CLINICAL ~~EFFECTS~~ APPLICATIONS OF THE PROGESTERONE RECEPTOR ANTAGONIST MIFEPRISTONE DURING EARLY PREGNANCY AND ITS APPLICATION IN THERAPEUTIC ABORTION.

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ABSTRACT

Mifepristone (RU 38,486) is a new synthetic steroid which is antagonistic to progesterone at the receptor level. Mifepristone has been demonstrated to cause spontaneous abortion of early pregnancies when taken orally. When used in combination with a prostaglandin analogue the termination success rate is increased. The studies performed in this thesis were designed to further investigate the clinical effects of mifepristone in the first and second trimesters of pregnancy, and to study the mechanism of action of the drug.

These studies examined:

1). The efficacy and side-effects of mifepristone (600mg) in combination with a prostaglandin E_1 1mg (Gemeprost) pessary for early termination of pregnancy.

2). The effect of mifepristone on prostaglandin metabolite levels in the first and second trimesters of pregnancy.

3). The effect of mifepristone on progesterone and oestrogen receptor concentrations in the decidua and placenta in early pregnancy.

4). The physiological and clinical effects of progesterone inhibition with mifepristone in the second trimester.

5). The placental transfer of mifepristone during the second trimester and its influence upon maternal and fetal steroid concentrations.

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PREFACE

This work was performed whilst the author was holding a clinical research fellowship financed by a grant from Roussel Laboratories Limited, Uxbridge, U.K., awarded to the Nuffield Department of Obstetrics and Gynaecology, University of Oxford, to examine the endocrine and clinical effects of mifepristone.

The author performed all clinical trials and helped the technical staff in performing the prostaglandin and receptor assays. All the work was performed in the Nuffield Department of Obstetrics and Gynaecology, University of Oxford, The John Radcliffe Hospital, Headington, Oxford, under the supervision of Mr. Ian Z. MacKenzie. The work was performed between January 1988 and December 1989.

All terminations and abortifacient agents were given on hospital premises within the Abortion Act, 1967, and informed consent was obtained from all the patients. Central Oxford Regional Research Ethics Committee approval was granted for each study before commencement.

Some of these results have already been submitted for publication in a modified form. These publications are listed below.

Hill NCW, MacKenzie IZ. (1989)
2308 Mid-trimester terminations using extra-amniotic or intra-amniotic PGE2 - An analysis of efficacy and complications. Br. J. Obstet. Gynaecol. 96: 1424-31.

Brice AL, Cheetham JE, Balton VN, Hill NCW, Schofield PN. (1989) Temporal changes in the expression of the insulin-like factor II gene associated with tissue maturation in the human fetus. Development 106: 543-554.

Hill NCW, MacKenzie IZ. (1990)
Early termination of pregnancy: medical induction with prostaglandins versus surgical aspiration under local anaesthetic. Int. J. Gynecol. Obstet. 32: 269-74.

Hill NCW, Ferguson J, MacKenzie IZ (1990)
The efficacy of mifepristone (RU 38,486) with a prostaglandin E_1 analogue for the termination of early pregnancy: Complications and patient acceptability.
Am. J. Obstet. Gynecol. 162: 414-8.

Hill NCW, Selinger M, Ferguson J, MacKenzie IZ. (1990)
The placental transfer of RU 38,486 (mifepristone) during the second trimester and its influence upon maternal and fetal steroid concentrations. Br. J. Obstet. Gynaecol. 97: 406-11.

Hill NCW, Lopez Bernal A, Rivera P, MacKenzie IZ. (1990)
The effect of RU 38,486 on progesterone and oestrogen receptor concentrations in the decidua and placenta in early pregnancy. Human Reprod. 5: 464-8.

Hill NCW, Lopez Bernal A, Ferguson J, MacKenzie IZ. (1990)
The effect of the anti-progestin mifepristone (RU 38,486) on plasma prostaglandin metabolite levels in early pregnancy. Acta Scand. Gynec. Obstet. 69: 321-5.

Hill NCW, Selinger M, Ferguson J, Lopez Bernal A, MacKenzie IZ. (1990) The physiological and clinical effects of progesterone inhibition with RU 38,486 (mifepristone) in the second trimester. Br. J. Obstet. Gynaecol. 97: 487-92.

Hill NCW, Selinger M, Ferguson J, MacKenzie IZ. (1991)
Transplacental passage of mifepristone and its influence upon maternal and fetal steroid concentrations in the second trimester of pregnancy. Human Reprod. 6: 458-62.

Hill NCW. (1991) The clinical applications of the anti-progesterone steroid mifepristone (RU 38,486) Contemp. Reviews Obstet. Gynaecol. 3: 93-100.

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Miepristone (RU 38,486 or Mifegyne) - 17ß-hydroxy-11 ß (4 - dimethylaminophenyl - 1) - 17 a (Prop-1-ynyl)-estra-4, 9-dien-3-one), Roussel-Uclaf, Paris, France, is a synthetic steroid which acts as an antagonist to progesterone and glucocorticoids at the receptor level. Unlike Epostane, a 3-ß hydroxysteroid dehydrogenase enzyme inhibitor, it does not cause a reduction in peripheral serum progesterone levels. The exact mode of action of mifepristone is not fully understood. The drug has a high affinity for the progesterone receptors and therefore blocks the normal receptor binding of progesterone. Withdrawal of progesterone stimulates uterine activity, possibly increases prostaglandin production, and therefore results in termination of early pregnancy (Baulieu, 1985a).

Initial studies using mifepristone for medical termination of early pregnancy alone showed a complete abortion success rate of between 60-80% (Kovacs et al, 1984; Shoupe et al, 1986). As the gestation of the pregnancy increased beyond 8 weeks gestation the complete abortion success rate fell to only 33% (Haspels, 1985). However, much better results are obtained when the drug is combined with a prostaglandin pessary. Rodger and Baird (1987) showed that a complete abortion rate of 90-100% could be obtained when mifepristone was combined with prostaglandin E_1 (Gemeprost). Various dose regimens were investigated, but the best results were obtained with the highest dose (600mg).

The clinical trials reported in this thesis were

designed to further investigate the clinical effects of mifepristone in both first and second trimester termination of pregnancy. These results were compared with retrospective data previousely obtained for early and mid-trimester prostaglandin termination of pregnancy, therefore possible advances in these techniques by the addition of mifepristone were identified. An attempt has been made to try to investigate the possible mechanism of action of the drug. This has been performed by: measuring prostaglandin metabolite levels in the first and second trimester; progestrogen and oestrogen receptor concentrations; and uterine activity after mifepristone treatment. One study was performed to try to quantify mid-trimester maternal-fetal transfer of mifepristone and to investigate possible effects on the fetus of drug treatment.

Clinical Trial 1 - was designed to assess the efficacy of mifepristone (600mg) given 48 hours prior to a prostaglandin E_1 pessary (Gemeprost) for termination of pregnancies of eight weeks gestation or under. This study was part of a larger multi-centre study undertaken on 1000 patients. Patients were given individual questionnaires to investigate patient acceptibility of the technique.

Clinical Trial 2 - was designed to investigate the effect of mifepristone treatment on prostaglandin EM and FM levels in early pregnancy and these results were compared with matched controls treated surgically.

Clinical Trial 3 - using the decidua and placentas

recovered from study 1, the progesterone and oestrogen receptor concentrations in the decidua and placenta were compared with matched controls treated surgically.

Clinical Trial 4 - this double blind placebo controlled trial was designed to assess the physiological and clinical effects of mifepristone upon myometrial contractility and sensitivity to ergometrine, syntocinon and prostaglandin E_2 during mid-trimester. As part of the study prostaglandin metabolite concentrations in the mifepristone and placebo groups were compared.

Clinical Trial 5 - this double blind placebo controlled trial was designed to investigate the placental transfer of mifepristone and to document any changes in fetal steroid levels after treatment.

SECTION 1.

A REVIEW OF THE CONTROL OF UTERINE ACTIVITY, PROGESTERONE AND THE ANTI-PROGESTERONES.

1.1. - PHYSIOLOGY OF PARTURITION.

1.2. - PROGESTERONE BIOCHEMISTRY.

1.3. - THE ANTI-PROGESTERONE STEROIDS.

1.4. - CLINICAL STUDIES ON THE ANTI-PROGESTERONES.

1.5. - TERMINATION OF PREGNANCY

1.1. PHYSIOLOGY OF PARTURITION

Introduction

The normal development and birth of a human fetus at term depends on the mechanism which ensures that the uterus remains quiescent during pregnancy and then, at the appropriate time, initiates uterine contractions, softening and dilatation of the cervix, and ultimately delivery of the mature infant. The sequence of events which precede labour have been the subject of much discussion. Whether the maintenance of pregnancy is a consequence of inhibition or the result of lack of stimulation of contractility remains uncertain. Extensive studies in animals have shown that pregnancy is maintained by a balance of controlling factors, including hormones and substances that suppress the myometrium, and those that stimulate the myometrium to contract (Csapo, 1981). Prior to labour the balanced system is upset, a situation leading to conditions which favour less inhibition and greater stimulation. However, because a similar balanced system does not appear to be present in humans, there has been reluctance to accept this hypothesis.

Recently advances have been made in our understanding of the factors that control uterine contractility during pregnancy. These advances include the observation that receptors, stimulants, and gap junctions change during labour and result in increased excitability, cell-to-cell coupling, and increased contractility. The endocrine

control of parturition and the systemic or local hormonal changes which are thought to be associated with the onset of labour are therefore reviewed.

Basis of Myometrial Contractility

The myometrium consists of at least two well defined muscle layers of different origin, an outer longitudinal muscle layer, orientated in the long axis of the uterus, and an inner circular muscle layer, arranged concentrically around the longitudinal axis.

The contractile or functional units of the myometrium are the smooth muscle cells, which are long (300-600u), narrow (5-10u) and spindle shaped (Garfield, 1984). The myofilaments, actin (5-8nm in diameter) and myosin (15nm) interact with calcium and adenosine triphosphate (ATP) as in skeletal muscle to contract and shorten the cell. Myosin, which is both a structural protein and an enzyme, converts the chemical energy of ATP into the mechanical energy of muscle contraction. The head of the myosin molecule contains sites for ATP-ase, actin combining, and phosphorylation. Enzymatic phosphorylation and dephosphorylation of the myosin light chains by myosin light-chain kinase, are the final elements in the regulation of smooth muscle contractility. Myosin light-chain kinase activity is regulated by calmodulin, a calcium dependent regulatory protein [MW 16,500 D] (Yagi et al, 1979; Steer, 1990), cellular calcium levels (Huszar & Roberts, 1982) and most importantly, cyclic adenosine

monophosphate (cAMP) mediated phosphorylation of the myosin light-chain kinase (Adelstein et al, 1978).

The ability of myometrial cells to contract depends upon their ability to maintain ionic gradients across their plasma membranes. The ionic distribution in the uterine smooth muscle cell is such that sodium and calcium ion concentrations are higher outside the cell than inside, whereas potassium ions are in higher concentration within the cell. These ionic gradients allow the muscle cells to respond when small changes in permeability result in significant movements of ions down their electrochemical gradients. Intracellular calcium levels are the key to regulation of contraction. At internal calcium concentrations of 10^{-8} to 10^{-7}M, the muscle is relaxed, and at 10^{-6}M calcium, the actin and myosin filaments interact and the muscle contracts (Garfield, 1984; Carsten and Miller, 1987). The mechanism responsible for the reduction in intracellular calcium levels to produce relaxation are thought to be due to the uptake of calcium by the smooth sarcoplasmic reticulum and extrusion of calcium across the plasma membrane by a calcium pump or a sodium-calcium exchange mechanism (Johansson & Somlyo, 1980; Wuytack et al, 1984; Somlyo, 1985).

In summary, when an action potential or similar stimulus causes a rise in the intracellular calcium concentration (both from an influx of calcium into the cell and calcium release from the sarcoplasmic reticulum),

calcium and calmodium form a complex which has a much higher affinity for the myosin light-chain kinase. Once bound to the kinase, phosphorylation can proceed and actin/myosin interaction occurs. When the calcium level subsequently falls again, the calcium-calmodium complex dissociates and the free calmodium dissociates from the kinase which is thereby inactivated (Steer, 1990).

Second messengers

The events leading from hormone binding to hormone action in the uterine muscle are thought to be mediated by second messengers. Hormones stimulating (or suppressing) uterine activity bind to their specific receptor at the outside of the cell membranes. The distribution and ligand specificity of the receptors determine the tissue response and production of a second messenger, which transposes non-specifically to the substrate inside the cell (Carsten and Miller, 1987). Previously it was thought that all hormones causing uterine contraction worked through the second messenger cyclic guanosine monophosphate (cGMP) and all hormones causing uterine relaxation worked through the second messenger cyclic adenosine monophosphate (cAMP). However, attempts to correlate cGMP levels with either contraction or relaxation have failed (Diamond, 1983). cAMP is still thought to play an important part in the mechanism of relaxation, but the compound is probably not an obligatory mediator of relaxation of the uterus (Smith and Marshall, 1986). There is increasing evidence that binding of agonists to receptors and subsequent calcium

mobilisation is mediated by hydrolysing phosphoinositides, which are components of all cell membranes (Schrey et al, 1988; Liggins and Wilson, 1989). Receptor binding activates phospholipase C which hydrolyses phosphatidyl 4,5-biphosphate to diacylglycerol and inositol trisphosphate. The latter acts to mobilise intracellular calcium from the sarcoplasmic reticulum (Steer, 1990).

Guanine nucleotides may also provide a level of control of uterine contractility by modifying the cAMP system (Carsten and Miller, 1987) A separate set of receptors communicates with a pair of guanine nucleotide-binding regulatory proteins (G proteins) in the cell membranes, one of which enhances the production of cAMP by stimulating adenylate cyclase, while the other inhibits it (Spiegal et al, 1985). G proteins are also involved in the regulation of the inositol trisposphate system (Cockcroft and Gomperts, 1985).

Control of Myometrial Activity

The electrical activity of any smooth muscle tissue may be controlled by myogenic, neurogenic, or hormonal mechanisms. Myogenic activity refers to spontaneous activity which occurs in the absence of any neural or hormonal input, and it includes the basic intrinsic excitability of the muscle, the ability of the muscle to contract spontaneously, and the mechanism for conduction to produce rhythmic contractions. Neurogenic and hormonal activity are attributed to the neural or humoral control

systems which are superimposed on the myogenic mechanisms to modulate and/or initiate myometrial activity. Stimulation or inhibition of myometrial activity depends on the influence of these three mechanisms.

Myometrial Control

The myometrium is able to generate spontaneous activity in vitro and in vivo without any hormonal or neural influences through its pacemaker cells (Marshall, 1962). Pacemaker cells are autonomously active smooth muscle cells in which the resting membrane potential is smaller than non-pacemaker cells (Lodge & Sproat, 1981). Spontaneous oscillations (pacemaker potentials) in the membrane potential of pacemaker cells leads to action potentials when the threshold for firing is reached. The electrical activity which arises from the pacemaker areas then excites surrounding areas, and propagation of electrical activity along the cell surface occurs by a local circuit mechanism (Garfield, 1984). The propagation of current between cells is thought to occur by the same mechanism and involves the flow of ions between cells through channels which connect the cell interiors, the gap junctions (Peracchia, 1980). Cells with gap junctions would be expected to be better coupled electrically than those without junctions, and when present the myogenic properties change such that electrical signals propagate further and excite more cells (Garfield et al, 1977). Studies of rats, guinea pigs, sheep and humans suggests that gap

junctions are absent or present in low concentrations throughout pregnancy, and at term the frequency and size of the gap junctions increases, but within 24 hours of delivery they begin to disappear (Garfield et al, 1980a; Garfield & Hayashi, 1981; Puri & Garfield, 1982). The absence of gap junctions throughout gestation may play some part in maintaining pregnancy by limiting electrical or metabolic communication between cells, and thereby preventing co-ordinated uterine contractions (Garfield et al, 1982b).

Neurogenic Control

Neurogenic activity of smooth muscle is that component of contractility which occurs in response to the discharge of nerves by the effects of transmitters on the muscle. It is generally believed that modulation by neurogenic control of uterine contractility is not important, because transection of the spinal cord or peripheral nerves has no consistent effect on implantation, pregnancy or parturition. Also there is abundant biochemical, histological and electron-microscopic evidence which indicates that adrenergic nerves in the uterine wall, but not the cervix, disappear during pregnancy in humans (Marshall, 1981).

These observations have led to the hypothesis that the adrenergic nerves degenerate at term, and the maintenance of pregnancy and the initiation of labour may be related to the disappearance of these nerves (Thorbett et al, 1977).

Myogenic activity could normally be suppressed by the adrenergic nerves throughout pregnancy, and with their disappearance near term, myogenic contractions may increase.

However, Thorbett et al (1979), have also shown that implantation, pregnancy, and parturition occur with subsequent pregnancies prior to regeneration of the adrenergic nerves, which tends to argue against the adrenergic nerves having a significant role in controlling parturition.

Hormonal Control

There is a wealth of evidence that myometrial contractility is under very dominant control by hormonal mechanisms, with the prostaglandins, oxytocin, vasopressin, relaxin, oestrogen and progesterone all playing a part in control of uterine contractility.

Prostaglandins

It is generally accepted that prostaglandins play an important role in the physiology of human labour. Evidence for this is derived from three observations: first, exogenous prostaglandins can induce labour (Karim et al, 1969); second, the decidua has the capacity to synthetise prostaglandins which increases in labour; third, that the prostaglandin inhibitors can suppress premature labour (Zuckerman et al, 1974; Wiqvist et al, 1975). However, it is uncertain whether prostaglandins are involved in the

initiation and maintenance of uterine contractions, or whether they are released as a consequence of the onset of labour and therefore serve simply as the final common pathway of other humoral factors (Steer, 1990).

Prostaglandins are detected in the amniotic fluid from mid-pregnancy, and their concentration when measured at amniotomy increases throughout the remainder of pregnancy (Dray and Frydeman, 1976). However, measurement of prostaglandins in the amniotic fluid is influenced by sampling method with prostaglandin levels higher in samples collected at amniotomy compared with amniocentesis, and Turnbull (1989) has reported no increase in PGF concentrations collected by amniocentesis in the amniotic fluid with increasing gestation from 34 weeks. Both the prostaglandin and arachidonic acid concentrations in the amniotic fluid are higher during labour than before labour, and these concentrations increase with labour (MacDonald et al, 1978). Measurement of prostaglandins in maternal peripheral plasma is hampered by their very low concentrations, due to their rapid clearance, especially in lung. Measurement of prostaglandin metabolite concentrations provide a more accurate measurement, and using this technique no increase in 13,14-dihydro-15-keto-PGF (PGFM) concentrations in the peripheral plasma occurs up to the onset of labour (Mitchell, 1981), but levels increase dramatically during labour (Sellers et al, 1981; Mitchell, 1984). PGFM concentrations have also been found to increase concomitant with cervical ripening without

uterine activity before the onset of labour (Keirse et al, 1983). Measurement of the stable 11-16 bicyclo-PGEM metabolite shows that its concentration does not change significantly during pregnancy or with the onset of progression of labour to delivery (Demers et al, 1983).

The formation of prostaglandins in human parturition begins in the fetal membranes (amnion [Mitchell, 1987a] and chorion [Olson et al, 1983]), and also in the decidua (Skinner & Challis, 1985). Prostaglandin production is greatest in amnion (Okazaki et al, 1981; Casey and MacDonald, 1986). The first stage is release of free arachidonic acid, which is the obligatory precursor of prostaglandins of the "2" series. Arachidonic acid accumulates in human fetal membranes in esterified form in glycerophospholipids. The major pathway of arachidonic acid release is by phospholipase A_2 action, which hydrolyses glycerophospholipids at the C2 position resulting in formation of free fatty acid and lysoglycerophospholipid. PGE_2 and PGF_{2a} are formed from arachidonic acid in a multistep process, the first stage of which is catalysed by the enzyme cyclo-oxygenase or PGH synthase, which results in the formation of the cyclic endoperoxide prostaglandins PGG_2 and PGH_2. These are transformed by appropriate enzymes into three important groups of active prostaglandins. The first, prostacyclin (PGI_2) produced by prostacyclin oxycyclase, and the second, thromboxane (TXA_2) produced by thromboxane synthetase, are primarily vasoactive. The third group comprises the prostaglandins of PGE_2, PGF_2 and PGD_2,

of which PGE_2 and PGF_{2a} are the major uterotonic compounds.

The rate of prostaglandin production is controlled more by the rate of release of arachidonic acid than the activity of the cyclo-oxygenase enzymes. As prostaglandins are not stored in cells, but are immediately released after their synthesis and rapidly metabolised, and since only free arachidonic acid can be used by the prostaglandin synthetase system, its release from tissue phospholipids may be a regulatory step in prostaglandin biosynthesis.

Early studies on the sites of prostaglandin production suggested that the main source of PGF_{2a} was the myometrium. However, Liggins and Greaves (1971) showed that in the sheep prostaglandin in the myometrium had diffused there from the uterine epithelium. Both in the pregnant sheep uterus (Campos et al, 1980) and in the non-pregnant human uterus (Abel et al, 1980) the uterine epithelium or endometrium predominantly converts arachidonic acid to PGF_{2a} and PGE_2, whereas the myometrium produces mainly PGI_2. An additional site of prostaglandin production is the cervix. Ellwood et al (1980) demonstrated that prostaglandin production in cervical tissues obtained during the first trimester was less than prostaglandin production in tissues obtained at Caesarean hysterectomy in the third trimester. Therefore, the human cervix may produce prostaglandins which could act locally during pregnancy and contribute to cervical softening and dilatation over the last weeks of pregnancy as well as contributing to the accelerating dilatation of labour.

Prostaglandin production in the fetal membranes and decidua probably plays a central role in the initiation of labour. In the amnion, PGE_2 is the most important prostaglandin produced, and since there is little or no 15-keto-prostaglandin dehydrogenase in this tissue, the large quantities of PGE_2 formed are not metabolised (Casey and MacDonald, 1986). However, the human myometrium is capable of actively metabolising the PGE_2 to PGF_{2a} because it is an important source of PG-9-oxo-reductase activity (Canete Soler et al, 1987). The ability of amnion to produce PGE_2 has also been shown to increase during labour (Lopez Bernal et al, 1987a). Although the chorion also synthesises PGE_2 and the decidua synthesises PGE_2 and PGF_{2a}, both tissues have considerable prostaglandin dehydrogenase activity which tends to quickly inactivate the prostaglandins produced (Turnbull, 1989).

Except for the availability of precursors, the factors which initiate or regulate endogenous production of prostaglandins have not been fully documented. A variety of substances have been identified which may inhibit or stimulate prostaglandin production, and hence play a part in controlling the initiation of parturition.

Endogenous Inhibitors of Prostaglandin Synthesis

Robinson et al (1978) suggested that the onset of labour was associated with withdrawal of an inhibitor of prostaglandin biosynthesis. The discovery of an endogenous inhibitor of prostaglandin synthetase (EIPS) in several

animal species led to speculation that it may play a part in suppression of prostaglandin biosynthesis during pregnancy (Saeed et al, 1977). EIPS has now been found in maternal and fetal plasma and in the amniotic fluid (Saeed et al, 1982). Brennecke et al (1982, 1985) investigated EIPS in human pregnancy but found similar activities in the plasma of non-pregnant women; men; women in the first and second trimesters of pregnancy, and at full term and postpartum. Although there appeared to be a small but significant decrease in EIPS activities in plasma samples obtained from women in the third trimester and from those at full term, this was not maintained in labour or after deilvery, suggesting that a decrease in EIPS is not the cause of the increased prostaglandin production during labour.

Mortimer et al (1985) demonstrated that amnion obtained from pregnant women could contain an endogenous prostaglandin synthetase inhibitor which was no longer present in amnion obtained from women during labour. Wilson et al (1985) demonstrated in amniotic fluid a 58-kd protein (chorionic inhibitory protein) which specifically inhibits arachidonic acid release from decidual cells. This protein works by inhibiting decidual cell phospolipase A_2, and although found in the amniotic fluid of women with ongoing pregnancies, it disappears in amniotic fluid obtained in labour (Wilson, 1988). It has been suggested that the protein is secreted by the chorion throughout pregnancy, and by diffusing into nearby tissues it inhibits

prostaglandin synthesis and contributes to the maintenance of pregnancy (Liggins, 1989). At term the protein is inactivated and inhibition of prostaglandin production ceases thereby facilitating the onset of labour.

Endogenous stimulators of Prostaglandin Production

It is now thought that increased amnion prostaglandin production may play a critical role in the onset of human labour (Mitchell, 1984). A substance has been isolated from fetal urine, and amniotic fluid, which stimulates prostaglandin synthetase (Strickland et al, 1983). This prostaglandin synthetase stimulating substance increases the rate of production of prostaglandin E_2 in the amnion by 5 to 50 fold (Casey et al, 1983). It acts via the epidermal growth receptor and its activity is markedly enhanced by the presence of calcium ions (Mitchell, 1987b). Since this substance is formed in the fetus and communicates with the amnion, it is a strong candidate for a role in the timing of labour. Furthermore, this factor may provide a link between fetal development and growth, and the mechanism of labour. However, since fetal urine samples obtained before labour also exert this effect, other regulatory mechanisms must be involved.

Another prostaglandin stimulating substance has been reported by Bleasdale & Johnston (1984), who showed that platelet activating factor (PAF) is present in amniotic fluid from women in active labour, although it is absent before labour. PAF is a phospholipid that increases calcium

concentrations in a variety of cell types, especially platelets from which its name is derived. Calcium dependent enzymes activated by increased calcium include phospholipase A_2 and phospolipase C both of which regulate release of arachidonic acid and promote prostaglandin synthesis. PAF in amniotic cell culture has been reported to increase the output of PGE_2 by two to three fold (Challis, 1985). The source of PAF in amniotic fluid is uncertain, but amnion has the capacity to synthesise it (Billah et al, 1985). PAF is present in fetal urine and in the surfactant fraction of amniotic fluid suggesting an origin in fetal lung (Liggins, 1989). Increased secretion of PAF in tracheal fluid has been postulated to link fetal lung maturity to the initiation of parturition. However, in pregnancies with tracheal stenosis, in which tracheal fluid can not escape from the lungs, no associated with prolonged pregnancy occurs and in sheep total fetal pneumonectomy does not prevent labour (Wagner and Ellendorf, 1986). Therefore, the exact place of platelet activation factor in the initiation of parturition is unclear.

Premature labour secondary to chorio-amnionitis is associated with the presence in amniotic fluid of substances with the capacity for stimulation prostaglandin synthesis (Liggins, 1989). Endotoxins are liberated by the bacteria in amniotic fluid of patients in premature labour, which stimulate the formation of cytokines by macrophages that are part of the defensive response to infection. In particular, interleukin-1 and tumour necrosis factor are

found in infected amniotic fluid obtained from women in premature labour and both are able to stimulate amnion production of PGE_2 (Romero et al, 1986). While these cytokines are likely to contribute to the onset of premature labour in the presence of chorio-amnionitis, their absence in amniotic fluid at term excludes a possible physiological role.

Oxytocin

Oxytocin is one of the most potent endogenous uterine stimulants. It is released in a pulsatile manner from the posterior lobe of the pituitary, and has a half-life of approximately 3-17 minutes (Fuchs & Fuchs, 1984; Chard, 1989). Oxytocin release by the neurohypophysis is controlled by a neuroendocrine reflex originating from tactile nipple stimulation and a reflex arising from stretch receptors in the lower genital tract (Ferguson's reflex).

Dawood et al (1979) and Sellers et al (1981b) have both reported a rising trend in oxytocin concentrations in the maternal plasma with gestation. However, technical difficulties with the assay, fluctuating patterns, and wide scatter of obtained oxytocin values have made these results questionable (Carsten and Miller, 1987). The overall levels of oxytocin may increase progressively during pregnancy, but if this occurs at all, it is probably of minor significance (Chard, 1989). Fuchs et al (1983a) reported that oxytocin levels in the first stage of

spontaneous labour were significantly higher than in non-labouring controls. However, Leake et al (1981) and Leake (1983) showed no rise in plasma oxytocin concentrations until late in the second stage of labour, and Gibbens and Chard (1976) reported that the frequency of oxytocin spurts (and hence the mean levels of oxytocin) increased with the onset of labour and reached a maximum at the time of delivery. The same authors found no obvious relationship between the timing of the oxytocin spurt and uterine contractions.

An alternative explanation for the effect of oxytocin would be that simple plasma levels are not as important in controlling labour as the oxytocin receptor levels in the uterus (Fuchs et al, 1984). Oxytocin receptors have been identified in the rat (Alexandrova & Soloff, 1980), ewe (Crankshaw et al, 1982), rabbit (Riemer et al, 1986) and human uterus (Fuchs et al, 1982). The receptors are located in the myometrial cell membrane and their concentration rises towards the end of pregnancy, is maximal during labour and then declines (Fuchs & Fuchs, 1983a). The increased levels of receptors allows for an increase in the concentration of oxytocin binding in the presence of the relatively low concentrations of circulating oxytocin which are found during the early stages of labour. In the rat and rabbit, the concentration of oxytocin receptors appears to be regulated by oestrogen and progesterone, with oestrogen increasing the number of receptors and progesterone inhibiting this increase (Nissenson et al, 1978; Fuchs et

al, 1983b). These findings correlate with observed physiologic function and suggest that the increase in uterine sensitivity to oxytocin is related to an increase in myometrial oxytocin binding sites (Carsten and Miller, 1987).

The relationship between oxytocin and prostaglandin is complex. Oxytocin stimulates release of arachidonic acid and PGF_2a from the decidua, but not from the myometrium (Fuchs et al, 1982), and this ability increases with labour (Wilson et al, 1988). The mechanism by which oxytocin stimulates prostaglandin release is probably by increased phospholipase A_2 and C activity mediated by calcium (Liggins, 1989). Oxytocin stimulates the turnover of phoshatidylinositol and the formation of inositol triphosphate in human decidua (Schrey et al, 1986), which in turn, mobilises intracellular calcium and may also increase the flux of calcium into the cell (Kuno and Gardner, 1987). In rats, administration of PGF_2a near term stimulates the formation of oxytocin receptors, and treatment with prostaglandin antagonists (indomethacin) inhibits receptor formation (Chan, 1987). Whether this occurs in humans is unknown, but the observation that treatment with PGF_2a in late pregnancy sensitises the uterus to oxytocin after a latent period of several hours suggests that it may occur (Liggins, 1989). Oxytocin therefore appears to have a dual role in the mechanism of parturition. Firstly, it stimulates myometrial contractions by activating myometrial oxytocin receptors, and secondly,

it stimulates prostaglandin production in the decidual tissues.

A plausible hypothesis incorporating all the evidence relating to prostaglandin and oxytocin suggests that the initiating event in human parturition is the release of a small quantity of a prostaglandin (either PGF_2a or PGE_2) from the fetal membranes (probably amnion) or decidua (Liggins, 1989). This results in an increase in oxytocin receptors, which in turn, further stimulate prostaglandin synthesis. A positive feedback loop involving prostaglandin synthesis and oxytocin receptors will then be activated, allowing accelerated uterine activity. A preliminary report that an oxytocin analogue which competitively antagonises oxytocin inhibits labour (Akerlund et al, 1985; Akerlund et al, 1987), suggests that oxytocin and prostaglandin are dependent on each other for their full expression as uterotonic agents.

Oxytocin is produced by the fetal pituitary from 14 weeks gestation, and its output gradually increases during pregnancy (Khan-Dawood and Dawood, 1984). The fetus releases substantial amounts of oxytocin in association with labour, which progressively increases as labour progresses, and reaches a maximum during the expulsive phase of labour (Chard et al, 1971; Dawood et al, 1983; Pochard and Lutz-Burcher, 1986). Fetal oxytocin levels are also significantly lower in fetuses delivered by caesarean section compared with vaginal delivery (Kuwabara et al,

1987). The oxytocin levels in the umbilical artery are significantly higher than in the umbilical vein, confirming fetal origin of the oxytocin (Chard et al, 1971; Leake et al, 1981; Dawood, 1983), and both fetal umbilical artery and venous plasma levels are significantly higher than maternal plasma (Sellers et al, 1981b; Fuchs et al, 1982; Fuchs, 1985). The stimulus to fetal oxytocin secretion is unknown, and it is possibly a reaction to anoxia, although at present no direct evidence exists to confirm this theory (Chard, 1989). The significance of the fetal oxytocin release is also unknown. Certainly the hormone is able to cross the placenta and is also present in fetal urine, both of which may produce local effects on the uterus to increase uterine activity. Apart from a direct effect, oxytocin may also produce a vasoconstrictor effect, since a reduction in uterine blood flow may also stimulate uterine activity (Chard, 1989). However, fetal release of oxytocin is probably not essential for human parturition, because delivery still occurs in anencephalic fetuses, which lack a posterior pituitary, and in which oxytocin can not be detected in the umbilical circulation (Otsuki et al, 1983). It would therefore appear that if fetal release of oxytocin plays a role in human parturition, it may be as a signal from the fetus that alters the balance of other factors in favour of uterine activity (Chard, 1989).

Vasopressin

The non-pregnant uterus is more sensitive to vasopressin than to oxytocin (Fuchs and Fuchs, 1984), and

vasopressin can be measured in fetal pituitary extracts from the second trimester of pregnancy (Burford and Robinson, 1982). In early pregnancy, there is a high vasopressin to oxytocin ratio, which gradually decreases towards unity in the neonate (Dicker and Tyler, 1953). Although high vasopressin levels are demonstrated in human umbilical cord blood at delivery (Chard et al, 1971), no correlation has been found between the maternal and fetal vasopressin levels (DeVane and Porter, 1980). Vasopressin concentrations are not elevated in women having a spontaneous delivery compared with a caesarean section (DeVane, 1985), and vasopressin levels are similar during the first and second stages of labour (Gibbens and Chard, 1976). From this evidence it would appear that vasopressin may not be involved in the initiation or maintenance of parturition.

Relaxin

The role of relaxin in parturition is unclear, but this polypeptide hormone is thought to promote connective tissue refashioning during pregnancy, inhibit myometrial contractility until late pregnancy and help cervical ripening at parturition (MacLennan, 1981 and 1983). The role of relaxin can now be further investigated as recently a radioimmunoassay has been developed for the hormone (Eddie et al, 1986). Relaxin was not detected in non-pregnant women, but was found in pregnant patients. Levels were highest in the first trimester, and consistently low after 24 weeks gestation. Maclennan et al

(1986) also reported lower relaxin levels in the third compared with the first or second trimesters of pregnancy. However, Quagliarello et al (1980) and Eddie et al (1986) found no consistent changes in levels towards term.

Relaxin has been isolated from cultured human decidua and placenta (Bigazzi et al, 1980; Yamamoto et al, 1981) and localised immuno-histochemically in the decidua and chorionic cytotrophoblast (Bryant-Greenwood et al, 1987). A paracrine action of relaxin has been suggested by the demonstration of specific receptors in amnion and chorion, and by increased production of plasminogen activator and collagenase activity by these tissues in response to relaxin in vitro (Koay et al, 1986). Recently Lopez Bernal et al (1987b) have demonstrated that relaxin, present in the chorion and decidua, may have a paracrine effect on the amnion, inhibiting prostaglandin production during continuing pregnancy but favouring its production in labour.

Prolactin

Prolactin is found in large amounts in the amniotic fluid of humans and other primates (Orgen and Talamantes, 1988; Bonney and Franks, 1989), and immunocytochemical studies have shown that production of the hormone is predominantly by the parietal decidua cells and to a lesser degree the chorionic cytotrophoblast (Bryant-Greenwood et al, 1987). Amniotic fluid levels rise progressively after the 14th week of gestation and decline slightly during the

third trimester (Kletzky et al, 1985). However, the ability of decidua to produce prolactin appears to be similar in both trimesters (Tomita et al, 1982), therefore the fall in amniotic fluid levels in late pregnancy may reflect decreased permeability of the fetal membranes to prolactin (Tyson et al, 1985).

Prolactin secretion by the decidua appears to be regulated by a different mechanism to pituitary prolactin. The hormone is not stored in secretory granules prior to release (Handwerger and Capel, 1985), and neither decidual nor amniotic fluid prolactin are affected by dopamine or dopamine agonist drugs (Healy and Hodgen, 1983). Although oestrogen has a strong stimulatary effect on pituitary prolactin secretion, it has minimal effects on decidual prolactin production (Rosenberg and Bhatnager, 1984). However, progesterone stimulates decidual prolactin secretion (Ying et al, 1988), which is inhibited by the anti-progesterone agent mifepristone (Chen et al, 1989).

The precise role of decidual prolactin is unclear. The hormone may be important in the regulation of water and ion transport across the amnion (Davis, 1990), and prolactin stimulates progesterone production by the placenta (Barea et al, 1989). Prolactin has also been reported to reduce production of prostaglandin E_2 by the fetal membranes in late gestation, which has suggested that decidual prolactin may have a role in inhibiting labour (Tyson et al, 1985).

Placental steroids

Parturition in the human, unlike in the sheep and in some other species, is not preceded by changes in maternal peripheral plasma levels of oestrogen or progesterone, and although the evidence is suggestive that these steroids may be important in human parturition, their exact role remains uncertain. In human peripheral venous blood and amniotic fluid, the levels of both steroids increase up to the onset of both term and pre-term labour (Turnbull et al, 1977; Bibby et al, 1980). Recently, Darne et al (1987) reported an increase in the salivary oestriol:progesterone ratio most marked five weeks before the onset of spontaneous human labour, and as salivary steroid concentrations reflect the circulating concentrations of the free hormone, this might be more biologically relevant than the plasma levels. However, this was not confirmed in a similar study by Lewis et al (1987).

These findings do not exclude the possibility that changes in oestrogens or progesterone in target cells may play a part in the initiation of labour. Mitchell et al (1982) have shown that oestrogens may be capable of both stimulating prostaglandin synthesis in cell preparations of fetal membranes, and also of inhibiting progesterone synthesis in the decidua and chorion. Therefore, these tissues could be subjected to local progesterone withdrawal and increased oestrogen action great enough to initiate labour without any detectable changes in peripheral plasma hormone levels.

Generally, oestrogen and progesterone have opposing effects on the myogenic properties of the myometrial cells, with oestrogen stimulating and progesterone inhibiting uterine activity. Oestrogen has been found to increase oxytocin receptors in the myometrium (Fuchs et al, 1983b), and evidence from studies in rats and sheep have shown that the steroid also aids in gap junction formation between adjoining myometrial cells (Garfield et al, 1980b; Kawarabayashi & Marshall, 1981; Sanfilippo et al, 1983). Both these findings may affect uterine activity.

Csapo (1956) proposed the progesterone block theory when he demonstrated that a fall in progesterone converted the myometrium from inactive to an active and reactive muscle. The theory stated that the maintenance of pregnancy was determined by the ability of progesterone to suppress myometrial contractility, and when the hormone declined myometrial activity gradually intensified to produce labour contractions. The theory was later expanded to the "see-saw theory" to include the effects of stimulants (prostaglandins, oestrogens and oxytocin) of myometrial activity during the onset of parturition (Csapo, 1977). The see-saw theory predicted that the maintenance of pregnancy involved a balance in the myometrium of opposing forces, between stimulants and suppressors, whereas the onset and progression of labour depended upon a regulatory imbalance (decrease in progesterone) in favour of the stimulants (increase in oestrogen and prostaglandins).

