UTERINE EFFECTS OF INHIBITION
of
PROGESTERONE SYNTHESIS
by a specific
3β HYDROXYSTEROID DEHYDROGENASE INHIBITOR

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SUMMARY

The steroid hormone progesterone is considered indispensible for pregnancy - it is the 'hormone of pregnancy' as described by Corner (1928). It is generally agreed that the role of progesterone in all species is to prepare the endometrium for pregnancy and to contribute to the maintenance of myometrial quiescence from implantation to the end of pregnancy. There is however, a conflict of opinion concerning its role in pregnancy termination. In some species, for example the sheep, there is considerable evidence that progesterone provides an important link between fetal cortisol and the control of uterine activity. But in human pregnancy the evidence is conflicting.

The present study investigates the role of progesterone in the initiation of uterine activity in;

- late pregnant ewes,
- early human pregnancy and
- the luteal phase of the human menstrual cycle.

The study was designed following the development of a 3 β-hydroxysteroid dehydrogenase (3 β-HSD) inhibitor (Epostane) by Sterling Winthrop Research. In vitro, Epostane acts competitively to inhibit the conversion of pregnenolone to progesterone. This provides a method of investigating the role of progesterone in the classical manner - depressing the rate of secretion.

METHOD

1. Late pregnant ewes

Six animals were studies at 131 ± 2 days of pregnancy.

(i) Progesterone production was inhibited by administration of Epostane.

(ii) Hormone levels in the ewe were monitored to detect changes early in parturition.

(a) Maternal catheters were placed into a carotid artery and a jugular vein.

(b) The concentrations of progesterone, oestradiol-17β cortisol and 13, 14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (PGFM) were measured by radioimmunoassay.

(iii) Hormone levels in the fetus were monitored to measure fetal responses to falling concentrations of progesterone.

(a) Fetal catheters were placed in a carotid artery or a jugular vein.

(b) The concentrations of progesterone and cortisol were measured by radioimmunoassay.

(iv) Uterine activity was monitored by an intra-amniotic pressure transducer.

(v) The fetus and the fetal adrenals were weighed after delivery.

2. Early Human Pregnancy

Sixty-five pregnant women between five and 18 weeks of pregnancy were studied.

(i) Progesterone production was inhibited by oral administration of Epostane.

(ii) Hormone levels were monitored to assess the effect

of inhibition of 3 β -HSD on ovarian and placental steroidogenesis.

(a) Serial venous blood sampling was performed via an indwelling forearm catheter.

(b) The concentrations of progesterone, cortisol, oestradiol-17β and PGFM were measured by radioimmunoassay.

(iii) A detailed history, physical examination, haematological and biochemical assays were performed prior to and on completion of the study to exclude adverse effects.

(iv) The effect of inhibition of progesterone secretion on uterine activity was determined by pressure monitoring and/or direct questioning.

3. Human Menstrual Cycle (Luteal Phase)

Thirty-three women in the luteal phase of a menstrual cycle were studied.

(i) Progesterone production was inhibited by oral administration of Epostane.

(ii) Hormone levels were monitored to assess the effect of inhibition of progesterone secretion on ovarian steroidogenesis.

(a) Serial venous blood sampling was performed, usually via an indwelling forearm catheter.

(b) The concentrations of progesterone, oestradiol-17β and cortisol were measured by radioimmunoassay.

(iii) Detailed history, physical examination, haematological tests and biochemical assays were

performed prior to and on completion of the study to exclude adverse effects.

(iv) The effect of inhibition of progesterone secretion on the timing of the subsequent menstruation was recorded.

RESULTS

1. Late Pregnant Ewes

(i) In all animals, detectable uterine contractions were recorded via the intra-amniotic catheter four to six hours after treatment.

(ii) Three of the six animals delivered within 36 hours and the remaining three were in established labour when the experiment was terminated.

(iii) The fetal condition as determined by arterial pO_2 and pH were normal throughout labour.

