tissue within the cervix, with elastin representing 1.4%. The highest ratio of elastin to collagen is at the



internal os. The zones of collagen blend smoothly into each other on passing radially outward from the canal. Adjacent to the canal and in the outermost zone the fibrils are oriented predominantly longitudinally, i.e. parallel to the canal. In the middle zone the fibrils have a preferred orientation in a circumferential direction.

Figure 3: The collagen network in cervical tissue in a non-pregnant woman. The plane of this image is perpendicular to the longitudinal direction of the endocervical canal. (From Myers K et al. Changes in the biochemical constituents and morphologic appearance of the human cervical stroma during pregnancy. *Eur J Obstet Gynecol* 2009;S82-S89).

About 80% of the cervical collagen is type I collagen and almost 20% type III. These proportions are similar to those found in other fibrous connective tissues. The muscle content is 29% in the upper third of the cervix, 18% in the middle third and 6% in the lower third, whereas it is 69% in the corpus.^{2,3} The tensile strength and firmness of the cervix derives from the collagen structure/component. Intercalculated among the collagen molecules are glycosaminoglycans, which are large, unbranched polysaccharide chains. There are a variety of glycosaminoglycans in the cervix, predominantly dermatan sulphate, hyaluronic acid and heparin sulphate.¹

3.2 CERVICAL RIPENING

The uterus had its own hieroglyph under the Third Dynasty in Egypt over 5500 years ago. The Egyptians understood the uterine cervix to be a strong structure with the capacity to resist the forces of pregnancy. The cervix must remain closed and firm

throughout pregnancy to ensure that the fetus is retained until it can survive outside the uterus. Some softening of the cervix (Goodell's sign) and especially of the isthmus (Hegar's sign) can be palpated about six weeks after the last menstrual period.² At term, however, the cervix changes from a firm, tough tissue to one that is soft and able to dilate to about 10 cm, to allow delivery of the baby. The transformation process – thinning, softening, relaxing and opening of the cervix - is termed 'cervical ripening' and has been observed for centuries.⁴ 'Priming' and 'maturation' of the cervix are also commonly used terms for this physiological change. An old concept was that cervical ripening was caused by uterine contractions but it is now known to be a series of biochemical processes.⁵ The complex biochemical processes and the mediators involved are still largely unknown.⁶⁻⁹ Research in this area is challenging, partly due to difficulties in obtaining cervical tissue prior to, during and after delivery in healthy fertile women without damaging the reproductive tract, or jeopardising the pregnancy. Results from animal studies do not reliably carry over to humans because of structural and hormonal differences between species. From the beginning of the 1980s, cervical tissue in the nonpregnant state began to be studied,¹⁰ but so far there have not yet been any histological studies of the ripened nonpregnant cervix.

Although the underlying mechanisms of cervical ripening are incompletely understood, there is increasing evidence to suggest that it is an inflammatory reaction.¹¹⁻¹⁴ There appears to be a cascade of synergistically interconnected biochemical events, that ultimately lead to the softening of the cervix in which collagen seems to be an important regulator. Studies have proposed that ripening of the uterine cervix relates to a reduction in collagen density together with a remodelling of the collagen fibre alignment, decreased collagen fibre strength, and diminished tensile strength of the extracellular matrix (although the collagen skeleton remains).¹⁵⁻²⁰ Cervical ripening is accompanied by the influx of neutrophils; the neutrophil is a ready source of collagenolytic enzymes, such as matrix metalloproteinases.¹¹ In addition, changes in the glycosaminoglycan composition (the ground substance between the collagen fibrils and other structural components of the cervix) and activation of inflammatory cytokines are associated with cervical ripening. The concentration of collagen decreases while the concentration of hyaluronic acid

increases during pregnancy.²¹ When compared with non-pregnant women, the concentration of collagen is 70% at ten weeks of gestation, and 30-50% at term. The collagenolytic activity increases with advancing gestational age. During labour, the enzyme collagenase breaks down collagen. Cervical biopsies from women after protracted labour show higher concentrations of collagen than in women with normal labour.²² It has been proposed that smooth muscle cells undergo programmed cell death (apoptosis) and that this also may play a role in the cervical ripening process.⁴

Connective tissue biopsy specimens from the lower part of the uterine cervix in pregnant women have been studied and show that prostaglandin application to cervical tissue in early pregnancy causes similar structural and physiological changes to those that naturally occur towards the end of pregnancy and during labour.^{10,23,24}

3.3 METHODS OF ACHIEVING CERVICAL RIPENING

Ripening or softening of the cervical tissue may be desirable earlier in pregnancy or in non-pregnant women if intrauterine surgery is indicated.^{25,25} The cervix may be ripened mechanically with osmotic dilators²⁷ or balloon catheters,²⁸ or biochemically with prostaglandins,²⁹ antiprogestins³⁰ or nitric oxide donors.³¹⁻³³ Osmotic dilators are impractical to use and are associated with a higher incidence of complications, compared with prostaglandins.^{34,35} Mechanical methods to ripen the cervix or to induce labour were first developed in the eighteenth century. Doctors used tents made from the stems of *Laminaria japonica* or *Laminaria digitata* (dried brown sea weed) that were inserted into the cervix and swelled because of tissue fluid. Balloon catheters, introduced into the cervical canal to permit the extra-amniotic instillation of estrogens and prostaglandins, were found to be effective without the pharmacological agent.³⁶

3.4 ROLE OF VARIOUS HORMONES IN CERVICAL RIPENING

A complex cascade occurs whereby various hormones stimulate the chemical reactions critical for cervical ripening. The key hormones associated with cervical ripening in pregnancy and parturition are prostaglandins and proinflammatory cytokines.^{8,37-39} Tissue levels of prostaglandin synthetases are increased during cervical ripening.¹³ Increased formation of prostaglandins in turn stimulates the collagenolytic

enzyme activity and induces alterations of the ground tissue glycosaminoglycans, contributing to an altered binding affinity to collagen, tissue hydration and cervical extensibility.^{2,40,41} Cervical fibroblasts induce glycosaminoglycan production, including hyaluronic acid.⁴²

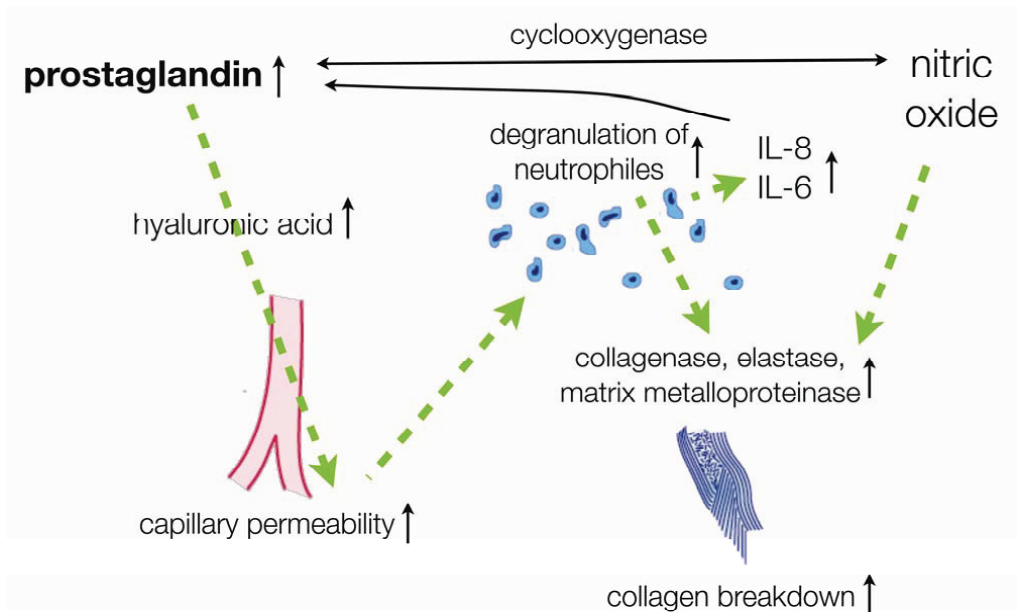


Figure 4: A simplified proposal of the inflammatory cascade in cervical tissue during the ripening process

A marked increase in the level of hyaluronic acid during the latent phase of labour promotes tissue hydration, release of collagenase, leukocyte migration and involvement of inflammatory mediators.⁴³⁻⁴⁵ Rising concentrations of hyaluronic acid in cervical tissue can be a potent inducer of cytokines by various leukocyte populations, such as interleukin (IL)-6⁴⁶ and (IL)-8.^{47,48} (IL)-8 has been identified as a key factor in the ripening process,⁴⁷ not only in late pregnancy⁴⁹ but also in the first trimester.²³ Interleukin (IL)-1 β has been shown to induce expression of the enzyme cyclooxygenase-2, which leads to a local increase of prostaglandin E2 (PGE2) in the cervix.⁵⁰ Nitric oxide is linked to almost all functions of male and female reproduction. It is thought that prostaglandins may also