Part of the reluctance to accept Csapo's theories comes from the fact that progesterone does not decline significantly prior to human labour and progesterone administration does not inhibit labour (Thorbert, 1979). Recent studies show that anti-progesterone drugs, especially mifepristone, are efficient abortifacients at any time during human pregnancy. These studies would support the fundamental role of progesterone in the maintenance of pregnancy, and a functional progesterone withdrawal could occur at the receptor level without a decline in tissue or plasma levels. This would explain why progesterone withdrawal is unnecessary in humans and why administration of progesterone does not stop uterine contractility, thus confirming Csapo's see-saw theory.

Progesterone has been shown to antagonise some of the oestrogen mediated activities by inhibiting the replenishment of cytosolic oestrogen receptors (Fuchs & Fuchs, 1984) and preventing the development of gap junctions in the myometrium (Garfield & Hayashi, 1981). However, in humans it does not effect the oxytocin receptor levels in the uterus. Progesterone also diminishes cell wall permeability to calcium and increases intracellular calcium binding (Mitchell, 1987b; Huszar and Walsh, 1988).

In summary, progesterone withdrawal is such an important feature of the initiation of labour in so many species that local progesterone inhibition and its release remain a regulatory option for human pregnancy maintenance and the initiation of labour, but although this

possibility has not been finally excluded it is now largely discounted (Turnbull, 1989). It therefore appears that although progesterone is essential for the maintenance of human pregnancy, parturition begins and progresses to delivery without progesterone being withdrawn.

1.2. - PROGESTERONE BIOCHEMISTRY

Introduction

Progesterone is a unique reproductive hormone, which is essential for the establishment and maintenance of pregnancy in humans. Progesterone causes the endometrium to undergo decidualisation, which is necessary for implantation of the blastocyst that occurs during the second week after fertilisation. The hormone helps decrease the responsiveness of the uterine myometrium to contractile, excitatory agents such as prostaglandin or oxytocin, and it also firms the cervix and favours formation of a mucous plug (Baulieu, 1989).

Subsequent to ovulation, progesterone is secreted by the corpus luteum. The corpus luteum is partly under the control of pituitary LH during the menstrual cycle, and its life span is remarkedly constant at 14 days. The functional demise of the corpus luteum is associated with a rapid fall in progesterone and oestradiol concentrations, and endometrial disintegration and shedding. However, if a fertilised ovum implants, human chorionic gonadotrophin, produced by embryonic chorionic cells, prolongs the life span of the corpus luteum and therefore continued progesterone production occurs. The corpus luteum ceases to be essential for continuing pregnancy after 7 weeks gestation, when its endocrine function has been taken over by the placenta (Csapo et al, 1973; Navot et al, 1986), although it may continue to produce hormone in small

amounts throughout pregnancy (Weis et al, 1977). The placenta continues to produce progesterone throughout pregnancy, which inhibits further ovulation via a negative feedback effect on the hypothalamic-pituitary LH release system.

Progesterone is also involved earlier in the cycle with follicle development and ovulation. Folliculogenesis depends in part on intra-ovarian progesterone, which is not secreted into the blood but is active locally. A small increase in plasma progesterone levels occurs prior to ovulation, which reinforces the positive feedback effect of oestradiol in the triggering of the midcycle LH surge. During the normal luteal phase progesterone is released in a pulsatile pattern, probably controlled by intermittent secretion of luteinising hormone from the anterior lobe of the pituitary (Filicari et al, 1984). However, the exact mechanism of progesterone secretion during a pregnancy cycle is incompletely understood as hCG alone cannot induce progesterone secretion from the normal corpus luteum for more than a few days (Wilks & Noble, 1983), so how progesterone secretion is controlled beyond this time is unknown (Crowley, 1986).

Progesterone Structure and Synthesis

Progesterone is a cyclopentanoperhydro-phenanthrene steroid (also known as a sterone), and like other steroid hormones has a methyl group attached to carbon 13. Progesterone, like androgens, glucocorticoids and

mineralcorticoids, also has a second methyl group attached to the carbon 10 atom. All these hormones, except androgens, have a 2 carbon side chain attached to the carbon 17 atom. The variation in position and number of attached ketone or alcohol groups, and the position of the double bonds between the carbon groups within the basic structure, gives rise to the spectrum of steroid hormones produced, and accounts for the variety of effects (Martin, 1985).

Progesterone is synthetised by the placenta from cholesterol which is derived from at least two sources (Conley and Mason, 1990). It can be formed de novo from two carbon units in a series of reactions. Alternatively, low-density lipoproteins (LDL) in the maternal circulation bind to specific LDL receptors on the trophoblast cell surface and are internalised. Subsequent degradation of the protein component of LDL and hydrolysis of the cholesterol ester yields free fatty acids and cholesterol. LDL receptors are detectable in villous trophoblast from six weeks gestation, and at least two forms of mRNAs are responsible for receptor synthesis (Conley and Mason, 1990). The larger a 5.9kb mRNA is more abundant than the 3.7kb mRNA species, but while levels of the 3.7 kb fragment remain constant throughout pregnancy, the relative levels of the 5.9 kb mRNA species declines with increasing gestation (Furuhashi et al, 1989).

Cholesterol is converted to pregnenolone in

placental mitochondria by the action of the enzyme cytochrome $P450_{scc}$ (Mason and Boyd, 1971). Cytochrome $P450_{scc}$ catalyses the hydroxylations occurring at the C_{20} and C_{22} positions as well as the subsequent cleavage of 20,22R-dihydoxycholesterol to form pregnenolone and isocaproic aldehyde. At each step a pair of electrons from nicotinamide-adenine dinucleotide phosphate (NADPH) are transferred to $P450_{scc}$ via two accessory electron transfer proteins, adrenodoxin and adrenodoxin reductase. Finally, pregnenolone is readily converted to progesterone by steroid oxidoreductase and isomerase enzymes, which are commonly known as the 3-beta hydroxysteroid dehydrogenase enzymes (Ryan et al, 1966). Pregnenolone synthesis has been considered to be the rate limiting step in progesterone production, but it is more likely that this is due to a limiting substrate accessibility to $P450_{scc}$ than to a lack of enzyme (Conley and Mason, 1990).

Progesterone Binding and Plasma Levels

Progesterone binds to a variety of plasma proteins, mainly albumin, and transcortin or corticosteroid binding globulin (Rosenthal et al, 1969). It also binds to a lesser extent to sex hormone binding globulin and thyroxine binding globulin (Gurpide & Holinka, 1980). The levels of these proteins increase in pregnancy under the influence of oestrogens. The obvious result of the increase in binding protein levels is an increase in the plasma levels of progesterone bound to them, but the percentage of unbound progesterone remains unchanged during pregnancy at between

2-11% (Yannone et al, 1969). Also the percentages of progesterone distributed amongst the various binding globulins is relatively unchanged by pregnancy (Rosenthal et al, 1969). Since only unbound progesterone is thought to bind with the receptors at a cellular level, control of secretion and feedback regulation may depend on the concentration of unbound hormone level (Gurpide & Holinka, 1980).

Pregnancy is associated with a marked increase in total peripheral plasma progesterone levels. The total progesterone level rises from a mid-luteal (non-pregnant) range of 8-17 ng/ml. to a third trimester range of 120-180 ng/ml. (Tulchinsky & Okada, 1975). The unbound level rises much less from a non-pregnant level of 0.7-0.8 ng/ml., to 1.1-2.3 ng/ml. in the first trimester, and finally 2.2-10 ng/ml. in the third trimester (Rosenthal et al, 1969; Batra et al,1976). After termination or delivery, the plasma progesterone falls very rapidly. Fetal death is not associated with a significant reduction in the maternal plasma progesterone level, indicating that the fetus plays little part in the maintenance of the concentration of progesterone in maternal plasma (Lurie et al, 1966).

Fetal Progesterone Levels

The fetus is exposed to high plasma concentrations of progesterone from the placenta. The mean ± (SEM) umbilical artery progesterone concentration at term is 500 ± (80) ng/ml., compared with a mean ± (SEM) of 1020 ± (100) ng/ml.

in the umbilical vein (Mathur et al, 1980). Tulkinsky & Okada (1975) showed that the mean concentrations of total and unbound plasma progesterone at term were five and sevenfold higher respectively in the umbilical vein than in maternal plasma.

The difference in umbilical vein and artery plasma progesterone levels suggests that the fetus clears about 50% of the steroid it receives through the umbilical vein, equivalent to 35 mg. of progesterone each day (Maynard et al, 1980). The fetus uses much of the progesterone for steroid synthesis, but the necessity for such large amounts remains unclear, as does the control of placental progesterone secretion.

Unlike maternal levels, the progesterone concentration in amniotic fluid is highest during the second trimester of pregnancy, and after 20 weeks gestation the levels gradually fall (Johansson & Jonasson, 1971). This may be due to a combination of declining placental transfer to the fetus, increased utilisation and metabolism by the maturing fetus, reduced entry to the amniotic fluid, or dilution of the amniotic fluid progesterone level by increasing amniotic fluid volumes (Warne et al, 1977).

Progesterone Receptors

Few physiological studies have been performed on progesterone receptor action in the human uterus, and most of the information on receptors comes from work on the

oviduct of the chicken. This receptor is a dimer of 2 distinct proteins: Protein A (M.W. 80,000 daltons) and Protein B (M.W. 110,000 daltons). Each component can bind separately with progesterone. Protein A binds to DNA, whilst Protein B binds preferentially with the chromatin and nonhistone proteins of the chromosomes (Schrader et al, 1972). The human progesterone receptor also consists of two parts: the A form [M.W. 84,000 daltons] and the B form [M.W. 114,000 daltons] (Lessey et al, 1983; Horwitz, 1985). Progesterone binds with high affinity to the complete receptor, but the resultant complex is very labile and rapidly dissociates (Healy, 1985).

Investigators have studied endometrial steroid hormone receptor kinetics in the natural menstrual cycle in humans (Bayard et al, 1978; Kreitman et al, 1979; Pollow et al, 1981; McRae et al, 1984) and primates (Martel et al, 1980; West & Brenner, 1983). The results are variable but there is general agreement that both oestrogen and progesterone receptor concentrations increase during the follicular phase of the cycle under the influence of oestradiol stimulation (Milgrom et al, 1973; Levy et al, 1980). The protein progesterone receptors are produced by the endometrial epithelial cells, and approximately 12,000 progesterone receptors per endometrial cell are present at mid-cycle (Walters & Clark, 1979). The progesterone receptor level falls progressively throughout the luteal phase, as increasing progesterone produced by the corpus luteum "down regulates" the receptor concentration (Brenner

et al, 1974; Hsueh et al, 1975; Tseng & Gurpide, 1975; Clark et al, 1977; West et al, 1986).

The action of a steroid is mediated by its receptors, however the exact mechanism of receptor action has recently been questioned. The traditional theory is that steroids diffuse into the cytoplasm where they combine with receptor proteins. Following steroid binding, the receptor undergoes configurational changes and translocates into the nucleus where it binds to chromatin and therefore gene transcription is initiated (O'Malley et al, 1971; Jensen & De Sombre, 1973; Gorski & Gannon, 1976; Muldoon, 1980). The cytoplasmic receptor might be considered as available receptor and the proportion of nuclear to cytoplasmic receptor would be expected to increase as the circulating hormone concentration increased. However, recent studies have raised doubts on this traditional "two step" theory of receptor action (Schrader, 1984). King & Greene (1984), and Welsons et al (1984), demonstrated that the steroid receptors reside primarily in the nucleus both in the presence and absence of steroid, and they concluded that the cytosolic receptor was an extraction artefact caused by tissue homogenisation. These results have recently been confirmed by others (Gasc et al, 1984; Perrot-Applanat et al, 1985; Gravinis & Gurpide, 1986).

There can now be little doubt that the unoccupied receptor is located in the nucleus, but the unoccupied receptor which is recovered in the low salt fraction of a tissue homogenate (cytosolic receptor) may represent

receptor that is loosely bound with nuclear components (King, 1987). Binding of the receptor results in tighter nuclear association, and it is this phenomenon which has previously been interpreted as translocation from the cytoplasm to the nucleus (Greene & Press, 1986). Furthermore, although the majority of unoccupied receptor resides in the nucleus, it is possible that some still exists in the cytoplasmic form (King, 1986). The new thoeries of steroid receptor binding are still compatible with the "two-step" theory of receptor action, in that an inactive unassociated progesterone receptor is activated after binding to progesterone and the steroid-receptor complexes regulate nuclear function (Schrader, 1984; Agarwal & Lazar, 1987).

Research on progesterone receptor antagonists has further investigated the structure of the receptor. Baulieu (1989) suggests that prior to ligand binding, nuclear steroid progesterone receptors are hetero-oligomeric in the so called 8S form as detected in gradient centrifugation experiments. The receptor includes a hormone binding unit and a non-steroid binding, non-DNA binding heat shock protein with a relative molecular mass of 90-kD (hsp-90). In the absence of progesterone, the ligand-binding domain of the receptor is capped by hsp-90, which also caps the DNA-binding domain of the receptor. Therefore, the receptor is a nuclear, non-DNA binding protein complex. Progesterone binding induces a conformational change of the ligand-binding domain, resulting in dissociation of hsp-90,

thereby permitting the receptor binding to the hormone response elements of regulatory genes and DNA activation.

THE ANTI-PROGESTERONE STEROIDS

The development of the progesterone antagonists has highlighted the fundamental role of progesterone in maintaining pregnancy, because one of the most important tools used to study the physiology of a hormone is an antagonist to its action. So far, two main types of antiprogesterones have been developed; the progesterone synthesis inhibitors; and the progesterone receptor blockers.

Progesterone Synthesis Inhibitors

Trilostane (WIN 24,540)

Trilostane has been used successfully in the treatment of Cushing's syndrome (Komanicky et al, 1978), primary hyperaldosteronism (Semple et al, 1982), and low renin hypertension (Liddle et al, 1976). Although trilostane has been shown to inhibit progesterone production in early human pregnancy, the dosage needed to produce abortion also significantly suppresses adrenal steroid production as the drug is a preferential adrenal steroid inhibitor (van de Spuy et al, 1983).

Azastene (WIN 17,625)

Creange et al, (1978) showed that azastene inhibited 3 beta-hydroxysteroid activity, suppresses progesterone production, induces progesterone withdrawal and thereby disrupts pregnancy when given to rats. They also showed that concurrently given progesterone completely abolishes

the effect of azastene on pregnancy. This effect has also been documented in rhesus monkeys (Schane et al, 1978). In rats, but not monkeys, it also antagonises cortisol production. However in the presence of hCG, azastene does not induce luteolysis in women and therefore the drug appears to have little potential for human fertility control (Chatterton, 1981).

Epostane (WIN 32,729)

Epostane (4,5 epoxy-17 hydroxyl-4, 17 dimethyl 3 oxo androstane-2 carbonitrile) is an orally effective 3 beta-hydroxysteroid dehydrogenase inhibitor both in vitro and in vivo (Pattison et al, 1984). The compound does not have any intrinsic hormone activity and is non-androgenic. It has been shown to inhibit progesterone production in rats and rhesus monkeys at a level that does not affect adrenal corticosteroid production (Creange et al, 1981). Unpublished research data by the Sterling Winthrop company, who developed the drug, have shown that epostane is free from toxicity in acute studies on mice, rats, rabbits and healthy male volunteers, and in chronic studies over 6 months, in rats and monkeys.

Epostane produces a significant lowering of peripheral progesterone levels in the luteal phase of the menstrual cycle (Birgerson & Johansson, 1983; Pattison et al, 1984), and in the first trimester (Birgerson & Johansson, 1983; Van der Spuy et al, 1983; Pattison et al, 1984; Webster et al, 1985a; Webster et al, 1985b; Birgerson

& Odlind, 1986; Crooij et al, 1986a), and second trimester of pregnancy (Pattison et al, 1984; Selinger et al, 1987a).

Epostane does not appear in cord blood or amniotic fluid after maternal ingestion, but the fetal progesterone level falls by 95% after treatment (Selinger et al, 1988). This suggests that epostane may be able to cross the placenta, and its subsequent effects on the fetus are unknown.

Progesterone Receptor Antagonists

Gestrinone (RU 2323)

Gestrinone, a derivative of 19-nortestosterone, exhibits a potent antiprogesterone activity in animals, as well as some androgenic activity and moderate pituitary inhibition. The mechanism of its antiprogestagen action seems to be due to the inability of the nuclear steroid-receptor complex to activate the chromatin template (Robyn et al, 1984).

If given in large doses before implantation gestrinone has a low contraceptive efficacy, probably mediated mainly through ovulation suppression, but the drug has no abortifacient effect if given after implantation (Sakiz et al, 1974; Mora et al, 1975).

More recently gestrinone has been shown to be very effective in the medical treatment of endometriosis where it inhibits pituitary activity, leading to a suppression of

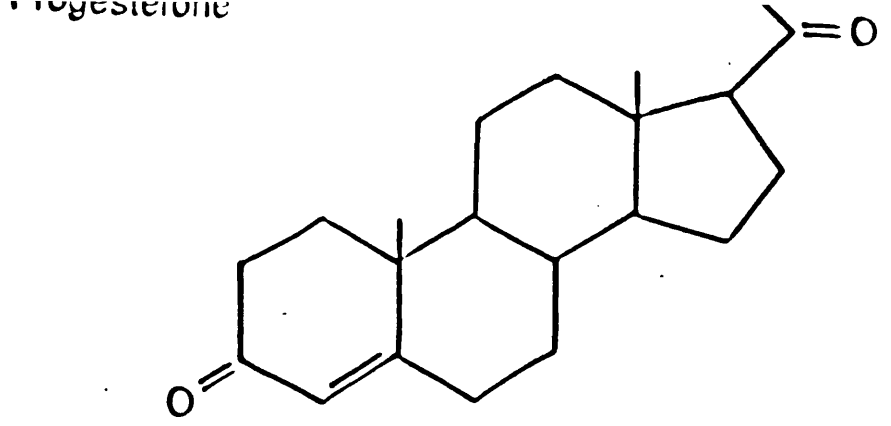
gonadal action, and also a direct antiprogestin effect in peripheral target tissue (Azadian-Boulanger et al, 1984). The drug has recently been granted a product licence in the United Kingdom for its use in the management of endometriosis.

MIFEPRISTONE (RU 38,486)

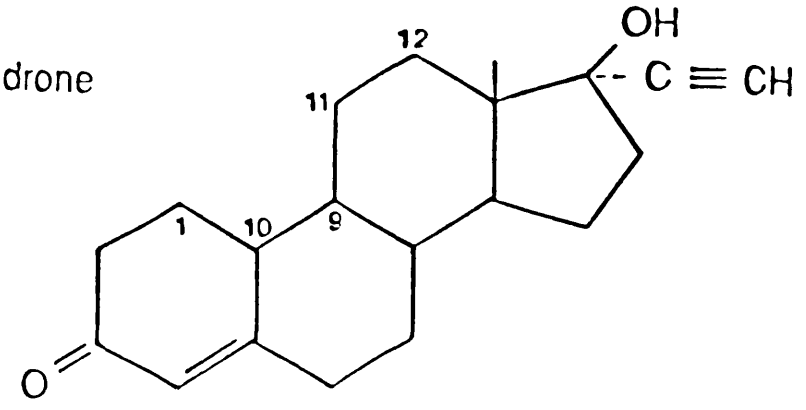
Pharmacological and Biological Effects

Mifepristone (17 beta-hydroxyl-11 beta-4 dimethylamino -pheny-17a-1 propynyl esta-4, 9 dien-3-one, Roussel-Uclaf, Paris) is a synthetic derivative of norethindrone with an additional side-chain at C17 to increase progesterone receptor affinity, and a b-phenyl group cycle at C11 which gives the determining role in conferring antagonistic activity (Baulieu, 1985b) [figure 1]. The drug binds to the progesterone receptor in the rabbit uterus more avidly than progesterone (Philibert et al, 1985), but its affinity for the human progesterone receptor is approximately equal to progesterone (Grievance et al, 1985). Mifepristone also binds to the glucocorticosteroid receptor, for which it has greater affinity than dexamethasone (Moguilewsky & Philibert, 1985; Schneider et al, 1988), and to the androgen receptor, but with lower affinity than testosterone. Mifepristone does not bind to oestrogen or mineralcorticoid receptors (Healy, 1985).

Mifepristone has a bioavailability of 30-56% in humans, with 85% of the steroid being absorbed orally (Deraedt, 1985). Peak plasma concentrations are achieved in



Norethindrone



Ru 38,486

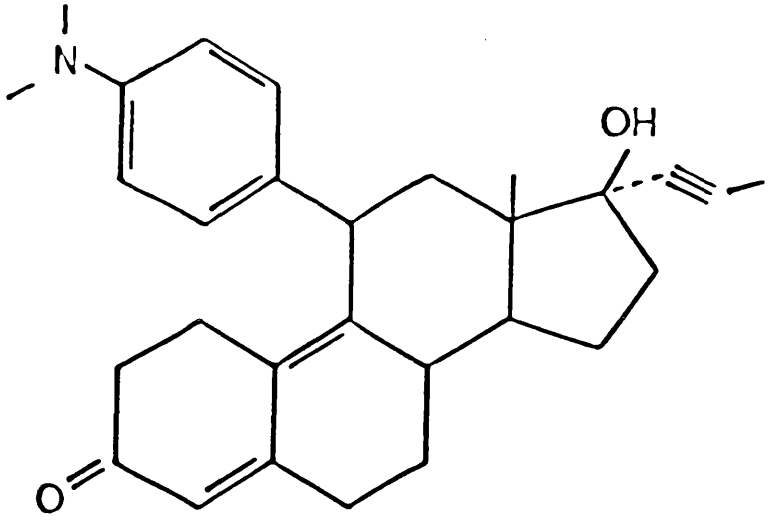


Figure 1 - Structure of progesterone, norethindrone and mifepristone

1-2 hours, thereafter the serum levels remain stable (approximately 2.5 umol/l) for the next 24-48 hours, and are measurable for up to 5-7 days after ingestion (Swahn et al, 1986; Liu et al, 1988, Heikinheimo, 1990). The pharmacokinetics of mifepristone are non-linear: within the dose range of 100-800mg, neither the peak nor the steady state serum levels of the drug correlate with the dose ingested (Heikinheimo 1990). After absorption, the drug binds avidly to alpha-1 acid glycoprotein (AAG) and albumin (Heikinheimo et al, 1987a) and therefore has a long half-life of 24 hours (Kawai et al, 1987) to 54 hours (Liu et al, 1988; Heikinheimo, 1989). It does not bind to transcortin or sex hormone binding globulin (Baulieu, 1985a; Nieman et al, 1985). The correlation between the principle serum transport protein (AAG) and the serum concentrations of mifepristone are highly significant. At serum concentrations below 2.5umol/l, the free fraction of mifepristone is only 2.3%, however, with increasing concentrations, the fraction of free mifepristone, which is available for excretion or tissue distribution, increases rapidly (Heikinheimo, 1990).

The drug is metabolised by two-step demethylation and by hydroxylation in the liver (Deraedt et al, 1984) and the major metabolite, RU 42,633 (17b-hydroxyl-11b-[4-monomethylaminophenyl]-17a[1-propynyl-estra 4, 9-dien-3-one) is a N-demethylated form of the compound (Appendix 1), which also has antiprogesterone and antiglucocorticoid activity (Heikinheimo et al, 1987b) [Appendix 2]. Over 90%

of the drug is excreted in the faeces, with recycling via the enterohepatic circulation occurring (Liu et al, 1987).

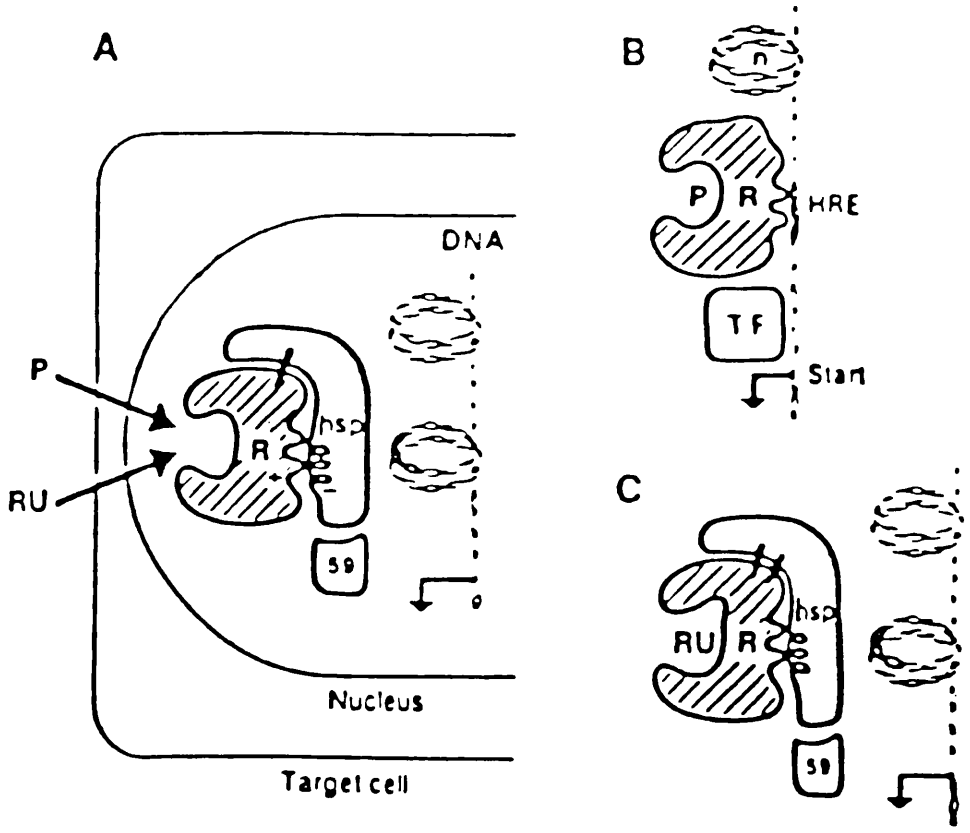
Toxicology studies in mice and rats showed no acute toxic effects after mifepristone treatment, but after 30-day toxicology tests in both rats and monkeys evidence of adrenal suppression occurred (Healy, 1985). Lamberts et al (1991) investigated the endocrine effects of long-term therapy of mifepristone 200mg daily for one year in ten patients with meningiomas. Treatment with mifepristone activated the hypothalamic-pituitary-adrenal axis, resulting in a resetting of the system at a higher level at which the cortisol diurnal rhythm was maintained. Secondarily, the production of androstenedione and oestradiol increased significantly, probably related to induction of partial cortisol receptor resistance.

Mifepristone has teratogenic effects in the rabbit but not in any other species (Jost, 1986), and it may have been associated with a malformed fetus aborted in the second trimester after the mother took the drug to terminate an early pregnancy (Henrion, 1989). Lim et al (1990) reported normal development in three fetuses after exposure to mifepristone in early pregnancy. However, in view of the sparsity of data, a teratogenic effect in humans can not be excluded.

The action of mifepristone is via competitive antagonism with progesterone for receptor sites. The progesterone receptors consist of a hormone-binding unit, a

non-steroid-binding non-DNA-binding 90-kD heat shock protein (hsp-90) and a nuclear protein (p59) [Baulieu EE, 1989a]. The hsp-90 binds to the ligand-binding domain of the receptor and caps the DNA-binding receptor domain. Therefore, in the absence of progesterone, the receptor is a nuclear non-DNA-binding protein complex [figure 2a]. Progesterone binding induces a conformational change of the ligand domain, resulting in dissociation of the hsp-90, therefore allowing receptor binding to the hormone response elements of regulator genes. These in turn allow binding of a transcription factor and hence DNA transcription [figure 2b]. When mifepristone binds to the progesterone receptors, the binding of the hsp-90 is stabilised and the receptor remains in a non-DNA-binding form [figure 2c] (Baulieu EE, 1989b). Alternatively mifepristone may be able to interact with human progesterone receptors, thereby allowing receptor transformation. Evidence from work performed in vitro on mouse mammary tumour virus suggests that progesterone receptors transformed by mifepristone exhibit no impairment in binding to specific DNA sites of target genes, but when bound to DNA assume a structurally different form compared with the receptor-agonist complex (El-Ashry et al, 1989). Therefore the failure of mifepristone-receptor complexes to activate transcription may result from this structural alteration in progesterone receptors, which does not permit the protein-protein interactions required for receptor-mediated induction of gene transcription.

Figure 2 - The receptor mechanism of action of mifepristone



Mifepristone also blocks the glucocorticoid receptor, therefore the drug can act as an anti-glucocorticoid agent, but a 50-fold difference between a significant endometrial response and a significant anti-glucocorticoid action has been reported (Healy, 1985). In rhesus monkeys Healy et al (1985) have demonstrated a dose and time dependent increase in ACTH and cortisol after mifepristone treatment. However in the human, although increased ACTH and cortisol levels have been reported (Kovacs et al, 1985; Couzinet et al, 1986; Shoupe et al, 1986; Nieman & Lorieux 1988b), the hormone rise has been modest. The mean levels have not exceeded the normal range (Bygdeman & Van Look, 1988), and no disruption of the diurnal pattern occurred (Liu et al, 1988). Besides ACTH and cortisol, an increase in prolactin has been reported after mifepristone treatment (Swahn et al, 1988a). Similarly a transient increase in aldosterone and 17ß-oestradiol occurs during the first 2-3 days following the start of the drug treatment (Bygdeman & Van Look, 1988). No other oestrogenic or mineralocorticoid effects and no androgenic or thyroid effects of mifepristone have been observed in humans (Baulieu, 1985a).

In an attempt to discriminate between progesterone and glucocorticoid receptor binding, several structurally related analogs of mifepristone have been synthetised (Teutsch et al, 1988), and studies on these drugs are awaited.

1.4. - CLINICAL STUDIES ON THE ANTI-PROGESTERONE STEROIDS

Epostane

Due to the suspension of the development of epostane in 1987 only a limited number of studies have been performed using this drug in humans.

Webster et al (1985a) treated 20 women between 8-11 weeks gestation in a double blind, placebo controlled trial with epostane 100mg or placebo for three days. In the patients treated with epostane the peripheral plasma progesterone level fell, and was associated with increased uterine activity compared to the placebo controls. However they failed to demonstrate a difference in uterine activity following oxytocin stimulation between the 2 groups. These workers also showed in a further 20 women an increased reactivity to PGE_2 pessaries in patients treated with epostane over controls (Webster et al, 1985b). This effect was dose related and the highest dose (600mg) was able to induce abortion in early pregnancies without affecting cortisol levels. At this dose the progesterone concentration in the epostane treated group had fallen by 92%.

Other studies have confirmed the ability of epostane to cause abortion in early pregnancy. Birgerson & Odlind (1986) reported a success rate of 73% in 26 women of less than 7 weeks gestation who were treated with 200mg of epostane 8 hourly for 7 days. Crooij et al, (1986) found

that the success rate of termination with epostane was gestation related. They treated 50 women between 6-12 weeks gestation with 800mg of epostane per day in divided doses. The overall abortion rate was 82%, but the group with 7-8 weeks amenorrhoea had the highest success rate of 87%. They also found that the success rate in inducing abortion was higher in multigravidae.

Webster et al, (1987) showed that the abortion success rate with epostane was also dose related. Thirty women of less than 7 weeks gestation were treated with epostane 200mg 8 hourly for 7 days. These were compared with 24 women of similar gestation who received epostane 200mg 6 hourly for 7 days. Although in both groups the serum progesterone levels fell and the serum cortisol level was not affected, the success rate in achieving abortion was 70% and 87% respectively. The authors observed that spontaneous uterine activity only developed after the progesterone level had fallen below a critical value of 1-2ng/ml.

Selinger et al, (1987a) showed that the mean peripheral progesterone level fell by 74% after treatment with epostane in the aid second trimester. In this double-blind, placebo controlled trial they demonstrated that epostane significantly decreased the mean induction-abortion interval in the treated group over controls. In the same study intra-uterine pressure recordings demonstrated increased sensitivity of the uterus to prostaglandin E_2 after epostane treatment, but no change in

oxytocin sensitivity.

MIFEPRISTONE (RU 38,486)

During the Menstrual Cycle

Herrmann et al (1982) were the first to demonstrate that mifepristone treatment during the luteal phase of the cycle induced menstruation. Patients received mifepristone 200mg/day for 4 days, and the cycle was interrupted with endometrial bleeding starting on the second or third day of treatment. There was irreversible luteolysis with rapidly falling progesterone and oestrogen levels, associated with a decrease in the luteinising hormone (LH) and follicular stimulating hormone (FSH) levels. These results were interpreted in terms of anti-progesterone activity at the endometrium causing menstruation, and at the hypothalamus and/or pituitary causing a decrease in LH and subsequent luteolysis.

Schaison et al, (1985a) gave mifepristone (25-100 mg/day) to 32 women with a normal menstrual cycle from days 19-23 and induced bleeding within four days in 29 of 32 women. In 15 a further episode of bleeding occurred at the expected time of menstruation implying that mifepristone acted directly on the progesterone receptors in the endometrium in the absence of luteolysis. Also, menstruation could not be prevented by administration of hCG in the mid-luteal phase of the cycle (Nieman et al, 1987). Kelly et al, (1986) demonstrated that treatment with

mifepristone led to release of prostaglandin from the endometrial stromal cells. They proposed that the generation of prostaglandins, in synergism with any direct action of the antiprogestin on the myometrium, should stimulate myometrial contractility, vasoconstriction, ischaemia and tissue death, which accounts for the menstruation inducing properties of the drug.

The effect of mifepristone on the menstrual cycle depends on the timing of administration. If the drug is given in the first three days of the follicular phase of the menstrual cycle then no discernible impact upon follicular recruitment, LH surge, luteal phase length, or progesterone secretion occurs (Stuenkel et al, 1990). Although no impairment of the overall integrity of the cycle occurs, there is a functional decrease in oestrogen levels. This thought to be due to impaired folliculogenesis (Luukkainen et al, 1988) but may be due to a direct inhibitory action on steroidogenesis (Dimattina et al, 1987).

If mifepristone is given during the follicular phase on days 11-13 of the cycle and therefore after emergence of the dominant follicle, endometrial bleeding does not occur (Herrmann et al, 1985; Swahn et al, 1988b). A decrease in oestradiol concentration occurs followed by a rebound increase in gonadotrophin, renewed folliculogenesis, and commencement of a new cycle (Liu et al, 1987). The mid-cycle surge of LH is delayed (Spitz et al, 1989), and the fall in circulating oestradiol is

associated with collapse of the dominant follicle (Shoupe et al, 1987).

Mifepristone treatment in the first half of the luteal phase inhibits glandular secretory activity (Graham et al, 1991), accelerates degenerative changes and induces vascular changes in the endometrium (Li et al, 1988). However, menstruation induction has variably been reported to occur in 42% (Li et al, 1988) to 73% (Nieman and Loriaux, 1988) of treated patients. This bleeding occurs despite high circulating levels of oestradiol and progesterone and presumably represents a direct effect on the endometrium (Shoupe et al, 1987). In approximately one third of patients, this represents the only bleeding episode and is associated with reduction in serum FSH, oestradiol and progesterone concentrations, and a shortened cycle length (Spitz et al, 1989). In the remaining patients, no changes in hormone values occurs and after some days a further bleed occurs which is associated with spontaneous regression of the corpus luteum.

If given in the late luteal phase, when normal endogenous progesterone levels are decreasing, mifepristone treatment results in consistent induction of menstruation in 1-3 days in 28 of 30 treatment cycles (Croxatto et al, 1987). Although the luteal phase of the cycle was shortened, the follicular phase in the subsequent cycle was lengthened, so no overall disruption of the menstrual rhythm occurred. However since luteolysis occurs normally

after day 26 in the non-pregnant cycle, its occurrence cannot be unequivocally ascribed to mifepristone treatment.

It now appears from combining all the studies on mifepristone treatment in the luteal phase of the cycle, that if the drug is given after the sixth luteal phase day then endometrial shedding occurs in 93% of cases, but in only 73% if given before the fifth luteal phase day (Nieman & Loriaux, 1988). The minimum dose for this effect has not been established but Shoupe et al, (1987) found a single mid luteal dose of mifepristone 50mg to be effective.

Mifepristone has also been reported to modify ovarian steroidogenesis, with a dose dependent suppression of progesterone synthesis from cultured granulosa cells (DiMattina et al, 1986), and inhibition of 17-hydroxylase activity (DiMattina et al, 1987) both being reported. It has also been suggested that the drug may influence luteal function either directly or through an effect on pituitary gonadotrophin secretion. Schaison et al (1985b) reported a decrease in LH levels and the disappearance of the pulsatile pattern in a dose-dependant way after mifepristone treatment in the mid-luteal phase of the cycle. Shoupe et al (1990) confirmed a significant fall in LH secretion and LH pulse amplitude after mifepristone, but failed to demonstrate any significant change in LH pulse frequency after drug treatment. However, Asch & Rojas (1985) in the rhesus monkey, and Croxatto et al (1987) and Swahn et al (1988a) in the human female, could not demonstrate any decrease in mean gonadotrophin levels. But

in none of these three studies was the design suitable for measuring the pulsatile release of LH.

From these results it has been proposed that mifepristone has potential use as a once-a-month fertility controlling agent (Baulieu, 1985a; Spitz & Bardin, 1985). Before this can be achieved much more work is necessary, especially as work on monkeys has shown that if the drug is used for a prolonged period its effect influences the subsequent cycle (Nadler et al, 1985), and an ovulatory delay of 32 days has been reported following luteal-phase menstrual induction with mifepristone in rhesus monkeys (Hodgen, 1985). However, Croxatto et al (1987) have investigated the effect of luteal administration of mifepristone in 10 women for three consecutive cycles, and found that all but three cycles were normal.

Initial results using mifepristone as a monthly contraceptive have been discouraging. Van Santen & Haspels, (1987a) treated 7 women with mifepristone 400mg on day 27 of the cycle in a total of 24 cycles. Three pregnancies occurred, demonstrating a fertility rate of 12.5%. These results have since been confirmed by Couzinet et al (1990), who reported a pregnancy rate of 18.2% after a single dose of mifepristone 600mg given the day before the expected date of menses and a further dose eight days later in case of continuing pregnancy. These poor results may be due to the unpredictable time of ovulation in subsequent cycles after drug treatment causing imprecise timing of

drug administration. This failure rate may occur because, although mifepristone administration in the luteal phase of the cycle may induce menstruation, this may not be associated with shedding of the functional layer of the endometrium, and therefore pregnancy may continue undisturbed despite treatment (Li et al, 1988).

Kekkonen et al (1991) treated seven patients with mifepristone 25mg/day on days 1-14 of the follicular phase of the menstrual cycle, followed by norethisterone 5mg in the luteal phase for three cycles. Although levels of FSH and LH were normal, ovulation was suppressed. Evidence of ovulation and follicle growth occurred during norethisterone treatment in the first treatment period, however, no ovulation occurred in subsequent cycles. Although this study is small and of short duration, it suggests that sequential treatment with mifepristone and norethisterone could possibly be used as a contraceptive.