(iv) Maternal progesterone in both the uterine vein and peripheral plasma fell precipitously to levels of less than 1.0 ng/ml and remained at this level until immediately prior to delivery.

(v) Plasma concentrations of oestradiol-17β were not significantly altered in either the uterine vein or peripheral plasma.

(vi) Maternal cortisol concentrations followed the expected diurnal variation.

(vii) There was a dramatic and progressive rise in PGFM concentrations which were significantly different from the pretreatment values at four hours.

(viii) Fetal plasma progesterone concentrations fell

below measurable levels by the time of the first post-treatment sample.

(ix) Cortisol concentrations in the fetal plasma rose significantly throughout the study in parallel to the rise in PGFM levels in the maternal plasma.

2. Early Human Pregnancy

(i) Epostane suppressed progesterone production without significant side effects. The fall in the serum progesterone levels was both dose- and gestation- related.

(ii) Serum oestradiol-17β levels fell in parallel to serum progesterone. The fall was both gestation- and dose- related.

(iii) Serum cortisol levels were not significantly affected by Epostane (300 mg daily for five days).

(iv) Despite a decline in serum progesterone to levels below the minimal value (10 ng/ml) said to be necessary for the maintenance of human pregnancy (Csapo and Pulkkinen, 1978), there was no clinical effect in four of the five treated women.

3. Human Menstrual Cycle (Luteal Phase)

(i) Epostane suppressed progesterone production without side effects. The fall in the serum progesterone concentration was dose - dependent.

(ii) Serum oestradiol-17β levels were not affected.

(iii) Serum cortisol levels were not significantly affected.

(iv) The fall in serum progesterone levels was accompanied by early menstruation in five out of eight treated women.

CONCLUSIONS

1. Late Pregnant Ewes

(i) Epostane inhibits the synthesis of progesterone, probably by inhibiting the activity of 3 β-HSD (hydroxysteriod dehydrogenase).

(ii) A fall in the concentration of progesterone alone is sufficient to initiate the chain of events leading to parturition.

(iii) A decline in the concentration of progesterone stimulates the synthesis of $PGF_{2\alpha}$

(iv) A substantial and prolonged decline in the level of progesterone is required to initiate parturition in the ewe.

(v) Although the decline in the concentration of progesterone achieved in this study did initate parturition it is unlikely that progesterone acts in isolation to initate parturition in the ewe.

2. EARLY HUMAN PREGNANCY

(i) Epostane inhibits the synthesis of progesterone, probably by inhibiting the activity of 3 β- hydroxysteriod dehydrogenase.

(ii) A rapid decline in the concentration of progesterone is not the trigger to the initiation of uterine activity in human pregnancy.

(iii) It is unlikely that a physiological change in the concentration of progesterone leads to parturition in women.

(iv) A substantial and prolonged decline in the level of progesterone achieved by pharmacological means may initiate uterine activity in early human pregnancy.

3. HUMAN MENSTRUAL CYCLE (Luteal Phase)

(i) Epostane inhibits the synthesis of progesterone, probably by inhibiting the activity of 3 β- hydroxysteriod dehydrogenase.

(ii) A rapid decline in the level of progesterone is not the trigger to menstruation women.

(iii) A substained and prolonged decline in the level of progesterone is required to initiate menstruation.

CHAPTER I

INTRODUCTION

"In general, oestrogen is the hormone of the woman; it assures the development of the genital tract and the mammary apparatus: progesterone is the hormone of the mother; it is indispensible for reproduction" (Corner and Allen, 1930).

The mechanism by which parturition is initiated in the human is largely unknown. Clinical observations and experiments in animals have implicated the steroid hormone progesterone as having an important role in the complex train of hormonal changes which precede parturition. In the sheep, large portions of this train have been explored and clarified (Liggins, Fairclough, Grieves, Kendall and Knox, 1973; Thorburn and Challis, 1979). However, the expectation that knowledge of the physiology of parturition in one species would explain parturition in another has been unfulfilled. Although there are undoubted similarities between species there are major differences particularly in the role played by progesterone.