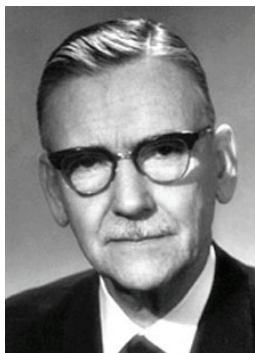
stimulate the production of nitric oxide, which may further activate the collagenolytic system.⁵¹

3.5 ESTROGEN

Estrogen appears to be essential for cervical ripening to take place. Women with placental sulphatase deficiency (resulting in relatively low circulating estrogens) do not undergo normal ripening of their cervix.^{5,52-54} The inflammatory cascade during the cervical ripening process involves leukocytes and the presence of estrogen receptors on cervical leukocytes,⁵⁵ and suggests that estrogen may directly regulate leukocyte functions in the cervix. Estrogen can be shown to trigger eosinophile invasion. The estrogen effect is blocked by progesterone and this inhibition is reversed by mifepristone.⁵⁶ It has been demonstrated that estrogen enhances cervical ripening⁹ and local application of estrogen for induction of labour has been tried.⁵⁷ Ripening does occur, but estrogens appear to be less effective than prostaglandins for inducing labour and delivery. However, there are insufficient data to draw any conclusions.⁵⁸ The effect of estrogen alone on cervical ripening on postmenopausal women has not been investigated. Although one study has investigated the ripening effect of misoprostol on the estrogen-pretreated cervix in postmenopausal women,⁵⁹ the authors concluded that misoprostol on its own was not effective for cervical ripening but that it was effective in 22 women after they had first used estriol vaginal cream for 14 days.

3.6 PROSTAGLANDINS

In 1930, the American gynaecologists Raphael Kurzrok and Charles Lieb first described the stimulatory effects of seminal fluid on human uterine muscle tissue at the Department of Obstetrics and Gynecology at Columbia University, while trying out artificial insemination in women. They found that in some cases there was a violent reaction of the uterus after instillation of semen and noticed that semen would also contract human uterine muscle *in vitro*.⁶⁰ These findings were independently corroborated two years later by British pharmacologist M.W. Goldblatt⁶¹ and Swedish physiologist and neurochemist Ulf Svante von Euler.



Ulf Svante von Euler

In 1934, von Euler, working at the Karolinska Institute in Stockholm, Sweden, found that a lipid extract from monkey, sheep and goat vesicular glands dramatically lowered blood pressure when injected into animals.⁶² Human seminal fluid also seemed to contain this unidentified substance. In 1935, von Euler dubbed this substance “prostaglandin”, in the mistaken belief that it originated from the prostate gland.⁶³ The field of prostaglandin research was then abandoned for over two decades.

In 1945, Sune Karl Bergström met von Euler at a meeting of the Physiological Society of the Karolinska Institute. Von Euler asked Bergström if he might be interested in studying some of his lipid extracts of sheep vesicular glands. Bergström began purifying the acid von Euler had extracted, in collaboration with Jan Sjövall. In 1957 they had isolated crystals of alprostadiol (prostaglandin E1) from sheep prostate glands, and by 1962, the structures of the “primary” prostaglandins had been determined by Bergström, Bengt Samuelsson, Sjövall and co-workers at the Karolinska Institute.⁶⁴



Sune Karl Bergström

Prostaglandins were then deduced to be products of polyunsaturated acid metabolism, particularly arachidonic acid (AA). They are very easily released from membrane phospholipids by the action of phospholipase A2 in response to a variety of physical, chemical, and neurohormonal factors. AA is rapidly metabolized to oxygenated products by two distinct enzymatic pathways: cyclooxygenase and lipoxygenase. The intermediate cyclooxygenase products are converted to primary prostaglandins, while the lipoxygenase products are converted to leukotrienes.⁶⁵



An unexpected finding was that prostaglandins could be made in all tissues (except red blood cells), not just male reproductive organs.⁶⁶ Prostaglandins of the E series and Prostaglandin I₂ are generated by the endothelium and the vessel wall to maintain the microcirculation and to counteract the vasoconstrictive and proaggregatory actions of thromboxane A₂ (TXA₂).

Bengt Samuelsson

Exogenous prostaglandins of the E and I series are potent vasodilators in various vascular beds, and result in decreased systemic blood pressure and reflex stimulation of heart rate. Prostaglandins of the E series and prostaglandin I₂ increase renal blood flow and provoke diuresis and natriuresis, partly by modulating the renin-angiotensin-aldosterone system. Prostaglandins of the F series contract the bronchial and gut muscle, while prostaglandins of the E series and prostaglandin I₂ have opposite effects. Prostaglandins of the E and F series, but not prostaglandin I₂, cause a strong contraction of the uterine muscle. Prostaglandins of E series relax bronchial muscle, whereas prostaglandins of the F series cause bronchoconstriction; their imbalance may contribute to the high bronchial tone in bronchial asthma. Prostaglandins of the E and I series and TXA₂ are generated by the gastrointestinal mucosa and released into the lumen upon neural or hormonal stimulation; it is likely that they participate in the maintenance of mucosal integrity and microcirculation. Exogenous prostaglandins of the E and I series inhibit gastric acid secretion and stimulate alkaline secretion while increasing mucosal blood flow.⁶⁵

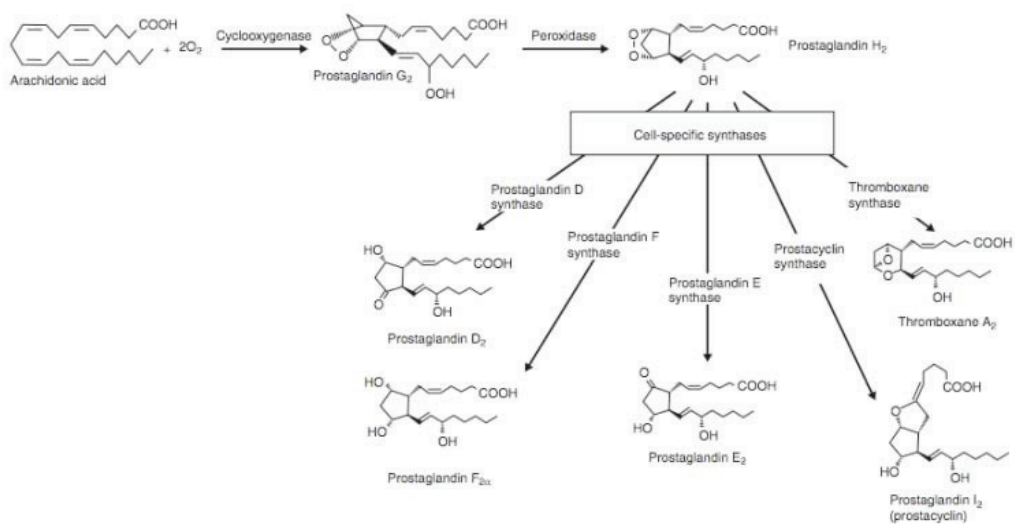


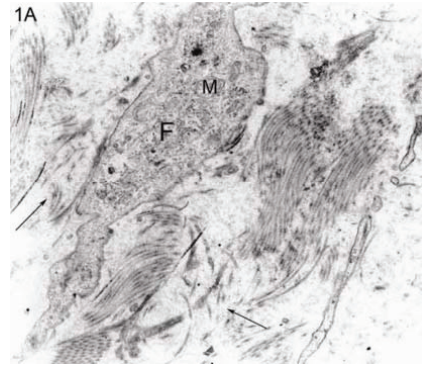
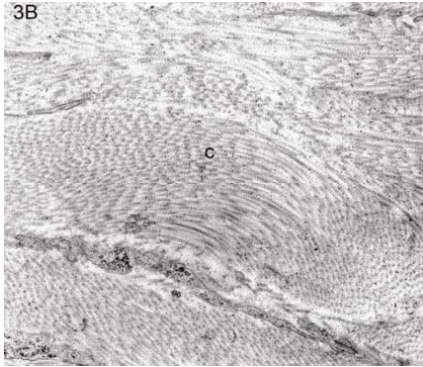
Figure 5: Synthesis of the five principal prostaglandins from arachidonic acid. (From: Chandrasekharan NV, Simmons DL. The cyclooxygenases. *Genome Biology* 2004 **5**:241).