Postcoital Interception

A single episode of mid-cycle unprotected intercourse leads to a 35-67% chance of pregnancy (W.H.O., 1983). In postcoital interception, high-dose oestrogens (Haspels, 1985) and a low-dose combination of oestrogens and progestins (van Santen & Haspels, 1985) are effective if given within 72 hours postcoitum. The efficacy of treatment is not established, and depending on the studies, the failure rate (ratio of number of pregnancies to the number of expected pregnancies) is 3-32% (Yuzpe & Lanlee, 1977;

Dixon et al, 1980; Schilling et al,1987). A postcoital IUCD can be used up to five days after coitus as an abortifacient (Black et al, 1980), because all hormone interceptive regimens are ineffective once implantation has started, but this is not a suitable technique for nulliparous women (Editorial, 1983).

Van Santen & Haspels (1987b) treated 62 women who had unprotected mid-cycle intercourse with mifepristone 150mg/day from day 25-28 of the cycle. Although only limited beta-hCG screening was performed, a conception rate of 1.6% occurred as only 1 patient remained pregnant after treatment. In view of the limited beta-hCG screening, it is difficult to interpret the efficacy of the treatment from this study.

Lahteenmaki et al (1988) treated 30 women who had unprotected mid-cycle intercourse with a single oral dose of mifepristone 600mg one day before the expected onset of menses. Eighteen (60%) patients had conceived as tested by raised beta-hCG concentrations, and menstrual bleeding was induced in 29 (97%), demonstrating a true conception rate of 5.6%. The treatment was well tolerated, but it lengthened the post-treatment menstrual cycle by on average four days.

Dubois et al (1988a) report a multicentre study on 139 women who were treated with mifepristone (400 or 600mg) the day before their expected menses. Forty-eight (34.5%) were pregnant and an ongoing pregnancy after treatment was found

in nine (18.8%) cases. No disturbance in the menstrual cycle occurred.

Glaiser et al (1991) compared mifepristone 600mg as a single dose to the standard Yuzpe regimen (100ug ethinyl oestradiol and 1mg norgestrel taken twice, 12 hours apart) in 379 women. No patient treated with mifepristone conceived but two pregnancies (rate 1.1%) occurred in the patients who received the Yuzpe's regimen. However, no beta-hCG testing was performed on any of the patients.

In conclusion, when it is too late in the cycle for other postcoital contraceptives and too early for a vacuum aspiration, the late luteal administration of mifepristone constitutes a technique with an efficacy comparable to that of conventional forms of postcoital contraception.

Early termination of Pregnancy

In 1982 the results of the first study of mifepristone in early pregnancy termination were published (Herrmann et al, 1982). Mifepristone 200mg was given orally for 4 successive days to 11 women who were shown to be pregnant. Abortion occurred in 9 of the patients, establishing conclusively that the drug had the ability to block the progesterone effects in the early stages of pregnancy.

A subsequent collaborative study by the WHO included 37 women who were less than seven weeks pregnant (Kovacs et al, 1984). The patients received mifepristone 25, 50, or

100mg twice daily for 4 days. Twenty-two (61%) of the patients had a complete abortion within 2 weeks of starting treatment. Eleven (31%) had an incomplete abortion and in 3 (8%) there was apparent continuation of pregnancy. Two (5%) patients required a blood transfusion for heavy vaginal bleeding. The clinical efficacy was not dose related. Plasma progesterone levels did not significantly decline until after abortion had occurred, although plasma cortisol levels did increase after treatment. The authors concluded that mifepristone used alone had little to offer compared with surgical termination of pregnancy.

Haspels (1985) reported a trial of 33 women seeking termination treated with mifepristone 200mg orally per day for 4 days. The patients were divided into 2 groups by gestation: Group 1 (n=24) with amenorrhoea of under 8 weeks; and Group 2 (n=9) with amenorrhoea of 8-10 weeks. Although all patients bleed after treatment the success rate of termination in group 1 was 79% compared with 33% in group 2. Two (6%) needed a transfusion for heavy vaginal bleeding. Unlike the previous study, the cortisol levels in the patients were not statistically affected by mifepristone treatment.

Birgeson & Odlind (1986) reported a 61% complete abortion rate after mifepristone 50mg or 100mg in women who received the drug for 7 days. No difference in efficacy was seen between the 2 dose regimens. Shoupe et al, (1986) compared a high dose, short duration course of mifepristone (400mg/day x 4 days, or 200mg/day x 4 days,

n=10) with a medium dose, long duration course of treatment (100mg/day x 7 days, n=50). The complete abortion rate in group 1 was only 10% compared with 72% in the lowest dose group. The authors postulated that the results could be due to the drug either acting as a partial agonist at high doses, or that when high doses of the drug were given there may be more destruction to the endometrium thus preventing prostaglandin activity. Perhaps more likely is that only a small number of patients were treated with the high dose regimen, so the results may have occurred by chance, especially because when the dose of mifepristone was lowered to 50mg/day x 7 days the abortion success rate fell to 50% (Mishell et al, 1987).

Couzinet et al (1986) treated 100 women within 10 days of the expected onset of the missed period. The patients were divided into groups receiving mifepristone 400mg in 4 days (n=34), 600mg in 4 days (n=26), or 800mg in 2 days (n=40). The percentage of women with a complete abortion (85%) was similar in all groups. Only the highest drug dose had any effect on plasma cortisol.

Baulieu & Ulmann (1986) reported an 81% success rate for abortion if mifepristone 400-600mg was given as a single dose, therefore making drug administration simpler. Baulieu (1986) also proposed a model for how mifepristone actually causes an abortion. Blockage of progesterone activity on the decidualised endometrium causes detachment of the embryo, leading to a fall in ß-hCG and to secondary

luteolysis. This also leads to prostaglandin production, uterine contractility and hence abortion. Another primary effect of the drug is that it facilitates the opening of the cervix (Baulieu, 1985b). Mifepristone has also been shown to competitively inhibit the progesterone action in the trophoblast, where it impairs the production of ß-hCG, HPL, and progesterone (Das & Catt, 1987). The drug also has a direct inhibitory effect on decidua where it decreases production of prolactin (Wu et al, 1990) and the progesterone dependent placental protein 12 (Olajide et al, 1989).

Marie et al (1988) [n=150] and Grimes et al (1988) [n=50] report an 88% complete abortion rate in early pregnancies with amenorrhoea up to 42 days from a single dose of mifepristone 600mg. The latter study concluded that the efficacy of mifepristone as an abortifacient appeared inversely related to gestational age and thus ß-hCG concentration at time of treatment.

All these studies using mifepristone report "mild" side-effects of nausea, vomiting, and uterine pain in some patients. None of the studies were placebo controlled and none were "blind", but they all suggest that the drug induces complete abortion in 85% of patients with a menstrual delay of up to 14 days. When pregnancy is more advanced (up to 56 days amenorrhoea) the average rate of complete abortion falls to 58-63% (Ulmann, 1987; Van Look, 1988). Pregnancy gestation and ß-hCG concentrations were the only parameters which were related to treatment outcome

(Birgerson & Odlind, 1988; Sitruk-Ware et al, 1990). The reason for this is unclear, but it may be that in early pregnancy the main source of progesterone is from the corpus luteum, the plasma concentrations are relatively low, and therefore can be more effectively antagonised by mifepristone. When the gestation is slightly more advanced, and implantation is better established, the placenta produces large amounts of progesterone locally which may be harder to antagonise (Bygdeman & Van Look, 1988). Although, Heikinheimo et al (1990) concluded that failure to abort after mifepristone treatment was not related to altered pharmacokinetics or metabolism in individual patients, Grimes et al (1990) found a higher failure rate in obese patients, but this finding has not been reported in other studies.

The abortion success rate can be increased by combining mifepristone treatment with a prostaglandin. Bygdeman & Swahn (1985) treated 32 women with mifepristone 100mg/day for 4 days and then gave the patients a PGE2 analogue (16-phenoxy-tetronor-PGE methyl sulfonylamide, sulprostone). They reported a complete abortion rate of 94% and found an increase in uterine contractility, and sensitivity to prostaglandins, measured by an intra-uterine pressure catheter in patients treated with mifepristone (n=5) over controls (n=5). In another study, Swahn & Bygdeman (1988) also demonstrated that treatment with mifepristone (50mg daily x 4 days) led to the appearance of regular uterine contractions at 24 hr in two of five

patients, and in all patients 36 hr, 48 hr, and 72 hr after the start of treatment. They also demonstrated increased sensitivity to PGE2 analogue (Sulprostone) in the patients treated with mifepristone and that this increased sensitivity was already apparent 24 hr after starting treatment. No significant increase in sensitivity to prostaglandin was demonstrated at the longer time intervals.

A number of clinical trials have confirmed the effectiveness of mifepristone and prostaglandin for termination of pregnancy. Cameron et al (1987) improved the termination rate of women who were treated with mifepristone 150mg/day x 4 days from 60% to 95% by giving vaginally a PGE_1 analogue (gemeprost) to patients 48 hours after starting treatment with the anti-progestin. In a second study, Rodger & Baird (1987) gave 100 women of less than 8 weeks amenorrhoea mifepristone 150mg daily for four days or a single administration of 400, 500 or 600mg of the drug followed 48 hours later by a gemeprost 1mg vaginal pessary. The effectiveness of the four treatments were similar with an overall complete abortion rate of 95%. Even at the higher dose of mifepristone 600mg the plasma cortisol levels were unaffected by treatment.

The combined use of mifepristone with a subtherapeutic dose of prostaglandin analogue increases the complete abortion rate to approximately 95%, and there are now a number of reports which testify to the efficacy of

mifepristone with: intramuscular PGE2 analogue [sulprostone] (Swahn and Bygdeman, 1989; WHO Task Force, 1989; Silvestre et al, 1990); intravaginal PGE1 analogue [Gemeprost] (Baird et al, 1988; Dubois et al, 1988b; Shu-rong, 1989; Hill et al, 1990); intravaginal PGE2 analogue [meteneprost] (Maria et al, 1990); and oral 9-methylene PGE2 (Swahn et al, 1990). Recently the results of the first 580 patients in the United Kingdom Multicentre Trial (1990) have been published where patients with amenorrhoea of up to nine weeks duration were treated with a single oral dose of mifepristone 600mg 48 hours before treatment with a single gemeprost pessary. The overall complete abortion rate was 94%, with 0.5% requiring surgical evacuation for treatment failure and 5.5% for incomplete expulsion of the products of conception. The efficacy of the treatment was gestation related, with none of the 46 patients at <43 days gestation requiring surgical evacuation. Opiate analgesia was required after gemeprost treatment by 37% of nulliparae and 13% of multiparae, with 26% vomiting and 13% having diarrhoea. In 9% bleeding was described as severe at the time of abortion, and 1% of patients required a transfusion. Rodger and Baird (1989) reported that the median blood loss during 222 medical terminations with mifepristone and prostaglandin was 75 (range 14-512) ml, and the median duration of bleeding 13 (range 1-44) days. This bleeding pattern is similar to that after vacuum aspiration (Baird et al, 1988). The actual blood loss was unrelated to the dose of mifepristone or prostaglandin used, but was significantly greater with increasing

gestations, especially after 56 days. This is probably related to the increased volume of products and the larger vascular area in the uterus, or may be related to an increased incidence of retained trophoblast.

The combination of mifepristone and prostaglandin offers a safe, effective method for early pregnancy termination. The treatment regimen is acceptable to the patients (Urquhart and Templeton, 1991). Expulsion of the products of conception is quite rapid, with 88% and 96% of patients aborting within 4 or 6 hours respectively of prostaglandin treatment (see later), therefore the procedure can be performed as a day case. Most patients are able to avoid instrumental intervention with the concurrent risks of uterine perforation and cervical injury. The treatment requires strict medical supervision to monitor cases of excessive bleeding and to ensure follow-up in cases of treatment failure. Patients should be warned of the possibility of incomplete abortion and advised to return if they have problems.

Missed abortion

A logical approach to ending pregnancies where a missed abortion or a blighted ova has occurred would be to use mifepristone, which will block the action of progesterone at the endometrium. Asch et al, (1990) published a case report of three missed abortion pregnancies successfully terminated with a single oral dose of mifepristone 600mg. All patients expelled the products

of conception without complication. A prospective study on the use of mifepristone versus expectant management would therefore be justified.

Mid-trimester termination of pregnancy

Urquhart and Templeton (1987) randomly allocated 40 patients between 16 and 18 weeks gestation to receive prostaglandin alone or mifepristone (200mg) 24 hours before prostaglandin treatment. The induction-abortion interval was significantly shorter and the dose of prostaglandin required to cause abortion was significantly lower in the patients who received mifepristone. These results have been confirmed in a trial comparing mifepristone 600mg with placebo 36 hours before second trimester termination of pregnancy using vaginal gemeprost pessaries (Rodger and Baird, 1990). In this study patients treated with mifepristone had a significantly shorter induction-abortion interval, required fewer gemeprost pessaries to achieve abortion, and experienced significantly less pain than patients treated with placebo. Mifepristone 600mg has been administered 24, 36, and 48 hours prior to extra-amniotic infusion of prostaglandin in an attempt to elucidate the optimal time interval for treatment (Urquhart and Templeton, 1990). In this study, there was a significant reduction in the induction-abortion interval, dose of prostaglandin required and attendant side effects in all three treated groups compared to controls. However, there was no significant difference among the treatment groups.

It would therefore appears that pre-treatment with mifepristone before second trimester prostaglandin termination of pregnancy represents a significant improvement in terms of efficacy and safety.

Intra-uterine fetal death

Mifepristone has been used in two trials to induce labour in patients who had a second trimester missed abortion or an intra-uterine fetal death. Cabrol et al (1985) reported an non-placebo controlled trial of 18 patients with an intra-uterine fetal death at varying gestations between 18 and 38 weeks gestation. Patients were treated with mifepristone either 200mg twice daily for 2 days or 100mg/day for 3 days. Success was defined as fetal expulsion within 72 hours of starting treatment. Statistically more patients in the high-dose group (82%) delivered in this time compared with the low-dose group (29%). The authors suggested a dose related labour inducing effect, although they did recognise the methodological shortcomings of the trial.

Padayachi et al (1988) have since confirmed the ability of mifepristone to induce labour in a randomised placebo controlled trial of 24 patients with recent onset intra-uterine fetal death in the latter half of pregnancy. Patients received mifepristone 200mg twice daily or placebo for three days. Of the patients treated with mifepristone, 67% delivered within 72 hours of treatment compared with 17% in the control group ($p<0.02$). These results have been

confirmed by Cabrol et al (1990) who report a double-blind placebo controlled multicentre study involving 94 patients with an intra-uterine fetal death. Patients received mifepristone 600mg/day or placebo for two days. In the mifepristone group 29/46 (63%) delivered within 72 hours of first tablet intake compared with 8/48 (17%) in the placebo group ($p<0.001$).

Labour Induction

Frydman et al (1988) treated 35 women whose fetuses had major malformations with either mifepristone 150 mg or 450 mg, 48 hours before scheduled prostaglandin in the second and third trimesters of pregnancy. Pre-treatment with mifepristone significantly modified the Bishop's score and in three cases labour was induced. However, the study was not placebo controlled.

Data in non-human primates suggest that mifepristone could promote labour induction and sensitise the uterus to exogenous oxytocin stimulation. In cynomalogus monkeys (Macaca fascilicularis) a single dose of mifepristone (25mg) was effective in promoting cervical dilatation by simultaneously shortening and ripening the cervix (Wolf et al, 1989), but such treatment did not reliably induce the uterine contractions necessary for parturition. However, administration of oxytocin advanced the time of delivery in the mifepristone group over controls.

Frydman et al (1991) have recently reported a

randomised double-blind placebo controlled trial on the effects of mifepristone at term for induction of labour in 62 women. Gestation ranged from 37.5 to 42 weeks. Patients received mifepristone 200mg/day or placebo for two days, with induction of labour planned for day 4. In the mifepristone group 18/31 women laboured before the induction date compared with 7/31 in the placebo group ($p<0.05$). The mean time between the first day of treatment and the start of labour was significantly shorter in the mifepristone group (49.5 vs 68.5 hours; $p<0.05$). The need for prostaglandin and oxytocin treatment were also significantly less in the mifepristone patients ($p<0.05$).

Mifepristone has teratogenic effects in the rabbit, but not in other species (Jost, 1986) and it may have been associated with a malformed fetus aborted in the second trimester after maternal ingestion of the drug to terminate an early pregnancy (Henrion, 1989). Lim et al (1990) reported a small series of three human fetuses exposed to mifepristone in early pregnancy, where all fetuses continued to develop normally and were normal at birth. However, as the transplacental passage of mifepristone has already been demonstrated (Frydman et al, 1985), fetal toxicity studies should be performed before further labour induction studies are considered.

Cervical priming prior to termination of pregnancy

Lefebvre et al (1990) studied 180 patients requesting therapeutic abortion in the first trimester of pregnancy

and randomly allocated them to receive placebo or mifepristone in a dose range of 50-600mg. The degree of cervical dilatation was assessed by the size of the largest dilator that could be inserted through the internal cervical os without resistance which was measured before treatment, 24 and 48 hours later. Significant increases in cervical diameter were observed at 48 hours with all mifepristone doses greater than 50mg. The increases in cervical diameter were significantly greater in patients with a gestational age of >10 weeks, but parity had no influence on dilatation. These results have been confirmed by a World Health Organisation (1990) multicentre study in 230 primigravid women between 10-12 weeks gestation. Patients received 50-200mg mifepristone or placebo 24 and 12 hours prior to surgical termination. In mifepristone treated women the cervix was on average between 0.9 and 1.2mm more dilated at operation, but the effect was not dose related. The ease of dilatation assessed subjectively by the operating surgeons was not only improved by the antiprogestin, but was also dose related. Only two (1%) patients had vaginal bleeding prior to surgery.

The effect of mifepristone on the pregnant and non-pregnant cervix has been further investigated by Gupta and Johnson (1990), who assessed cervical dilatation after mifepristone administration with a pressure strain gauge. Treatment with mifepristone increased mean operative cervical dilatation in both pregnant and non-pregnant treatment groups compared with placebo. The force required

to dilate the cervix was also decreased in the mifepristone group. Urquhart and Templeton (1990) found that 35% of patients did not require any further dilatation of the cervix to perform surgical termination between 10-13 weeks gestation after a single oral dose of mifepristone 600mg 48 hours prior to surgery. In this study the peri-operative blood loss was also reduced in the mifepristone group.

Recently, Henshaw and Templeton (1991) reported a placebo controlled, randomised, single blind trial comparing mifepristone 200mg or identical placebo 36 hours prior to surgical termination at 63-91 days amenorrhoea with gemeprost 1 mg four hours prior to surgery at a similar gestation. Both drugs were significantly more effective than placebo, but there were no significant differences in the baseline cervical dilatation, the force required to dilate the cervix or the volume of intraoperative blood loss between the active treatment groups. However, significantly fewer patients in the mifepristone group had adverse side effects than in the gemeprost group.

Other studies report cervical changes after mifepristone treatment before suction termination of pregnancy (Lefebvre et al, 1987; Durlot et al, 1988; Radestad et al, 1988; Johnson and Bryce, 1990; Radestad et al, 1990), and mifepristone appears to result in slower cervical dilatation, possibly of a smaller magnitude, compared with cervical priming with prostaglandins. However, the cervical priming effects of mifepristone is

painless and free of the gastrointestinal side effects usually associated with prostaglandins. As mifepristone dilates and softens the non-pregnant cervix, this effect may facilitate many gynaecological procedures including hysteroscopy, endometrial sampling and insertion of intra-uterine contraceptive devices.

The exact manner in which mifepristone results in cervical softening and dilatation is unclear at present, but two mechanisms are thought to be involved: a direct action of the drug on the cervix and an indirect effect related to stimulation of uterine contractions. Electron microscopic study of cervical biopsies from patients treated with mifepristone has demonstrated an accumulation of mast cells, dissolution of collagen fibres and an outgrowth of blood capillaries, possibly related to local prostaglandin release (Radestad et al, 1990). In addition, in the pregnant rabbit, treatment with mifepristone results in a dramatic fall in cervical alpha-adrenoreceptors (Falkay, 1990). In pregnancy, these receptors are thought to be important in maintaining the tone of the internal cervical os. The increased uterine contractility after mifepristone is likely to enhance these direct cervical effects of the compound.

Ectopic pregnancy

Although ectopic pregnancy is traditionally managed surgically, one study reports the use of mifepristone (200 mg/day x 4 days) for treatment in eight women with

laparoscopically proven ectopic pregnancies (Paris et al, 1984). The outcome of treatment was assessed one week later at a further laparoscopy. Complete regression occurred in one patient; in four the clot had decreased in size but was removed surgically; in the remaining three the haematosalpinx had increased in size and was therefore removed surgically. The series lacks a control group, and as spontaneous resolution of ectopic pregnancy has been reported (Garcia et al, 1987; Pansky et al, 1991), the use of mifepristone for the medical management of ectopic pregnancy is controversial. This is further highlighted by Levin et al (1990) who reported the successful abortion of an intra-uterine pregnancy but the failure to terminate a concurrent heterotopic ovarian pregnancy. Finally, Kenigsberg et al (1987) treated a residual tubal pregnancy with mifepristone 650mg. No decrease in serum beta-hCG or progesterone occurred and this pregnancy was later terminated with systemic methotrexate treatment.

Progesterone receptor containing tumours

Progesterone receptors have now been demonstrated in a number of tumours, including breast, endometrial and ovarian malignancies and meningiomas. There remains the potential that the progesterone receptor blocking agent mifepristone may be useful in treatment of these conditions. Studies in vitro on cultured human breast cancer (Read et al, 1988; Bagchi et al, 1988) and meningioma (Blankenstein et al, 1983; Olsen et al, 1986)

cell lines show that mifepristone inhibits tumour cell growth, but few human studies have been performed.

Romieu et al (1987) conducted an open trial in 27 menopausal or castrated women with advanced breast cancer containing progesterone receptors. They were treated with mifepristone 200mg day for 1-3 months. In six the concentration of carcinoembryonic antigen decreased or plateauxed suggesting a transient remission in tumour growth. However, Lamberts et al (1991) reported a compensatory increase production of androgens and oestrogens after long-term mifepristone treatment. This increase in oestrogens might limit the use of mifepristone in the treatment of oestrogen dependent cancer (Klijn et al, 1989).

Meningiomas do not contain oestrogen receptors and recently a case report describes the successful treatment of a recurrent, inoperable meningioma treated with mifepristone (Haak et al, 1990). Further studies on mifepristone treatment for progesterone receptor containing tumours are necessary before the role of the drug is certain in these conditions.

Mifepristone has potent antiglucocorticoid activity, which has been confirmed in humans (Bertagna et al, 1984; Bertagna et al, 1988). This effect has been used in the successful palliative treatment of hypercorticism (Nieman et al, 1985), but few other studies have investigated this potential of the drug.

Halbreich (1990) has suggested that because premenstrual tension syndrome (PMS) may be associated with increased levels of progesterone and/or its fluctuations during the luteal phase of the cycle, mifepristone may be helpful in its treatment. However, Schmidt et al (1991) reported that neither the timing nor the severity of PMS symptoms were altered by mifepristone-induced menses or luteolysis.

Already potent new analogues of mifepristone with more dissociated antiprogesterone activity have been developed (Philibert et al, 1989), which may be more suitable for a variety of long-term therapies.

1.5. - TERMINATION OF PREGNANCY

It is estimated that between 30 - 55 million induced abortions are performed world-wide each year (Grimes & Cates, 1979), and since the introduction of the Abortion Act 1967 almost 3 million legal terminations have been performed on women resident in England and Wales. Official figures from notification forms calculate the abortion rate in England and Wales to be gradually increasing from 10.5/1000 women aged 15 - 44 in 1976 to 13.4/1000 in 1986 (OPCS, 1987).

Although the incidence of therapeutic abortion has risen since the introduction of the 1967 Act, this has been associated with a dramatic reduction in maternal mortality caused by abortion. In the three years 1964 - 1967 abortion was the most important cause of maternal death. Of 133 deaths, 98 followed illegal termination and 25 "spontaneous" abortion. By contrast in the triennium 1982 - 1984, 11 maternal deaths were associated with abortion, seven after legal termination, and four after spontaneous abortion. For the first time no deaths were attributed to illegal abortion (OPCS, 1989).

Medical termination

The technique of "menstrual regulation" has been promoted because of its simplicity, low cost, and low complication rate (W.H.O., 1987). For pregnancies of less than 49 days amenorrhoea, vacuum aspiration has been performed without cervical dilation using small, disposable

flexible cannulae, but the technique is not without complications, particularly incomplete evacuation of the uterus and continuation of the pregnancy. In a W.H.O. multicentre trial the frequency of these complications was 4.9% and 3.7% respectively (W.H.O., 1979). Therefore the procedure, although regarded as simple, needs a skilled and experienced surgeon.

A medical method of menstrual regulation provides an attractive alternative, especially as the procedure could be managed on an out-patient basis or even self administered. It should result in a high incidence of complete evacuation of the uterus, and be safe for both non-pregnant and pregnant women.

Until recently only prostaglandins have been investigated clinically on a large scale for menstrual regulation. The use of these compounds is based on their ability to stimulate contractility and induce abortion, even during very early pregnancy. The initial results using PGE2 and PGF2a were discouraging with the only effective route being intra-uterine (Bygdeman, 1979). The prostaglandin analogues improved the effectiveness of menstrual regulation in terminating pregnancy and offered the possibility of intra-uterine, vaginal or intra-muscular administration (Karim et al, 1977a; Takagi et al, 1977). Several studies have shown a frequency of complete abortion of over 90%, with side-effects being limited to occasional vomiting and diarrhoea in approximately 50% of patients,

and uterine pain requiring analgesia in approximately 30% of patients (Karim et al, 1977b; Takagi et al, 1978; Smith & Baird, 1980; W.H.O., 1982; Bygdeman et al, 1983). Studies have also shown that vaginal or intra-uterine administration of prostaglandins is as effective as vacuum aspiration if performed at the same gestation (Ragab & Edelman, 1976; Lundstrom et al, 1977; Rosen et al, 1984: W.H.O., 1987).

With the new approach of combining the progesterone receptor antagonist mifepristone with a prostaglandin analogue, it is possible that increasing numbers of patients will be treated medically rather than surgically for early termination of pregnancy.

Suction termination

Suction termination using flexible Karmen cannulae is the technique employed for over 80% of all terminations (OPCS, 1987). The operation is usually performed under general anaesthetic, although local anaesthetic techniques have become increasingly popular especially at earlier gestations (Meyer, 1983).

Although the technique may be regarded as relatively simple, it is not without its complications and 10% of 6105 patients have been reported to present with morbidity related to the termination within the first 21 days following operation (RCGP & RCOG, 1985). However major complications, including haemorrhage requiring transfusion, uterine perforation, salpingitis, complications requiring

laparotomy, and deep venous thrombosis or pulmonary embolism, only occurred in 0.8% of the study patients.

The main factors which independently affected the morbidity were the gestation at termination and site of operation, the private sector having the lower morbidity. Therefore, probably the most efficient way of decreasing the morbidity of first trimester suction termination is performing all operations at the earliest possible gestation. It remains to be ascertained whether medical terminations with mifepristone and a prostaglandin analogue will be associated with a lower morbidity.

Second trimester termination

Since the introduction of the Abortion Act 1967 in England and Wales, the number of terminations during the second trimester has declined from 22% in 1968 (OPCS, 1970) to 19% in 1977 (OPCS, 1979). During the subsequent years, the proportion has remained relatively unchanged (OPCS, 1987). Figures from a survey by the Royal College of Obstetricians and Gynaecologists in 1982 (Stanwell-Smith, 1984) indicated that the methods of termination used include 27% performed by the minor surgical methods and 68% using prostaglandins, with the majority involving intra-uterine instillation techniques.

Virtually all reports have indicated that terminations in the second trimester result in greater morbidity than occurs with first trimester termination, and vacuum

aspiration alone is not suitable for terminations after 13 weeks gestation. There is some controversy about which method of termination should be used after this gestation. Reports from the U.S.A. suggest that cervical dilatation and surgical evacuation (D&E) of the uterus is the safest method of termination up to 20 weeks gestation (Hodari et al, 1977; Barr, 1978; Grimes et al, 1980; Cadesky et al, 1981; Beckhuizen et al, 1982; Berger et al, 1982; Cates et al, 1982; Grimes & cates, 1982; Peterson et al, 1983; Castadot, 1986). The operation usually involves some form of cervical preparation, usually with lamineria tents passed transcervically, and then evacuation of the uterine contents using a combination of crushing forceps and large (12-14mm) suction catheters. Although the technique is widely used, the impact on long-term injury (cervical incompetence and premature labour) remains to be ascertained.

In our unit prostaglandin instillation, either extra- or intra-amniotically or vaginally is the preferred method of second trimester termination. The technique and its side-effects will be discussed in more detail later in this thesis.

SECTION 2 - TERMINATION OF PREGNANCY RESULTS

SECTION 2.1. - MEDICAL INDUCTION WITH PROSTAGLANDINS FOR EARLY TERMINATION OF PREGNANCY

INTRODUCTION

In the United Kingdom almost a third of all terminations are performed at eight weeks gestation or under, usually by cervical dilatation and surgical evacuation of the uterus under general anaesthesia (OPCS, 1987). With the development of the new progesterone antagonists considerable interest has been re-awakened in the advantages of medical termination of pregnancy.

To address this issue, the records of the patients who have been treated with prostaglandins for early termination over 10 years in The Nuffield Department of Obstetrics and Gynaecology, University of Oxford have been reviewed.

PATIENTS AND METHODS

Between 1973 and 1983, 820 patients were referred by family practitioners or local family-planning clinics for consideration of early termination of pregnancy within the terms of the Abortion Act 1967. The decision regarding which treatment method was used for individual patients depended on the availability of prostaglandins. Data on patient characteristics, gestation, and abortion method were recorded on standard record sheets. These data are illustrated in table 1.

Pregnancy was suspected from the clinical history and examination, and was confirmed with a positive hCG result

Table 1 - Patient characteristics and gestation of 820 women undergoing early termination of pregnancy

Patient details			(%)
Age			
Median (range)	27	(15-46)	
< 16	29		(3.3)
16 - 24	310		(37.6)
25 - 39	431		(52.2)
> 40	50		(5.9)
Parity			
0	435		(53.0)
1+	385		(47.0)
Previous TOP.	102		(12.4)
Marital Status			
Single	340		(43.8)
Married	373		(45.5)
Prev. Marriage	102		(10.7)
Menstrual Delay (days)			
Median (range)	19	(0-43)	
0 - 7	26		(3.2)
8 - 14	144		(17.6)
15 - 21	353		(43.0)
22 - 28	252		(31.0)
29 - 35	34		(4.0)
> 36	11		(1.2)

prior to treatment, and by examination of the products of conception that were expelled. In all cases haemoglobin, blood group and rhesus state were determined prior to termination, and anti-D was given when indicated at termination.

Table 2 shows the regimens employed in the patients treated with prostaglandin and the number of patients treated in each group. Prostaglandins when given by intra-uterine instillation were dissolved in 4 ml of 5% tylose gel which was introduced into the uterine cavity via a size 12 French-gauge Nelaton catheter passed transcervically which was removed immediately after injection (MacKenzie et al, 1978). Lipid based pessaries were used for vaginal treatment. Patients were allowed home after prostaglandin treatment and were seen and examined 1, 2 and 6 weeks later. The success of treatment was indicated by vaginal bleeding at least equivalent to a normal menstrual loss persisting for at least 4 days, and confirmed by vaginal examination at post treatment follow-up, and a negative pregnancy test. Surgical evacuation by aspiration under general anaesthetic was arranged for those patients in whom prostaglandin treatment was unsuccessful.

All patients were given a questionnaire which was returned following the first subsequent menstrual loss reporting any complications and the acceptability of the termination method.

Table 2 - Drug regimens and success rates in 820 patients undergoing a medical termination of pregnancy

Treatment Schedule	No treated	No Pregnant	No successful	(%)
Intra-uterine				
E_2 (0.1-0.5mg/4ml gel)	43	38	29	(76)
15 Me.F_2a (100-150ug/4ml gel)	493	452	388	(86)
Sulprostone (25-50mg/4ml gel)	40	40	33	(83)
Intravaginal				
E_2 (10-30mg/10ml gel)	34	27	15	(56)
15 Me. F_2a (1.5mg/10ml pessary)	129	116	99	(85)
16:16 dime. E_2 (2-4mg pessary)	46	37	35	(94)
E_1 (2mg pessary)	35	34	30	(88)
TOTAL	820	744	629	

RESULTS

Seven hundred and forty-four (90%) of the patients treated were subsequently found to have been pregnant at the time of the termination procedure. The median (range) menstrual delay was 19 (0-43) days.

Efficacy

Of the 744 patients with confirmed pregnancies treated with prostaglandins, 629 (85%) were successfully terminated without any further treatment. Success rates for the different prostaglandin regimens are illustrated in table 2. Sixty-three (8.5%) required a subsequent surgical evacuation of the uterus for excessive bleeding in 37 and prolonged bleeding in 26. The gestation (figure 3) and parity (figure 4) did not appear to influence either the success rates, or duration of uterine bleeding with the various regimens.

Side-Effects

Thirty-nine percent of the pregnant patients treated with prostaglandins vomited and 20% had diarrhoea (table 3). The extent of the gastrointestinal side effects was not related to gestation, analgesic requirement, or drug regimen.

Oral analgesia was required by 476 (64%) following prostaglandin administration, 168 (22%) required an opiate injection, and 100 (14%) did not require any analgesia. Opiate analgesia was more frequently required by nulliparae

Figure 3 Influence of gestation upon success in inducing abortion by local administration of prostaglandin

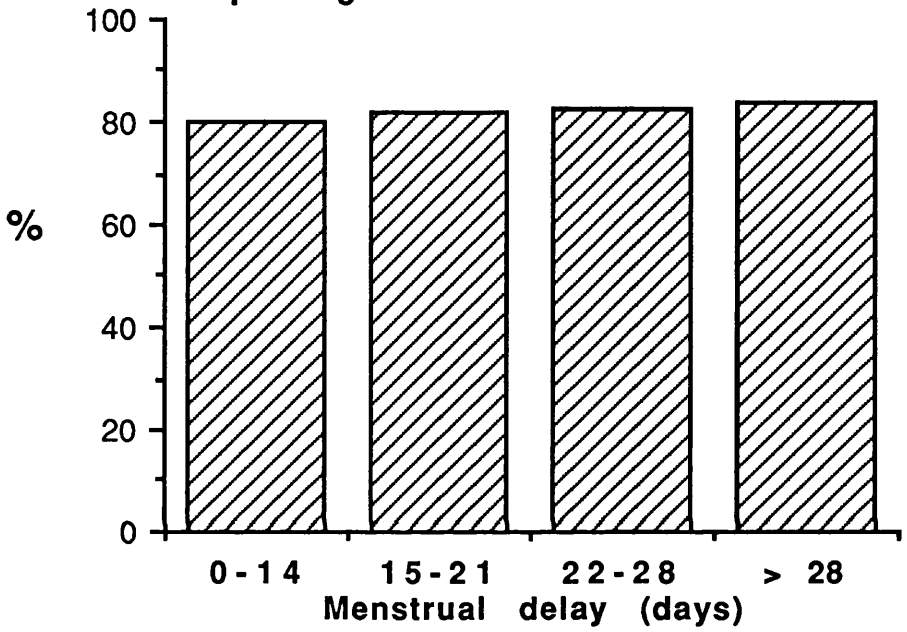


Figure 4 Influence of parity on success in inducing complete abortion after prostaglandin

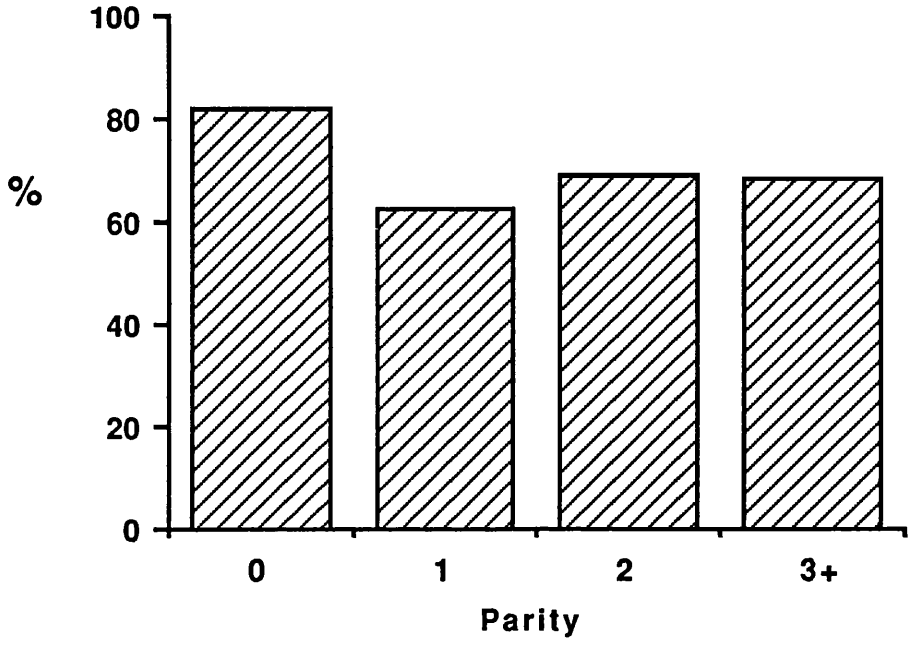


Table 3 - The incidence of side-effects and complications in 820 treated patients

	PREGNANT (N=744)	(%)	NON-PREGNANT (N=76)	(%)
Vomiting	290	(39)	22	(30)
Diarrhoea	149	(20)	17	(22)
Blood transfusion	10	(1.3)	0	(0)
Pelvic infection	7	(0.9)	0	(0)
ERPC	63	(8.5)	0	(0)

(31%) than multiparae (14%) patients ($p \leq 0.001$). Gestation did not influence analgesic requirement.

Morbidity

Blood Loss - Ten (1.2%) patients pregnant at time of treatment with prostaglandins required a blood transfusion (table 3). Eight patients of the first 520 (1.5%) were transfused, compared with only two of the last 300 (0.6%) patients. In all instances, transfusions were given at the time of re-admission for heavy bleeding 2-6 weeks after treatment. Gestation did not affect the transfusion requirement.

Pelvic Infection - Pelvic infection was suspected in 7 (0.9%) of the prostaglandin group. One patient treated with intra-uterine prostaglandins underwent a laparotomy for drainage of a pelvic abscess. The infection was considered secondary to the continued presence of an IUCD.

Genital trauma - Genital tract trauma was not encountered in any patients managed with prostaglandins.

Two patients required a laparotomy 10 and 14 days respectively after prostaglandin treatment for a tubal ectopic pregnancy.

Non-pregnant subjects

None of the non-pregnant patients had any complications after prostaglandin treatment (table 3). The incidence of side-effects and the analgesic requirement

were similar to the pregnant patients.

Follow-up

Sixty-five (7.9%) patients failed to attend follow-up. Attempts were made to contact the patients via telephone or by their General Practitioner, and details relating to outcome and complication were unobtained in 24 (2.9%) patients.

Patient Acceptability

Of the patients treated with prostaglandins, 74% stated they would repeat the procedure if necessary on a future occasion, 20% would not, and 6% were unsure.

INTERPRETATION OF RESULTS

It is generally considered that pregnancy termination is more acceptable to the patient and results in fewer complications the earlier in pregnancy it is performed. The wish to avoid general anaesthesia with its potential risks has led to the investigation of possible less traumatic, non-surgical techniques for termination of pregnancy. The use of low dose prostaglandins administered locally is a method with the potential to achieve these goals. It avoids general anaesthesia and thus can be performed without the need for hospital admission, and is acceptable to the majority of patients (Stringer et al, 1975; Rosen et al, 1979; Rosen et al, 1984). The results of this analysis are similar, with 74% of patients treated with prostaglandins stating they would repeat the termination method if

required. But success of a termination method depends on several factors apart from patient acceptability, and these include the efficacy, safety, extent of side-effects, availability and long term sequelae.

The overall success rate using prostaglandins was 85%, with variation between 56% and 94% according to the regimen used. These results are similar to other reports of series using prostaglandins for early medical termination (Mocsary and Csapo, 1975 ; Smith and Baird, 1980; Brenner et al, 1983; Foster et al, 1985). To achieve high rates of expulsion of the conceptus the doses of prostaglandin required are often associated with gastrointestinal side-effects. The rates of vomiting (39%) and diarrhoea (20%) are consistent to those reported by others (Bygdeman et al, 1976; Wan et al, 1981).

However, the termination method is not without complications. The overall transfusion rate was 1.2% which is higher than previously reported from our unit (MacKenzie et al, 1978), and the rate of incomplete abortion (8.5%) was also relatively high. The pelvic infection rate was similar to that reported from studies of early pregnancy termination by suction aspiration under local anaesthetic (Goldthorp, 1977; Irani et al, 1975). As the termination method usually avoids surgical intervention, no cases of genital trauma occurred. This is of considerable advantage as a uterine perforation rate of 0.7% in 1030 first trimester aspiration terminations under general anaesthesia

has been reported from our unit (MacKenzie & Miller, unpublished data).

The data presented in this chapter is by its very nature retrospective and the patients were treated some years ago. However, the data was recorded on purpose designed forms at the time of termination and it was these original forms that were used for the analysis. It is recognised that with the changes that occurred in staff and unit practice, errors in interpretation may have been introduced into this analysis. However, as the patients were treated in the same unit under the supervision of the same consultant, the data presented in this chapter will be used later in this thesis as the basis to compare the outcome of medical termination of early pregnancy with prostaglandins alone or with a combination of mifepristone and prostaglandin.

SECTION 2.2. - AN ANALYSIS OF EFFICACY AND COMPLICATIONS OF INTRA-AMNIOTIC AND EXTRA-AMNIOTIC MID-TRIMESTER TERMINATION OF PREGNANCY

INTRODUCTION

Progesterone inhibition with epostane has been reported to significantly decrease the induction-abortion intervals during mid-trimester prostaglandin termination (Selinger, 1988). Recently a similar effect has been described with mifepristone, which was also associated with a decrease in the dose of prostaglandin needed for termination (Urquhart & Templeton, 1987). This may have potential benefits on side-effects, as these are known to be dose related. Therefore the records of 2308 consecutive mid-trimester abortions induced using intra-uterine PGE_2 conducted over a twelve year period in the Nuffield Department of Gynaecology have been reviewed to assess the induction-abortion intervals and complication rates. These figures will be compared later in this thesis to the results obtained from the mifepristone second trimester studies.

PATIENTS AND METHODS

Between January 1976 and December 1987, 2308 patients admitted for pregnancy termination in the second trimester under grounds 2 and/or 3 of the Abortion Act 1967 were managed using either extra-amniotic or intra-amniotic injections of PGE_2. Most of the patients were treated extra-amniotically, but the decision upon route of

administration was generally made according to the gestation and surgeons preference, with other individual patient factors taken into consideration. The intra-amniotic route tended to be used for terminations of a greater gestation, due to the procedure being technically more difficult before 14 weeks. Data on patient characteristics, gestation, and abortion method were recorded on purpose designed record sheets. The same forms were used for recording outcome and complications.

All patients were treated with prostaglandins on the day of admission. Extra-amniotic administration was performed by giving PGE_2 1.5mg or 2.5mg dissolved in 10ml of 6% viscous methyl hydroxy-ethyl cellulose as a single injection via a 14 French gauge Nelaton catheter passed through the cervix and immediately withdrawn following the injection (MacKenzie & Embrey, 1975). Intra-amniotic administration involved the technique described by MacKenzie et al (1974a). PGE_2 5mg or 10mg was injected as a single dose, following which the needle was withdrawn. All the patients received an intravenous infusion of oxytocin (100mU/min) beginning six hours after PGE_2 administration, and this was maintained until the abortion. The following morning, if abortion had not occurred or was not imminent, contractions were augmented with more prostaglandins or by doubling the oxytocin infusion rate. Premedication was not given to patients treated extra-amniotically, but some treated intra-amniotically received diazepam 5-10mg intravenously shortly before the prostaglandin injection.

Papaveretum 20mg and chlorpromazine 25mg were given intramuscularly during the abortion process for analgesia when required.

Following expulsion of the fetus, ergometrine 500Ìg or oxytocin 5 units were given intramuscularly. Surgical uterine evacuation was only performed if the placenta was retained or was found to be incomplete, or if placental tissue was palpated through the cervix at routine post-abortion vaginal examination. Blood loss at abortion was estimated to the nearest 100ml, and transfusions were only given when clinically indicated. Antibiotics were administered if infection was suspected on clinical grounds after the appropriate bacteriological specimens had been collected, or if the induction abortion interval (IAI) was unduly prolonged, or if indicated for some pre-existing condition. Non-sensitised rhesus negative patients routinely received anti-D immunoglobulin during the first 24 hours following abortion. Patients were not followed-up routinely but all re-admissions were directed to our unit, the gynaecological unit serving more than 95% of the local population.

Statistical analyses were performed using the Chi-squared test and Mann-Whitney test.

SECTION 7.3. - RESULTS

One thousand six hundred and eight (70%) were treated extra-amniotically and 700 (30%) intra-amniotically. There

were no statistically significant differences between the two treatment groups with regard to age, marital status, parity or previous termination (table 4). The median (range) pregnancy gestation in the intra-amniotic group of 17 (12-22) weeks was greater than in the extra-amniotic group of 15 (12-22) weeks ($p \leq 0.001$).

In no patient was the prostaglandin technique unsuccessful in provoking abortion, so resort to another termination method was not required.

Efficacy

Cumulative abortion rates for the two administration routes and different PGE_2 doses are illustrated in figure 5. The median (range) IAI for the extra-amniotic group was 14.8 (3-59) hours, and for the intra-amniotic group was 14.8 (4-60) hours. Gestational period did not influence this (figure 6), but the median (range) IAI was significantly longer for nulliparae at 15.7 (4-60) hours compared with multiparae at 12.9 (4.5-48) hours ($p \leq 0.01$). Oxytocin augmentation was given to 92.5% of the intra-amniotic group compared with 88.5% of the extra-amniotic group ($p \geq 0.05$). In the extra-amniotic group, treatment with PGE_2 2.5mg in 1308 (81.3%) resulted in a significantly shorter median (range) IAI at 14.0 (3-59) hours than with PGE_2 1.5mg in 250 (18.7%) at 15.7 (4.3-58) hours ($p \leq 0.001$). Similarly PGE_2 10mg intra-amniotically in 429 (61.3%) resulted in a mean (SD) IAI of 14.6 (4.1-45) hours compared with PGE_2 5mg in 271 (38.7%) of 15.5 (4.1-60) hours

Figure 5 Induction abortion interval (IAI) according to route and dose of prostaglandin in mid-trimester termination of pregnancy

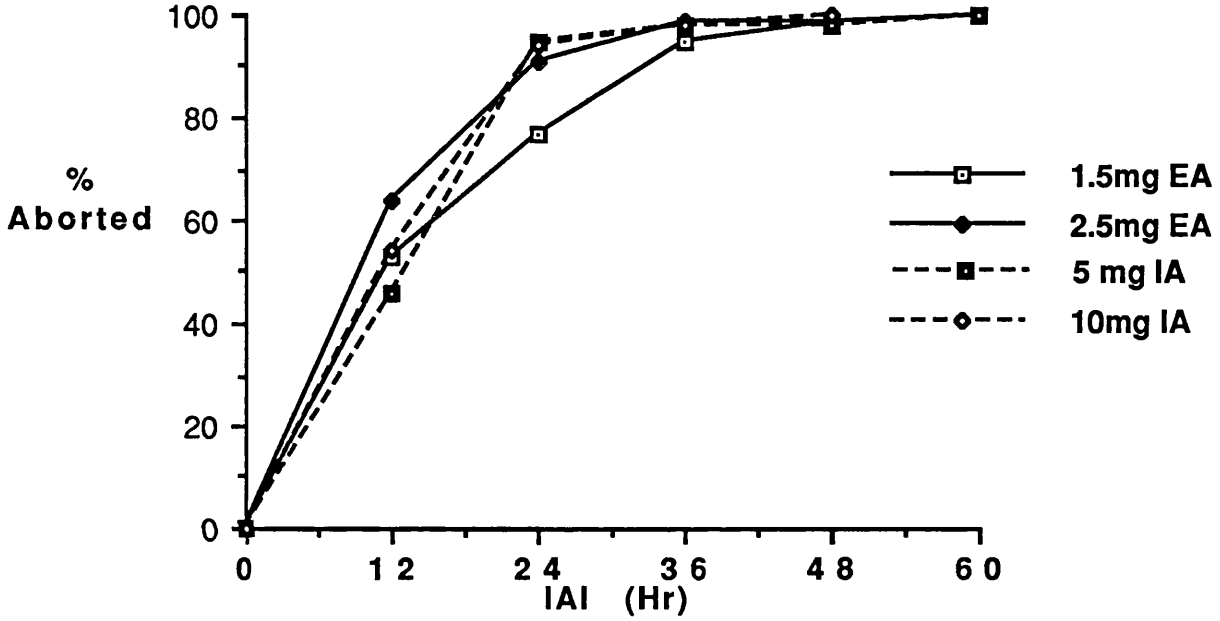


Figure 6 Effect of gestation (weeks) upon efficacy of mid-trimester termination of pregnancy

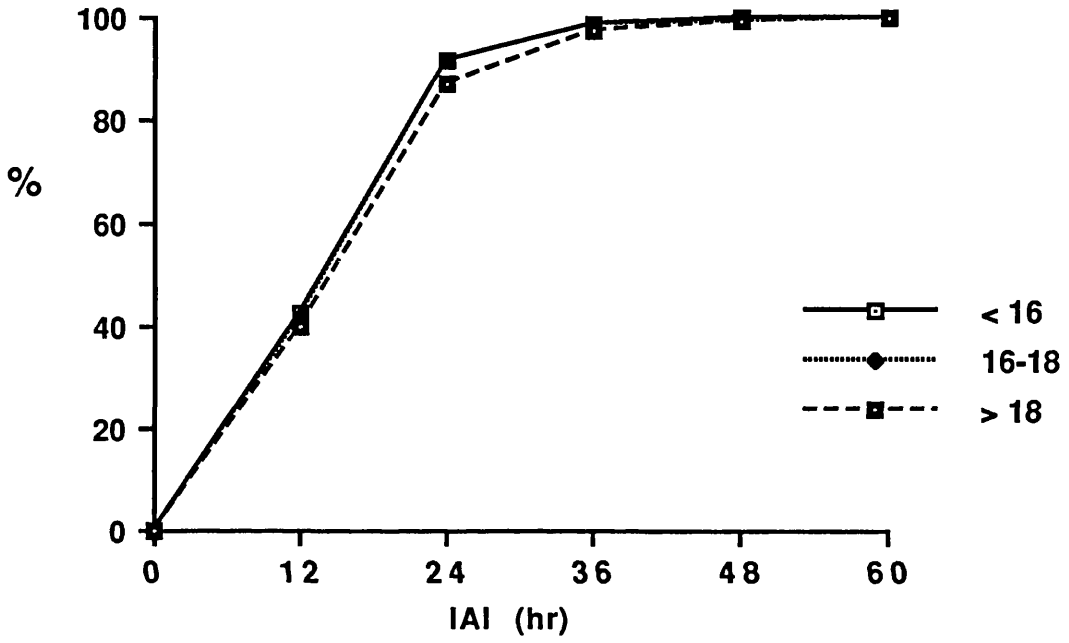


Table 4 - Patient characteristics of 2308 patients undergoing mid-trimester prostaglandin termination

	INTRA-AMNIOTIC	(%)	EXTRA-AMNIOTIC	(%)	TOTAL	(%)
Number	700	(30)	1608	(70)	2308	
Age Median [range]	22	[13-48]	22	[13-46]		
< 16	33	(5)	109	(7)	142	(6)
16 - 20	332	(46)	708	(44)	1040	(45)
21 - 25	174	(25)	340	(21)	514	(22)
26 - 30	66	(10)	200	(12)	266	(11)
31 - 35	49	(7)	127	(8)	176	(8)
35 - 40	34	(5)	80	(5)	114	(5)
> 40	12	(2)	44	(3)	56	(3)
Parity						
0	480	(69)	1028	(64)	1508	(65)
1 - 3	201	(29)	530	(33)	731	(32)
4+	50	(3)	19	(2)	69	(3)
Prev. top	80	(11)	170	(11)	250	(11)
Marital Status Single	508	(73)	1062	(66)	1570	(68)
Married	125	(18)	386	(24)	511	(22)
Pr. marriage	67	(9)	160	(10)	227	(10)
Gestation						
Mean	17	[12-22]	15	[12-22]		
12 - 13	12	(2)	180	(11)	192	(8)
14 - 15	129	(18)	681	(42)	810	(35)
16 - 17	230	(33)	590	(37)	820	(36)
18 - 19	260	(37)	139	(9)	399	(17)
20+	69	(10)	18	(1)	87	(4)

($p \leq 0.01$).

Abortion within 24 hours occurred in 89.4% of the intra-amniotic group compared with 83.2% of the extra-amniotic group ($p \leq 0.01$). In all, only 2% of the intra-amniotic group and 3.1% of the extra-amniotic group took longer than 36 hours to abort; 10% of patients in each group required additional oxytocic augmentation to reach abortion.

Incomplete abortion

Figure 7 illustrates the incidence of complete abortion according to gestation and route of PGE_2 administration. Doses of PGE_2 used, patients' age and parity did not influence the results. There was an association between route of PGE_2 administration and the incidence of incomplete abortion, with 35% of the extra-amniotic patients needing a surgical evacuation of the uterus compared with 25% of the intra-amniotic group ($p \leq 0.01$).

Side effects

Vomiting was the most common side-effect encountered with 732 (45.5%) extra-amniotic patients experiencing at least one episode compared with 274 (39.0%) of the intra-amniotic patients ($p \leq 0.01$) [table 5]; the respective figures for diarrhoea were 284 (17.6%) and 36 (5.1%) ($p \leq 0.001$). These side-effects were not related to pregnancy gestation, parity, IAI, or dose of PGE_2 given.

Figure 7 Complete abortion rate of intra-amniotic (hatched) and extra-amniotic (plain) mid-trimester termination

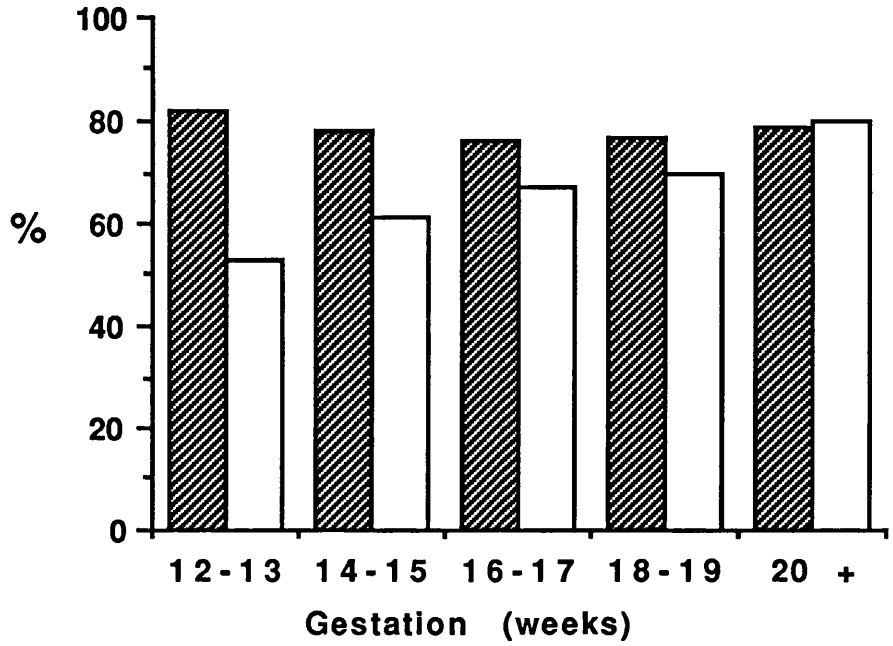


Table 5 - Incidence of side-effects and complications after intra-amniotic and extra-amniotic termination

	INTRA-AMNIOTIC	(%)	EXTRA-AMNIOTIC	(%)	TOTAL	(%)
Vomiting	274	(39)	732	(45)	1006	(44)
Diarrhoea	36	(5)	284	(17)	320	(14)
Blood loss > 500ml	11	(1.5)	29	(1.8)	40	(1.7)
Transfusion	5	(0.7)	8	(0.5)	13	(0.6)
Genital trauma	2	(0.3)	2	(0.1)	4	(0.1)
Pelvic Infection	3	(0.4)	5	(0.2)	8	(0.4)
Re-admission for ERPC	8	(1.1)	25	(1.1)	33	(1.4)
					1424	(61.7)

A pyrexia of 37.5C on at least one occasion during the abortion process was recorded in 15% of the total population studied, only 2% of which developed a higher temperature. Four patients experienced a severe "prostaglandin reaction" which responded to supportive treatment alone, and abortion was induced in the usual way. Analgesia was given to 2030 (88%) patients during the contractile phase. The need for analgesia was not influenced by pregnancy gestation, patients age, PGE_2 dose or route of administration. More nulliparae (92.2%) required analgesia than multiparae (80.0%), ($p \leq 0.001$).

Morbidity

Blood loss

In total 40 (1.7%) patients lost more than 500ml and 13 (0.6%) required a transfusion (table 5). These cases were evenly distributed between the extra- and intra-amniotic groups. The number of patients losing more than 500 ml at termination was not significantly affected by gestation, parity, or IAI. Five percent of patients who had an incomplete abortion lost more than 500ml of blood.

Infection

Forty-five (1.9%) patients received antibiotics whilst in hospital, but only two (0.08%) had proven sepsis. This was due to a ß-haemolytic streptococcus infection in one case, and a clostridium welchii infection in the other. Both patients recovered uneventfully after antibiotic

treatment. In six (0.3%) patients pelvic infection was later suspected.

Genital tract trauma

Four (0.17%) patients sustained damage to the genital tract during termination: in three this was due to a broad ligament haematoma; and in one a cervical tear. The incidence of genital tract trauma was not significantly different between the extra- and intra-amniotic groups.

Duration of hospital admission

There was no significant difference in the duration of hospital stay between the two groups (Table 6). Sixty-seven percent of the patients were in hospital for only 1 night. Fourteen patients had prolonged hospitalisation having undergone a tubal ligation immediately following the abortion.

Re-admission

Forty-one (1.8%) patients were re-admitted to hospital after the termination. Thirty-three (1.4%) had excessive bleeding managed by uterine curettage, and six (0.3%) with suspected, but unproven, pelvic infection. Patients were spread between both administration groups. Half the re-admissions occurred within seven days of termination, and the remainder over the subsequent five weeks.

INTERPRETATION OF RESULTS

The results represent a marked improvement upon those

Table 6 - Duration of hospitalisation in 2308 patients undergoing termination according to route

LENGTH OF STAY (Days)	INTRA-AMNIOTIC	(%)	EXTRA-AMNIOTIC	(%)
1	453	(65.2)	1075	(67.0)
2	223	(32.1)	473	(29.5)
3	19	(2.7)	56	(3.5)
Tubal Ligation	5		4	
TOTAL	700		1608	

that were published in 1974 of 626 consecutive mid-trimester abortions induced with intra-uterine PGE_2 and PGF_2a using a variety of dosage protocols from our unit (MacKenzie et al, 1974b). The median IAI of 14.8 hours for both extra-amniotic and intra-amniotic administration is markedly less than the 16.7 and 17.2 hours respectively reported in 1974. Morbidity rates have similarly improved with a reduction in excessive haemorrhage from 6.1% to 1.9%, transfusion requirement from 1.3% to 0.6%, genital tract trauma from 0.5% to 0.2%, and proven clinical sepsis from 0.3% to 0.08%. The very low rate of confirmed cases of pelvic sepsis (0.08%) and the overall use of antibiotics (1.9%) for prophylaxis or in cases when a pyrexia developed is testament to the approach not to give antibiotics as a routine (MacKenzie & Fry, 1981a).

There is a lack of similar large series of mid-trimester terminations using intra-uterine prostaglandins with which to compare these results. The report by the RCOG (Stanwell-Smith, 1984) on 2766 second trimester abortions which included intra-amniotic and extra-amniotic prostaglandin instillation methods, does not provide any figures which allow adequate analysis by termination method. It was however concluded that the need for re-admission and re-evacuation of the uterus was more common following extra-amniotic prostaglandin administration compared with other methods. The figures of 1.2% for the extra-amniotic group and 2.0% for the intra-amniotic group, resulting from a policy of selective curettage immediately

following expulsion of the conceptus rather than routine curettage are much lower than those reported for the total group in the RCOG study of 1.4% - 5.8% (Stanwell-Smith, 1984). Indeed, a re-admission rate for re-evacuation of 2.7% following 670 first trimester aspiration terminations under general anaesthesia has been reported from our unit (MacKenzie & Fry, 1981b). The recommendation of that report for routine post-abortal curettage following prostaglandin terminations can therefore be challenged.

Overall there were few differences in IAI and morbidity rates achieved with the two administration routes using the dosage regimens employed. As expected however, patients treated with the larger doses of PGE_2 with both routes had shorter IAI's. The intra-amniotic technique resulted in a greater proportion of abortions within 24 hours, with a lower incidence of gastrointestinal side-effects, which represents genuine advantages. For technical reasons, the intra-amniotic route was infrequently used before 15 weeks gestation, but with the introduction of small mobile real-time sonar scanning machines, this route is more readily employed at the beginning of the second trimester. With continuous vision it is possible to avoid the "prostaglandin reaction" that occasionally occurs immediately following prostaglandin injection, due to inadvertent instillation into decidual blood vessels, leading to a large dose of prostaglandins reaching the systemic circulation and causing acute circulatory collapse.

Delayed morbidity following pregnancy termination represents an important aspect of any termination procedure. A number of prospective long-term studies have been reported after a mid-trimester termination using prostaglandins which have failed to show any significant adverse affect upon subsequent menstrual function (MacKenzie & Hillier, 1975), fertility (MacKenzie & Fry, 1988), and pregnancy outcome (MacKenzie & Hillier, 1977). There is a dearth of similar information following second trimester pregnancy termination performed by D&E, and there must remain some anxiety about possible loss of cervical integrity and subsequent pregnancy outcome compromise following this procedure.

In recent years, vaginal administration of prostaglandin analogues for second trimester abortion has been reported (Mandelin & Kajanoja, 1978; Embrey, 1982; Cameron & Baird, 1984). To date there do not appear to be any definite advantages over the techniques described here. By avoiding intra-uterine injections of prostaglandins, post-abortal sepsis might be reduced, and inadvertent systemic injection will be circumvented. A more exciting new approach combines the use of an anti-progestational agent given prior to a reduced dose of prostaglandins. Although the data presented in this chapter is retrospective, therefore errors in interpretation may have been introduced secondary to changes in practice and staff, the numbers presented are very large (2308 patients). The data in this chapter will therefore be used later in this

thesis to compare the effects of the combination of mifepristone and low dose prostaglandin for termination of pregnancy in the second trimester.

SECTION 3 - CLINICAL RESULTS OF THE USE OF MIFEPRISTONE IN PREGNANCY TERMINATION

SECTION 3.1. - THE EFFECTIVENESS OF MIFEPRISTONE IN CONJUNCTION WITH A PROSTAGLANDIN E_1 ANALOGUE IN THE TERMINATION OF FIRST TRIMESTER PREGNANCY

INTRODUCTION

In the England and Wales 172,286 therapeutic abortions were performed in 1986, and of these 55,798 (32.4%) were performed at under 64 days gestation (OPCS, 1987). Although vacuum aspiration of the uterus can be regarded as a relatively simple procedure, complications do occur and attempts have been made to develop a non-surgical means of terminating early pregnancy. Previously this could only be performed using prostaglandins, but this was associated with gastrointestinal side-effects and an unacceptably high incidence of incomplete abortion (see section 2.1.). The development of the progesterone antagonist mifepristone offers the possibility of improvement on these results.

Preliminary studies using mifepristone by itself for termination of early pregnancy found the abortion success rate to be only 60-85% (Kovacs et al, 1984; Verest & Haspels, 1985; Shoupe et al, 1986; Couzinet et al, 1986; Mishell et al, 1987; Cameron & Baird, 1988), however this could be increased to 95% by the addition of a small dose of prostaglandin (Bygdeman & Swahn, 1985; Cameron et al, 1986). Rodger & Baird (1987) studied various mifepristone doses with prostaglandin E_1 for early termination, and found the best results were obtained using a single dose of

mifepristone (600mg).

TRIAL DESIGN AND METHODOLOGY

As part of the United Kingdom Multi-centre Trial 100 patients were treated with mifepristone 600mg orally and 48 hours later a single prostaglandin E_1 analogue (gemeprost) 1mg pessary for termination of pregnancy within the terms of the Abortion Act, 1967. Gestational age was calculated using menstrual dates and confirmed by ultrasound measurement of the fetal crown-rump length.

Patients were included in the trial if their menstrual delay was 63 days or under at time of taking mifepristone, if at least 18 years old, if they had no significant past or current medical disorder, and if they had not taken steroids in the past year.

Before treatment a peripheral venous blood sample was taken for haematological (haemoglobin, white cell count and blood group), biochemical (urea, electrolytes, uric acid, creatinine, and liver function tests), and hormone (progesterone and ß-hCG) analyses. These analyses were repeated immediately prior to insertion of the prostaglandin pessary 48 hours later, and at follow-up visits seven, 14 and 28 days after termination.

On day 1, the patients attended the hospital and received a single dose of mifepristone (3 x 200mg) with water in the presence of the supervising physician. Before treatment and hourly after treatment, for four hours, the

patients were asked specific questions about uterine pain, vaginal bleeding, and any symptoms that could have been due to side-effects or drug reaction. The patient's temperature, pulse, blood pressure and analgesic requirement were also checked hourly for four hours. All data were recorded on prepared study data recording sheets. The patients were discharged four hours after taking tablet ingestion with a diary to record the presence and severity of specified symptoms which they completed each morning for the subsequent 48 hours (appendix 3).

Forty-eight hours later the patients were admitted to hospital. The presence or absence of any side-effects, abdominal pain, vaginal bleeding or drug therapy since mifepristone treatment were recorded. If any vaginal bleeding had occurred a repeat ultrasound was performed to check for fetal viability. If abortion was thought to have occurred, a vaginal examination was performed to assess the size of the uterus and the state of the cervical os, and an ultrasound scan performed. If the abortion was complete, no further treatment was undertaken, but the patient was still followed-up at the same time intervals. If abortion had not occurred the patient was treated with gemeprost 1mg pessary placed in the posterior vaginal fornix.

After insertion of the pessary, the temperature, pulse, blood pressure, degree of uterine pain, onset and severity of vaginal bleeding were recorded hourly for four hours. The time of expulsion of products of conception and

analgesic requirement were also noted. In all patients a speculum examination was performed four hours after prostaglandin pessary insertion and the vagina inspected for products of conception. If abortion had occurred the patients were then discharged, but if no products of conception had been seen an ultrasound scan was performed. If this showed an intra-uterine gestational sac then the patient remained in hospital for a further two hours for observation before being discharged. Rhesus negative patients routinely received anti-D prophylaxis. Each patient was given another diary, for the first two weeks after termination, to record any unusual symptoms, abdominal pain and vaginal bleeding pattern and severity (appendix 3).

All patients were followed up seven, 14 and 28 days after termination. At each visit the diaries were inspected for the presence and severity of possible side-effects of mifepristone, for the occurrence of vaginal bleeding and abdominal pain. Any drug therapy especially analgesic or antibiotic was recorded, along with any surgical evacuations performed. All patients had an ultrasound scan at the first follow-up visit and this was repeated at subsequent visits if the patient was still bleeding. If abortion had not occurred by the first week follow-up visit a surgical evacuation of the uterus was arranged under general anaesthetic.

All patients were asked at the follow-up visit if they would repeat the termination method in the future if

necessary. The last 50 treated patients were also given a purpose designed questionnaire enquiring about the acceptability of the termination method and the nature of their first subsequent menstrual period. This was to be completed and returned after the period had finished (appendix 4).

The Mann-Whitney and chi-squared test were used for statistical analysis.

SECTION 4.3. - RESULTS

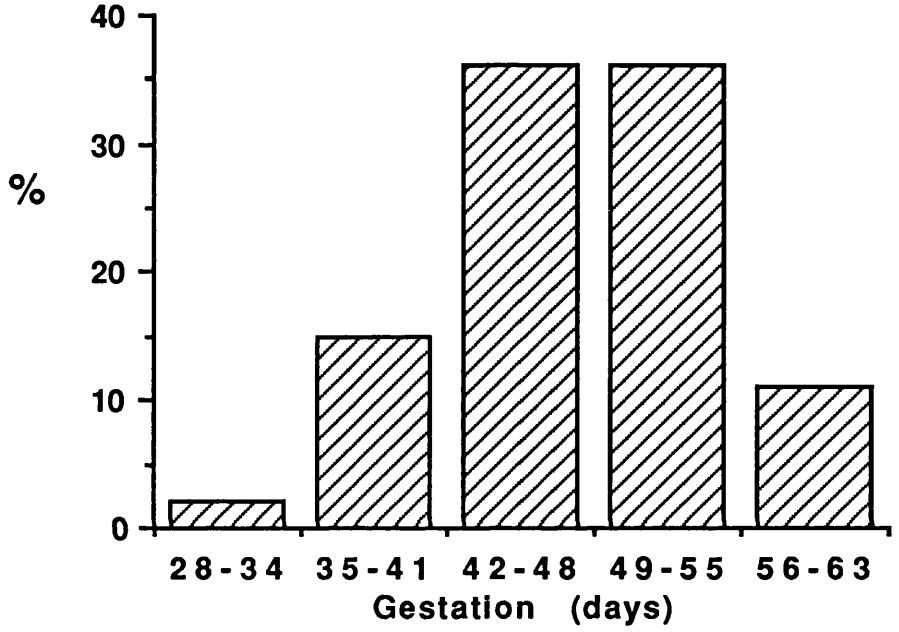
One hundred and sixty-five women of less than 63 days gestation who were referred for termination were invited to take part in the trial. One hundred and five (64%) subsequently agreed, although 4 patients changed their mind and withdrew from the study prior to treatment with mifepristone. Sixty patients (36%) stated that they would prefer a termination under general anaesthetic which was arranged in the usual way. One patient recruited to the study miscarried before being treated, leaving a total of 100 patients for inclusion in the trial.

Patient details relating to age and parity are shown in table 7. The median (range) age of the patients was 26 (18-43) years. Sixty-three percent of the patients had never had a term pregnancy, 18% had previously had a termination of pregnancy, and 6% a previous spontaneous abortion. The median (range) menstrual delay was 19 (3-35) days. Individual pregnancy gestations are shown in figure 8.

Table 7 - Characteristics of treated group

	Number of patients	%
N	100	
AGE		
18 - 20	21	21
21 - 25	37	37
26 - 30	16	16
31 - 35	15	15
36 - 40	7	7
> 40	4	4
PARITY		
0	63	63
1+	37	37
Previous TOP	18	18
Spontaneous abortion	6	6

Figure 8 Gestation of pregnancy in patients treated with mifepristone and PGE1



Efficacy

Three patients aborted completely between 36-48 hours after mifepristone treatment, and these patients were therefore not treated with a prostaglandin pessary. All pregnancies were successfully terminated with the regimen employed, but only 95% were complete.

One patient failed to expel the products of conception after prostaglandin treatment, although she did have mild pelvic pain and light vaginal bleeding. When seen for follow-up one week later ultrasound examination showed a missed abortion and the ß-hCG and progesterone concentrations were still raised at 16,420 mIU/L and 36 ng/ml respectively. As the patient was no longer bleeding a surgical evacuation of the uterus was performed under general anaesthesia on the tenth day after pessary insertion.

Four patients had an evacuation of the uterus because of an incomplete abortion. These were as follows:

A 23 year old multipara, gestation 42 days, who had expelled products of conception three hours after prostaglandin insertion, was re-admitted 3 days after prostaglandin treatment with a temperature of 39°C and heavy vaginal bleeding. She was treated with antibiotics for a suspected pelvic infection and a surgical evacuation of the uterus was performed the following day. The histology of the curettings confirmed the presence of products of conception.

A 31 year old multipara, gestation 53 days, who had expelled products of conception four hours after pessary insertion, when seen at follow-up seven days later complained of heavy vaginal bleeding for the previous 24 hours. At vaginal examination an open cervical os was noted and an ultrasound scan showed mixed echogenic areas in the uterus consistent with retained products of conception. Surgical evacuation of the uterus under general anaesthesia was arranged that day and the histology confirmed the diagnosis. The ß-hCG was subsequently reported at 3330 mIU/ml, but the progesterone was only 9 ng/ml.

A 22 year old nullipara, at 48 days gestation, who had expelled the products of conception four hours after pessary insertion, returned six days later complaining of heavy vaginal bleeding since the termination. An ultrasound examination suggested retained products and a surgical evacuation under general anaesthesia was performed that day. The histology confirmed the presence of products of conception and the ß-hCG and progesterone were later reported as 2110 mIU/ml and 24 ng/ml respectively.

A 30 year old nullipara, at 54 days gestation, who had expelled the products of conception five hours after pessary insertion, had persistent light bleeding for six weeks after termination. Both vaginal examination and ultrasound scan were normal, and at uterine curettage under general anaesthetic a few chorionic villi were removed, which were histologically confirmed.

The success rate of the termination process was not affected by the parity of the patient, gestation of the pregnancy (table 8), or ß-hCG level prior to termination (table 9). Eighty-eight percent and 96% of the patients expelled the products of conception within four and six hours respectively after prostaglandin treatment (figure 9). Only four patients failed to abort whilst in hospital. All patients aborted within the first 24 hours after discharge except the one patient with the missed abortion (see above).

Progesterone and ß-hCG plasma levels did not statistically change in the 48 hours after mifepristone treatment, but both had significantly decreased when measured at the follow-up visits (table 10).

Side-effects

No biochemical or haematological abnormality was detected after treatment with mifepristone (table 10). However 25% of the patients complained of nausea and 15% vomited after tablet ingestion. One patient developed a generalised papular rash 24 hours after receiving the tablets, which lasted for 48 hours. No patients had any diarrhoea after mifepristone treatment. After insertion of the prostaglandin pessary 13% of the patients vomited and 10% had diarrhoea. Pregnancy gestation did not appear to influence the incidence of gastrointestinal side-effects.

Table 8 - Efficacy of mifepristone and gemeprost by gestation

Gestation (days)	Number of Patients	Number of Successes	Rate (%)
28 - 34	2	2	100
35 - 41	15	15	100
42 - 48	36	34	95
49 - 56	36	34	95
57 - 63	11	10	91
Total	100	95	95

Table 9 - Influence of ß-hCG concentration upon success in inducing abortion

ß-hCG level (IU/l)	Number of Patients	Number of Successes	Rate (%)
< 10,000	23	23	100
10 - 20,000	51	47	92
> 20,000	26	25	96
Total	100	95	95

Figure 9 Cumulative abortion interval

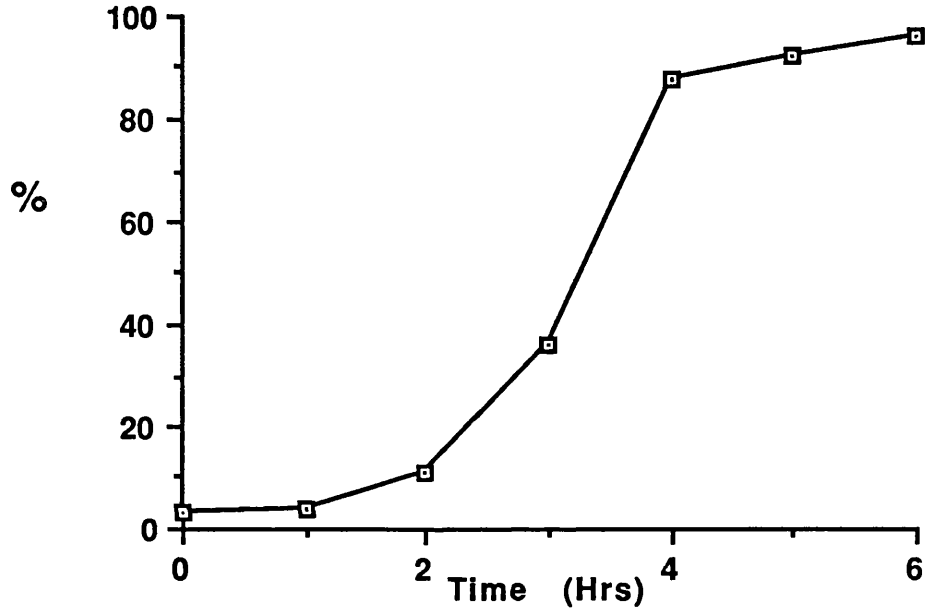


Table 10 - Median plasma concentrations after mifepristone and gemeprost treatment

	Pre mifepristone	Post mifepristone	Follow-up (days) 7	14	28
Haematology					
Hgb (g/dl)	13.3	13.0	12.7	12.9	13.2
Wbc ($x10^9$/l)	9.7	9.9	7.5	7.8	8.0
Liver function					
Tot. prot. (g/l)	69	67	69	70	70
ALT (IU/l)	45	44	46	47	46
Alk Phos. (IU/l)	49	51	49	56	52
AST (IU/l)	20	20	25	24	21
Gamma GT (IU/l)	12	12	13	14	13
Bilirubin (mmol/l)	8.3	8.3	7.0	9.0	10.2
Biochemistry					
Calcium (mmol/l)	2.35	2.34	2.36	2.36	2.35
Phosphate (mmol/l)	1.18	1.10	1.16	1.10	1.31
Creatinine (umol/l)	75	80	81	84	85
Urea (mmol/l)	2.9	2.4	3.4	3.7	3.5
Sodium (mmol/l)	138	138	141	140	141

Table 10 cont.