The first total synthesis of a natural prostaglandin was achieved by Philip Beal and his colleagues at Upjohn laboratories in 1965.⁶⁷ W.P. Schneider at Upjohn reported synthesis of Prostaglandin E_2 and $\text{F}_2\alpha$ in 1968⁶⁸ and the remaining primary prostaglandins E_3 and $\text{F}_3\alpha$ were synthesised within the next few years. Bergström and Samuelsson, together with John Vane at the Burroughs Wellcome Research Laboratories in Beckenham, UK, were jointly awarded the Nobel Prize in Medicine in 1982 for their work leading to the elucidation of the chemical structures of prostaglandins and their biosynthetic pathways.⁶⁹ Nils Wiquist and Marc Bygdeman at the Karolinska Institute demonstrated the extremely high activity of intravenously injected prostaglandins on the human myometrium.⁷⁰ Findings regarding cardiovascular effects included the strong dilatation of the local blood vessels after intra-arterial injection and the prevention of closure of ductus arteriosus after birth in certain types of blue babies. In addition, Andre Robert and co-workers at Upjohn Company found the E-type prostaglandins inhibited gastric secretion in 1967.⁷¹

As prostaglandins seem to modulate almost every biological function in the body, they were initially considered to have a broad spectrum of therapeutic utility e.g. in labour induction, asthma, arthritis, peptic ulcer disease, hypertension, platelet dysfunction and periodontal disease. At one point, they were declared the “steroids” of the 1970s.⁷² In fact, their use has mainly been confined to the induction of labour and cervical ripening and in the synchronization of estrus in farm animals. The naturally occurring prostaglandins in the female reproductive tract are prostaglandin E₂ (PGE₂) and prostaglandin F₂α (PGF₂α), which act by binding and activating their respective receptors.^{73,74} They are both efficient in producing cervical ripening and labour. In 1968, intravenous infusion of PGF₂α was found to be an effective way of inducing labour.⁷⁵ A group at Upjohn found that PGF₂α interrupted pregnancy in rats. The first medical interruption of pregnancy with a prostaglandin (PGF₂α) was done in Stockholm in 1969 and clinical trials on the use of prostaglandins for induction of abortion were initially reported in January 1970.^{76,77} PGE₂ was licensed in 1972 for the induction of abortion after first trimester pregnancy.⁷⁸ Prostaglandins are now widely used in the field of reproductive health, with PGE₁ analogues replacing PGE₂ and PGF₂α as the prostaglandin of choice in many high-income countries.^{29,79}

3.7 PROSTAGLANDINS USED CLINICALLY FOR CERVICAL RIPENING

Although cervical change during pregnancy has been studied to determine the mechanism of cervical ripening, it has not yet been conclusively established how prostaglandins actually produce it. It has been demonstrated that local application of prostaglandin E₂ induces cervical ripening by increased remodelling of the cervical connective tissue.⁷ Application of PGE₂ splits and dissolves the collagen fibres.⁸⁰



Figures 6 and 7: Electron micrographs of cervical tissue showing (left) intact well organised collagen fibrils and (right) disorganised collagen framework indicating disintegration of the collagen fibrils following treatment with misoprostol. (From Radulovic et al. Cervical priming in the first trimester: morphological and biochemical effects of misoprostol and isosorbide mononitrate. *Acta Obstet Gynecol Scan* 2009;88:43-51).

Vaginal treatment with misoprostol has been shown to increase cervical concentration levels of the matrix metalloproteinases MMP-8 and MMP-9 in the first trimester, signifying induction of collagenolysis.^{14,24} It has been suggested that the inflammatory cascade,³⁸ leukocyte infiltration,⁸¹ and collagen remodelling⁸² observed in the cervical tissue may be due to the increase in local prostaglandins. Cervical prostaglandin receptors have been demonstrated,⁸³ and the cervix can produce a variety of prostaglandins⁸⁴ that may affect fibroblast activity through their production of glycoproteins.⁵ Prostaglandins are capable of inducing the production of hyaluronic acid by cervical fibroblasts, causing increased hydration and alteration of the composition of glycosaminoglycans/proteoglycans.⁸⁵ Prostaglandins may act as chemotactic agents, promoting the infiltration of leukocytes and macrophages into the cervical stroma. These inflammatory cells could be the source of the specific degrading enzymes causing the changes in the extracellular matrix that are associated with cervical ripening.⁵

There is evidence for the existence of a nitric oxide system within the human uterine cervix and a role for nitric oxide in the control of cervical function and relaxation of cervical smooth muscle.⁸⁶ Nitric oxide may regulate the activity of metalloproteinases

responsible for collagen degradation and induce prostaglandin production by stimulating cyclooxygenase activity.⁸⁷ Ripening due to nitric oxide may be mediated in part via increased prostaglandin F₂alpha (PGF_{2α}) synthesis,^{88,89} prostaglandin E₂ (PGE₂) and cyclic guanosine monophosphate.⁹⁰ Nitric oxide acts synergistically with prostaglandin, possibly inducing cervical ripening by causing changes in the extracellular matrix that are associated with ripening of the cervix.⁹¹ Sodium nitroprusside applied into the cervical canal induces a rapid and significant softening of the cervix and isosorbide mononitrate and glyceryl trinitrate can effect cervical ripening similar to the prostaglandin analogue gemeprost.³²

The potent uterotonic activity caused by prostaglandins is due to increased intracellular calcium and the activating of myosin light chain kinase (MLCK). Actin and myosin muscle fibres then undergo conformational changes that allow them to slide over each other causing shortening of the muscle cell and thereby uterine contraction.^{5,73}

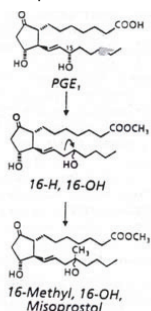
Clinically, the licensed PGE₂ preparations used for cervical ripening and labour induction are dinoprostone and sulprostone. These preparations have several disadvantages. Dinoprostone (Minprostin®, Cervidil®, Prostin E2®, and Propess®) is expensive, it is unstable at room temperature – making storage and transport difficult - and it is only available in a limited number of countries, due to political health issues. The PGE₂ derivative sulprostone has been associated with an augmented risk of myocardial infarction.⁹² The PGF_{2α} preparation Dinoprost® is only available as an injectable preparation and is associated with a high incidence of gastro-intestinal side effects.⁹³

The PGE₁ analogues used for cervical ripening and labour induction are gemeprost (Gemeprost®) and misoprostol (Cytotec®). Gemeprost has been widely used in early medical abortion. It is easily available and gynaecologists have a long experience with the drug as it is licensed for vaginal application in second trimester abortion. It has been superseded by misoprostol⁹⁴ as gemeprost has several disadvantages compared with misoprostol: it is only available as 1 mg pessary for vaginal use, the available pessary contains an unnecessarily high dose of prostaglandin for very early medical abortion (which can result in dose-related side effects), and it is expensive and unstable at room temperature.

3.8 MISOPROSTOL

Paul W. Collins discovered misoprostol (15-deoxy-16-hydroxy-16-methyl PGE₁) while working at the pharmaceutical company G.D. Searle (Skokie, Illinois, USA) in the

Figure 8: Discovery of misoprostol



70s.⁷² Misoprostol was developed as an anti-gastric ulcer drug since it had been shown in 1967 that naturally occurring prostaglandins of the E series inhibit gastric acid secretion.⁷¹ It was first approved in 1988 by the FDA for the prevention and treatment of gastric ulcers induced by NSAIDs (Nonsteroidal anti-inflammatory drugs) after studies demonstrated its efficacy^{95,96} and it is now approved in more than 80 countries, mostly under the brand name of Cytotec®. It is supplied in tablets containing 200 micrograms of misoprostol for oral use. A 100 microgram form is available in some countries. Before

Barry Marshall and Robin Warren showed a causal connection between the bacterium *helicobacter pylori*, gastritis, and peptic ulcer disease in 1984,^{97,98} the prevailing theory toward a cure for this disease was “no acid-no ulcer” and misoprostol was an immediate candidate for ulcer therapy. The uterotonic properties were noted as a worrisome side effect of natural prostaglandins and a cause for potential concern with synthetic analogues. Collins and his co-workers were unable to eliminate the stimulatory activity of misoprostol on uterine smooth muscle. Studies demonstrated that misoprostol could increase uterine contractile activity and endanger pregnancy. The uterine effects of misoprostol were not seen in animal studies, but two studies conducted using women in the first trimester of pregnancy, seeking legal abortion, showed that misoprostol was associated with uterine bleeding and partial or complete expulsion of the uterine contents.⁹⁹ Treatment and prevention of gastric ulcers are still the only licensed indications for misoprostol, with the exception of Gymiso® in France and a 25µg vaginal suppository for induction of labour available in Brazil and Egypt.¹⁰⁰ In the UK, a misoprostol tablet for labour induction (Isprelor™ - 25µg vaginal tablets) is currently undergoing phase III trials.