	Pre mifepristone	Post mifepristone	Follow-up (Days) 7	14	28
Potassium (mmol/l)	4.5	3.9	4.3	4.2	4.4
Urate (umol/l)	186	170	222	245	212
ß-hCG (mIU/ml)	22904	29100°	951*	122*	14*
Progesterone (ng/ml)	60.6	63.9	6.7*	5.0	8.6

Significant difference compared with previous result

° = $p \leq 0.01$

* = $p \leq 0.001$

Morbidity

Analgesic Requirement

During the first 24 hours following mifepristone treatment 5% of the patients complained of mild period like pelvic pain, but no patient required analgesia. During the second 24 hours after treatment 58% had pelvic pain, but in only 8% was the pain severe enough to warrant oral paracetamol analgesia.

Between prostaglandin insertion and expulsion of the products of conception 29% of patients required oral paracetamol 1000mg and 23% required intra-muscular pethidine 75mg for pain relief. The remaining 48% patients did not need any analgesia. Forty (63.5%) of the nulliparae required analgesia following prostaglandin treatment, which was statistically greater than the 12 (48%) multigravidae ($p \leq 0.05$). The analgesic requirement was not affected by pregnancy gestation.

Blood Loss

Six percent of the patients bled in the first 24 hours after mifepristone treatment, but in only one patient was this heavy. In the second 24 hours after treatment 63% of the patients had vaginal bleeding. The bleeding was described as mild in 35%, moderate in 25% and heavy in 3%. The remaining 37% of patients had no vaginal bleeding after mifepristone. Eleven (11%) patients had heavy bleeding at the time of abortion and two patients required an intra-

muscular injection of ergometrine 0.5mg to control the bleeding. The median (range) duration of bleeding after abortion was 13 (3-42) days (figure 10), and this was not affected by parity or pregnancy gestation. The haemoglobin concentration before and after abortion did not change significantly (table 10), and no patients required a transfusion.

Pelvic Infection

Nine percent of the patients were treated with antibiotics during the study, but only one case of pelvic inflammatory disease occurred (see earlier). Three patients were treated with antibiotics as a prophylactic measure prior to a surgical evacuation for retained products of conception. Three patients were treated after termination by their general practitioners because of asymptomatic vaginal discharge although none had any vaginal or endocervical swabs taken, and two patients received antibiotics because of prolonged bleeding and suspected endometritis.

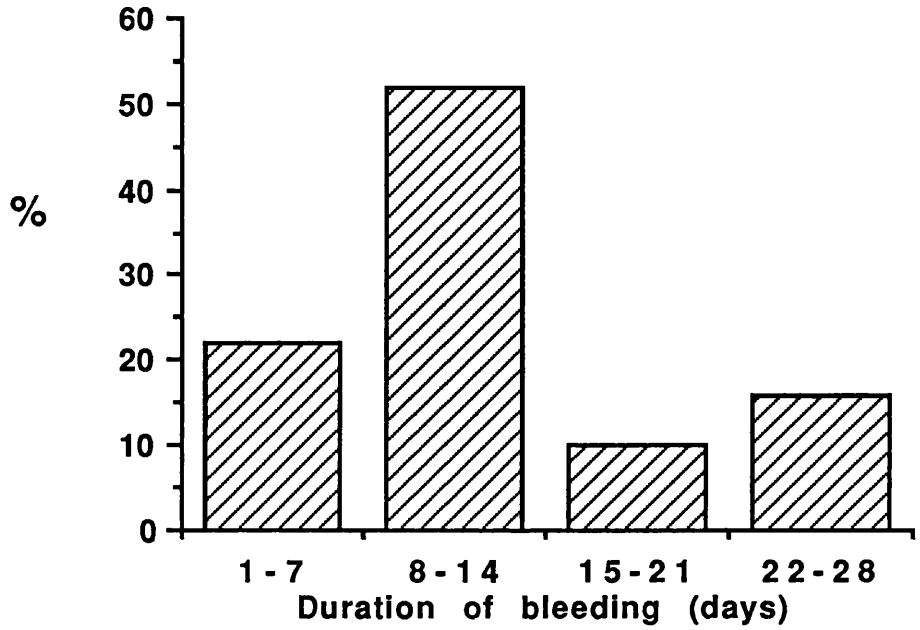
Genital Trauma

No cases of genital trauma occurred.

Length of Stay

Sixty-nine percent of patients stayed in hospital for four hours, 17% stayed for between 4-6 hours, and 14% were discharged between 6-8 hours after prostaglandin treatment. The length of the patients stay in hospital was not

Figure 10 Duration of bleeding after termination



affected by parity or gestation. No patient required overnight hospital admission.

Follow-up

Ninety-seven percent of patients were seen for follow-up on at least one occasion after termination. Attempts were made to contact the rest by writing to the patient or phoning the patient's general practitioner, and details on all patients except one were obtained. This patient passed products of conception whilst in hospital but left the country two days later.

Ninety-three percent of the patients were seen for follow-up one week after the termination. Of the patients seen 77% were still bleeding and 11% had taken oral analgesia during the preceding week for period like pain. When scanned, 11% had echogenic areas in the uterus consistent with blood clots or retained conceptual products. These patients were managed conservatively without any problems.

Eighty-five percent of the patients attended the second follow-up visit 2 weeks after termination. Only 25% were still bleeding and only 4% had abdominal pain during the preceding week. No patients had needed any analgesia. Ultrasound scans were performed on all patients who were still bleeding and on patients who had previously been shown to have echogenic areas in the uterus. All the ultrasounds scans were normal.

Eighty-seven percent of the patients were seen for follow-up 4 weeks after termination. Five percent were still bleeding slightly and 20% thought that they may have had a period before the visit. No patients complained of abdominal pain.

Subsequent Period

The questionnaire which was sent to the last 50 patients was returned by 38 (76%) patients. In 16 (42%) their first menstrual loss was the same as normal, in 14 (37%) heavier than normal, and in eight (21%) lighter than normal.

Patient Acceptability

Eighty-eight percent of the 93 patients who attended the first follow-up examination visit stated they would repeat the termination method, 9% definitely would not repeat this form of termination again and 3% were unsure on what they would do. Of the 9% who would not repeat the method, this was because of method failure or incomplete abortion (3%), and the termination being too painful (6%). All the patients who returned the questionnaire found the medical method of termination acceptable because it was "natural" and that they felt "more in control of what was happening". All 18 patients who had previously had a suction termination found the medical method of termination more acceptable.

All pregnancies were terminated with the combination of mifepristone and gemeprost, and the high incidence of complete abortion (95%) is similar to that reported by Bygdeman and Swahn (1985), Cameron et al, (1986) and Rodgers and Baird (1987). One patient failed to expel the pregnancy, the scan showed a nonviable pregnancy one week after treatment, and an evacuation was performed three days later. If left longer she may have aborted spontaneously. The rate of incomplete abortion was only 4%, and no serious complications occurred in these or any other patient.

These results represent a marked improvement to those analysed retrospectively in section 2.1. when a prostaglandin alone was used for termination. The pregnancy gestation and patient parity are similar in both groups of patients. In the patients treated with mifepristone and gemeprost the abortion failure rate (1%) and the rate of incomplete abortion (4%) were significantly lower than the respective rates of 14.5% and 8.5% for patients treated with a prostaglandin alone. Although heavy bleeding has been reported after mifepristone treatment (Kovacs, 1985) no patients required a transfusion, but 1.3% of patients were transfused when prostaglandin analogues were used solely for termination. Ulmann (1987) has since reported an incidence of transfusion after mifepristone treatment for termination of 0.4% in 1500 women. The median duration of bleeding after termination (13 days) is similar to previous studies (Couzinet et al, 1986; Mishell et al,

1987; Swahn et al, 1988). The results also represent an improvement on those reported by Webster & Gillmer (1989) for epostane alone for early medical termination. In this study of 54 women less than 49 days gestation the termination efficacy was 70-87%.

Despite 25% of the patients complaining of nausea after mifepristone treatment, the incidence of vomiting (13%) and diarrhoea (10%) is much less than the respective figures of 36% and 24% when prostaglandins alone are used for termination. However Swahn et al (1988) have reported no change in the incidence of nausea after mifepristone compared with before treatment. Both the pelvic infection rate (1%) and the percentage of patients treated with opiates (23%) were identical to the patients reported earlier in this thesis.

Gemeprost has been reported in two studies (n=30 in each) to provoke abortion in 87% (Smith & Baird, 1980) and 97% (Cameron & Baird, 1988) of pregnancies of less than 56 days duration. But the high doses of prostaglandin needed (gemeprost 1mg, 3 hourly for 12 hours) are associated with a high rate of gastrointestinal side-effects: vomiting (23-43%) and diarrhoea (33%) and in one study 53% of patients required opiate analgesia (Cameron & Baird, 1988).

To compare complication rates for medical termination with vacuum aspiration is not straightforward. The 4% incidence of incomplete abortion in this study is higher than the 0.9% for surgical evacuation under local

anaesthesia (Hill & MacKenzie, 1990), but this should be weighed against the uterine perforation rate of 0.6% and the need for a skilled operator for surgical evacuation. The incidence of pelvic infection and heavy bleeding are similar with both methods, but medical termination avoids a specific procedure with emotional advantages and permits anonymity.

The combination of mifepristone with gemeprost for medical termination of pregnancy appears to offer a safe, patient acceptable, efficient, out-patient alternative to surgical abortion. Its combination has the advantage of high complete abortion rates with low side-effect frequency. Patients should be warned of the possibility of incomplete abortion and advised to return if they have problems. At present only one fetal study has been performed on mifepristone in humans (see section 5), so the effect on the fetus of the patient changing her mind after treatment with mifepristone, but before abortion, remains a problem, and careful follow up is required to identify any ongoing pregnancies and ensure the patient is adequately counselled (Baird et al, 1988).

More studies are needed on the effects on the efficacy of termination of decreasing the mifepristone to prostaglandin treatment time interval and prostaglandin dose. Swahn & Bygdeman (1988) reported the results of a controlled trial measuring uterine activity after mifepristone treatment (50mg daily x 4 days) with a

pressure transducer in early pregnancy. They found a marked increase in myometrial sensitivity to exogenous 16-phenoxy-PGE_2 (0.05-0.25mg I.M.) administration 24 hours after treatment, but prolonging the duration of mifepristone treatment to 36 or 48 hours did not statistically increase the sensitivity. Also evidence from mid-trimester termination of pregnancy suggests that mifepristone is an effective anti-progestin 24 hours after oral ingestion (see section 3.2.). However if the time of drug ingestion to treatment is cut to 4 hours (see section 5), this time interval is too short for any anti-progestin activity to have developed. A study should therefore be performed to assess the efficacy of mifepristone (600 mg) and gemeprost 24 hours later for early termination of pregnancy.

SECTION 3.2. - THE CLINICAL EFFECTS OF MIFEPRISTONE IN SECOND TRIMESTER TERMINATION OF PREGNANCY

INTRODUCTION

Studies previously performed in this department demonstrated that when patients were treated orally with epostane the induction-abortion interval of mid-trimester prostaglandin termination was significantly decreased over patients treated with placebo (Selinger et al, 1987). No significant difference in side-effects or blood loss between the two groups occurred, but the epostane patients required less parenteral narcotic analgesia.

Urquhart & Templeton (1987) in a non-placebo controlled study treated 20 patients with mifepristone 200mg orally 24 hours prior to prostaglandin termination, and compared these results with 20 patients treated with prostaglandin alone. The induction-abortion interval significantly decreased in the mifepristone group and these patients needed a significantly lower dose of prostaglandin to achieve expulsion of the conceptus.

The aim of this study was to assess the clinical effects of mifepristone during mid-trimester prostaglandin termination.

TRIAL DESIGN AND METHODOLOGY

A double blind, placebo controlled study was performed including 20 primigravidae at 16-18 weeks gestation scheduled for pregnancy termination by extra-amniotic

injection of prostaglandin. Following entry into the trial, pregnancy gestation was determined by clinical and ultrasonic examination. Patients were treated with either mifepristone 600mg or identical placebo orally 24 hours before the planned termination. Randomisation was carried out by using a predetermined randomisation schedule balanced within groups of ten. Sealed copies of the code identifying the contents were held by the hospital pharmacy.

Before tablet treatment a peripheral venous blood sample was taken for haematological (Hb and blood group), and ß-hCG analysis. On admission to hospital a Gaeltec pressure transducer and a Foley catheter were passed transcervically. After myometrial contractility studies had been performed (see section 4.4), a single dose of PGE_2 (Upjohn) 1.5mg in 6gm of Tylose gel was injected into the extra-amniotic space, and the catheters removed one hour later. Six hours after the PGE_2 instillation, a constant infusion of syntocinon 100mu/ml was commenced and maintained until abortion. The following morning, if abortion had not occurred or was not imminent, contractions were augmented with prostaglandin E_1 1mg (gemeprost) pessaries. Papaveretum 20mg and chlorpromazine 25mg were given intramuscularly during the abortion process for analgesia when required. Following expulsion of the fetus, ergometrine 0.5mg was given intramuscularly and uterine evacuation was only performed if the abortion was thought to be incomplete on vaginal examination. Blood loss at

abortion was estimated to the nearest 100ml, and transfusions or antibiotics were only given when clinically indicated. Non-sensitised rhesus negative patients routinely received anti-D immunoglobulin during the first 24 hours following abortion.

Clinical progress, side-effects, vital signs and time of abortion were recorded on purpose designed record sheets. The induction-abortion interval was calculated from the time of prostaglandin instillation into the uterus to the time of expulsion of the products of conception.

RESULTS

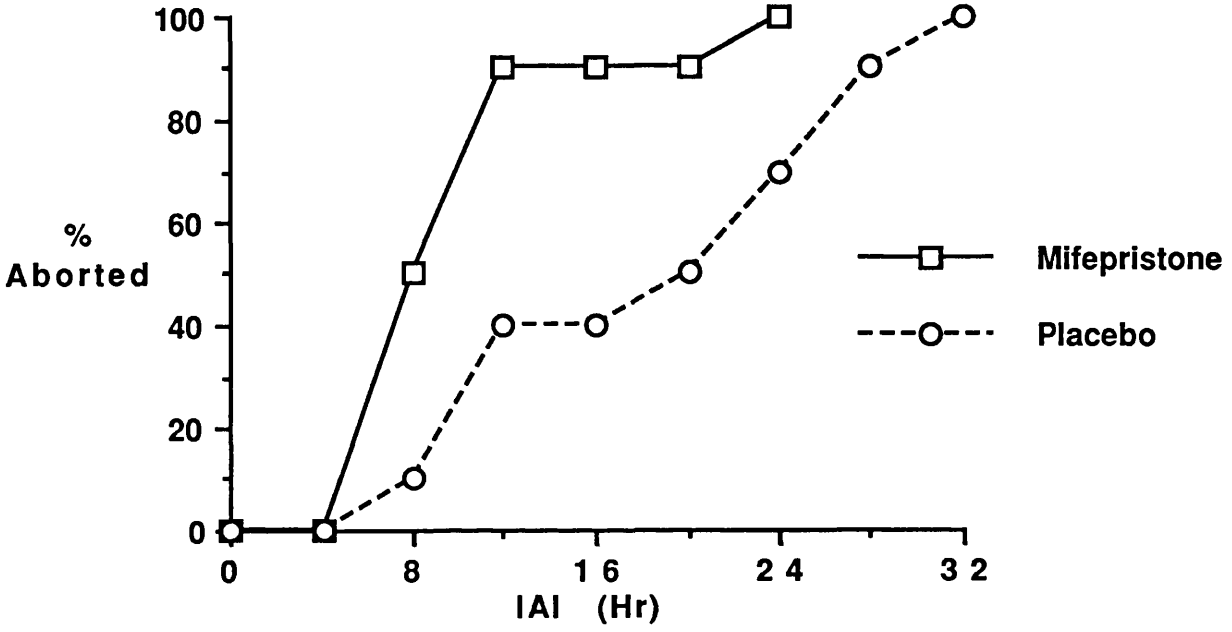
Patients details relating to age, height, weight, pregnancy gestation and ß-hCG concentration are shown in table 11. There were no significant differences between the two groups (Mann-Whitney U test). No drug reactions or side-effects were recorded after mifepristone treatment and no patient had vaginal bleeding or uterine pain before prostaglandin treatment.

The median (range) induction-abortion interval in the patients treated with mifepristone was 512 (245-1400) min. which was statistically less than the median (range) interval of 1128 (345-2100) min. in the control group ($p \leq 0.02$, Mann-Whitney U test). Ninety percent of the mifepristone patients aborted within 12 hours of prostaglandin instillation in the uterus, compared with 40% in the control group (figure 11). Four (40%) patients in

Table 11 - Median (range) characteristics of patients

	Mifepristone (n=10)	Control (n=10)
Age (year)	22 (18-27)	21 (18-26)
Height (cm)	163 (151-171)	161 (148-175)
Weight (kg)	58 (50-76)	57 (52-75)
Gestation (weeks)	17 (16-18)	17 (16-18)
ß-hCG (IU/l)	12,605 (6664-20,693)	14,407 (6645-23400)

Figure 11 - Induction abortion interval (IAI) after mifepristone or placebo



the mifepristone group aborted without the need for a syntocinon infusion compared with one (10%) in the placebo group. Three (30%) patients in the placebo group required augmentation with further prostaglandin, but no patients in the mifepristone group needed further prostaglandin treatment. All patients aborted without complication and were discharged from hospital within 48 hours of admission.

No patient in either group had heavy vaginal bleeding or required a blood transfusion during the abortion process, and no patient required antibiotic treatment for suspected pelvic infection. Three (30%) patients vomited after prostaglandin treatment in the mifepristone group compared with four (40%) in the placebo group. There was no difference in the incidence of need for evacuation of the uterus after abortion between the two groups. There was also no significant difference in the analgesia requirement between the mifepristone or placebo groups.

INTERPRETATION OF RESULTS

The dramatic reduction in the induction-abortion interval by more than half from 1128 to 512 min. in the patients treated with mifepristone, with 90% aborting within 12 hours of prostaglandin treatment, was of considerable benefit to patients in reducing the need for an oxytocin infusion and further prostaglandin augmentation. This reduction was achieved with a single treatment with a small dose of PGE_2 (1.5mg). This has the advantage of ease of administration and low incidence of

gastrointestinal side-effects. As these side-effects are directly related to the dose of prostaglandin (MacKenzie & Embrey, 1976), it is possible that extra-amniotic treatment with an even smaller dose of prostaglandin (0.5-1.0mg) could potentially further decrease their incidence without necessarily affecting abortion efficacy. A larger dose of extra-amniotic prostaglandin (2.5mg) may lead to a further decrease in the induction-abortion interval, and this should also be investigated.

This study was by necessity small and the number of patients involved were too few to demonstrate any significant differences in the incidence of incomplete abortion and need for analgesia during the abortion process. Further studies on a larger number of patients are planned to address these issues. Increasingly more of the patients in our unit undergoing mid-trimester termination of pregnancy are treated with PGE_1 1mg (gemeprost) pessaries rather than either extra-amniotic or intra-amniotic prostaglandin, and it would be interesting to investigate the effect of mifepristone treatment prior to the termination on these patients and see if a similar decrease in induction-abortion interval occurs.

With the potential of reduced inpatient stay, day-case prostaglandin termination of mid-trimester pregnancy becomes a realistic possibility after mifepristone priming of the uterus. The induction-abortion interval in second trimester termination is parity related (Hill & MacKenzie, 1989), therefore this study was only performed on

primigravidae, however it confirms that similar reductions in induction-abortion intervals are obtained with mifepristone and epostane (Selinger et al, 1987).

Progesterone inhibition with mifepristone has been demonstrated as an effective "priming" procedure which has been used in the treatment of mid-trimester termination of pregnancy. The drug has also been demonstrated to have a similar priming effect on patients having labour induced after an intra-uterine fetal death (Cabrol et al, 1985; Padayachi et al, 1988). The possibility of another agent to help improve the outcome of induced term labour becoming available in a few years may now be realised.

SECTION 4 - THE MECHANISM OF ACTION OF MIFEPRISTONE

SECTION 4.1. - THE EFFECT OF MIFEPRISTONE ON PROGESTERONE AND OESTROGEN RECEPTOR CONCENTRATIONS IN THE DECIDUA AND PLACENTA IN EARLY PREGNANCY

INTRODUCTION

Although mifepristone interrupts the luteal phase of the menstrual cycle (Herrmann et al, 1982) and terminates early pregnancies (Kovacs et al, 1984), the exact mode and site of action is not fully established. There is evidence that the antagonistic effects of mifepristone are related to the essential biochemical steps following progesterone receptor occupancy which leads to the biochemical response: activation of the cytosolic progesterone receptors and nuclear translocation (Heuber et al, 1985).

As progesterone receptors (PR) are thought to be influenced by oestrogen action which in turn is related to oestrogen receptor (ER) levels (Levy et al, 1980), the concentration of progesterone and oestrogen receptors in the decidua and placenta of patients undergoing medical termination of early pregnancy with a combination of mifepristone and a prostaglandin E_1 analogue have been studied. These results are compared with the receptor concentrations in the decidua and placenta from a control group of patients undergoing surgical evacuation of the uterus at the same gestational period.

TRIAL DESIGN AND METHODOLOGY

Twenty-five women whose pregnancy was confirmed by an

ultrasound scan were recruited into the study. The pregnancy gestation was calculated from the fetal crown-rump length. In all cases haemoglobin, blood group and rhesus state were determined prior to termination, and anti-D immunoglobulin was given when clinically indicated.

The women were treated within one of two groups. In 15 women of eight weeks gestation, medical termination was performed using a combination of mifepristone 600mg orally, and followed 48 hours later by vaginal administration of a single pessary of prostaglandin E_1 1mg analogue (gemeprost). Specimens of placenta and decidua were collected at the time of expulsion of the products of conception. A control group of 10 women at the same gestation were treated with a single vaginal pessary of PGE_1 1mg and 2-4 hours later, surgical evacuation of the uterus under general anaesthesia was performed and specimens of placenta and decidua collected.

Immediately after collection, the tissue was cut into small fragments with scissors and washed several times in ice cold isotonic saline. Samples (1-2 g) were blotted dry, quickly frozen in liquid nitrogen and stored at -70°C for up to three 3 months: under these conditions, no detectable loss of receptor occurs (Giannopoulos & Tulchinsky, 1979).

Preparation of cytosol and nuclear fractions

Before assay, the tissue was allowed to thaw in 10 volumes of ice cold TED-buffer (10 mM Tris buffer, pH 7.5,

containing 1.5mM EDTA, 5mM sodium molybdate and 1mM dithiothreitol). All subsequent steps were performed at 0-4°C. The tissue was disrupted with two 15 sec. bursts using a Silverson homogeniser set at maximum speed, followed by three passes in a glass-teflon Potter-Elvejhem homogeniser. The homogenate was then centrifuged at 800g for 15 min. The cytosolic fraction was prepared by centrifuging the 800g supernatant at 105,000g for 60 min in a Beckman ultracentrifuge, and an aliquot was assayed for total soluble protein by the method of Bradford (1976). Cytosolic fractions were diluted to 1-2 mg protein/ml before measuring cytoplasmic receptor concentrations. The 800g pellet, containing the nuclear fraction, was gently washed twice with TED buffer, and rehomogenised with a glass-glass homogeniser in 10 volumes of TED buffer containing 0.6 M potassium chloride and 1 mg/ml bovine serum albumin. Salt soluble receptors in the nuclear fraction were extracted at 0°C for 60 min, with sporadic stirring. The suspension was then centrifuged at 105,000g for 30 min. and the supernatants were used for the measurement of oestrogen and progesterone nuclear receptors. The DNA content of the pellets was estimated by the method of Burton (1968).

Oestrogen and progesterone receptor immunoassays

Oestrogen and progesterone receptor concentrations were measured by enzyme-linked immunosorbent assay (ELISA) using specific monoclonal antibodies (Abbott Laboratories, Diagnostic Division, Maidenhead, Kent, U.K.). The assays

were optimised for decidual and placental cytosolic fractions and salt-soluble nuclear extracts. The monoclonal antibody used was able to recognise both occupied and unoccupied forms of the receptors (Stowers et al 1988).

0.1 ml aliquots of each sample were incubated with plastic beads coated with an anti-receptor monoclonal antibody (anti-PR or anti-ER) for 18 h at 4°C. The complex antibody-receptor was then incubated with a second peroxidase-conjugated anti-receptor antibody for 60 min at 4°C for PR and at 37°C for ER. This was followed by a further 30 min incubation with a peroxidase substrate solution (hydrogen peroxide plus orthophenyldiamine) in the dark at room temperature. The colour reaction was stopped by the addition of 1 ml sulphuric acid solution. The absorbance was measured at 492 nm and the concentration of receptors calculated from a standard curve prepared with receptor standards provided by the manufacturer (0, 5, 25, 100 and 250 fmol/ml).

In each assay positive controls were included, and reproducibility of both PR and ER assays was checked with positive and negative controls. The mean coefficient of variation for PR and ER was 8.3% and 9.7% respectively. In no case (6 experiments) did a negative control give values above the sensitivity reported by the manufacturer (1.0 fmol/ml for PR and 1.5 fmol/ml for ER). The standard curves for PR and ER are shown in figures 12 and 13 respectively.

Figure 12 Standard curve for progesterone receptors

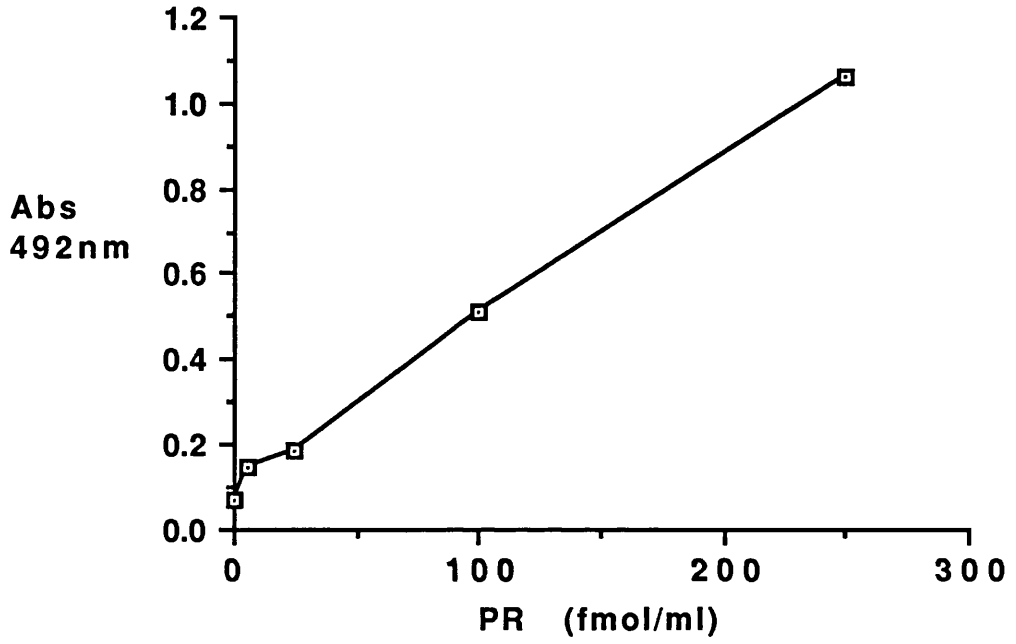
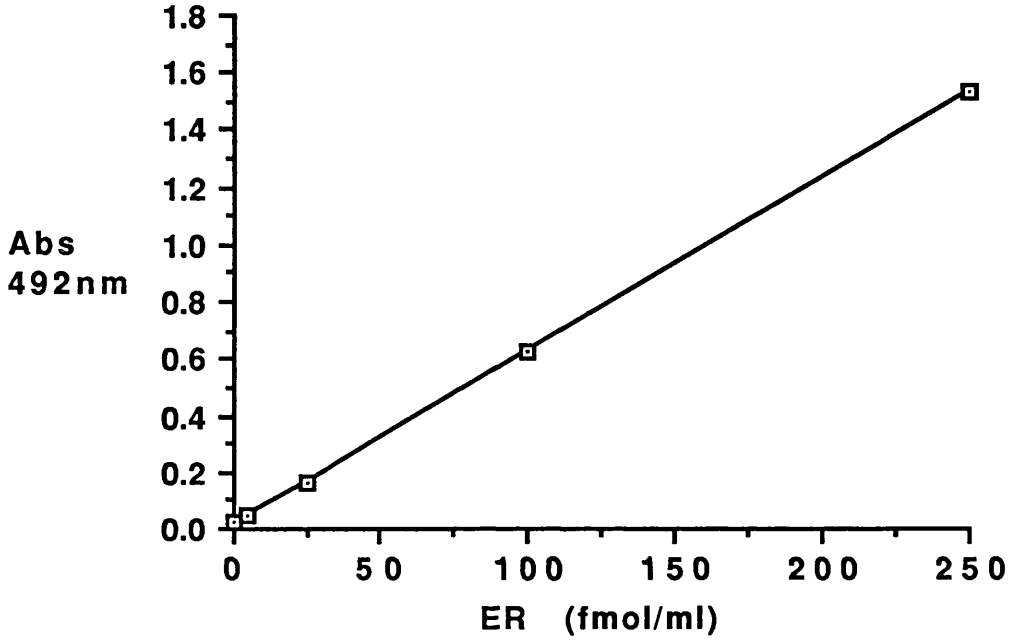


Figure 13 Standard curve for oestrogen receptors



Patient details relating to age, parity and pregnancy gestation are shown in table 12. There were no significant differences in these indices between the patients treated with mifepristone or by surgery (Mann-Whitney, U-test).

Of the patients undergoing medical termination nine (60%) had lower abdominal period like pain and vaginal bleeding after mifepristone treatment, but no patient aborted before prostaglandin treatment. All patients aborted completely within four hours of prostaglandin treatment and no subsequent complications were encountered.

Of the patients treated surgically, all pregnancies were successfully terminated without complications.

Progesterone Receptor (PR) Concentrations

Decidua

The decidual PR concentrations in both groups of patients are shown in figure 14. The median (range) cytosolic PR concentration of the patients treated with mifepristone of 2.1 (0.8-22.3) fmol/ugDNA was significantly lower than in the surgical group of 18.3 (3.0-41.2) fmol/ugDNA ($p \leq 0.02$, Mann-Whitney U-test). The median (range) nuclear PR concentrations in the mifepristone group of 1.0 (0.4-17.0) fmol/ugDNA was not significantly different from the respective values of 2.9 (1.0-11.2) fmol/ugDNA in the surgical group. The median (range) total PR concentration of 3.3 (1.6-23.4) fmol/ugDNA in the

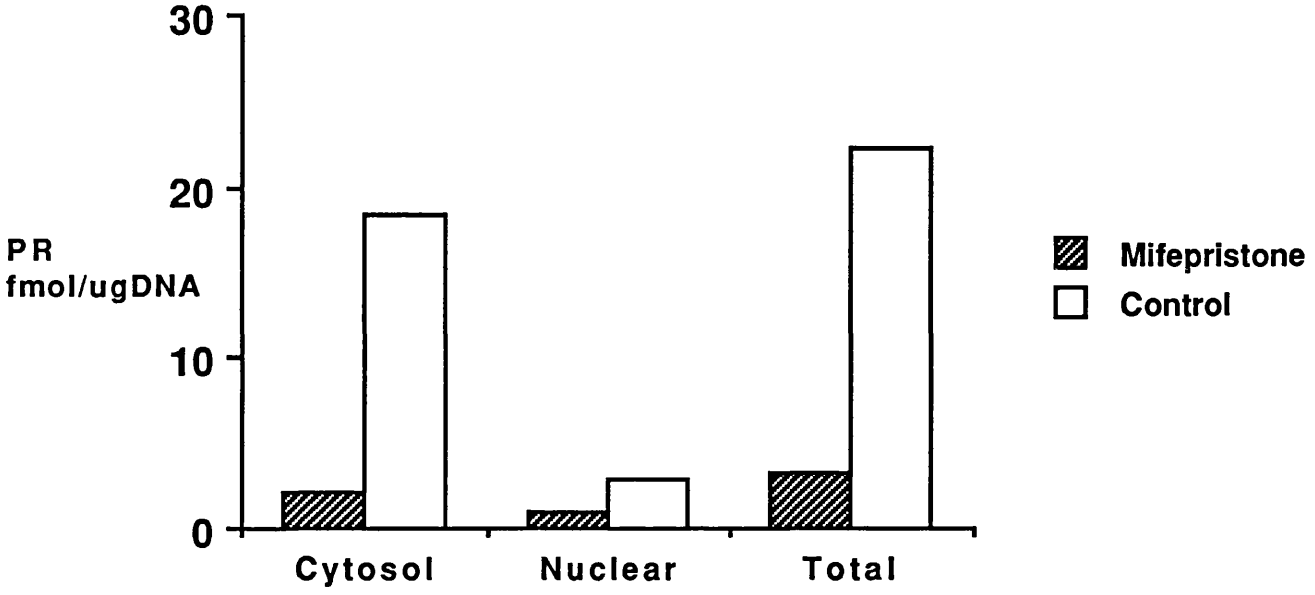
Table 12 - Details of patients undergoing termination with mifepristone or surgery

	Mifepristone		Surgery	
Age (years)	24 [18-32]		23 [19-31]	
Gestation (days)	52 [51-58]		54 [50-59]	
Parity (%)				
0	9	(60)	6	(60)
1 +	6	(40)	4	(40)

[ ] = Range.

( ) = Percentages

Figure 14 Median progesterone receptor levels in the decidua after mifepristone and suction termination



mifepristone group was significantly less than in the surgical group of 22.3 (4.2-51.2) fmol/ugDNA ($p \leq 0.02$, Mann-Whitney U-test).

The decidual PR concentrations were not significantly different in the patients who had abdominal pain and vaginal bleeding after mifepristone from patients without pain or bleeding (figure 15).

Placenta

No cytosolic or nuclear PR were detected in the placentae obtained from patients treated with mifepristone or surgery.

Oestrogen receptor (ER) concentrations

Decidua

The decidual ER concentrations in the patients treated with mifepristone or surgery are shown in figure 16. There was no significant difference in the cytosolic, nuclear or total ER concentrations between the mifepristone and surgical group. In patients who had abdominal pain and vaginal bleeding after mifepristone the ER concentrations were not significantly different from the asymptomatic patients (figure 17).

Placenta

In 10 (75%) patients treated with mifepristone and five (50%) treated surgically no ER was detected in the placenta: this was not a significant difference. In the

Figure 15 Median decidual progesterone receptor levels after mifepristone

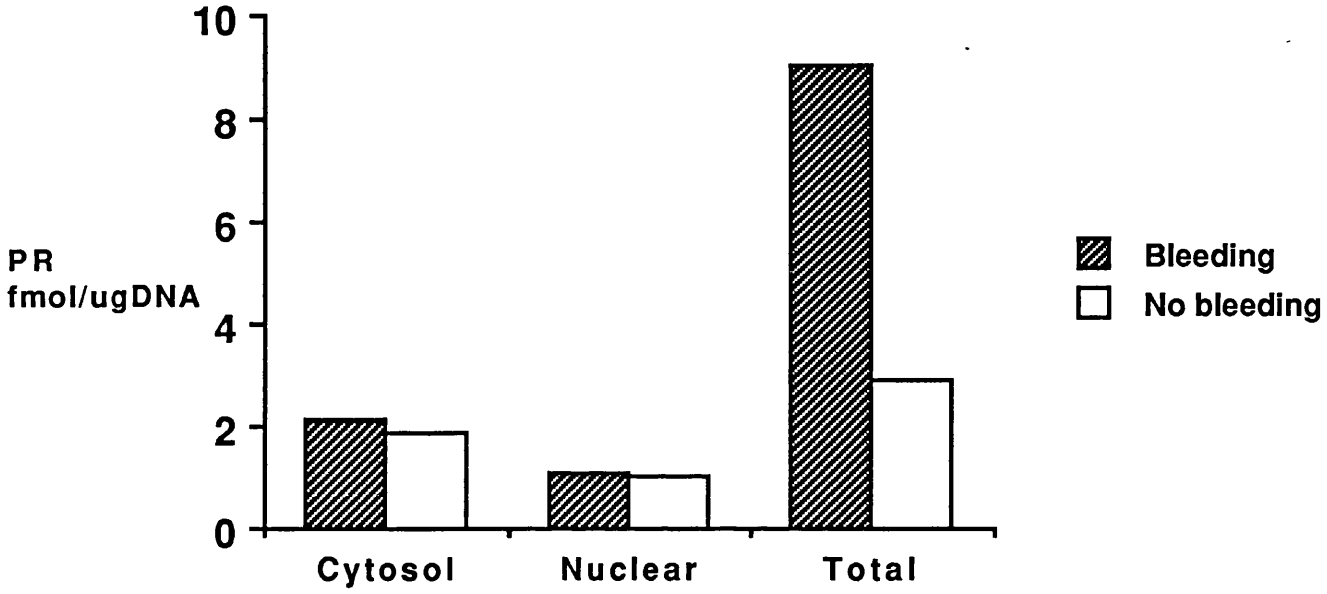


Figure 16 Median decidual oestrogen receptor levels after mifepristone or surgery

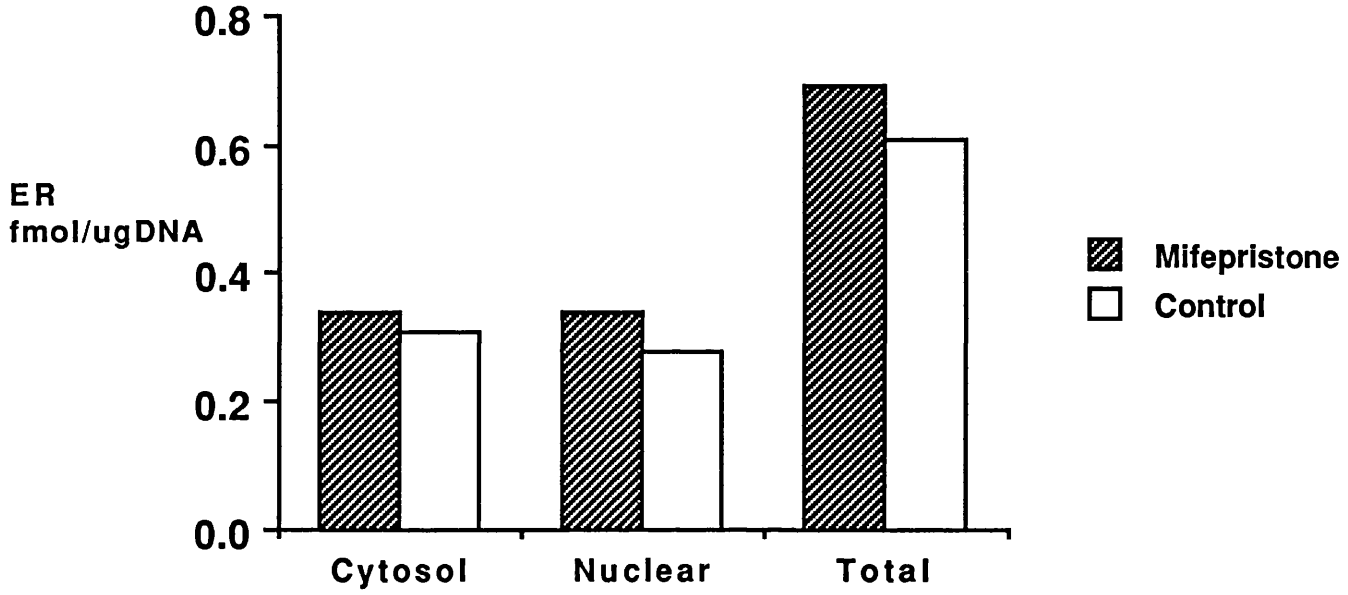
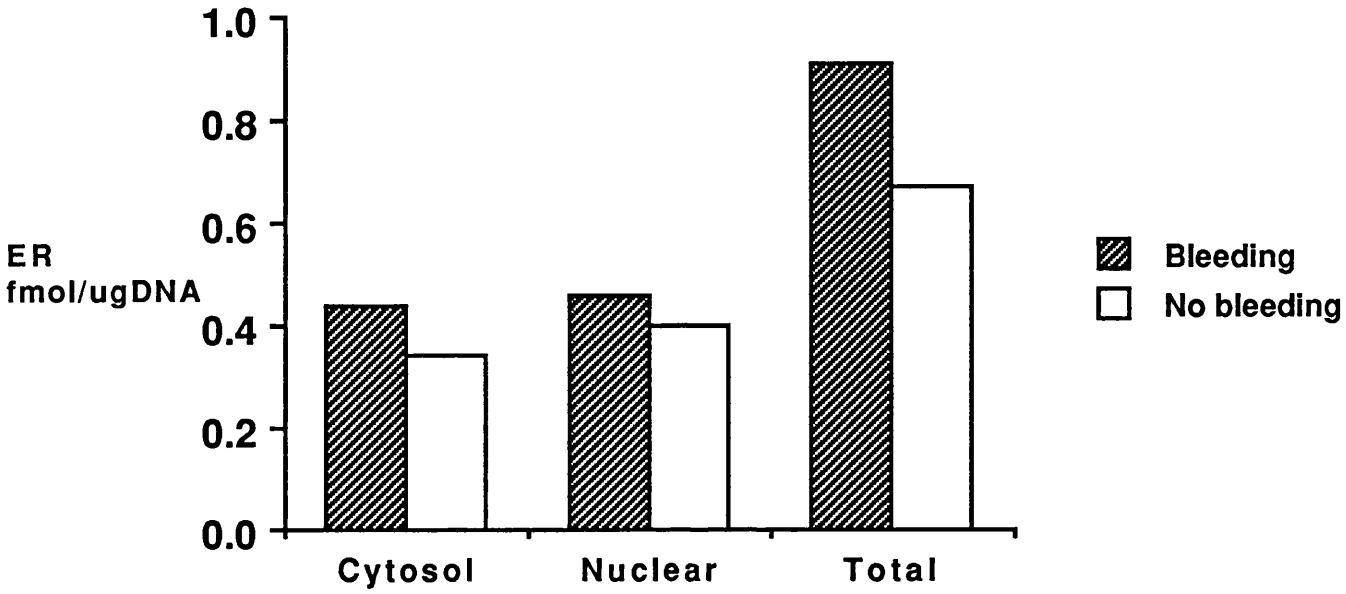


Figure 17 Median decidual oestrogen receptor levels after mifepristone



patients where ER was detected there were no difference between the mifepristone and surgical group.

INTERPRETATION OF RESULTS

This is the first study measuring progesterone and oestrogen receptor concentrations in human decidua and placenta after treatment with mifepristone. Progesterone regulates the expression of specific genes by increasing the efficiency of transcription (Bagchi et al, 1988). This transcriptional activity is triggered by the interaction of the activated PR with specific DNA sequences called progesterone responsive elements [PRE] (Yamamota, 1985). However, the exact mechanism of interaction between receptor and PRE is unknown. A two stage model of steroid hormone action proposed that the unoccupied steroid receptors resided in the cytoplasm (Toft et al, 1967; Jensen et al, 1968; Gorski et al, 1968; Baulieu et al, 1971). Hormone binding resulted in migration of the receptor-hormone complexes into the nucleus, and binding to DNA (Yamamota et al, 1972). However, recent studies have demonstrated that probably the unoccupied receptors are nuclear proteins (King & Greene, 1984; Welshons et al, 1984; Rajendran & Parikh, 1987; Stowers et al, 1988), which are merely artifactually transferred to the "cytosolic" fraction, possibly because they dissociate in the high ionic strength medium used for extraction of the nuclear receptors (King, 1987; Renoir et al, 1990).

In humans, mifepristone is known to bind to PR with a

samilar affinity as progesterone (Gravinis & Gurpide, 1986), and this binding results in the formation of high affinity receptor-PRE complexes. However, mifepristone as opposed to progesterone, or agonists such as R 5020, may not elicit transcriptional responses (Heuber et al, 1985; Groyer et al, 1987; Miller et al, 1988). Therefore anti-hormone receptor complexes may bind to PRE, but are unable to promote the necessary changes in chromatin to stimulate transcription (Webster et al, 1988).

The results from this study may suggest another possible mechanism of mifepristone anti-progesterone receptor action, namely that the drug is able to down regulate the progesterone receptor concentration in the decidua of early human pregnancy. There is evidence from in vitro studies that progesterone down regulates PR concentrations in target cells (Wei et al, 1988; Read et al, 1988) and it is possible that mifepristone has a similar effect. In human breast cancer cell lines, mifepristone treatment in vitro results in a significant decrease in both PR protein and PR-mRNA (Read et al, 1988). This effect is also elicited by the progesterone agonist R 5020 and suggests that receptor down regulation is a consequence of ligand-PR interaction. The data presented here shows that women treated with mifepristone have a significant loss in decidual PR concentrations. The loss of receptor protein together with the lack of transcriptional activation following PR-mifepristone binding may explain part of the overall anti-progestogenic effect of

mifepristone. This down regulating ability of mifepristone was specific for PR, and no effects on oestrogen receptor concentrations occurred, despite the drug previously having been reported to increase nuclear oestrogen receptor concentrations in rats (Fuentealba et al, 1988a; Fuentealba et al, 1988b) and monkeys (Haluska et al, 1990; Neulen et al, 1990).

Berthois et al (1991) have shown that if mifepristone is given in the immediate postovulatory period it blocks tissue evolution in the follicular phase and PR levels actually increase compared with placebo. However, Swahn et al (1990) reported a significant fall in PR 12 hours after mifepristone at a similar period in the menstrual cycle. Mifepristone acts by blocking the effects of progesterone at a cellular level by blocking PR, and as progesterone is known to down regulate PR levels, theoretically mifepristone treatment should result in an increase in PR as reported by Berthois et al (1991). Why then did the PR concentrations fall in the patients treated with mifepristone in this study? Schindler et al (1985) have reported oedema, necrosis and capillary damage in the decidua 24h after mifepristone treatment, and it is possible that the fall in PR may have been secondary to cell damage and not necessarily due to down regulation. Also, in view of the time delay, the placental and decidual tissue obtained after treatment with mifepristone may have been degraded more than the "fresh" tissue obtained by surgical curettage. Perhaps a better control group of

patients would have been patients undergoing medical termination of early pregnancy with gemeprost alone, so less errors in interpretation would have been introduced. However, further studies are required to fully elucidate the molecular antagonistic action of mifepristone.

SECTION 4.2. - THE EFFECT OF MIFEPRISTONE ON PLASMA PGEM AND PGFM LEVELS IN EARLY PREGNANCY

INTRODUCTION

The synthetic anti-progestin mifepristone administered orally has been shown to induce abortion in 60% of women (Kovacs et al, 1984), and if a low dose of prostaglandin is administered vaginally 48 hours later, the abortion rate increases to 95% (Rodger & Baird, 1987). The mechanism whereby the anti-progestin enhances the action of the prostaglandin is uncertain. Mifepristone blocks the progesterone receptors, which in turn may lead to increased prostaglandin production. Therefore, to investigate the mechanism of action of mifepristone further, changes in prostaglandin E metabolite (PGEM) and F metabolite (PGFM) concentrations in peripheral blood have been measured following the oral administration of the drug.

Prostaglandin radioimmunoassay (RIA) was first developed by Levine & Van Vunakis, (1970) and the technique was used for measurement of primary prostaglandins in plasma and urine. The results were often of limited value, because of collection and storage of samples problems (Jubiz & Frailey, 1974). Furthermore both PGE_2 and PGF_{2a} undergo rapid degeneration to the 15-keto-13,14-dihydro metabolite with one circulatory pass through the lungs (Ferreira & Vane, 1967).

To overcome these problems it has been suggested that plasma PG metabolite assays should be used to study PG

levels (Granström, 1978). RIA's for PGFM have proved successful (Granström & Kindahl, 1976), but PGEM is chemically unstable in blood and rapidly breaks down to PGAM, which in turn can bind to albumin (Fitzpatrick et al, 1980; Granström et al, 1980). However all of these metabolite forms are converted under alkaline conditions to a stable compound 11-deoxy-13,14-dihydro-15-keto-11ß,16ð-cyclo-PGE_2 (bicyclo-PGEM) which can be measured by RIA (Granström & Kindahl, 1980; Demers et al, 1983).

TRIAL DESIGN AND METHODOLOGY

Twenty-five women accepted for termination gave consent to be included in the study. Pregnancy was confirmed by an ultrasound scan and in all cases haemoglobin, blood group and rhesus state were determined prior to termination. Anti-D was given when clinically indicated.

The women were distributed for treatment into three groups. In 10 patients, peripheral plasma was collected prior to treatment, and at 4, 24 and 48 hours after mifepristone 600mg administration orally. In a further 10 patients, plasma were assayed before and 48 hours after mifepristone 600mg treatment orally. Immediately after the last plasma sample was collected in both these groups, a PGE_1 (gemeprost) 1mg pessary was given to induce abortion. In the third group of five patients, plasma samples were collected at time 0 and 48 hours later, following which their pregnancies were terminated by uterine aspiration

under general anaesthesia. All blood samples were subsequently analysed for PGEM and PGFM concentrations. In addition, blood samples were collected for ß-hCG and progesterone concentrations prior to any treatment.

The blood samples for prostaglandin metabolite levels were collected into chilled bottles containing acetylsalicylic and ethylene diamino-tetra-acetic acids (Mitchell et al, 1978). Plasma was separated immediately by centrifugation at 1500g at 10°C, and divided into 3 ml samples for PGFM assay and 1 ml samples for PGEM assay. All samples were stored at -70°C until assayed (Sellers et al, 1981).

PGEM Assay Details

The PGEM radioimmunoassay procedure employed was based on an assay described by Demers et al (1983), who also provided the antisera. PGEM (80 Ci/mmol) was purchased from the Radiochemical centre (Amersham).

The label was prepared by evaporating to dryness under nitrogen, then reconstructed in ethanol (9ml) and stored in ethanol at -20°C. To make bicyclo-PGEM 1 ml of stock solution was evaporated under nitrogen and redissolved in distilled water, and the solution brought to pH 11.0 (NaOH; 50ul; 0.25 mol/l) and incubated at 37°C for 24 hours. The solution was brought to pH 3 with citric acid and tritiated bicyclo-PGEM was extracted 3 times with ethyl acetate (2.4 ml), and evaporated to dryness under nitrogen.

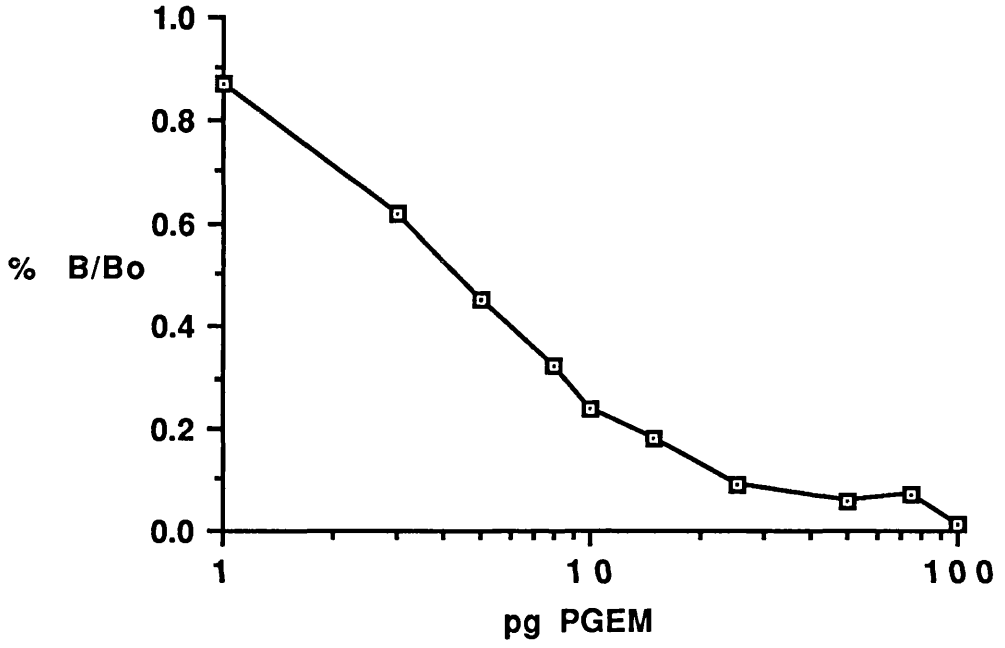
The bicyclo-PGEM was stored in ethanol at -20°C.

Plasma samples (1 ml) were initially incubated for 48 hours at pH 11.0 after the addition of NaOH (30 ul, 1 mmol/l). The sample pH was then neutralised to 7.4 by the addition of KH_2PO_4 (70Ìl, 1 mmol/l). The standard curve was prepared from a 1 mg/l bicyclo-PGEM stock stored in ethanol at -20°C and was made up in T.G.Buffer (pH 7.4), with added bovine serum albumin [BSA],(40 g/l) to approximate the plasma sample matrix. Standard quantities of bicyclo-PGEM (0-100pg) [figure 18] and treated plasma sample unknowns were added in duplicate in 100 Ìl aliquots to plastic RIA tubes. Antiserum and label diluted in the assay phosphate buffer were also added in 100 Ìl aliquots such that the final antiserum dilution was 1:6000, and each tube received 5000 cpm label. After vortex mixing, the incubation was left overnight at 4°C. Separation of bound from free bicyclo-PGEM was accomplished by the addition of 1 ml of a chilled dextran-coated charcoal mixture, which was incubated at 4°C for 14 min. The assay tubes were then centrifuged at 1000g and 4°C for 10 min. The supernatant was decanted into 10 ml scintillant and counted in the scintillation counter.

PGFM Assay Details

The assays utilised antisera donated by DR. F. Dray (Pasteur Institute, Paris) and the procedures employed were based on his published methodology (Dray et al, 1975; Sors et al, 1977), with some modifications (Mitchell et al,

Figure 18 Standard curve for bicyclo-PGEM

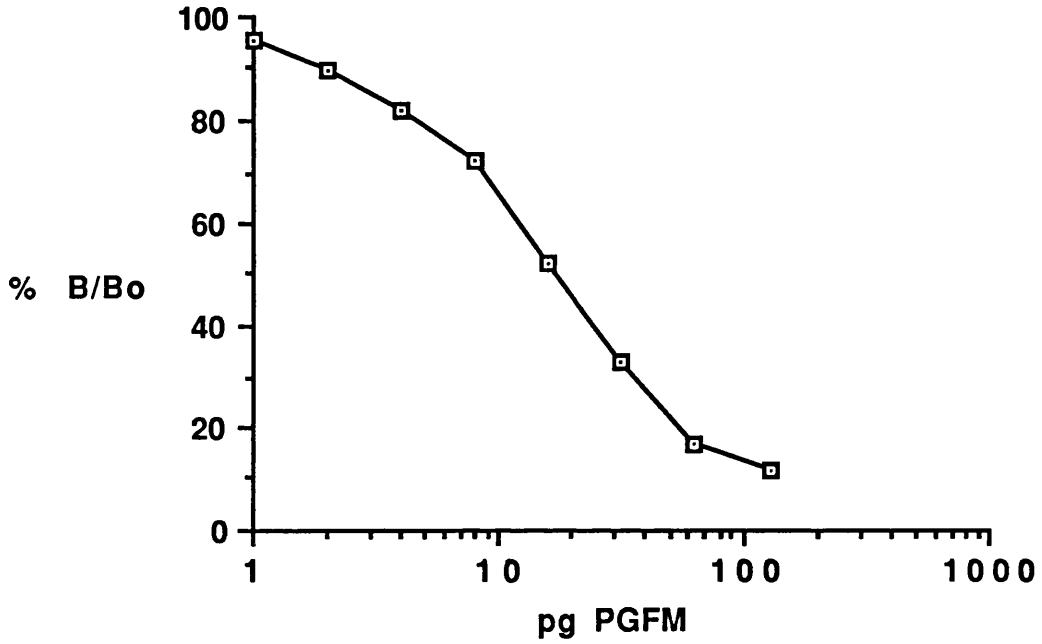


1978). All solvents were distol reagent grade (Fisons Scientific Apparatus, Leics.) and silicic acid was 100 mesh (Mallinckrott Co., MA).

After adding approximately 2000 cpm tritiated PGFM to monitor recoveries, plasma samples (3 ml) were extracted with an acidified mixture of cyclohexane and ethyl acetate (1:1, vol/vol). Samples were applied to microcolumns containing silicic acid (0.5g) in cyclohexane-ethyl acetate-methanol (60:40:20, vol/vol). After a wash procedure using cyclohexane-ethyl acetate (60:40, vol/vol; 4 ml), prostaglandins were eluted in a 5 ml fraction of cyclohexane-ethyl acetate-methanol (60-40-20, vol/vol). All procedures were performed under nitrogen pressure and all of the glassware was siliconised. The column fractions were evaporated under nitrogen and then dissolved in 1 ml T.G.buffer (1M K_2HPO_4, 80ml; 1M KH_2PO_4, 20 ml; 0.9% NaCl, 900ml: NaN_3,1g; and gelatin, 1g). A known volume (0.1 ml) was taken in duplicate for the determination of radioactivity to estimate recoveries.

Duplicate samples (0.1 ml) were taken for radioimmunoassay of PGFM. Final dilutions were 1:200,000, and tritiated tracers were used for PGFM assays (specific activity > 157 Ci/mmol; Radiochemical Centre, Amersham). Prostaglandin standards were in the range 1-128pg/tube (figure 19). Bound and free prostaglandin was separated by the addition of 1 ml of a chilled dextran-coated charcoal mixture, which was incubated at 4°C for 14 min. The assay tubes were then centrifuged at 1000g at 4°C for 10 min.,

Figure 19 Standard curve for PGFM



and the supernatant was decanted into 10 ml scintillant and counted in a Beckman LS 7000 liquid scintillation counter (Beckman Instruments, Palo Alto, USA).

The mean coefficient of variation for both the PGEM and PGFM assays was 8-11%. The relative cross reactivity of both PGEM and PGFM is illustrated in appendix 5.

Statistical analysis was performed using the Mann-Whitney U-test and Kruskal-Wallis analysis of variance test.

RESULTS

There were no significant differences in the patient details between treated and control patients (table 13). The mean ß-hCG and serum progesterone concentration in the two groups were not significantly different.

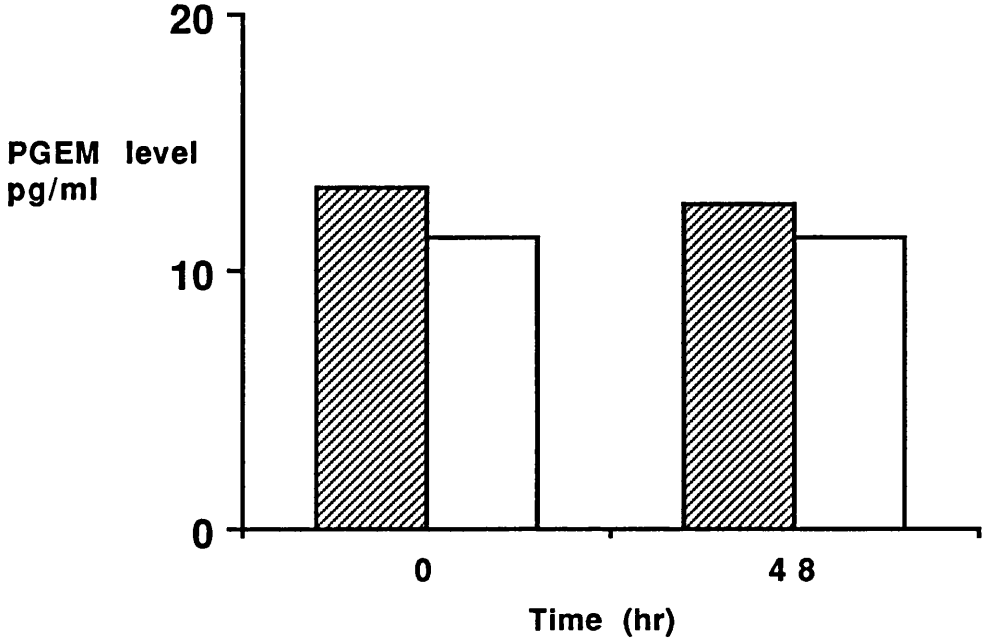
Of the 20 patients treated with mifepristone, 66% experienced uterine bleeding, less than a normal menstrual loss, and associated lower abdominal menstrual during the first 48 hours. No patients aborted prior to prostaglandin treatment. All patients aborted completely within four hours of prostaglandin treatment, and were managed without complication.

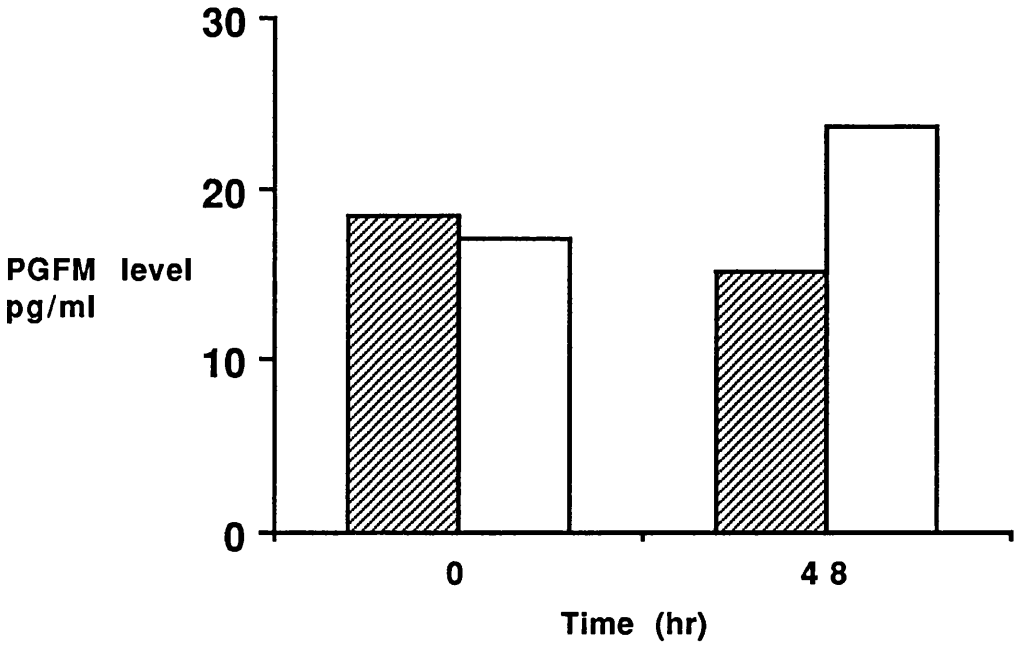
For all the patients treated with mifepristone, the median (range) PGEM concentrations at 0 and 48 hours were 13.3 (10.2-22.1) and 12.7 (10.9-18.2) pg/ml respectively, compared with 11.3 (10.6-13.7) and 11.3 (11.0-12.1) pg/ml for the control patients (figure 20). There were no

Table 13 - Median (range) patient characteristics of 25 women undergoing early termination of pregnancy

	Mifepristone (n=20)	Controls (n=5)
AGE (years)	28 (18-40)	24 (19-39)
Gestation (days)	45 (33-56)	46 (42-56)
Parity [%]		
0	60 %	60 %
1+	40 %	40 %
ß-HCG (IU/l)	24,964 (11,318-56,991)	17,279 (8,760-61,212)
Progesterone nmol/l	56 (39-96)	63 (41-78)

Figure 20 Median PGEM & PGFM levels after mifepristone (hatched) or surgery (plain)





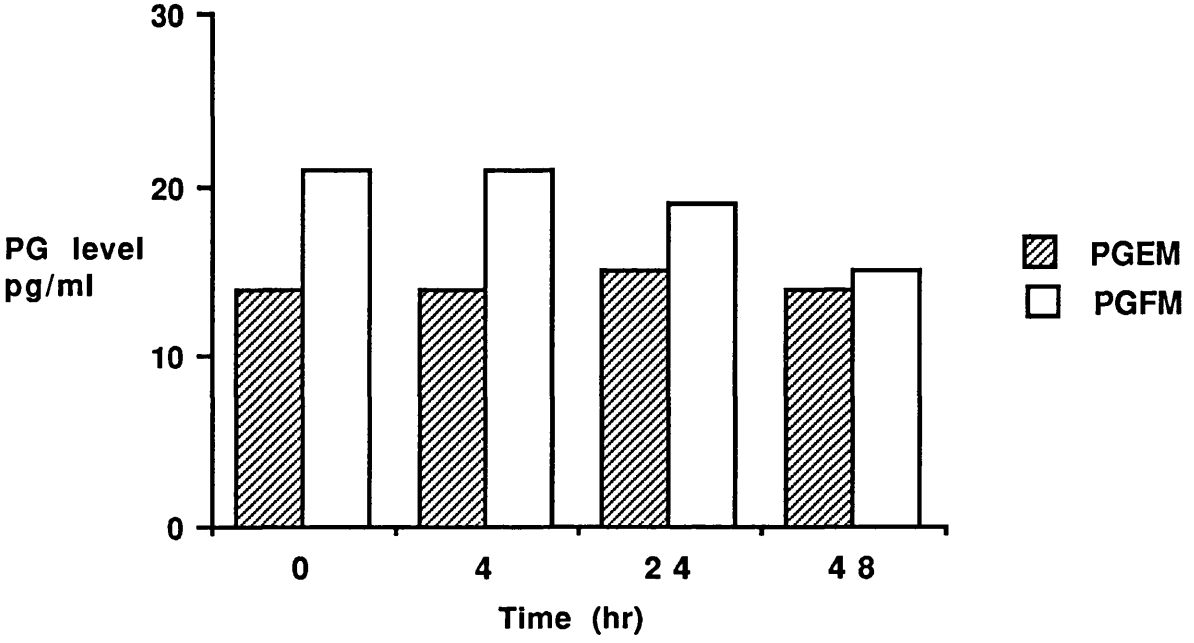
significant differences in these results at the two sampling times between the groups. The median (range) PGFM concentrations for the treated patients were 18.4 (9.1-61.0) and 15.0 (9.2-35.4) pg/ml at 0 and 48 hours compared with 17.2 (9.9-47.4) and 23.8 (17.4-37.1) pg/ml for the control group (figure 20). These differences did not reach statistical significance. In the 10 patients who had PGEM levels measured prior to and at 4, 24 and 48 hours after treatment with mifepristone, the median (range) prior to treatment 13.5 (12.0-22.1) pg/ml and the respective values over the study period of 14.2 (11.8-16.9), 14.8 (11.6-20.5), and 14.4 (11.4-18.2) pg/ml did not significantly change (figure 21). Similarly the median (range) PGFM value of 21.3 (12.7-40.1) pg/ml prior to treatment and the respective values of 21.2 (11.1-40.1), 19.5 (13.7-39.1) and 15.4 (9.0-35.2) pg/ml did not significantly alter.

There were no significant differences in the PGEM and PGFM levels in patients who had abdominal pain and vaginal bleeding and patients without symptoms after mifepristone treatment.

INTERPRETATION OF RESULTS

Mifepristone has been shown to stimulate the synthesis of PGE2 and PGF2a in vitro from the glandular, but not stromal, cells in early human decidua in a dose dependent manner (Smith & Kelly, 1987). It has also been shown to stimulate prostaglandin production from endometrial stromal cells during the menstrual cycle (Kelly et al, 1986). It is

Figure 21 Median PGEM & FM levels after mifepristone



possible that part of action of mifepristone is mediated by increased prostaglandin production and therefore increased myometrial contractility. Herrmann et al (1985) reported an increase in PGF2a concentrations in peripheral plasma 24 hours after oral administration of mifepristone in six out of seven patients. Although this increase was very variable, it was statistically significant. Somell et al (1990) reported an increase in PGF2a metabolite after mifepristone in 13 women, however this increase did not occur until six days after tablet ingestion. Finally, Yang and Wu (1988) reported an increase in PGFM 48 hours after mifepristone in seven women having a complete abortion after mifepristone. In this study one patient had an incomplete abortion and her PGFM concentrations were much lower than the corresponding concentrations in the patients who aborted completely. From these studies it would appear that prostaglandin levels increase after mifepristone. However, no increase occurred in our patients.

The plasma half-life of both PGEM (Husslein et al, 1984) and PGFM (Spona et al, 1983; Sellers, 1985) is approximately 30 minutes. However, mifepristone has a relatively long half-life of over 24 hours (Baulieu, 1985), and women who abort after treatment have increased uterine activity over at least 48 hours (Swahn & Bygdeman, 1988). Therefore blood sampling over this period should have detected any increase in the prostaglandin metabolite levels related to uterine activity, and failure to demonstrate a prostaglandin rise is therefore unlikely to

be due to infrequent sampling. The study performed by Somell et al (1990) suggests that it may takes at least six days for any significant increase in prostaglandin metabolite levels to occur after mifepristone, therefore in this study the time intervals may have been to short for any significant increase in metabolites to have developed.

The failure to demonstrate a significant change in peripheral plasma prostaglandin metabolite concentrations after mifepristone may also be due to the low levels which are found in peripheral plasma. Measurement of prostaglandin concentrations in the uterine efferent vessels would provide a more accurate way of investigating the local action of mifepristone on prostaglandin production. Although this has naturally never been performed in humans, in the guinea-pig a fall in uterine vein prostaglandins occurs after antiprogestin treatment (Elger et al, 1987). This suggests that no increase in prostaglandin production occurs in vivo after mifepristone. Kelly and Bukman (1990) have recently reported that in the guinea-pig treatment with mifepristone results in a significant decrease in prostaglandin metabolism to their inactive dihydro-15-keto form. Since prostaglandins are considered to exert their action locally, the control over breakdown as well as synthesis must be considered as an appropriate regulator of their function.

Csapo (1977) proposed that uterine activity was controlled by the balance between the intrinsic suppressor, progesterone, and the stimulant, prostaglandin. As no

increase in prostaglandin metabolite levels can be demonstrated in the plasma after mifepristone treatment, blockage of the progesterone receptors and therefore withdrawal of progesterone at a cellular level, may act by increasing the myometrial response to the prostaglandins already present, and not necessarily increasing basal production. An increased myometrial response to exogenous prostaglandin after mifepristone treatment has been demonstrated in both the first (Bygdeman & Swahn, 1985; Swahn & Bygdeman, 1988) and second (see later) trimesters. In spontaneous abortion an increase in prostaglandin production plays an essential role in the abortion process. The increased abortion rate in early pregnancy termination observed when mifepristone is used in combination with a prostaglandin analogue indicates that local progesterone withdrawal may not be as effective a trigger to prostaglandin production as the factors regulating early spontaneous abortion (Bygdeman & Van Look, 1988). However this increased efficacy may partly be due to the cervical priming effect of the antiprogestin (Lefebvre et al, 1987, Ulmann & Dubois, 1988).

Few studies have been performed measuring prostaglandin metabolite levels during termination. Wolfe et al (1987) demonstrated an increase in PGFM levels immediately after surgical termination of pregnancy. A similar increase was not detected in PGEM levels. The increase in PGFM may have been secondary to mechanical stimulation which occurs during the termination process

(Mitchell et al, 1977). No changes in PGFM concentrations were demonstrated after epostane treatment in the first trimester (Webster et al, 1985).

It would therefore appear that although mifepristone is very effective at priming the uterus to contract after a small prostaglandin dose and to expel a conceptus, no increase in prostaglandins levels are consistently found in the peripheral circulation. The results imply that the enhanced results obtained by menstrual induction with prostaglandins with prior treatment by the antiprogestin mifepristone may not necessarily be the consequence of a stimulation of prostaglandin production.

SECTION 4.3. THE EFFECT OF MIFEPRISTONE ON PLASMA PGEM AND PGFM LEVELS IN THE SECOND TRIMESTER

INTRODUCTION

Mifepristone acts by blocking progesterone receptors and it is possible that the increased uterine activity observed after treatment with this drug will be associated with an increase in prostaglandin production. In a study previously presented in this thesis no increase in prostaglandin E or F metabolite concentrations occurred after mifepristone treatment in the first trimester. To investigate further the effects of the drug on prostaglandin production, changes in PGEM and PGFM concentrations after treatment with mifepristone have been investigated in the second trimester of pregnancy.

TRIAL DESIGN AND METHODOLOGY

A double blind, placebo controlled study was performed including 20 primigravidae at 16-18 weeks gestation scheduled for prostaglandin termination of pregnancy. Patients were treated with either mifepristone 600mg or identical placebo orally 24 hours before the planned termination. Randomisation was carried out using a predetermined randomisation schedule, and sealed copies of the code identifying the contents were held by the company.

Before tablet treatment a peripheral venous blood sample was taken for PGEM and PGFM analysis [pre-treatment sample]. The PGEM and PGFM levels were repeated 24 hours

later when the patient was admitted to hospital [post-treatment sample], and again six hours after extra-amniotic instillation of prostaglandin E2 1.5mg in 6gm of 6% tylose gel via a Foley catheter passed transcervically [post-PG sample].

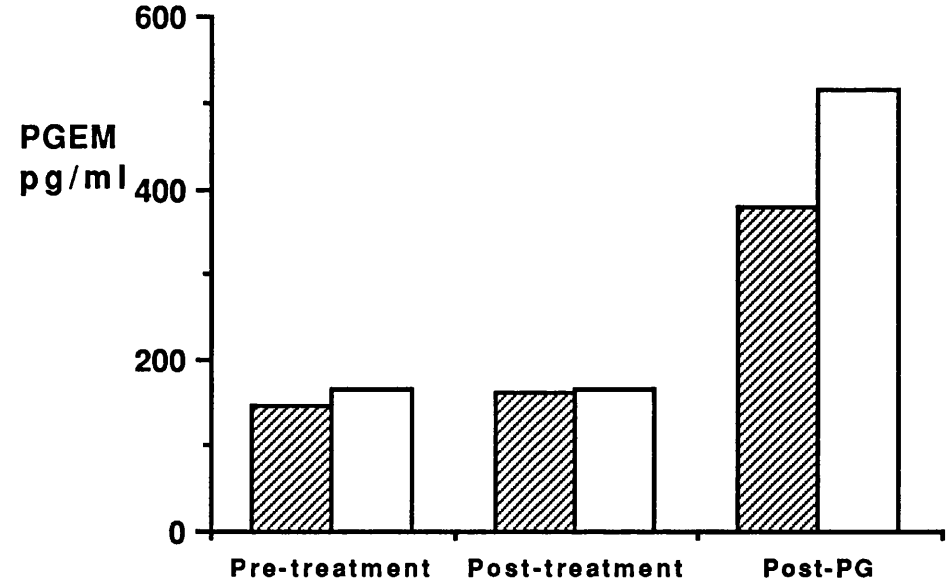
The maternal samples were collected in chilled plastic bottles containing acetylsalicyclic and ethylene diamo-tetra-acetic acids. The plasma was immediately separated by centrifugation at 1500g at 10°C and the samples stored at -70°C until assayed. Prostaglandin metabolite concentrations were analysed by radioimmunoassay as previously described (section 4.2.), and the mean coefficient of variation for both assays was 8-11%. The cross reactivity of PGEM and PGFM is illustrated in Appendix 5.

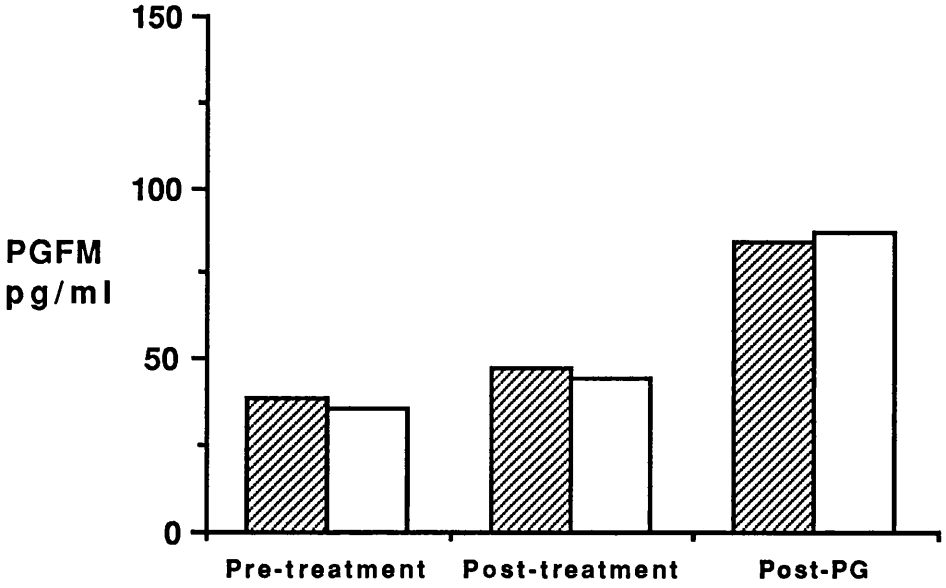
RESULTS

Patients details relating to age, height, weight, pregnancy gestation and ß-hCG concentration are shown in table 11. There were no statistically significant differences between the two groups (Mann-Whitney U test). No patients had vaginal bleeding or uterine pain prior to prostaglandin treatment.

The median (range) PGEM and PGFM concentrations in the patients treated with mifepristone or placebo were not significantly different at any of the time intervals studied (figure 22). There was no significant change in the

Figure 22 Median PGEM & FM levels 24 hours after mifepristone (hatched) or placebo (plain)





PGEM and PGFM concentrations prior to prostaglandin injection into the uterus in either the mifepristone or placebo group. In the patients treated with mifepristone the median (range) PGEM and PGFM concentrations following the therapeutic injection of PGE2 were 379 (98-620) and 77 (61-126) pg/ml respectively, which were significantly higher than the pre-treatment concentrations of 143 (105-275) and 38 (15-66) pg/ml, and the post-treatment concentrations of 161 (110-209) and 46 (29-71) pg/ml ($p \leq 0.001$, Mann-Whitney U-test). Similarly in the patients treated with placebo the median (range) PGEM and PGFM concentrations after prostaglandin injection of 516 (102-1017) and 75 (41-171) pg/ml were significantly higher than the pre-treatment concentrations of 165 (145-196) and 38 (19-54) pg/ml, and the post-treatment concentrations of 160 (109-251) and 40 (25-74) pg/ml ($p \leq 0.001$).

There was no correlation between the prostaglandin metabolite concentration, either before or after prostaglandin treatment, and the induction-abortion interval in either of the groups.