Compared with other prostaglandins – natural or synthesised – misoprostol is very stable and can be prepared from a solid dispersion on hydroxypropyl methylcellulose as

solid tablets with a shelf life of several years at room temperature. It has fewer side effects than PGE₂¹⁰² and is a safe and well-tolerated drug. It has shown neither estrogenic, progestogenic, nor androgenic agonism nor antagonism. Misoprostol is neither fetotoxic nor carcinogenic. No clinically significant adverse haematological, endocrine, biochemical, immunologic, respiratory, ophthalmic, platelet, mental or cardiovascular effects have been reported. It is very inexpensive, costing approximately 100 times less than other prostaglandins.¹⁰² The oral tablets are effective when administered orally, vaginally, sublingually, buccally or rectally.¹⁰³⁻¹⁰⁸

The manufacturer and holder of the patent for misoprostol, Searle (now incorporated into Pfizer), has never applied for licences for any gynaecological or obstetric indication, despite the abundant literature on its safe and effective use in these fields. The reason is probably to avoid potentially damaging publicity concerning the drug's properties as an abortion-inducing drug.¹⁰⁹ Misoprostol is contraindicated in pregnant women, or those wishing to become pregnant. The absence of registration for its obstetrical and gynaecological applications is an important problem. The pharmaceutical industry is normally responsible for informing physicians about drugs' indications, effectiveness, and correct dosages, route of administration, dosage interval, contraindications, precautions, side effects and management of complications. However, as misoprostol is generally not registered for reproductive health indications, the industry has neither provided this information for physicians nor packaged the drug in appropriate doses. The result is that this drug is used in many different ways according to informal local protocols.¹¹⁰

In January 2006, Pfizer stopped selling Cytotec® in Germany without prior notice. The official reason given by the company was that misoprostol was an outdated treatment regimen and no longer "state of the art". The company also stated that this decision was limited to Germany. However it is known that Pfizer (and formerly Pharmacia and Searle) were unhappy with the use of Cytotec® in OB/Gyn. It is open to speculation how much this withdrawal is a way to get rid of the product and whether or not other countries will follow.¹¹¹

3.9 PHARMOKOKINETICS OF MISOPROSTOL

Misoprostol is licensed for the oral treatment of peptic ulcers, the tablet having been designed for oral absorption. After oral administration, it is quickly absorbed and undergoes rapid de-esterification to misoprostol acid, its biologically active metabolite. Plasma concentrations of misoprostol acid peak approximately 12 minutes after oral ingestion and decline rapidly thereafter with a terminal half-life of 20-40 minutes. Elimination $t_{1/2}$ values are not significantly different in elderly (≥ 65 years) and young (18-40 years) subjects. The bioavailability of misoprostol is delayed after ingestion of antacids or food, delaying the plasma peak to 20 and 64 minutes respectively. Misoprostol is primarily metabolised in the liver and less than one percent of its active metabolite is excreted in urine. Misoprostol has no known drug interactions and does not induce the hepatic cytochrome P-450 enzyme system.⁷²

Gastrointestinal side effects are common following oral administration of misoprostol.¹¹² Furthermore, the oral route's limited pharmacokinetic profile (and short clinical efficacy) has stimulated research into alternative delivery routes in order to prolong its activity and minimize side effects. As previously mentioned, misoprostol can be administered via oral, vaginal, sublingual, buccal, or rectal routes.¹⁰³⁻¹⁰⁸ The vagina is considered an ideal site for drug administration.¹¹³ Vaginal administration of misoprostol has the advantage of a prolonged interval to plasma peak concentration and a slower half-life compared with oral administration.¹⁰⁴

The individual vaginal absorption of misoprostol varies greatly. In addition, it has been suggested that the vaginal route may not be acceptable to women.¹¹⁴ It has also been suggested that nursing staff responsible for vaginal application of misoprostol prefer the oral, buccal or sublingual routes.^{108,115,116} Misoprostol acid levels peak approximately one hour after vaginal application. After one dose of vaginal misoprostol, the plasma concentration gradually decreases and persists in levels significantly higher than oral or sublingual routes for at least six hours. The area under the misoprostol acid concentration versus time curve indicates that the systemic bioavailability of sublingual misoprostol is significantly greater than oral and vaginal misoprostol. There is no significant difference between sublingual and vaginal administration.¹⁰³ Rectal misoprostol is associated with a

qualitatively similar absorption curve to that of the vaginal route, but with a lower bioavailability.¹⁰⁷

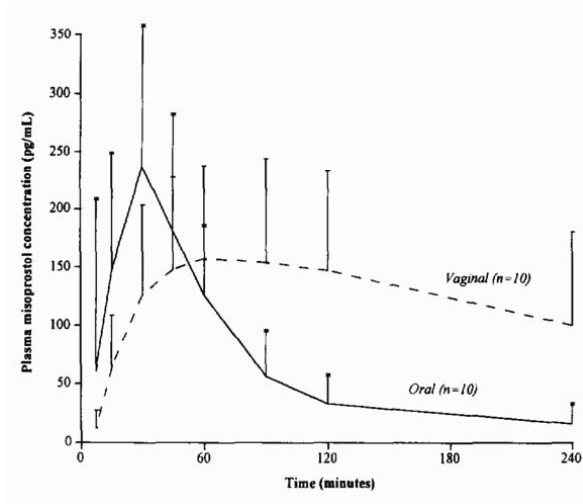


Figure 9: Mean plasma concentrations of misoprostol acid over time with oral and vaginal administration. (From: Ziemann et al. Absorption kinetics of misoprostol with oral or vaginal administration. *Obstet Gynecol* 1997;90:88-92).

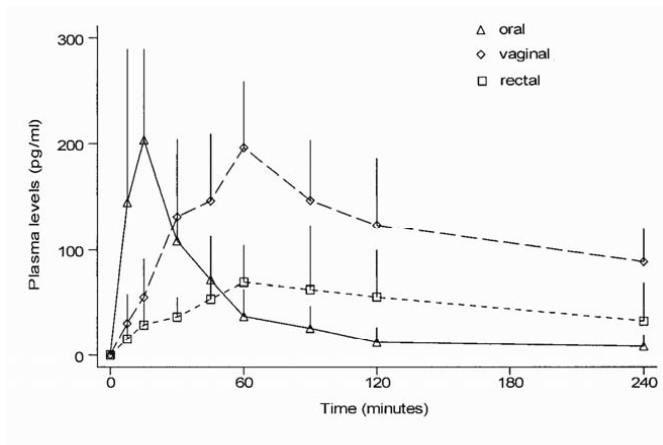


Figure 10: Mean plasma concentrations of misoprostol acid over time with oral, rectal and vaginal administration. (From: Khan et al. Oral, rectal, and vaginal pharmacokinetics of misoprostol. *Obstet Gynecol* 2004;103:866-870).

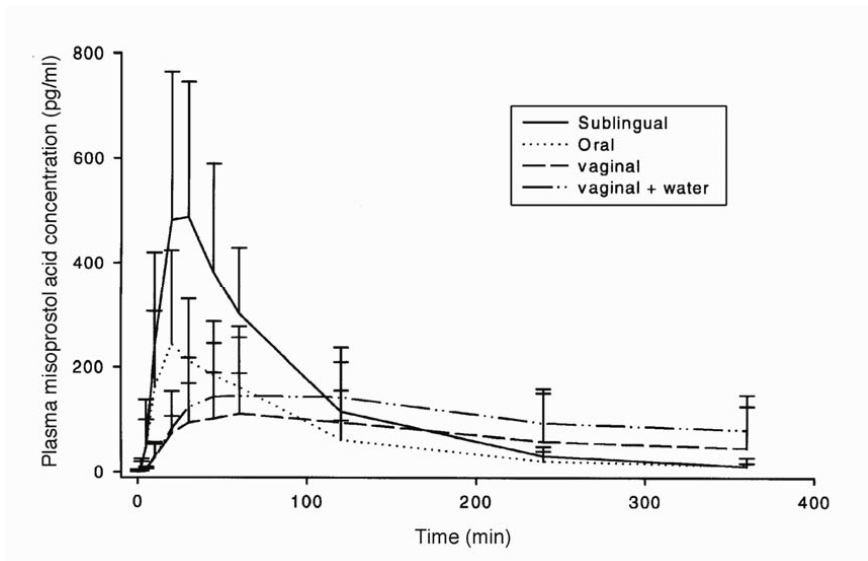


Figure 11: Mean plasma concentrations of misoprostol acid over time with sublingual, oral, vaginal and vaginal + water administration. (From Tang et al. Pharmacokinetics of different routes of administration of misoprostol. *Hum Reprod* 2002;17:332-336).

Remnants of the tablets may be found many hours after vaginal administration.^{104,117} This is due to incomplete dissolution of the misoprostol tablets. No clinical benefits have resulted from trying to enhance tablet dissolution by adding acetic acid (with a pH of 2) to the 200 microgram tablets of vaginal misoprostol given 3-4 hours before vacuum aspiration for termination of first trimester pregnancies.¹¹⁸

3.10 CERVICAL RIPENING BEFORE SURGICAL ABORTION

Cervical ripening is recommended in pregnancy before surgical abortion to facilitate cervical dilatation.¹¹⁹ Reduction in the incidence of cervical injury and uterine perforation during termination of pregnancy has been demonstrated by preoperative cervical ripening.^{25,26} This is particularly important for women with risk factors for complications during cervical dilatation; women with cervical anomalies/previous surgery, young women, nulliparous women before nine weeks and parous women after twelve weeks have been identified as being at high risk.¹²⁰

Many different methods for cervical ripening have been studied, to try and find a method that is practical, safe and with a low frequency of side effects. The cost/benefit factor is also important. In 1973, the first article describing the effect of a prostaglandin on preoperative cervical dilatation was published.¹²¹ The first trial using misoprostol for preoperative cervical ripening was reported in 1994.¹²² Misoprostol is a cheap and effective prostaglandin E1-analogue that is used worldwide for cervical ripening without sanction from the patent holders. This is why the usual phase II and III trials conducted for establishing the optimal dosage, dosage route and timing interval were not conducted for indications in obstetrics and gynaecology.