INTERPRETATION OF RESULTS

Mifepristone has been demonstrated to increase prostaglandin production in vitro from the glandular cells in early human decidua (Smith & Kelly, 1987) and from the endometrial stromal cells during the menstrual cycle (Kelly et al, 1986). Although an increase in primary PGF_{2a} (Herrmann et al, 1985) and PGFM (Yang and Wu, 1988; Somell

et al, 1990) have both been reported after mifepristone in the first trimester, no studies have reported the effects of this drug on prostaglandin metabolite concentrations in the second trimester. This study confirms the results of the previous chapter that no significant increase in either PGFM or PGEM levels occur in the peripheral plasma after mifepristone treatment. Again, the failure to demonstrate any significant increase in peripheral plasma PGEM or PGFM concentrations after mifepristone in this study could have been because the time interval between drug administration and metabolite measurement was too short. However, the dramatic reduction in the induction abortion interval in the patients treated with mifepristone, suggests that significant anti-progesterone activity had occurred in these patients. The results suggest that progesterone withdrawal at a cellular level caused by mifepristone may act by increasing the myometrial response to the prostaglandins already present and not necessarily by increasing basal production, or if increased prostaglandin production occurs it can not be demonstrated in peripheral plasma.

In both the mifepristone and placebo groups the prostaglandin EM concentration significantly increased after PGE2 treatment. This is as expected and represents either uptake of the PGE2 into the maternal circulation or increased prostaglandin production secondary to the abortion process. As there was no significant difference in the PGEM concentrations between the two groups, the

increase was not related to mifepristone treatment. Similarly as there were no significant differences between the PGFM concentrations after PGE2 injection into the uterus between the mifepristone and placebo groups, the PGFM increase was not related to mifepristone treatment. This increase in PGFM is almost certainly related to increased prostaglandin production as part of the abortion process, as such a large increase could not be explained by cross reactivity with the PGEM assay. It also confirms that it is possible to demonstrate increased prostaglandin metabolite levels in the peripheral circulation.

The results of studying prostaglandin metabolite concentrations after treatment with mifepristone in the first and second trimesters of pregnancy, suggest that although the drug enhances the results obtained with prostaglandin therapy, either for menstrual induction or mid-trimester termination, this is not necessarily the consequence of stimulation of prostaglandin production. It remains to be seen whether mifepristone has any effects on prostaglandin concentrations in the last trimester, as this may have implications for labour induction.

SECTION 4.4. - THE EFFECT OF MIFEPRISTONE ON MYOMETRIAL ACTIVITY IN THE SECOND TRIMESTER OF PREGNANCY

INTRODUCTION

The activity of the pregnant uterus has been the subject of scientific investigation for many years and now several methods have been devised for the quantification of uterine activity. This has been helped by the introduction of solid state catheter tipped pressure transducers. These soft, non-fluid filled devices are not prone to the complications of blockage or uterine perforation (Steer et al, 1978). It has been shown that, individually, neither the frequency nor the active pressure of contractions are adequate to quantitate uterine activity (Caldeyro-Barcia et al, 1957; Steer & Carter, 1977). The Montevideo unit (mean active pressure of contractions multiplied by frequency of contractions per 10 min) was introduced to combine both variables (Caldeyro-Barcia et al, 1957). But this is difficult to quantitate automatically and does not take into account the duration of the contractions. El Sahwi et al (1967) modified this method by adding a measurement of the duration of contractions formulating the Alexandria unit. However, advances in electronic technology have permitted the development of on-line quantification using uterine activity units (UAU) as described by Hon & Paul (1973): defined as a 1 mmHg increase of intra-uterine pressure lasting for 1 minute. The UAU therefore measures the contraction area above atmospheric pressure rather than

the active contraction area (contraction area above baseline tone) as described by (Steer et al, 1984).

Mifepristone has been shown to increase uterine contractility and sensitivity to prostaglandin analogues in the first trimester (Bygdeman & Swahn, 1985; Swahn & Bygdeman, 1988), but to date no human contractility studies have been performed using this drug in later pregnancy. The aim of this study was therefore to assess the influence of mifepristone on myometrial contractility and response to oxytocics in the second trimester of pregnancy.

TRIAL DESIGN AND METHODOLOGY

A double blind, placebo controlled study was performed including 20 primigravidae at 16-18 weeks gestation scheduled for pregnancy termination by extra-amniotic injection of prostaglandin. Patients were treated with either mifepristone 600mg or identical placebo orally 24 hours before the planned termination. Randomisation was carried out with a predetermined randomisation schedule balanced within groups of ten. Sealed copies of the code identifying the contents were held by the hospital pharmacy.

On admission to hospital an intravenous infusion of dextrose/saline was commenced and a Gaeltec pressure tip catheter passed transcervically. This was secured by a 14G Foley catheter with a 5ml balloon which was passed alongside the pressure catheter to retain it in the correct position. Following a 20 minute recording of "baseline"

intra-uterine pressure, a 30 minute metered infusion of syntocinon 100 mu/min (oxytocin phase) was commenced, followed after a 30 minute interval by a 30 minute infusion of PGE_2 2ug/ml (PG phase). In order to exclude any bias, the order of the oxytocin/prostaglandin was alternated. PGE_2 (Upjohn) 1.5mg in 6gm of 6% Tylose gel was then injected into the extra-amniotic space via the Foley catheter and uterine activity recorded for a further 60 minute period prior to removal of the catheters. Subsequent management is as previously described (section 3.2.).

Continuous recordings of uterine activity and pressure was performed throughout the infusion and rest periods. Uterine pressures were recorded directly onto computer discs, and ten minute epochs were later analysed using a programme quantifying in uterine activity units (Selinger, unpublished data).

Three-way analysis of variance was used to assess differences between the mifepristone and control groups for each phase of uterine activity (baseline, oxytocin, prostaglandin, and prostaglandin gel).

RESULTS

Patients details relating to age, height, weight, pregnancy gestation and ß-hCG concentration are shown in table 11. There were no statistically significant differences between the two groups (Mann-Whitney U test). No patient had vaginal bleeding or uterine pain before

prostaglandin treatment, and during the pressure studies no patient bled or expelled the transducer.

The baseline uterine activity in both groups and the effects of the oxytocin and prostaglandin treatment are shown in figure 23.

Baseline Phase

The baseline uterine activity per 10 minute epoch in the patients treated with mifepristone was significantly greater than the baseline activity in the placebo group ($p \leq 0.0001$, $F=18.5$). There was no significant difference in the activity with time.

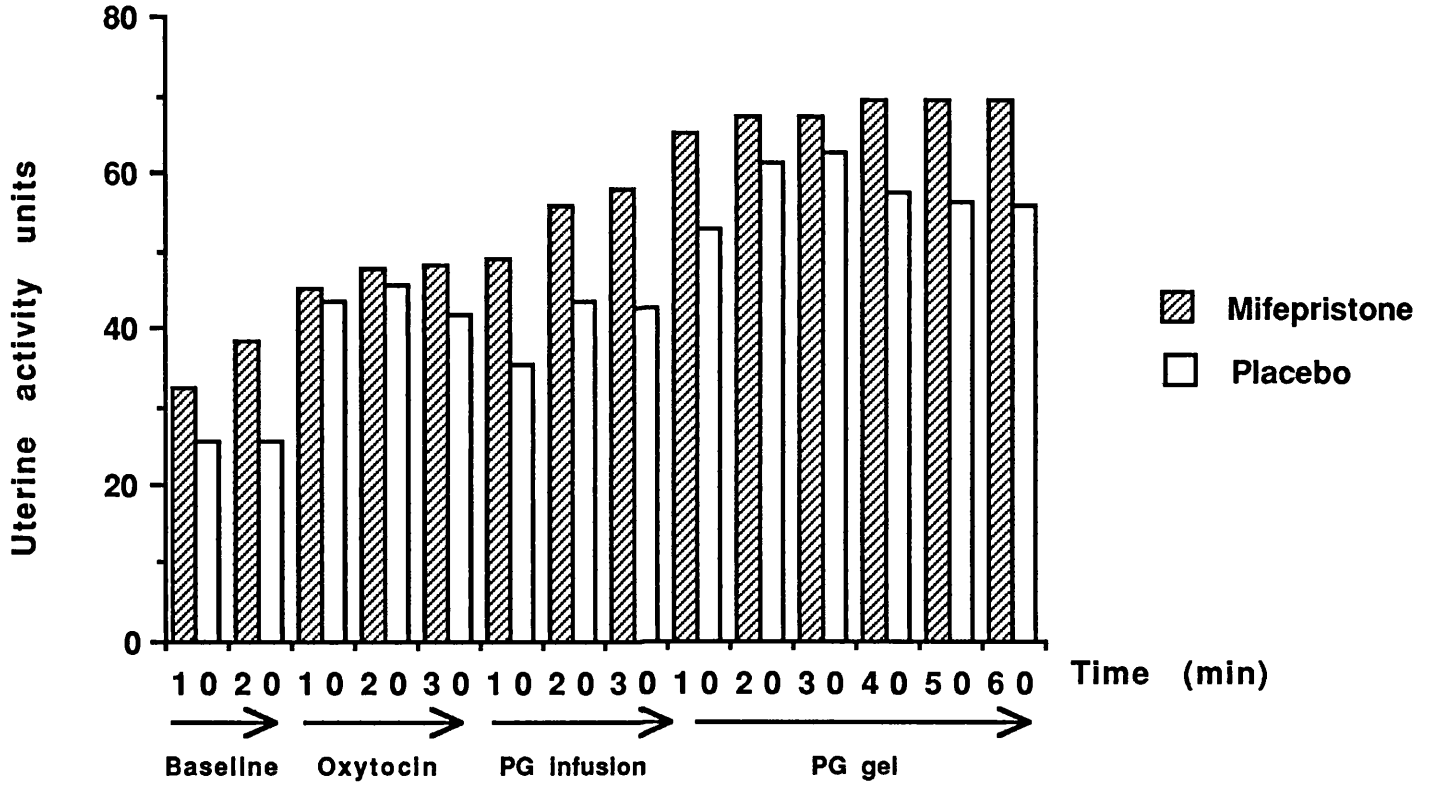
Oxytocin Phase

Although the uterine activity per ten minute epoch during the oxytocin infusion in the mifepristone group was higher than the activity in the placebo group, this difference failed to reach statistical significance ($p \leq 0.33$, $F=0.95$), and there were no significant differences with time.

Prostaglandin Phase

The uterine activity per 10 minute epoch during the prostaglandin infusion in the mifepristone group was statistically greater than the activity of in the placebo group ($p \leq 0.002$, $F=10.5$). Similarly the uterine activity after extra-amniotic prostaglandin injection into the uterus in the mifepristone group was significantly greater

Figure 23 Median uterine activity in ten minute epochs during baseline, oxytocin & prostaglandin phases



than in the placebo patients ($p \leq 0.002$, $F=9.84$). There were no significant differences with time.

INTERPRETATION OF RESULTS

The quantification of myometrial activity by intra-uterine pressure recordings analysed by computer, thus eliminating any error due to free-hand interpretation, suggests that mifepristone allows an increase in baseline uterine activity and increased sensitivity to PGE_2 during the second trimester of pregnancy. A similar increase in baseline uterine activity has been reported after epostane in the second trimester (Selinger et al, 1987a). The lack of sensitivity to oxytocin in this study confirms similar results obtained when epostane was used in the first (Webster et al, 1985) and second trimester (Selinger et al, 1987a). This might be due to the dose of oxytocin being too small or the duration of the infusion too short, or more likely due to the low levels of oxytocin receptors found in the uterus at this stage in pregnancy (Fuchs et al, 1984).

Although the pressure transducer was calibrated prior to insertion into the uterus in each patient, this study measured total contraction area above atmospheric pressure and not the contraction area above the baseline tone as advocated by Steer el al (1984). Therefore differences in resting tone in between patients may have introduced errors which may have affected the results. Also the position of the catheter tip relative to the level of the fundus may have varied between patients. Steer (1977) has shown that

measurement of uterine activity is the measurement which has the closest correlation with rates of cervical dilatation during the active phase of induced labour. As in this study, this is because measurement of UAU reflects all the contraction variables i.e. frequency, uterine pressure and duration of contractions. However, inaccuracies may be introduced using this technique, because one variable may counteract another variable to give the same UAU results. For example a patient with short duration frequent contractions may ultimately produced similar UAU results to a patient with strong infrequent contractions. Previous publications have stressed the importance of measuring UAU over a long period of time to minimise fluctuations due to contractions falling just in, or out, of a measurement period (Steer et al, 1975; Steer & Carter, 1977). Seitchik (1980) has shown that contraction by contraction analysis of UAU is not a useful index of uterine activity. Caldeyro-Barcia et al (1957) selected 10 min epochs for the measurement of the Montevideo unit, as was chose in this study, but Steer et al (1984) prefer 15 min epochs as this coincides with the length of time taken to establish a stable response to short-term changes in oxytocin infusion rates.

The increased spontaneous activity and sensitivity of the myometrium to prostaglandin after mifepristone treatment probably results from progesterone withdrawal at a cellular level after blockage of the progesterone receptors (Baulieu, 1985). The data supports the hypothesis

that the pregnant uterus is an organ suppressed by progesterone, and when this is withdrawn by mifepristone it becomes more active and sensitive to exogenous oxytocics. The increase in uterine activity and sensitivity, and its subsequent clinical effects, depend on the pregnancy gestation. In early pregnancy (<42 days) abortion is induced in 60-85% of patients (Kovacs et al, 1984; Couzinet et al, 1986; Baulieu & Ulmann 1986), but after this (< 63 days) a high abortion efficacy is only achieved with the addition of a prostaglandin. In the second trimester the induction-abortion interval of prostaglandin termination is decreased, associated with a smaller dose of prostaglandin required to achieve abortion (Urquhart & Templeton, 1987). The clinical effects of mifepristone in the last trimester are less well known, but a recent report describing the use of mifepristone to induce labour suggests that the anti-progesterone activity of the drug is still present in the third trimester (Frydman et al, 1991).

SECTION 5 - THE PLACENTAL TRANSFER OF MMIFEPRISTONE DURING THE SECOND TRIMESTER AND ITS INFLUENCE UPON MATERNAL AND FETAL STEROID CONCENTRATIONS

INTRODUCTION

Mifepristone 600mg orally 24 hours prior to mid-trimester extra-amniotic prostaglandin termination has been shown to significantly decrease the induction-abortion interval by over 50%, with 90% of patients aborting within 12 hours of prostaglandin treatment (see section 3.2.). It is possible therefore that this augmenting effect upon prostaglandin treatment could extend into the third trimester and mifepristone might be used to improve the outcome of induced labour. The drug is known to have both anti-progesterone (Baulieu, 1984) and anti-glucocorticoid (Bertagna et al, 1984) effects, both of which may have significant effects on the fetus. In view of this potential for mifepristone inducing labour and possible adverse effects upon the fetus, a study was designed to assess placental transfer of mifepristone and its major metabolite RU 42,633 (Appendix 1) to the fetal circulation, and observe possible effects upon fetal plasma steroid concentrations.

TRIAL DESIGN AND METHODOLOGY

Twenty-four women at 16-19 weeks gestation who were scheduled for pregnancy termination by intra-amniotic injection of prostaglandin were included in the study. The patients menstrual dates were confirmed by ultrasound prior

to recruitment. The study was performed in two parts: the first 12 patients were randomly allocated to receive either mifepristone 600mg or identical placebo tablets and fetal blood sampling was performed four hours after tablet ingestion. The second 12 patients received mifepristone 600mg orally but had fetal blood sampling at 24 or 48 hours after treatment.

Patients were treated with mifepristone 600mg or placebo orally at 09.00 on the day of admission. Before treatment and hourly for four hours, the patients were questioned about uterine pain, vaginal bleeding and symptoms which could have been due to the drug. A peripheral venous blood sample was collected immediately prior to taking the medication (pre-treatment sample) and analysed for haematological (haemoglobin, group) and hormonal (progesterone, oestradiol, cortisol and aldosterone) concentrations. Four, 24 or 48 hours after tablet administration and immediately prior to fetal blood sampling, a further maternal blood sample (post-treatment sample) was collected to measure mifepristone, RU 42,633, progesterone, oestradiol, cortisol, and aldosterone levels.

A 5ml aliquot of amniotic fluid was taken for analysis from each patient prior to fetal blood sampling under intravenous diazepam 10mg sedation using the technique described by Westgren et al (1988), as modified by Selinger et al (1987). Fetal blood sampling was performed under continuous ultrasound vision and the primary target for the

needle was the left ventricle. Heparinised syringes were used to aspirate 3ml of fetal blood which was later analysed for mifepristone and RU 42,633, progesterone, oestradiol, cortisol and aldosterone concentrations. Following collection of the samples, PGE_2 5mg was injected into the amniotic cavity.

If abortion had not occurred six hours later an infusion of syntocinon (Sandoz) 100mu/min was commenced, which was continued until abortion, and further management was as described in section 3.2. Clinical progress, side-effects, vital signs and time of abortion were recorded on purpose designed record sheets. The induction abortion interval was calculated from the time of prostaglandin treatment to expulsion of products of conception.

All plasma samples were stored at -20°C until the clinical studies had been completed and each steroid assay was carried out as a single run. Progesterone, oestradiol, cortisol and aldosterone levels were assayed using commercial radioimmunoassay kits supplied by Diagnostic Products U.K. Ltd., Abingdon, Oxford. (Appendices 5, 7, 8 and 9 respectively). Mifepristone and RU 42,633 assays were performed by Roussel-Uclaf, Romainville. France (Appendix 10) using a previously described technique (Salmon & Mouren, 1985).

RESULTS

Patient details relating to age, height, weight, pregnancy gestation, and parity are shown in table 14.

Table 14 - Characteristics of patients

	Placebo	Mifepristone		
Sampling time (h)	4	24	48	
N	6	6	6	6
Age (years)	22 (21-29)	22 (19-28)	21 (18-40)	22 (18-25)
Gestation (weeks)	16 (16-19)	17 (16-18)	17 (16-18)	17 (16-19)
Abortion time (h)	15.2 (7-31)	16.7 (8-30)	10.7 (8-17)	11.6 (5-13)

Results are median (ranges).

There were no statistically significant differences between the groups (Mann-Whitney U-test). No drug reactions or side-effects were recorded after mifepristone treatment and all patients aborted without complications and were discharged from hospital within 48 hours of admission.

Mifepristone and RU 42,633 concentrations

The maternal, fetal and amniotic fluid concentrations of mifepristone and RU 42,63 are illustrated in figure 24, 25 and 26 respectively. At all the time intervals studied maternal mifepristone levels were significantly higher than fetal plasma concentrations, with the fetal levels of mifepristone being 10-15% and RU 42,633 6-12% of the maternal levels. Mifepristone and RU 42,633 concentrations in the amniotic fluid were also significantly lower being 5-12% and 3-13% respectively of the maternal plasma levels. The highest maternal plasma mifepristone and RU 42,633 levels of 2.2 (range 1.1-7.2) umol/l and 3.4 (range 1.8-6.3) umol/l respectively occurred 4 h after treatment. In fetal plasma the highest mifepristone level of 0.34 (range 0.14-0.44) umol/l occurred at 4 h, but the highest RU 42,633 level of 0.30 (range 0.07-0.47) umol/l was not reached until 48 h after treatment. In amniotic fluid the highest mifepristone and RU 42,633 levels of 0.20 (range 0.08-1.3) umol/l and 0.17 (0.02-0.2) umol/l respectively both occurred 24 h after tablet ingestion. There did not appear to be any relationship between the maternal mifepristone or RU 42,633 concentrations and the induction-abortion intervals.

Figure 24 Maternal plasma mifepristone & RU 42,633 levels after oral ingestion

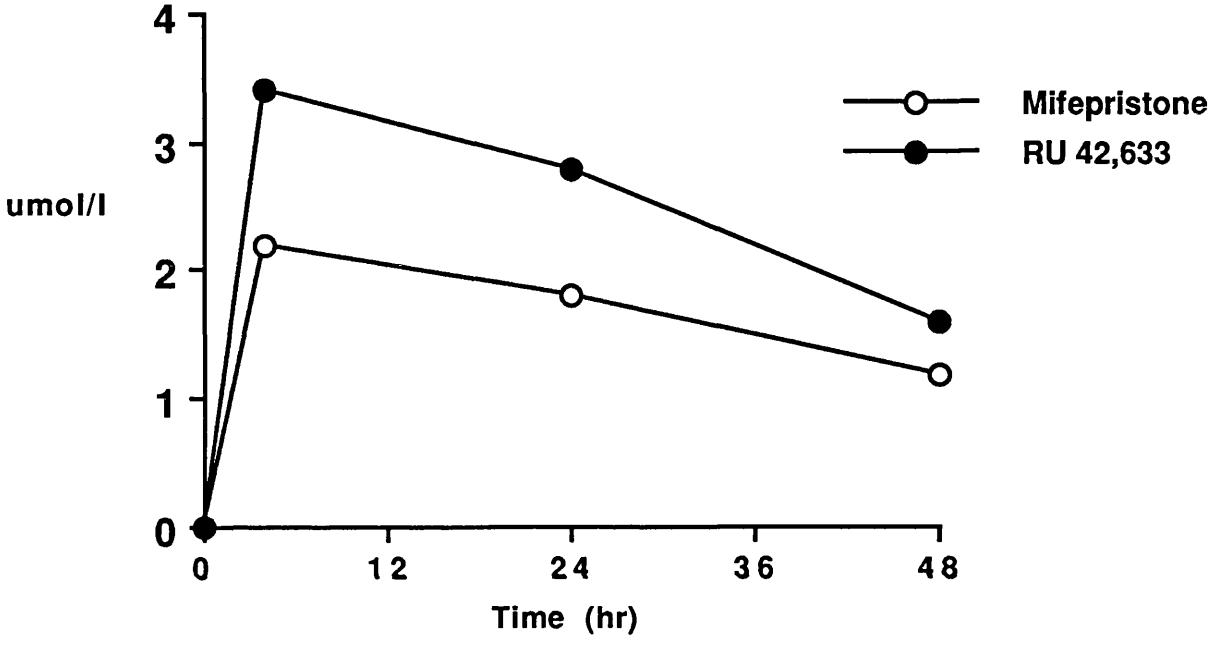


Figure 25 Fetal plasma mifepristone & RU 42,633 levels after maternal oral ingestion

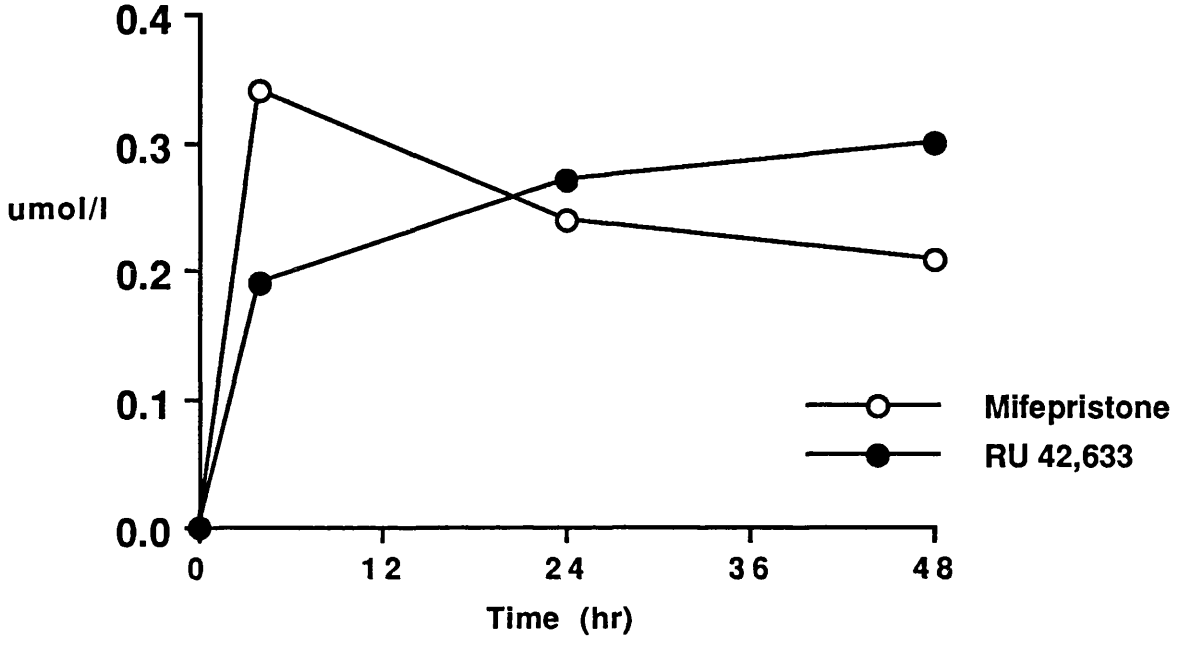
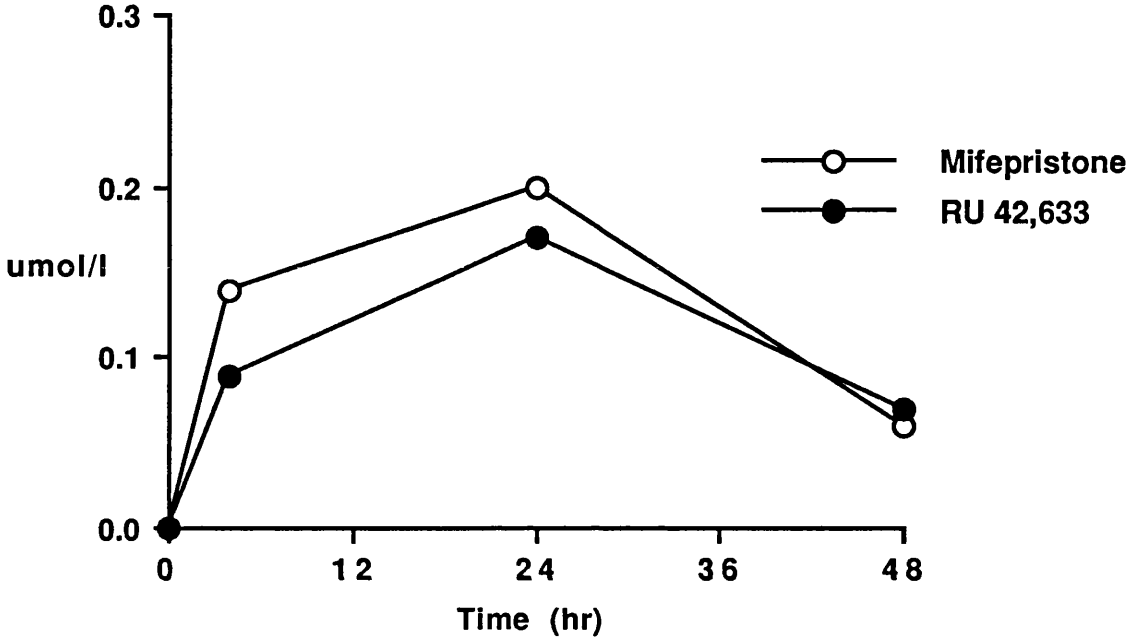


Figure 26 Amniotic fluid Mifepristone & RU 42,633 levels after maternal oral ingestion



Maternal steroid levels

Table 15 illustrates the median (range) concentrations of steroid in the maternal plasma at the two sampling times for the two groups. There were no significant differences between the pre- and post-treatment concentrations for treated or control patients (Wilcoxon's two-sample test). There were also no significant differences in progesterone, oestradiol, cortisol, or aldosterone between the patients who received mifepristone and the placebo group (Mann-Whitney U-test).

Fetal steroid levels

Table 16 gives the median plasma steroid levels in the fetal blood samples. The fetal progesterone levels in the patients treated with both mifepristone or placebo were significantly higher than the respective progesterone concentrations in the maternal circulation ($p \leq 0.05$, Mann-Whitney U-test) [figure 27]. There was no significant difference in the progesterone concentrations in the fetus between the mifepristone and placebo group.

Fetal oestradiol concentrations in both groups were not significantly different to the respective values in the maternal circulation (figure 28), and there were no significant differences in the oestradiol concentrations in the fetus between the patients treated with mifepristone or placebo.

The fetal cortisol concentrations in the patients who

Table 15 - Maternal peripheral venous steroid levels after treatment with mifepristone or placebo

	Placebo		Mifepristone	
Sampling time (h)		4	24	48
Progesterone (nmol/ml)	122 (96-200)	130 (100-150)	184 (155-218)	157 (136-228)
Oestradiol (nmol/l)	11.9 (8.2-18.6)	14.9 (10-24.4)	12.1 (6.3-18.4)	18.0 (4.2-27)
Cortisol (nmol/l)	579 (399-1105)	631 (425-1010)	830 (573-1141)	956 (464-1385)
Aldosterone (pmol/ml)	827 (407-1520)	818 (591-927)	937 (561-1847)	501 (264-1154)

Results are median (range) values.

Table 16 - Fetal steroid levels after maternal treatment with mifepristone or placebo

	Placebo		Mifepristone	
Sampling time (h)		4	24	48
Progesterone nmol/l	1269 (854-1808)	1636 (1172-2312)	1186 (1009-4090)	1140 (703-1755)
Oestradiol nmol/l	10.9 (6.9-17.4)	14.2 (9.7-18.3)	11.6 (9.2-33.1)	15.4 (10.8-37.2)
Cortisol nmol/l	40.5 (24-73)	52.0 (37-74)	64.6 (53-91)	58.5 (46-117)
Aldosterone pmol/l	959 (815-1282)	1809 * (914-2320)	1371 * (1046-2118)	1124 (840-1516)

(* $p<0.05$, Mann-Whitney U-test)
Results are median (range) values.

Figure 27 Median progesterone levels after mifepristone or placebo

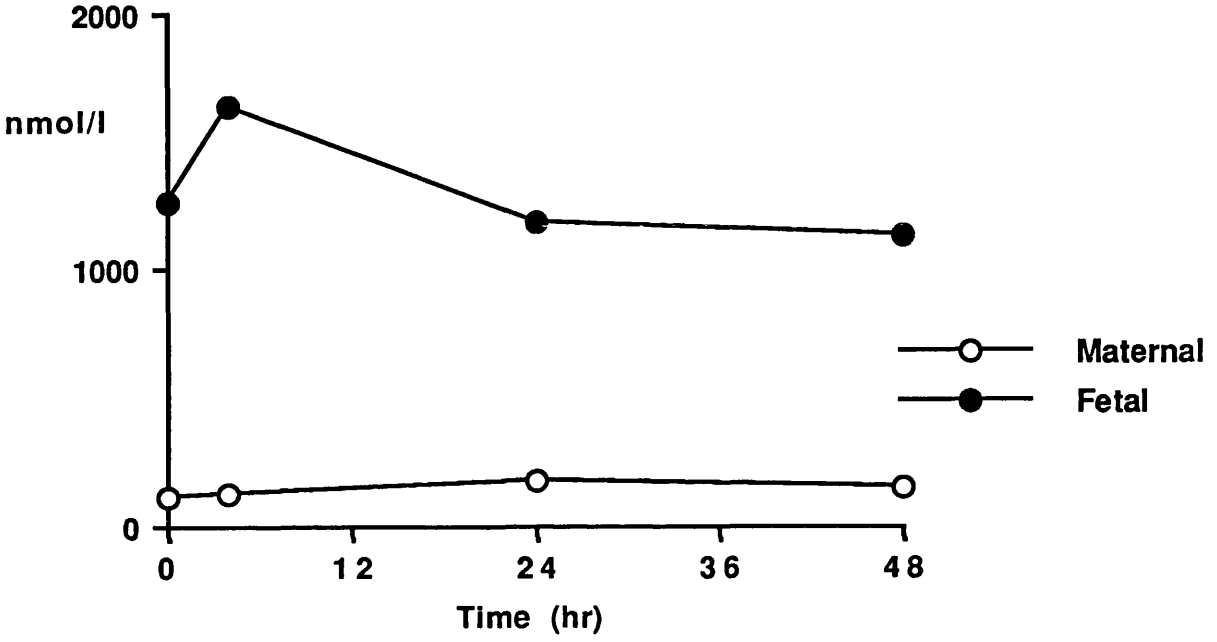
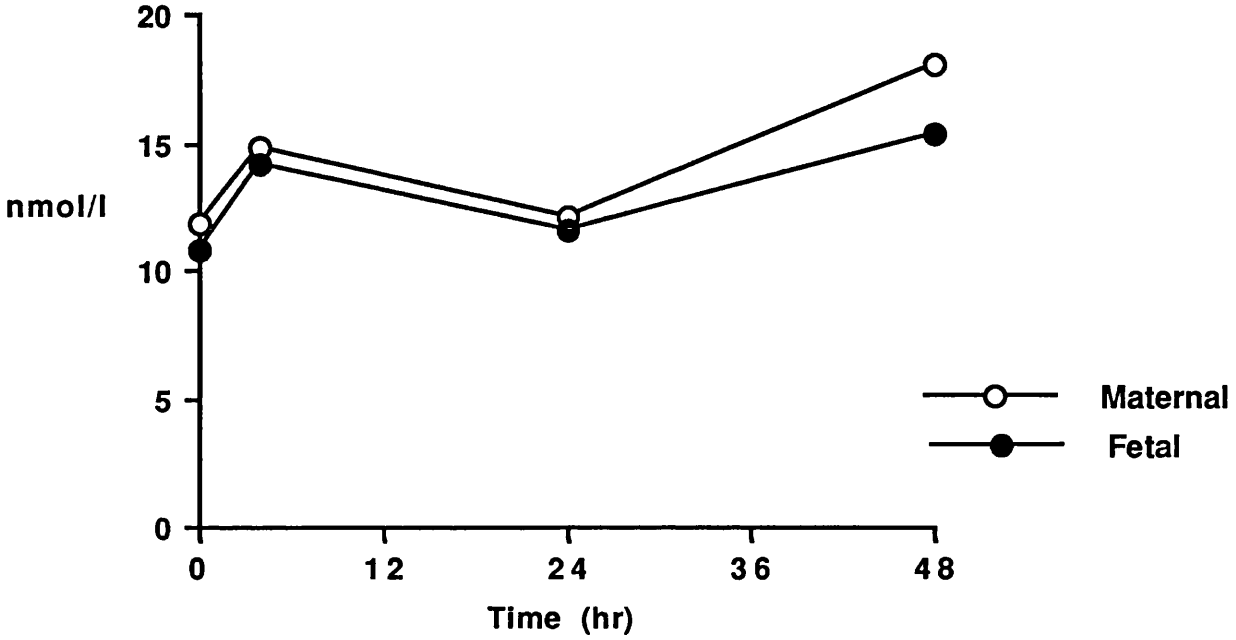


Figure 28 Median maternal and fetal oestradiol levels after mifepristone or placebo



received mifepristone or placebo were both significantly lower than the respective cortisol concentrations in the maternal circulation ($p \leq 0.05$, Mann-Whitney U-test) [figure 29]. There was no difference statistically between the fetal cortisol concentrations in the mifepristone and placebo groups.

The relationship between maternal and fetal aldosterone concentrations in both groups of patients is illustrated in figure 30. The fetal aldosterone concentrations in the mifepristone or placebo group were not statistically different from the respective maternal plasma concentrations. In patients treated with mifepristone, the fetal aldosterone concentration at 4 and 24 hours of 1700 (range 914-2320) and 1458 (range 1080-2118) pmol/l respectively was significantly higher compared with the placebo group concentration of 999 (range 466-1480) pmol/l ($p<0.05$, Mann-Whitney *U*-test).

There did not appear to be any relationship between maternal or fetal mifepristone or RU 42,633 concentrations and the fetal steroid levels.

There was no significant difference in the induction to abortion interval between the patients treated with mifepristone or placebo [table 14]. The analgesic requirements after prostaglandin treatment and incidence of incomplete expulsion of the products of conception were also not significantly different between the two groups.

Figure 29 Median maternal and fetal cortisol levels after mifepristone or placebo

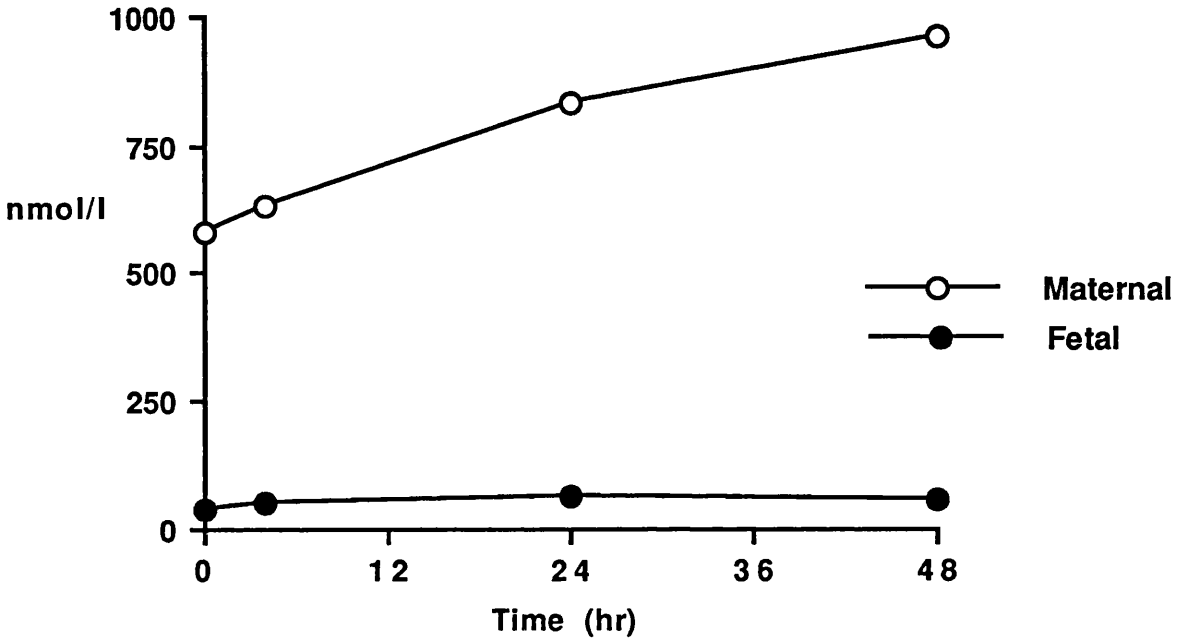
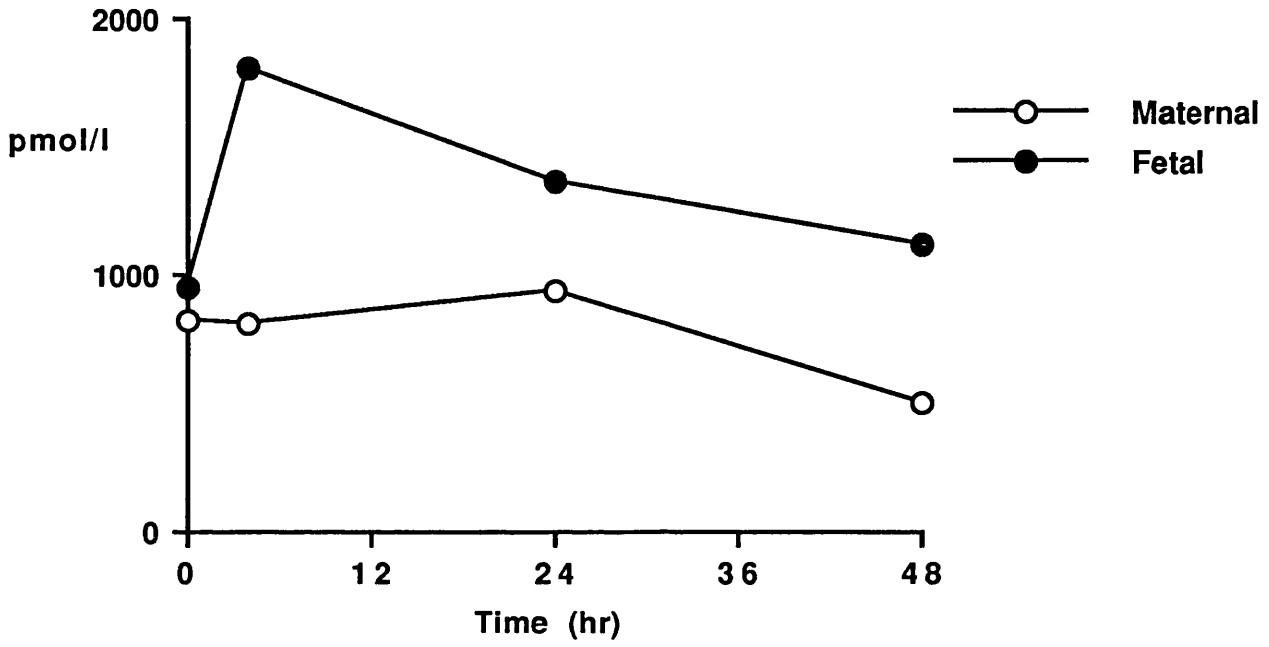


Figure 30 Median maternal and fetal aldosterone levels after mifepristone or placebo



Mifepristone has been shown to produce a ripening effect upon the cervix during the first trimester (Durlot et al, 1988; Frydman et al, 1988; Radestad et al, 1988; Ulmann & Dubois, 1988), and to result in spontaneous expulsion of the dead fetus in the second and third trimester of pregnancy (Cabrol et al, 1985; Padayachi et al 1988). It is therefore possible that mifepristone could be used to ripen the unfavourable cervix and induce labour in humans, and observations in primates are encouraging (Wolf et al, 1989). Recently, Frydman et al (1991) treated 62 women undergoing induction of labour at term in the presence of an unfavourable cervix with either mifepristone 200mg/day or placebo for two days. The mean time between the first day of treatment and the start of labour was significantly shorter in the treatment group (49.5 vs 68.5 h). The need for prostaglandin ripening and oxytocin stimulation were also significantly lower in the mifepristone group. No side-effects were found and all fetuses were normal. The authors suggest that mifepristone could be given to induce labour at term for women with an unfavourable cervix.