Two studies have compared laminaria with the PGE1 analogues gemeprost and misoprostol for cervical ripening before curettage and concluded that laminaria was inferior for cervical ripening, compared with the prostaglandins.^{35,123}

Oral misoprostol has been compared with the licensed prostaglandin gemeprost for cervical ripening and found to be superior in clinical studies, although the dosage of oral misoprostol used in these regimens is associated with a high frequency of side effects.^{124,125}

The antiprogesterin Mifepristone® has been compared with misoprostol for cervical ripening prior to surgical abortion and found to be superior. However, Mifepristone® is much more expensive than misoprostol and not as easy to acquire. The advantages of using mifepristone instead of misoprostol as a ripening agent are therefore doubtful.¹²⁶

Misoprostol has been used in combination with isosorbide mononitrate, but no advantage has been demonstrated compared with misoprostol alone for preoperative cervical ripening in the first trimester.¹²⁷ It has been shown to induce a more pronounced cervical softening than isosorbide mononitrate.¹²⁸

After our first study was completed in 2002, two review articles were published.^{29,79} A clinical consensus has emerged that preoperative treatment with misoprostol prior to vacuum aspiration facilitates the procedure and results in fewer complications. The optimal treatment regimen to use is 400-micrograms of misoprostol 3–4 hours preoperatively, with vaginal administration being superior to the oral administration.^{79,129-131}

3.11 CERVICAL RIPENING IN NON-PREGNANT WOMEN



10 mm hysteroscope

Many patients require cervical dilatation prior to operative hysteroscopy, depending on the size of the instrument used for the operation. An operative hysteroscope/resectoscope used to treat endometrial pathology typically has a diameter of 10 mm.

In women with a firmly closed and rigid cervix, dilatation can lead to considerable traumatization of the tissue. Furthermore, complicated cervical dilatation is attended by the risk of lacerations caused by the tenaculum, creation of false passages and an increased risk of uterine perforation. Maximum forces of more than 25 Newton have been measured electronically for dilatation up to Hegar 8.¹³² Nulliparous and postmenopausal women are particularly at risk of complications.¹³³⁻¹³⁷

Prostaglandins have been tried out for cervical ripening prior to hysteroscopy since 1985.¹³² The analogues licensed for use in pregnancy are both expensive and impractical to use for this purpose (e.g. intracervical application of sulprostone gel). Moreover, the majority of the studies carried out have been too small to evaluate primary outcome measures and there was initially no clear consensus whether prostaglandins actually have any cervical ripening effect in non-pregnant women. There has therefore been an interest in trying out misoprostol as a cervical ripening agent prior to cervical dilatation in non-pregnant women prior to hysteroscopy.^{35,59,117,119,138-153} Misoprostol appears to be effective for cervical ripening in premenopausal, but not postmenopausal women.^{152,154} It has also been tried prior to endometrial biopsy sampling¹⁵⁵ and intrauterine inseminations.¹⁵⁶ The conclusions from these trials are that there does not seem to be any clinical advantage in using misoprostol for these indications. It has also

been tried out prior to IUD (intra uterine device) insertion, with the conclusion that it facilitated the procedure and reduced the number of difficult and failed attempts at insertion.¹⁵⁷

4. AIMS OF THE STUDY

Women in Norway who want to terminate their pregnancy in the first trimester have the option of either the medical or the surgical method.¹⁵⁸ All pregnant women are offered free health care, including termination of pregnancy. Until 2006, surgical methods were used exclusively for termination between nine and twelve weeks of pregnancy. In 2004, 96% of all terminations were performed within the first trimester, and 61% of first trimester terminations were done surgically.¹⁵⁹ This trend is now changing, with medical termination of pregnancy increasingly being recommended as the optimal treatment, regardless of gestation length.¹⁶⁰

The Women's Clinic at Haukeland University Hospital in Bergen has been a national pioneer in developing and improving regimes for termination of pregnancy in Norway. Although misoprostol for cervical ripening and medical abortion was introduced there in 1998,¹⁶¹ there have been no published prospective studies to determine the optimal dose, route and interval of misoprostol for cervical ripening conducted in Norway.

The Department of Obstetrics and Gynaecology at Oslo University Hospital Ullevål is the largest in the country and cares for more than 3000 women seeking termination of pregnancy every year. In August 2000, the department started offering women 400 micrograms of oral misoprostol for cervical ripening prior to surgical termination of pregnancy. The women were offered misoprostol in the form of oral intake of two tablets, each containing 200 micrograms of misoprostol, the evening before they were due to attend vacuum aspiration as day care patients. Six months later, an evaluation of this regime by the nursing staff concluded that there was an unacceptably high rate of side effects, particularly vaginal bleeding. There were also reports of women aborting at home, or being admitted during the night with cramping and bleeding. In addition, no studies had evaluated whether or not this new treatment was actually effective for cervical ripening.

Operative hysteroscopy is a routinely performed day care surgery at the Department of Obstetrics and Gynaecology at Oslo University Hospital Ullevål, with more than 400 hysteroscopies carried out there each year. After the benefits of using

misoprostol before termination of pregnancy became obvious, there was speculation as to whether the same benefit could be gained from offering misoprostol before hysteroscopy. Misoprostol was therefore sporadically used for cervical ripening before hysteroscopy, particularly if the woman had previously experienced a failed hysteroscopy due to cervical stenosis or scarring of the uterus. There had been no formal audit or evaluation of this treatment.

The aims of this Ph.D. project were therefore to evaluate and explore the cervical ripening effects of various doses and dosage routes in the clinical settings of first trimester termination of pregnancy, as well as before hysteroscopy in non-pregnant women.

Specifically, we wanted to investigate whether:

1. Four hundred micrograms of oral misoprostol taken the evening before surgical termination of pregnancy results in satisfactory preoperative cervical ripening compared with placebo.
2. Reducing the dose to 200 micrograms results in a comparable cervical dilatation and if this dose is associated with fewer side effects.
3. Four hundred micrograms of self-administered vaginal misoprostol results in a more effective preoperative cervical ripening with fewer side effects, compared with 400 micrograms oral misoprostol.
4. Self-administered vaginal misoprostol is an acceptable dosage route for women measured with a four-point Likert scale (1= completely acceptable; 2 fairly acceptable; 3 = fairly unacceptable; 4 = completely unacceptable), and if acceptability depended on whether the women are from the Nordic countries.
5. One thousand micrograms of self-administered vaginal misoprostol results in satisfactory cervical ripening in non-pregnant premenopausal and postmenopausal women, compared with placebo.

6. A high dosage of self-administered vaginal misoprostol is safe, and whether women consider the way in which the dosage administered is acceptable.
7. One thousand micrograms of self-administered vaginal misoprostol results in satisfactory cervical ripening in postmenopausal women, compared with a placebo, after 14 days pre-treatment with vaginal estradiol.



Misoprostol (Cytotec®) and estradiol (Vagifem®)

5. MATERIALS AND METHODS

This Ph.D. thesis consists of four randomised trials:

I. A prospective randomised placebo-controlled trial, designed to determine whether 400 micrograms of oral misoprostol taken the evening before surgical termination of pregnancy results in satisfactory preoperative cervical ripening, compared with placebo. We have investigated whether reducing the dose to 200 micrograms results in a comparable cervical dilatation and if this dose is associated with fewer side effects.¹⁶²

II. A prospective randomised controlled trial, designed to determine whether 400 micrograms of self-administered vaginal misoprostol results in a more effective preoperative cervical ripening with fewer side effects, compared with 400 micrograms oral misoprostol. We also investigated whether self-administered vaginal misoprostol is found to be an acceptable dosage route by women, and if acceptability depended on whether the women were from the Nordic countries.¹⁶³ (Note; for details about the original trial design and the changes made, please see Chapter 12. Appendix)

III. A prospective randomised placebo-controlled sequential trial, designed to determine whether 1000 micrograms of self-administered vaginal misoprostol results in satisfactory cervical ripening in non-pregnant premenopausal and postmenopausal women, compared with placebo. We also investigated whether a high dose of self-administered vaginal misoprostol is safe, and whether women consider the way in which the dosage is administered acceptable.¹⁵²

IV. A prospective randomised placebo-controlled sequential trial, designed to determine whether 1000 micrograms of self-administered vaginal misoprostol results in satisfactory cervical ripening in postmenopausal women, compared with a placebo, after 14 days pre-treatment with vaginal estradiol.

Clinical hospital setting:

The four separate prospective randomised one-centre studies were conducted at the Department of Department of Obstetrics and Gynaecology at Oslo University Hospital Ullevål, Norway.

Authorisations and trial registration:

Each trial was conducted in accordance with the Declaration of Helsinki and national as well as local regulations. The Regional Committee for Medical Research Ethics in Eastern and Northern Norway (Regional komité for medisinsk forskningsetikk, Øst-Norge, REK ØST, Nord-Norge, REK Nord) approved trials I and II, while The Regional Committee for Medical Research Ethics in Northern Norway (Regional komité for medisinsk forskningsetikk, Nord-Norge, REK Nord) approved trials III and IV. The EU Clinical Trials Directive 2001/20/EU,¹⁶⁴ which relates to the implementation of Good Clinical Practice in the conduct of investigational medical products, came into force on 1 May 2004. Trials III and IV were subsequently conducted in accordance with these regulations. Authorisation from the Norwegian Medicines Agency (Statens Legemiddelverk, SLV) and Oslo University Hospital, Ullevål's Personal Data Officer (Personvernombud) was granted for studies III and IV. Trials III and IV are registered with ClinicalTrials.gov, the European Clinical Trials Database, and the study protocols are published in BJOG. Written, informed consent was obtained from all patients before study inclusion and randomisation.

CONSORT guidelines:

All trials were designed and conducted in accordance with the recommendations in the CONSORT statement.¹⁶⁵

Primary study end points:

The primary study end point in all trials was the degree of cervical dilatation, assessed by passing Hegar dilators through the cervix in ascending order. The size of the largest dilator passed into the inner cervical ostium, without subjective resistance felt by the operator, was recorded as the preoperative degree of dilatation.



Hegar dilators

5.1 TRIAL I

Trial I (Paper 1): A comparison of two doses of oral misoprostol for cervical ripening before first trimester termination of pregnancy

This trial was a randomised double-blind placebo-controlled trial that included five hundred and fifty-one women undergoing surgical termination of pregnancy between 7 and 12 weeks of gestation between January and June 2002. The sample size was calculated using the preoperative variability (SD) from Ngai et al.¹⁶⁶ on the primary outcomes of cervical dilatation and bleeding, with the assumption of a type 1 error of 0.05 and a power of 0.90. We selected the study from Ngai as it was a large recent study with five arms, comprehensively investigating oral and vaginal doses of misoprostol in the same two doses we wanted to study, and also because it was one of the few studies which had measured blood loss. The time interval from misoprostol administration to operation in Ngai's study was only three hours, but we felt that the measurements presented were sufficient to calculate an estimate of the preoperative variability. The women were randomised by third party concealed randomisation to either 200 or 400 micrograms of

oral misoprostol 10–16 hours before vacuum aspiration. The hospital pharmacist prepared ground misoprostol and an inactive ingredient as a placebo into identical capsules according to the randomisation list, so that neither the personnel involved in administering the intervention and assessing the outcomes, nor the women receiving the treatment, were aware of the dosage they were receiving. The primary study end points were the preoperative degree of cervical dilatation prior to termination of pregnancy by vacuum aspiration, measured with Hegar dilators, and preoperative bleeding assessed by weighing sanitary towels. Preoperative pain recorded on a visual analogue scale and vaginal bleeding were measured as secondary outcome measures.

5.2 TRIAL II

Trial II (Paper 2): Oral misoprostol versus vaginal misoprostol, self-administered at home, for cervical ripening before first trimester termination of pregnancy

This trial was a randomised controlled trial that included three hundred and thirty-eight women undergoing surgical termination of pregnancy between 7 and 12 weeks of gestation between April and August 2004. The sample size was calculated using the same method as in the previous trial on the primary study end point of cervical dilatation with the assumption of a type 1 error of 0.05 and a power of 0.90. The women were randomised to either 400 micrograms of oral misoprostol the evening before or 400 micrograms of self-administered vaginal misoprostol at home the same day as vacuum aspiration. This trial did not use a placebo, so the women participating in the trial and the doctors assessing the outcome were aware of the treatment given after randomisation. The primary study end point was preoperative cervical dilatation measured with Hegar dilators. Acceptability of self-administered vaginal misoprostol at home and peroperative complications were measured as secondary outcome measures.

5.3 TRIAL III

Trial III (Paper 3): Vaginal misoprostol, self-administered at home, for cervical ripening before day-care operative hysteroscopy

The trial protocol was submitted to BJOG for peer review prior to recruiting trial participants.

This trial consisted of two separate but identical parallel randomised double-blind, placebo-controlled sequential trials, one in premenopausal women and one in postmenopausal women. The boundaries for the sequential trials were calculated on the primary outcomes of a difference of cervical dilatation ≥ 1 mm, with the assumption of a type 1 error of 0.05 and a power of 0.95, using the preoperative variability (SD) in cervical dilatation from a small pilot study performed in the same department in January 2006. Eighty-six women (21 postmenopausal and 65 premenopausal women) referred to day-care operative hysteroscopy between August 2006 and April 2007 were included in the trial. The women were randomised by third party concealed randomisation to either 1000 micrograms of self-administered vaginal misoprostol or placebo 12 hours before day-care operative hysteroscopy. The hospital pharmacist prepared ground misoprostol and an inactive ingredient as a placebo into identical capsules according to the randomisation list, so that neither the personnel involved in administering the intervention and assessing the outcomes, nor the women receiving the treatment, were aware of the dosage they were receiving. The primary study end point was preoperative cervical dilatation measured with Hegar dilators. Acceptability, complications and adverse effects, as well as the number of women who achieved a preoperative cervical dilatation ≥ 5 mm, were recorded as secondary outcomes.

5.4 TRIAL IV

Trial IV (Paper 4): A combination of estradiol and vaginal misoprostol, self-administered at home, for cervical ripening in postmenopausal women before day-care operative hysteroscopy

The trial protocol was submitted to BJOG for peer review prior to recruiting trial participants.

This study was a randomised double-blind placebo-controlled sequential trial. The boundaries needed for the statistical model were calculated on the preoperative variability (SD) in cervical dilatation in postmenopausal women from trial III. Sixty-seven postmenopausal women referred for day-care operative hysteroscopy between January 2008 and April 2009 were included. They were randomised to either 1000 micrograms of self-administered vaginal misoprostol or self-administered vaginal placebo the evening before day-care operative hysteroscopy. All women had administered a 25-microgram vaginal estradiol tablet daily for 14 days prior to the operation. The primary study end point was preoperative cervical dilatation measured with Hegar dilators. Acceptability, complications and adverse effects, as well as the number of women who achieved a preoperative cervical dilatation ≥ 5 mm, were recorded as secondary outcomes.

6. RESULTS

6.1 TRIAL I (Paper 1): A comparison of two doses of oral misoprostol for cervical ripening before first trimester termination of pregnancy

Two hundred micrograms as compared with 400 micrograms oral misoprostol, given 10–16 hours before first-trimester abortions, resulted in a reduction in the occurrence and degree of preoperative bleeding. The lower dosage also resulted in a reduction in the degree of preoperative cervical dilatation. The mean preoperative dilatation in the 200-microgram group was 5.4 mm (SD 1.5) compared with 5.7 mm (SD 1.7) in the 400-microgram group; this difference is, in our opinion, not clinically significant. We noticed that the degree of preoperative dilatation obtained in our study was lower than in all other studies where 400 micrograms of oral misoprostol was given. The degree of dilatation in both dosage groups was higher in multigravidae than in primigravidae. Thirty percent of the women in the 400-microgram group achieved a preoperative cervical dilatation 7 mm compared with 20% in the 200-microgram group.

6.2 TRIAL II (Paper 2): Oral misoprostol versus vaginal misoprostol, self-administered at home, for cervical ripening before first trimester termination of pregnancy

This study shows the efficacy of self-administered vaginal misoprostol at home for cervical ripening in surgical termination of pregnancy. Our trial shows that 400 micrograms misoprostol administered via the vaginal route will result in a cervical dilatation ≥ 7 mm in about half of multigravidae but is much less effective in primigravidae. The oral route does not lead to satisfactory dilatation in either group. Occurrence of bleeding was much more common in women taking oral misoprostol, compared with women using misoprostol vaginally.

Women of Nordic origin recorded significantly lower preoperative pain scores on a visual analogue scale score, compared with immigrant women. There were no differences

between non-immigrant and immigrant women in preoperative cervical dilatation, acceptability or occurrence of bleeding. Self-administered vaginal misoprostol at home was considered highly acceptable, regardless of ethnic group.

Self-administered vaginal misoprostol was shown in this trial to be a beneficial treatment option, providing cervical ripening in a shorter time interval and with fewer side effects than oral misoprostol. We noticed that the effect of misoprostol was more pronounced if a woman had previously been pregnant, than if she was pregnant for the first time.