Although Frydman's study reports the successful use of mifepristone for induction of labour in humans, little is known about the placental kinetics of mifepristone and its possible effects upon the fetus. Indeed, this is the first study describing the effects of mifepristone on fetal plasma steroid concentrations in humans in mid pregnancy.

Frydman et al (1985) have previously demonstrated transplacental passage of mifepristone in second trimester termination of pregnancy, and found an exponential increase in drug concentration in the fetus with time. The lowest level (20ng/ml) was found at 30 min and the highest (400ng/ml) at 18 hours after oral administration. Wolf et al (1988) reported transplacental passage of mifepristone within two hours of oral ingestion during the second or third trimester of pregnancy in cynomalogus monkeys. They concluded that there was only minimal resistance to "free" passage of mifepristone across the placenta. The maternal:fetal ratio increased from 3:1 in the second trimester to 6:1 in the third trimester, indicating that the efficiency of placental transfer may decrease as pregnancy advances. The authors postulated that the bioavailability of mifepristone may decrease significantly with gestation and the theoretical risks of mifepristone side-effects on the fetus could be reduced in late pregnancy.

The results of this study indicate that both mifepristone and probably its metabolite RU 42,633, rapidly cross the placenta and can be found in both fetal plasma and amniotic fluid after oral ingestion. Compared with other drugs such as PGE_2 (MacKenzie et al, 1989) and mefenamic acid (MacKenzie et al, 1985), where the maternal:fetal ratio is approximately 3:1, the ratio for mifepristone in the fetal circulation is low (9:1). This relatively low concentration in the fetal circulation

is probably due to receptor binding in the placenta or to maternal plasma binding.

At the time of fetal blood sampling no changes in the progesterone or oestradiol concentrations could be found in the fetus compared with control subjects. Selinger et al (1988) demonstrated a 89% and 91% reduction in maternal progesterone and oestradiol concentrations three hours after treatment with the anti-progestin epostane in the second trimester. They also demonstrated a significant decrease in progesterone, but not oestradiol, concentrations in the fetal circulation after epostane treatment, but could not however detect any epostane in the fetal circulation or amniotic fluid at this time. The failure to demonstrate any significant maternal or fetal changes following mifepristone treatment in the present study was expected and is surely due to the different mode of action between this drug and epostane.

Mifepristone is known to block the glucocorticoid negative feedback effect at the pituitary gland and therefore induces a compensatory increase in plasma ACTH and cortisol (Nieman & Loriaux, 1988). In this study although ACTH was not measured, no change in maternal or fetal cortisol concentrations were demonstrated, and the fetal levels were in the expected range (Lopez Bernal & MacKenzie, 1987). The failure to demonstrate any significant change in fetal cortisol levels may be of clinical importance because any withdrawal of cortisol at a

cellular level, due to blockage of the glucocorticoid receptors, could have a detrimental effect upon fetal lung maturation.

An unexpected finding was the significantly higher fetal aldosterone concentrations 4 and 24 h after mifepristone treatment. Swahn et al (1989) found a transient elevation in aldosterone levels after treatment with mifepristone, and Spitz et al (1985) reported a similar increase in aldosterone after mifepristone in dogs. This was thought to be due to blockage of the mineralocorticoid receptors, but no other studies have reported any change in aldosterone concentrations after mifepristone. Fetal aldosterone levels have been measured in the umbilical cord at the time of delivery, and found to be higher (Beittins et al, 1972) and lower (Sippell et al, 1979; Dorr et al, 1987) than the maternal levels depending on the study. However little is known about fetal aldosterone levels in the second trimester, and no significant changes in fetal aldosterone concentrations were demonstrated after treatment with epostane at this gestation (Selinger et al, 1988). The importance of the increased fetal concentrations in the mifepristone patients is uncertain. They may be due to the drug blocking the mineralocorticoid receptors in the fetus, although if this had occurred a corresponding increase in maternal concentrations might have been expected. The number of patients in each group was small and the increase in fetal aldosterone levels only just reached statistical

significance, so this may have occurred by chance. Similarly in view of the small number of patients studied, any affect of mifepristone on the other steroid parameters can not totally be excluded, therefore further studies are necessary to investigate the affect of the anti-progestin on fetal steroid levels. These should include investigating the affect on fetal steroids of a longer time interval between mifepristone treatment and fetal sampling, and if possible the effects of chronic dosage.

Studies of early first trimester termination of pregnancy suggest that 36-48 hours is necessary between mifepristone and prostaglandin treatment to achieve a high complete abortion rate (Rodgers & Baird, 1987). However, pre-treatment with mifepristone 24 hours prior to mid-trimester termination with prostaglandin significantly decreases the induction-abortion interval over controls (see section 3.2.), suggesting that the drug is an effective anti-progestin at this time interval. In this study although the abortion intervals were decreased by 40% at 24 and 48 hours after mifepristone treatment, these changes failed to reach statistical significance: this is probably due to the small numbers of patients studied.

SECTION 6.1. - CONCLUSIONS

This thesis was designed to investigate the clinical effects of mifepristone in the first and second trimesters of pregnancy. The mechanism of action of mifepristone has been explored by measuring prostaglandin metabolite levels, progesterone and oestrogen receptor concentrations, and uterine activity after drug treatment. Mifepristone efficacy in both first and second trimester termination of pregnancy has been compared with existing termination methods using prostaglandin. Finally, as it is possible that mifepristone may in the future be used to improve the results of induction of labour, the placental transfer of mifepristone and its effect on fetal steroid concentrations has also been investigated.

By necessity some of the studies contain small numbers of patients and ideally should be larger, but the time available and the clinical situation means that small numbers are necessary to achieve the objectives of this thesis. In view of the small numbers, where possible the studies were performed in a double-blind, placebo controlled manner, with the randomisation codes only being broken when the data were analysed. All the patients were managed by the author and one Clinical Research Sister. Consistent patient management gives weight to the validity of data collected from the trials, especially in areas such as assessment of analgesia requirements, blood loss, and side-effects.

The author recognises that problems exist when comparing the results from the mifepristone clinical termination studies in the first and second trimester with the retrospective data for prostaglandin termination methods (Section 2.1 & 2.2). The patients in these two sections were not treated by the author as the retrospective data covers almost ten years of patient care. Errors in interpretation can therefore be introduced secondary to changes in practice and staff treating patients. However, all these patients were treated in our unit under the supervision of the same consultant, the original data was recorded at the time of termination by the original staff involved, and the number of patients involved in the retrospective studies are very large, therefore it is hoped that these errors have been kept to a minimum. Also, the retrospective studies are included merely to form a basis for discussion and absolute value comparisons are only used when clinically relevant.

The clinical effects of mifepristone in the first trimester were investigated by combining the drug with 48 hours later a single vaginal prostaglandin pessary of PGE_1 analogue. Abortion occurred in all patients, but was only complete in 95%. The termination process was associated with a low incidence of gastrointestinal side-effects, and no patients required transfusion or sustained genital tract trauma. One case of suspected pelvic infection occurred. The results appear to represent a marked improvement on those obtained when prostaglandins alone were used for

early termination of pregnancy, with the efficacy of abortion higher and the incidence of side-effects and complications lower. These improvements are almost certainly a result of the anti-progesterone action of mifepristone priming the uterus, thereby increasing the uterine activity prior to prostaglandin treatment. In this study 3% of patients aborted and 63% threatened to abort after mifepristone treatment, demonstrating the importance of progesterone in controlling uterine activity in the first trimester. The combination treatment of mifepristone and prostaglandin appears to offer a safe, efficient, acceptable, non-surgical out-patient method of termination. Further studies on the efficacy of lower mifepristone doses and shorter time intervals between tablet ingestion and gemeprost treatment would be justified.

The clinical effects of mifepristone in the second trimester were investigated by treating primigravida patients in a double blind study with either mifepristone 600mg or identical placebo 24 hours prior to extra-amniotic injection of PGE_2 1.5 mg into the uterus. The induction-abortion interval in the mifepristone group was significantly less than in the placebo group. Ninety percent of the patients who received mifepristone aborted within 12 hours of prostaglandin, thereby making day-case prostaglandin termination of mid-trimester pregnancy a realistic possibility. The results again appear to represent a marked improvement on those obtained when prostaglandins were used alone for extra- or intra-amniotic

termination of mid-trimester pregnancy, although absolute comparisons are difficult because of the retrospective nature of the prostaglandin results. A larger placebo controlled study is required to further investigate mifepristone use in prostaglandin termination of mid-trimester pregnancy. The issues that need to be explored include smaller doses of mifepristone and prostaglandin, the effectiveness of shorter time intervals between tablet ingestion and prostaglandin treatment, efficacy of different prostaglandin administration routes, and incidence of side-effects.

In a further study patients received mifepristone or identical placebo four hours before intra-amniotic injection of PGE_2 5mg for mid-trimester termination of pregnancy. No significant differences in the induction-abortion intervals occurred, suggesting that this is too short a time interval for any significant anti-progesterone activity to have developed.

In order to further investigate the possible mechanism of action of mifepristone, the effect of the drug on progesterone and oestrogen receptor concentrations in the decidua and placenta in early pregnancy were investigated. These levels were compared with patients whose pregnancies were terminated surgically. The median total and cytosolic decidual progesterone receptor concentrations in the mifepristone group were significantly lower than in the surgical group. The nuclear progesterone receptor concentrations were not significantly different. No

significant differences in oestrogen decidual or placental receptor concentrations occurred. These results suggest that mifepristone may be able to down regulate the progesterone receptor concentration in the decidua of early pregnancy, which might add to the anti-progesterone activity of the drug. However, the fall in progesterone receptor concentrations may have been secondary to cell damage and necrosis in the specimens obtained after mifepristone treatment compared with the fresher samples obtained surgically. Probably a better way of investigating progesterone receptor concentrations after mifepristone would have been either to have a control group of patients undergoing medical termination with prostaglandin E_1 alone, or to obtain all the specimens from patients treated surgically. Unfortunately, due to constraints introduced by the company providing the drug this was not possible.

Two studies investigated the affect of mifepristone on prostaglandin production. The first in early pregnancy compared the affect of mifepristone 600mg on prostaglandin metabolite levels over a 48 hour period in women undergoing medical termination of early pregnancy. These results were compared with controls of similar gestation who were treated surgically. There was no significant difference in the mean PGEM and PGFM concentrations after mifepristone treatment, and no significant differences in the prostaglandin metabolite concentrations in the patients treated with mifepristone or surgically.

The second study was designed to investigate the affect of mifepristone on prostaglandin metabolite concentrations in the second trimester of pregnancy. Each patient was randomised to receive either mifepristone 600mg or identical placebo 24 hours prior to admission for termination. There were no significant differences in the PGEM and PGFM concentrations between the two groups prior to treatment, 24 hours later, and six hours after instillation of prostaglandin into the uterus.

Although measurement of prostaglandin metabolite concentrations in peripheral plasma may not be the ideal way of investigating the affect of mifepristone on prostaglandin production, the results suggest that progesterone withdrawal by mifepristone may not increase prostaglandin production. It is therefore possible that the mechanism of action of mifepristone is to increase the sensitivity of the uterus to the prostaglandins already present without necessarily increasing basal production, or by decreasing prostaglandin metabolism.

The last study investigating the possible mechanisms of action of mifepristone was designed to assess the influence on myometrial contractility and response to various oxytocic agents in patients treated with mifepristone 600mg or identical placebo 24 hours before admission for mid-trimester termination of pregnancy. The patients who received mifepristone had a significantly greater baseline uterine activity and increased myometrial

sensitivity to exogenous prostaglandins compared to the patients treated with placebo. However, no change in myometrial sensitivity to oxytocin was demonstrated after mifepristone treatment. This data was analysed by computer, thus eliminating any error due to free-hand interpretation. The total contraction area above atmospheric pressure was studied rather than the contraction area above baseline tone, which might have been more accurate but was not available on the computer programme. Errors in interpretation of measuring uterine activity by this method can occur because the method measures only total activity which can be effected by frequency of contractions, strength of contraction, resting tone, position of the catheter, and length of time interval epoch. However, this data supports the hypothesis that the pregnant uterus is an organ suppressed by progesterone, and when this is withdrawn by mifepristone it becomes more active and sensitive to exogenous prostaglandins.

The last study was designed to investigate the placenta transfer of mifepristone and its possible effects on fetal steroid concentrations in the second trimester of pregnancy. Cardiocentesis was the most reliable method of obtaining fetal blood samples at this gestation. The results show that mifepristone, and probably its major metabolite RU 42,633, are able to freely cross the placental and can be demonstrated in both fetal plasma and amniotic fluid after maternal ingestion. For practical reasons the numbers in this study were small, therefore the

effects of mifepristone on the fetal steroid parameters studied may not be totally accurate. Certainly the increase in fetal aldosterone levels after mifepristone was unexpected, but might be explained by anti-mineralocorticoid receptor activity of the drug which has been reported in some studies.

What then is the current hypothesis on the mechanism of action of mifepristone in pregnancy? Mifepristone binds to the progesterone receptor and either stabilises the non-transformed, inactive form of the receptor possibly by maintaining hsp-90 capping of the DNA binding domain of the receptor, or hsp-90 may be released from the receptor and formation of mifepristone-receptor complexes that are defective at the level of DNA binding (imperfect interaction) or at a post-DNA binding step (steric hindrance precluding transcription factor activity) may occur. It is possible that this process may be further enhanced by down regulation of progesterone receptor concentrations after mifepristone treatment. As a result of blockage of the progesterone receptors, progesterone is withdrawn at a cellular level. In early pregnancy mifepristone primarily affects the decidua causing oedema, necrosis and capillary damage resulting in detachment of the chorionic tissue of the embryo. Human chorionic gonadotrophin levels decrease only secondarily to embryo detachment and luteolysis follows, with finally a decrease in progesterone and oestrogen concentrations permitting the initiation of a new cycle. The abortion process is aided by

increased myometrial activity probably as a result of increased prostaglandin production. However, as no increase in prostaglandin metabolite levels can be demonstrated in peripheral maternal plasma after mifepristone treatment, the increased myometrial activity may be secondary to decreased prostaglandin metabolism or increased sensitivity to prostaglandin already present. Either as a result of increased myometrial activity or by direct action of mifepristone on the cervix, dilatation and softening of the cervix occurs which further helps to prime the uterus. Abortion occurs in 60-85% of cases under 42 days gestation, but when a small dose of exogenous prostaglandin is administered either intramuscularly or vaginally, the products of conception are rapidly passed and an overall complete abortion efficacy of 95% results up to 63 days gestation.

In the second trimester, abortion does not occur but the basal activity of the uterus increases and there is increased sensitivity to exogenous prostaglandins. Again there may be increased prostaglandin production, but this is not reflected in peripheral maternal plasma concentrations. These changes stimulate uterine activity in the uterus, reflected in a 50% reduction in the induction-abortion interval in second-trimester prostaglandin termination of pregnancy.

In the last trimester, there remains the possibility that this ability of mifepristone to induce uterine

activity will occur and the drug could be used to enhance the results of induction of labour. A dose related labour inducing effect of mifepristone has been reported in patients who have an intra-uterine fetal death. However, at present only one randomised placebo controlled trial on labour induction with mifepristone has been performed on normal human pregnancies. This confirmed the potential for mifepristone to be used for labour induction, but there remains the possibility that mifepristone, via its anti-progesterone and anti-glucocorticoid action, will have significant effects on the fetus. From this thesis, placental transfer of mifepristone, and significant effects on fetal steroid production have been demonstrated in the second trimester, therefore further fetal toxicology studies are essential before the drug can be considered for labour induction.

With the arrival of the progesterone receptor antagonist mifepristone we can finally agree, at least in part, with Csapo in concluding that the normal pregnant uterus is suppressed by the influence of progesterone. A few more of the pieces of the complex jigsaw which controls uterine activity and the onset of human parturition are now known, but we are still uncertain about what finally initiates labour. There can be no doubt that progesterone plays a fundamental role in suppressing uterine activity, and although in humans no fall in plasma progesterone concentrations can be demonstrated before labour, this is probably too crude a measurement. More important is what

is happening to the progesterone concentration in the decidua and fetal membranes, and there remains the possibility that local withdrawal of progesterone at this level may be involved in the cascade of events which initiates human parturition.

1). The combination of mifepristone and PGE_1 analogue for medical termination of early pregnancy offers a safe, acceptable, efficient, out-patient alternative to surgical abortion.

2). Mifepristone 48 hours prior to prostaglandin termination of early pregnancy significantly increases efficacy and decreases side-effects compared with prostaglandins alone. Other time intervals and dose schedules warrant study.

3). No increase in prostaglandin metabolite concentrations in the maternal plasma can be demonstrated after mifepristone treatment in the first or second trimester of pregnancy.

4). Mifepristone treatment appears to decrease cytosolic and total progesterone receptor concentrations in early human decidua.

5). Mifepristone treatment appears not to significantly affect decidual or placental oestrogen receptor concentrations in early human pregnancy.

6). Mifepristone treatment 24 hours before prostaglandin mid-trimester termination of pregnancy decreases the induction-abortion interval by 55%, but no significant change occurs if the time from mifepristone to prostaglandin treatment is decreased to four hours - other

time intervals and dose schedules warrant study.

7). Mifepristone increases basal uterine activity 24 hours after treatment in the second trimester of human pregnancy.

8). Mifepristone increases the uterine sensitivity to exogenous prostaglandins within 24 hours in the second trimester of human pregnancy, but does not change the uterine sensitivity to oxytocin.

9). A single dose of mifepristone 600mg does not significantly alter maternal steroid, beta-hCG, haematological, electrolyte or hepatic indices.

10). Mifepristone and probably its metabolite RU 42,633 are able to cross the placenta and can be demonstrated in fetal plasma and amniotic fluid.

11). Mifepristone does not affect fetal plasma progesterone, oestradiol, or cortisol concentrations, but does cause a significant increase in fetal aldosterone concentrations.

12). Although mifepristone can be recommended for use as an adjuvant to termination of pregnancy in the first and second trimesters, and for the management of intra-uterine fetal death, further studies are necessary before it can be used for inducing human term labour.

APPENDICES

Appendix 1 - Structure of mifepristone and metabolites.

Appendix 2 - Binding affinities of mifepristone and its metabolites.

Appendix 3 - Patient diaries.

Appendix 4 - Patient questionnaires.

Appendix 5 - Cross reactivity of PGEM and PGFM assays

Appendix 6 - Progesterone assay.

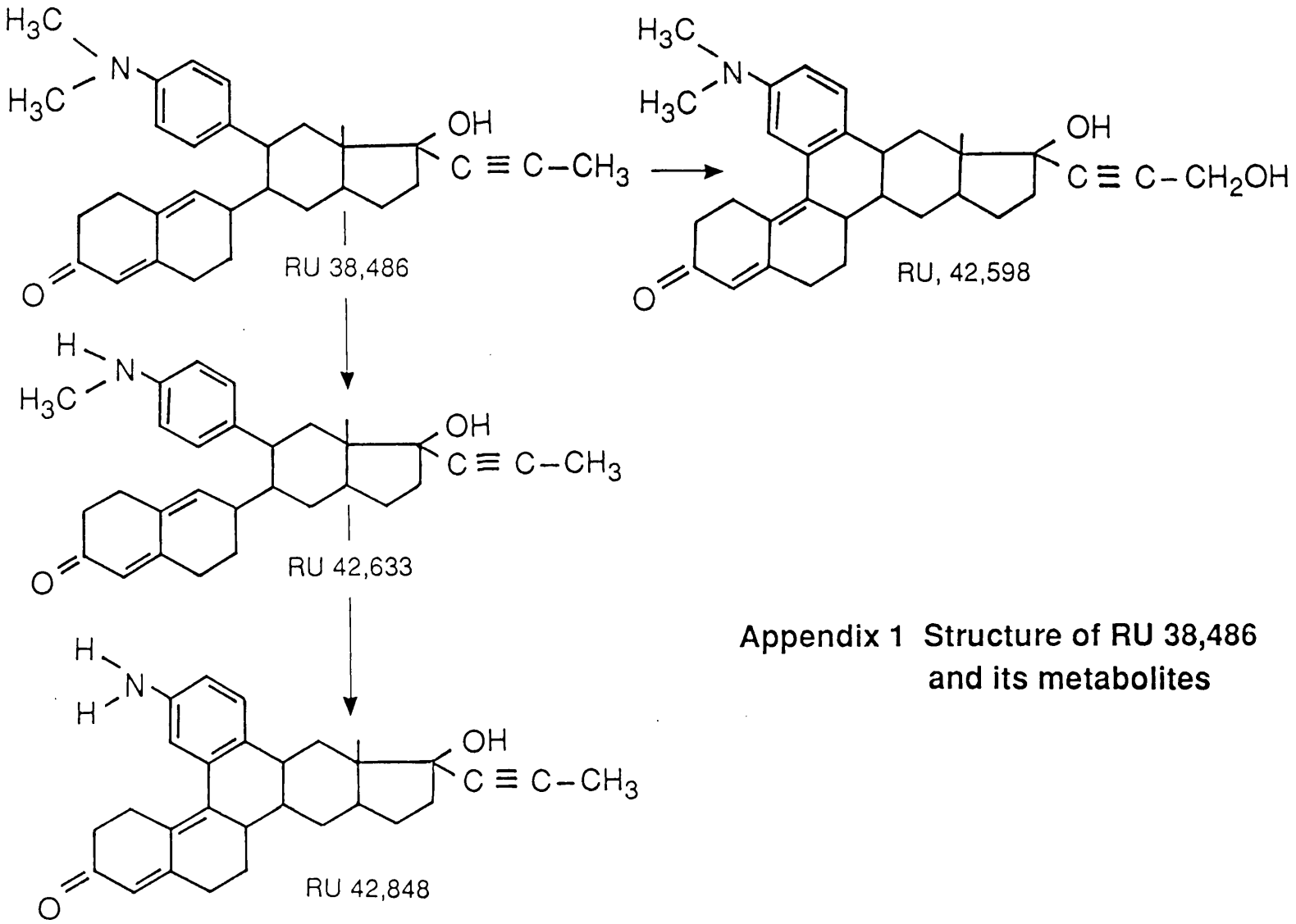
Appendix 7 - Oestradiol assay.

Appendix 8 - Cortisol assay.

Appendix 9 - Aldosterone assay.

Appendix 10 - Mifepristone & RU 42,633 assays.

Appendix 11 - ß-hCG assay.



Appendix 1 Structure of RU 38,486 and its metabolites

APPENDIX 2 - Binding affinities of mifepristone and its metabolites

Compound	Affinity progesterone receptors (%)	Affinity glucocorticoid receptors (%)
Mifepristone	100	100
Progesterone	43	-
RU 42,633	21	61
RU 42,698	15	48
RU 42,848	9	45
Dexamethasone	-	23
Cortisol	-	9

If you have had any of the symptoms below during the past 24 hours, please write the severity score in the box indicated.

Score each symptom 0 = None, 1 = Mild, 2 = Moderate, 3 = Severe.

If for some reason, you are not able to complete the chart on a particular day, then leave the spaces blank.

SYMPTOM	BEFORE TREATMENT [1] 17 Date _____ SCORE			ONE DAY AFTER TREATMENT [2] 17 Date _____ SCORE			TWO DAYS AFTER TREATMENT [3] 17 Date _____ SCORE		
Vaginal Bleeding			18			18			18
Headaches			19			19			19
Drowsiness			20			20			20
Nausea			21			21			21
Vomiting			22			22			22
Indigestion			23			23			23
Loss of Appetite			24			24			24
Diarrhoea			25			25			25
Constipation			26			26			26
Abdominal Pain			27			27			27
Abdominal Distention			28			28			28
Breast Pain			29			29			29
Weakness			30			30			30
Faintness			31			31			31
Tiredness			32			32			32
Pain whilst Urinating			33			33			33
Pelvic Discomfort			34			34			34
Hot Flushes			35			35			35
General 'unwell' feeling			36			36			36
Any Others? Please write them in here			37			37			37
			38			38			38

If you have had any of the symptoms below during the previous 24 hours, please write the severity score in the box indicated.

Score each symptom as 0 = None, 1 = Mild, 2 = Moderate, 3 = Severe.

If for some reason you are not able to complete the chart on a particular day, leave the space blank.

SYMPTOM	DAY NUMBER (Day 1 = Day after your last visit to the Doctor).						
	1 (17)	2 (17)	3 (17)	4 (17)	5 (17)	6 (17)	7 (17)
Vaginal Bleeding (18)							
Headaches (19)							
Drowsiness (20)							
Nausea (21)							
Vomiting (22)							
Indigestion (23)							
Loss of Appetite (24)							
Diarrhoea (25)							
Constipation (26)							
Abdominal Pain (27)							
Abdominal Distention (28)							
Breast Pain (29)							
Weakness (30)							
Faintness (31)							
Tiredness (32)							
Pain whilst Urinating. (33)							
Pelvic Discomfort (34)							
Hot Flushes (35)							
General 'Unwell' Feeling (36)							
Any Others? (37)							
Please write them in here (38)							

APPENDIX 4 - Patient questionnaires

The medical method of termination is relatively new and it is important that we document whether it is acceptable to the patients. I would be grateful if you could please complete and return this form after your first period.

Name :

1). When was the first day of your period?

2). How many days did it last?

3). What type of period was it?

Normal Heavy Light

4). Since last seen have you needed any medication?

Yes No

5). Since last seen have you needed re-admission to hospital?

Yes No

6). Have you had a termination before?

Yes No

If yes - did you prefer the medical method of termination.

Yes No

7). Have you any comments about the medical method of termination?

Appendix 5 - Cross reactivity of PGEM and PGFM assay

Specificity of the Bicyclo-PGEM antiserum

Compound	Relative cross reactivity (%)
PGEM	100
PGFM	< 0.1
PGA_2	< 0.05
PGE_1	< 0.05
PGE_2	< 0.05
PGF_{1a}	< 0.05
PGF_{2a}	< 0.05

Cross reactivity of PGFM antiserum

Compound	Relative cross reactivity (%)
PGFM	100
PGEM	< 0.2
PGF_{2a}	< 0.2
PGE_2	< 0.2
15-keto-PGF_{2a}	9.3
13,14-dihydro-PGF_{2a}	0.9

APPENDIX 6 - Progesterone assay

Progesterone concentrations were assayed by Technical Services International (TSI), Harley Street using commercially available "Coat-A-Count" radioimmunoassays supplied by Diagnostic Products Ltd., Abingdon.

Introduction

The "Coat-a-count" procedure is a solid-phase radioimmunoassay, wherein ^{125}I-labelled progesterone competes for a fixed time with progesterone in the patient sample for antibodies. The antibody being immobilised to the wall of a polypropylene tube, decanting the supernatant suffices to terminate the competition and to isolate the antibody-bound fraction of the radiolabelled progesterone. Counting the tube in a gamma counter then yields a number, which converts by way of a calibration curve to a measure of the progesterone present in the patient sample.

The antiserum is highly specific for progesterone, with very low crossreactivity to other compounds that might be present in patient samples. Neither haemolysis, lipaemia or bilirubin has any clinically significant effects on the assay.

Materials and methods

1). Label four plain (uncoated) polypropylene tubes T (total counts) and NSB (non-specific binding) in duplicate.

2). Label 14 Progesterone Antidody-Coated tubes A (maximum

binding) and B through G in duplicate. Label additional antibody-coated tubes in duplicate for controls and patient samples.

Calibrators	ng/ml	nmol/l
A (MB)	0	0
B	0.1	0.3
C	0.5	1.6
D	2	6.4
E	10	31.8
F	20	63.6
G	40	127.2

3). Pipet 100ul of the zero calibrator A into the NSB and A tubes, and 100 ul of each of the calibrators B through G into corresponding labelled tubes. Pipet 100ul of each control and patient sample into the tubes prepared.

4). Add 1.0ml of Buffered [^{125}I] Progesterone to every tube and vortex.

5). Incubate for 3 hours at room temperature.

6). Decant thoroughly.

7). Count for 1 minute in a gamma counter.

Specificity of the progesterone antiserum

Compound	Percentage crossreactivity
Progesterone	100
11-Deoxycortisol	2.4
20a-Dihydroprogesterone	2.0
11-Deoxycorticosterone	1.7
Corticosterone	0.4
17a-Hydroxyprogesterone	0.3
Oestradiol	ND
Cortisol	ND
Aldosterone	ND

Compounds flagged as "ND" were not detectable by the assay.

APPENDIX 7 - Oestradiol assay

Oestradiol assays were performed by TSI using coat-a-count radioimmunoassays supplied by Diagnostic Products Ltd, Abingdon. The antiserum was highly sensitive to oestradiol and the lower limit of sensitivity was 8pg/ml.

Materials and methods

1). Label tubes (see appendix 1).

2). Pipet 100ul of the zero calibrator into the A tubes, and add 100ul of each remaining calibrator, control and patient sample into the tubes prepared.

Specificity of oestradiol antiserum

Compound	Percentage crossreactivity
Oestradiol	100
Oestriol	0.2
Oestrone	1.3
Progesterone	ND
Cortisol	ND
Aldosterone	ND
Norethindrone	ND
11-Deoxycortisol	ND

3). Add 1.0ml of buffered [^{125}I] Oestradiol to every tube, vortex and incubate for three hours at room temperature.

4). Decant and count for one minute in a gamma counter.

APPENDIX 8 - Cortisol assay

Cortisol concentrations were assayed by TSI, using commercially available "coat-a-count" radioimmunoassays supplied by Diagnostic Products Ltd., Abingdon. The antiserum is highly specific for cortisol and can detected concentrations down to 0.2ug/dl.

Materials & methods

1). Label tubes (see appendix 1).

2). Pipet 25ul of the zero calibrator into the A tubes, and pipet 25ul of each remaining calibrator, control and patient sample into the prepared tubes.

Specificity of the cortisol antiserum

Compound	Percentage crossreactivity
Cortisol	100
11-Deoxycorticosterone	1.5
11-Deoxycortisol	0.25
Progesterone	0.15
Oestradiol	0.03
Oestriol	0.02
Oestrone	0.01
Aldosterone	0.01

3). Add 1.0ml of buffered [^{125}I] Cortisol to every tube, vortex and incubate for 45 minutes.

4). Decant and count for one minute in a gamma counter.

APPENDIX 9 - Aldosterone assay

Aldosterone concentrations were measured by TSI using commercially available "Coat-a-count" radioimmunoassays supplied by Diagnostic Products Ltd., Abingdon. The assay was highly specific for aldosterone and could detect concentrations as low as 16 pg/ml (1.6ng/dl).

Materials & methods

1). Label plain and coated tubes (see appendix 1).

2). Pipet 200ul of the various calibrators into the appropriate tubes, and pipet 200ul of control and patient sample into the tubes prepared.

Specificity of the aldosterone antiserum

Compound	percentage crossreactivity
Aldosterone	100
Progesterone	0.01
Oestradiol	ND
Oestriol	ND
Oestrone	ND
Cortisol	ND

3). Add 1.0 ml of buffered [^{125}I] Aldosterone to every tube and vortex.

4). Incubate for three hours at room temperature.

5). Decant and count for one minute in a gamma counter.

APPENDIX 10 - Mifepristone and RU 42,633 assays

Radioimmunoassay of mifepristone and RU 42,633 were performed by Roussel Laboratories, France using the technique described by Salmon & Mouren (1985). An antigen was prepared by coupling bovine serum albumin with the 3-carboxymethyloxime of mifepristone, and an anti-mifepristone antiserum was produced in rabbits.

Materials and methods

1). The standard curve was prepared from stock solution of mifepristone over concentrations of 0 - 2000pg/0.1ml.

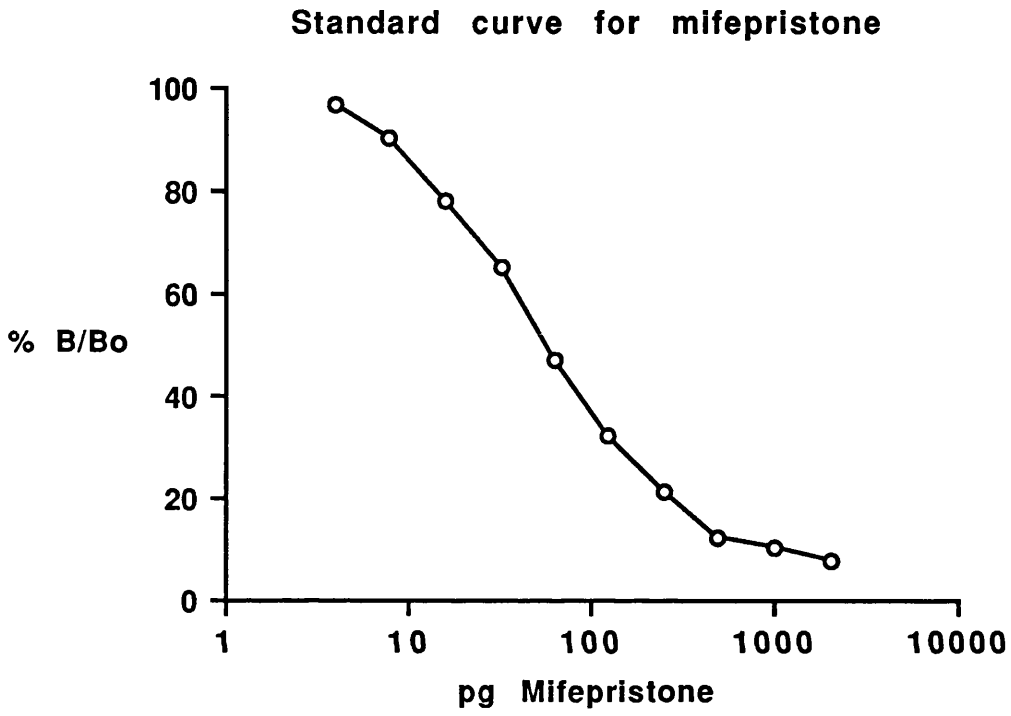
2). Dilute plasma samples by 1/200 to 1/4000 in phosphate-gelatin buffer (9g NaCl, 1g sodium azide and 1g gelatin in 1L of 0.1M phosphate buffer, pH 6.9).

3). Add 0.1ml of plasma dilutions or 0.1ml of standard solutions to 0.4ml distilled water in glass haemolysis tubes.

4). Extract the mifepristone by adding 3.0ml diethyl ether and agitate in a multivortexer for four minutes.

5). Freeze aqueous phases in a methanol-dry ice bath, decant ether phases and evaporate in a 40°C water bath.

6). Take residues up in 0.2ml of tritiated mifepristone solution (5,000 cpm), vortex and stand at room temperature for 30 minutes.


Standard curve for mifepristone
100
80
60
40
20
0
% B/Bo
1
10
100
1000
10000
pg Mifepristone

7). Add 0.8ml of antiserum diluted 1/100,000 in phosphate-gelatin buffer and incubate overnight at 4°C.

8). Add 0.75ml of ice cold dextran-coated charcoal suspension to each tube and incubate for 10 minutes.

9). Pellet charcoal at 3,000 rpm for 10 minutes at 4°C.

10). Transfer supernatants to polyethylene counting vials, add 10ml of scintillation fluid and count for two minutes in liquid scintillation counter.

A similar assay procedure was performed for measurement of RU 42,633 concentrations, and the cross reactivity of the assay is shown below.

Specificity of the anti-mifepristone antiserum

Steroid	Percentage
Mifepristone	100
RU 42,633	84
RU 42,698	60
Progesterone	0.8
Testosterone	< 0.01
Cortisol	< 0.01
17 ß-Oestradiol	< 0.01
Oestrone	< 0.01
Oestriol	< 0.01
Dexamethasone	< 0.01

APPENDIX 11 - ß-hCG assay

ß-hCG concentrations were calculated by TSI using immunoradiometric assays (Diagnostic Products Ltd, Abingdon) based on ligand-coated tubes and three monoclonal antibodies, two of them ^{125}I labelled and the other linked to a ligand hCG in the patient sample. The assay were highly sensitive and were able to detect ß-hCG as low as 0.5mIU/ml.

Materials and methods

1). Label 14 ligand-coated Tubes A (non-specific binding) and B through G (maximum binding) in duplicate, and label additional ligand-coated tubes for controls and patient samples.

2). Pipet 200ul of each calibrator, control and patient sample into the tubes prepared.

Specificity of the hCG antiserum

Compound	percentage crossreactivity
LH	0.20
FSH	0.17
TSH	0.10

3). Add 100ul of ligand-labelled monoclonal Anti-hCG to all the tubes and shake.

4). Incubate for 10 minutes at room temperature.

5). Add 25ul of Anti-ligand to all the tubes and shake for 10 minutes on a rack shaker.

6). Decant thoroughly, add 2ml of buffered wash solution and wait 1-2 minutes before decanting again.

7). Add 200ul of monoclonal [^{125}I] Anti-hCG tracer to every tube and shake for 10 minutes.

8). Decant, wash twice and count for 1 minute in a gamma counter.

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