6.3 TRIAL III (Paper 3): Vaginal misoprostol, self-administered at home, for cervical ripening before day-care operative hysteroscopy

One thousand micrograms of self-administered vaginal misoprostol taken 12 hours before day-care operative hysteroscopy results in a significant cervical ripening effect, compared with placebo. However, this effect is limited to premenopausal women; in postmenopausal women, there was no difference in cervical dilatation between the placebo and misoprostol groups. This trial was the first to allocate women referred to hysteroscopy according to their menopausal status and therefore provided a conclusion that was not subject to sub-group nor post-hoc analysis. In premenopausal women receiving misoprostol, a greater number had a satisfactory preoperative cervical dilatation, as compared with women receiving placebo. Dilatation of the cervix was easier and quicker in premenopausal women receiving misoprostol.

6.4 TRIAL IV (Paper 4): A combination of estradiol and vaginal misoprostol, self-administered at home, for cervical ripening in postmenopausal women before day-care operative hysteroscopy

One thousand micrograms of self-administered vaginal misoprostol taken 12 hours before day-care operative hysteroscopy results in significant cervical ripening in

postmenopausal women, compared with placebo, after 14 days pre-treatment with vaginal estradiol tablets.

Cervical dilatation in the postmenopausal study participants was easier and comparable to the premenopausal women from the previous trial. Self-administered vaginal misoprostol at home the evening before operative hysteroscopy is safe and highly acceptable. Few side effects were reported. There is a risk of moderate lower abdominal pain and light preoperative bleeding with this regimen, which is inexpensive and easy to use.

This trial design needed far fewer participants to reach a conclusion on the primary efficacy outcome, compared with a fixed sample size trial.

7. DISCUSSION

7.1 TRIAL I (Paper 1): A comparison of two doses of oral misoprostol for cervical ripening before first trimester termination of pregnancy

We found that giving oral misoprostol the night before resulted in a high rate of preoperative bleeding. However, contrary to the anecdotal evidence presented in the nurses' report before the study commenced, we did not find an increased risk of miscarriage and excessive bleeding during the six month trial period - although the amount of preoperative bleeding was larger in the 400-microgram group than in the 200-microgram group. We noticed that the effect of misoprostol was dependent on whether or not a woman had previously been pregnant, as primigravidae women had a lower preoperative cervical dilatation, and a lower frequency of bleeding compared with multigravidae women.

This trial indicated that the dosage regimen of 400 micrograms oral misoprostol was not optimal for cervical ripening before first trimester termination of pregnancy, as regards both the low efficacy and the higher occurrence of preoperative bleeding associated with oral misoprostol. Instead of choosing two different oral dosages routes, we could have compared an oral to a vaginal route for the first trial. However, the vaginal dosage route was novel at the time the study was planned in early 2001, although the first studies were beginning to emerge that vaginal misoprostol (used in combination with mifepristone) had a strong cervical ripening effect in pregnancy. El-Refaey and Templeton found that vaginally administered misoprostol was feasible in 1994.¹⁶⁷ Zieman and co-workers had studied the absorption kinetics of vaginal administration in 1997.¹⁰⁴ Danielsson and co-workers had also conducted a study in 1999 using vaginal misoprostol but this measured uterine contractility, and did not evaluate cervical ripening.¹⁰⁵ Vaginal misoprostol given alone for cervical ripening was virtually untested at the time and misoprostol was designed by the manufacturer to be given orally. We also had no idea of how effective the current dosage regime was, so the trial was, in effect, an audit of current practice.

Over half the women (55%) who requested surgical abortion were randomised and the groups were equal demographically. A population-based study might have been possible had we been able to recruit a greater proportion of the total number of women requesting a surgical abortion. Nonetheless, out of all the previous studies published, this was one of the largest to have been carried out and we feel the results are valid and robust for the primary outcome. Furthermore, the study included all women requesting termination of pregnancy, and was not confined to groups perceived to be at high risk of cervical injury. The dropout rate was low. Because of its size, it was also possible to estimate the rate of more rare complications. We noticed that complications still occurred in groups perceived to be “low-risk”, e.g. multiparous women. Although this did not occur frequently, we feel it still justifies offering a cervical ripening agent to every woman requesting a surgical termination of pregnancy.

The trial was designed to show a difference of one millimetre in cervical dilatation between the dosage groups and was designed to be sufficiently powered in order to ensure a valid conclusion regarding the primary efficacy outcome. It was designed using conventional sample size calculations using estimates of preoperative variability from a similarly designed and conducted study. The level of significance was set at $p < 0.05$ and means were compared with an independent samples t-test for normally distributed data. In retrospect, it is obvious that the conventional sample size calculations used in this trial was flawed. Our results as regards preoperative cervical dilatation did not resemble those of any other published studies, so assuming variability as in previously published studies was not a robust technique.

The method used to evaluate the primary efficacy outcome is subjective and the use of preoperative cervical dilatation as a primary outcome for assessing cervical ripening is open to discussion. The method has been used to assess the effect of prostaglandins on cervical ripening for almost as long as prostaglandins have been used for this purpose¹⁶⁸ and it is still very widely used in randomised trials. In 1981 a cervical tonometer was first used to try and provide a more objective analysis of cervical ripening.¹⁶⁹ A few studies have subsequently used a tonometer but we decided against using such an instrument because we felt that the clinical experience would deviate too much from normal clinical practice, making the results from the trial difficult to transpose.

Using other outcomes, such as “difficulty in dilating the cervix” or “pain experienced by the women”, might be open to even greater subjectivity and much wider variation in results.

A potential major flaw in the trial design of all the trials we conducted is the fact that the participants administered the treatment themselves, without any objective witnesses. Randomisation is designed to secure equal distribution among groups for participants who did not disclose that they had deviated from the treatment regimen. The trials were all designed to resemble the clinical setting as closely as possible. If the treatments proved to be impossible to administer, then the experiments would not have any clinical potential.

7.2 TRIAL II (Paper 2): Oral misoprostol versus vaginal misoprostol, self-administered at home, for cervical ripening before first trimester termination of pregnancy

Self-administered vaginal misoprostol was shown in this trial to be a beneficial treatment option, providing cervical ripening in a shorter time interval and with fewer side effects than oral misoprostol. We noticed that the effect of misoprostol was more pronounced when a woman had previously been pregnant, than when she was pregnant for the first time.

Almost three quarters of women requesting surgical termination of pregnancy were randomised. The sample size was large and the dropout rate was low, so we feel that the sample is representative of the population. However, the acceptance level of the treatment may have been lower among women who did not agree to participate, so the high acceptance result could have been biased. In the years following the trial, it has become clear that the self-administered vaginal misoprostol dosage is considered a natural and acceptable form of treatment. Abortion regimens in Norway using oral misoprostol were modified after this study, and it is apparent that this treatment regimen has a potential for medical termination of pregnancy.

Although this study compared two dosage routes and two time intervals, we

cannot make an unequivocal statement regarding the optimal dosage and timing. It could appear from our results that women who had previously been pregnant would only need 400 micrograms of vaginal misoprostol four hours preoperatively, but that primigravidae women would need stronger doses – perhaps up to 800 micrograms four hours preoperatively. In a large department, a compromise would be to offer 600 micrograms misoprostol four hours preoperatively to all women. We have used this treatment regimen at Ullevål since 2004 and the personnel responsible for the care of the women have reported no adverse effects similar to those reported when the oral misoprostol regimen was first implemented.

The results of the trial could have been more robust had it been designed as a placebo-controlled trial, but a large number of gynaecologists assessed preoperative cervical dilatation and we do not feel that observation of the primary efficacy outcome would have been biased towards a particular treatment regimen.

The sample size in this trial was calculated in the same way as in the first trial, with conventional sample size calculations using estimates of preoperative variability from a similarly designed and conducted study. We again found that our results were not comparable with those published elsewhere, so assuming variability as in previously published studies was not a robust technique.

7.3 TRIAL III (Paper 3): Vaginal misoprostol, self-administered at home, for cervical ripening before day-care operative hysteroscopy

This trial found that self-administered vaginal misoprostol at home the evening before operative hysteroscopy is safe and highly acceptable. It was the first to separate premenopausal and postmenopausal women, which made the design stronger than other trials that used sub-group analysis.

As regards the choice of dose, we chose a higher dose than had previously been used in almost all the other published trials, because there was no clear consensus as to whether misoprostol was effective for cervical ripening in nonpregnant women or not. We suspected one reason might have been that the doses previously chosen were too low, or

due to insufficient exposure to the drug. As we wished to use a dose that would enable us to draw a conclusion on the primary end point, we chose the largest dose previously used for preoperative cervical ripening before hysteroscopy. This was 1000 micrograms vaginal misoprostol given two to four preoperatively. The authors concluded that it was not effective.¹³⁸ We did not consider lower doses of misoprostol as we thought it illogical to assume that the cervical ripening effect of misoprostol would be similar in non-pregnant women as in pregnant women. In pregnant women, the effect of misoprostol on cervical ripening increases exponentially towards term. In non-pregnant women, there was no consensus from the literature as to whether there was a cervical ripening effect using doses commonly used before first trimester termination of pregnancy. We also wished to find a practical dosage regimen for these patients, if it were to have any clinical application. Four hours had previously been shown to be ineffective, compared with placebo. It would not be practical to ask the women to take the treatment during the night before their operation after they had gone to sleep. We feared that an even longer time-interval, or repeated doses over several days, would make the treatment too complicated. Thus twelve hours before the operation seemed a practical solution. We must emphasise that this study did not compare different doses, routes or timing intervals.

We thought the dose we chose was safe and the dosage route the one associated with the fewest side effects. There is abundant literature supporting the safety of misoprostol, even in high doses. Three out of 45 women who received misoprostol in this trial experienced severe lower abdominal pain. Although this side effect is not considered a serious adverse effect, we acknowledge that it caused distress to the women experiencing it. The dilemma faced in warning study participants of possible side effects is that many more may report side effects – even the group receiving placebo – leading to uncertainty regarding the actual frequency of side effects. We concluded that this dosage was safe overall for premenopausal women but would recommend reserving the higher dosage for primigravidae or nulliparous women and reducing the dosage to parous women. We would also recommend that women take a standard analgesic, e.g. paracetamol or ibuprofen at the same time as taking misoprostol, to minimise the severity of lower abdominal pain.

This trial design needed far fewer participants to reach a conclusion on the primary efficacy outcome, compared with a fixed sample size trial. The sequential model seems to be an exceptional method of reducing sample size while ensuring that the trial is adequately powered. However, it still requires an estimate of preoperative variability in order to calculate the boundaries. The ideal method of estimating preoperative variability would be to conduct a pilot study using identical subjects, operators and conditions as in the actual trial itself. For this trial, a small pilot study was carried out in order to do this, but the estimate of variability turned out to be quite different from that of the subsequent trial. One cannot therefore rule out the risk of a type I or type II error resulting - particularly in the post-menopausal group, which had a small sample size.

Over 80% of women referred to hysteroscopy were recruited to the study, resulting in a population-based study. In retrospect, it has become obvious that women who have undergone cervical surgery do not experience a cervical ripening effect from misoprostol. Also, women who were bleeding heavily before hysteroscopy probably did not benefit from vaginal misoprostol tablets. If we had excluded these women, we might have been able to reach a conclusion on the primary efficacy outcome using fewer study participants.

A separate problem beyond the scope of this thesis is whether women with symptom-free polyps should be offered operative hysteroscopy. The evidence is mounting that premenopausal women with symptom-free polyps do not need surgical treatment. The same recommendation may apply to postmenopausal women. The majority of women who partook in this trial had symptom-free polyps.

7.4 TRIAL IV (Paper 4): A combination of estradiol and vaginal misoprostol, self-administered at home, for cervical ripening in postmenopausal women before day-care operative hysteroscopy

This is the first trial to include only women who had not previously used hormone therapy, thus eliminating hormone therapy as a confounder for the effect of misoprostol. Furthermore, the study included all consecutive women referred to

outpatient hysteroscopy. Again, a majority of women included in the trial were referred for symptom-free polyps. As noted above, the controversy regarding whether women with symptom-free polyps should undergo treatment or not is beyond the scope of this thesis.

For this trial, we used the preoperative variability from similar patients from trial III to calculate the boundaries. Again, this method was not ideal: the patients in trial III were not identical as those in trial IV, as those in trial IV were all treated with estradiol before the primary outcome was assessed.

The prospective randomised trial design is used to limit bias and confounding. As far as the primary outcome is concerned, one can be reasonably sure that trial participants will be equally distributed between the two groups – and this can be checked by comparing the study demographics after the trial has ended. However, in trials II-IV, treatment acceptability was an important finding. If every patient referred for the operation had been recruited, then acceptability would be reliable. Those patients reluctant to participate might have changed the outcome negatively.

In the future, trials on misoprostol treatment should note whether postmenopausal women are using hormone therapy, or are morbidly obese.



One thousand micrograms misoprostol

8. CONCLUSIONS AND RECOMMENDATIONS

Based on the results and observations from our four trials, we draw the following conclusions and make the following recommendations:

Trials I and II (Papers 1 and 2) showed that both 200 and 400 micrograms of oral misoprostol were an inferior method of ripening the cervix in pregnant women, compared with self-administered vaginal misoprostol. The lower dosage of oral misoprostol did result in far fewer side effects while the preoperative cervical dilatation was only slightly less with the lower dose, compared with the higher dose. If it were culturally impossible to use vaginal misoprostol, then a low dose of oral misoprostol would be preferable to cervical dilatation without a cervical ripening agent. Our studies also demonstrated that women – regardless of age and ethnicity – are able to administer misoprostol vaginally themselves at home before coming to the clinic for their surgical procedure. Before our study was published, it was generally considered necessary for staff to administer misoprostol to women.¹⁷⁰ Even our own staff had misgivings as to whether women would be capable, and accepting, of such vaginal drug insertion. As a letter to the editor of BJOG pointed out, our study was helpful in the discussion to demedicalise termination of pregnancy.¹⁷¹ Women should be able to self-administer misoprostol before medical abortion just as easily as before surgical abortion.

We also showed in trial I (Paper 1) that reports of a high miscarriage rate associated with oral misoprostol were not substantiated during a major trial lasting six months. The main conclusion must therefore be that clinical practice can only be properly assessed by means of a trial and that there is an excellent opportunity to test clinical results each time new procedures are implemented in a department. It is risky to automatically accept findings from other studies and assume that the results have external validity.

Because we found that misoprostol had a different effect in primigravidae, as compared with multigravidae women, the best dosage for cervical ripening before first trimester termination of pregnancy would probably be 800 micrograms of vaginal misoprostol approximately four hours before the operation to primigravidae women.

Four hundred micrograms of vaginal misoprostol four hours before the operation for a woman who had previously been pregnant would probably be sufficient. To make the procedure practical to implement clinically, 600 micrograms inserted four hours preoperatively regardless of pregnancy history would probably be an adequate compromise. However, this has not been tested in a trial and as such, is a guideline based only on personal opinion.

Trials III and IV (Paper 3 and 4) had three key findings:

- i) Misoprostol does indeed have a cervical ripening effect in women who are not pregnant, although the effect seems to be much weaker in non-pregnant than in pregnant women.
- ii) The effect is relative to whether the woman is premenopausal or postmenopausal.
- iii) It seems possible to return a cervix to premenopausal misoprostol receptiveness in just 14 days. In response to our third paper, we received correspondence from colleagues who disagreed with our findings.¹⁷² However, we are reasonably sure that misoprostol will not have any cervical ripening effect unless there is enough estrogen present.

A frequent response from colleagues who have been shown that misoprostol has a cervical ripening effect beneficial to clinical practice is, "I've never had any problem dilating the cervix!" Citing personal experience as an argument to avoid using a safe, inexpensive and acceptable treatment, with the potential for simplifying a common procedure, cannot be justified. The goal is not only to avoid complications but also to simplify the procedure, thereby reducing the risk of adverse results for the patient. After all, hundreds of thousands of curettage abortions were performed before anyone thought to examine the risk involved and whether there was any way of lowering it.²⁵

9. FUTURE RESEARCH

Self-administered vaginal misoprostol has the potential to further demedicalise abortion. As the procedure no longer requires staff administration of the drug and inpatient care, future studies could investigate how to further reduce the need for follow-ups after the abortion.

One key, unanswered question arising from the last trial (Paper 4) is how local estradiol therapy is responsible for the remodelling effect of the cervix. This has, to our knowledge, never been previously described. It seems quite remarkable that cervical dilatation can be increased by two to three millimetres with two weeks of treatment. Cervical dilatation in postmenopausal women (trial IV, Paper 4) seemed much easier in the majority of women, compared with the postmenopausal women who had not used estrogen therapy in trial III (Paper 3). We wonder if it really is possible to reverse the effects of estrogen deficiency in a postmenopausal cervix in only two weeks. Future studies could include biopsy and electron microscopic examination of cervical tissue to confirm this.

Trial IV (Paper 4) is also, to our knowledge, the first time an association has been made between misoprostol's effect on cervical ripening and the levels of circulating estrogen. There is evidence that the effect of misoprostol is linked to the level of estrogen. In pregnant women, serum estrogen levels increase during the second half of pregnancy to reach levels near term more than 50 times non-pregnancy levels. In a postmenopausal woman of normal weight, the serum level of estrogen is extremely low. There is a potential to investigate whether estrogen levels are responsible for the effect of prostaglandins on cervical ripening. Estrogen may prove to be the most important factor in the inflammatory cascade reaction in the cervical ripening process. Alternatively, another mediator might exist, also needing estrogen to cause a local cervical inflammatory reaction with prostaglandins.

It is assumed that cervical ripening is achieved through the same biochemical and physiological processes in the nonpregnant state with prostaglandins but so far this has yet to be confirmed by histological examination of tissue. There is a potential for future studies in this area.

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11. PAPERS I-IV, CORRESPONDENCE