A role for progesterone in pregnancy termination was initially suggested following the luteectomy studies of Corner and Allen (1930). These experiments clearly demonstrated that in the rabbit the corpus luteum and its secretory product, progesterone, are indispensible for the pregnancy. These observations led to hypotheses postulating an all embracing role for progesterone in reproduction where not only was it essential for pregnancy maintenance but also

progesterone 'withdrawal' was considered an essential pre-requisite to pregnancy termination in all species - progesterone was said to be the 'defence mechanism' of pregnancy (Csapo, 1956).

Supportive evidence in some species followed. A fall in the concentration of progesterone in maternal blood precedes parturition in many animals, for example, in the sheep (Bassett and Thorburn 1969), in the goat (Thorburn and Schneider, 1972) and in the cow (Donaldson et al, 1970). Convincing evidence for the hypothesis has yet to be demonstrated in the human.

Progesterone has an established role in human reproduction. It is generally agreed that progesterone is essential for the preparation of the endometrium for implantation, for the transportation of fertilised ova and also for the development of the mammary glands. In women, progesterone is secreted during the luteal phase of the menstrual cycle by the theca lutein cells of the newly formed corpus luteum and is responsible for preparatory change in the endometrium essential for embryonic implantation. If fertilisation does not occur, the corpus luteum atrophies, progesterone secretion declines and the endometrium is shed. Should implantation occur, the functional life of the corpus luteum is maintained; progesterone production is continued and the support to the endometrium maintained. Progesterone secretion continues to rise throughout pregnancy, initially from the corpus luteum and later from the placenta to reach extremely high production rates (250 mg/day at term; Short

and Eton, 1959). However, the evidence implicating progesterone in the termination of human pregnancy is conflicting and is reviewed below.

Central to any discussion of the control of uterine activity must be the recognition that the smooth muscle and its connective tissue elements are the functional unit of uterine activity and therefore the focal point for regulatory influence. If progesterone does have a role in the termination of human pregnancy it must have an effect on this functional unit.

The contractility of the myometrium is controlled by the intracellular concentration of calcium ions. A flux of calcium ions into the cell generates a contraction by inducing a reaction between the myosin and actin filaments. Further, co-ordination of this muscular activity is essential for effective labour. Intercellular connections called gap junctions which enhance the electrical conductivity have been demonstrated in the myometrium during active labour and are possibly responsible for the co-ordination of uterine activity. Progesterone has been shown to be involved in calcium ion transport (Carsten, 1979) and in gap junction formation (Garfield, Kannan and Daniel, 1980).

THE EFFECT OF PROGESTERONE ON CALCIUM ION FLUX

Detailed reviews of the role of calcium ion in smooth muscle contractility are available (Stull, Blementhal and Cooke, 1980; Huszar 1981). The synopsis that follows is intended as a brief introduction to this thesis.

Uterine smooth muscle is composed of the contractile proteins, actin and myosin. Myosin with two heavy and two light polypeptide chains converts the chemical energy of adenosine triphosphate (ATP) into mechanical energy. The intracellular concentration of calcium ions regulates this reaction. Phosphorylation of the light chains by myosin -light chain kinase, an enzyme that requires calcium ions for its activity, promotes contraction. Dephosphorylation of the light chains by a phosphatase leads to muscle relaxation. Thus an increase in the intracellular free calcium ion concentration induces a uterine contraction and a decrease results in muscle relaxation.

Change in intracellular calcium ion concentration is brought about both by a movement of calcium ions between the cytosol and intracellular organelles (Van Breeman, Farinas, Gerba, Wuytock and Deth, 1972), such as the sacroplasmic reticulum and the mitochondria, and by flux between cytosol and extracellular water through calcium channels. Of these the sarcoplasmic reticulum rather that the cell membrane is considered to be the most important (Carsten and Millar,1987).

The calcium ion flux is controlled by local hormone concentrations (Carsten, 1974). Prostaglandins and oxytocin inhibit ATP - dependent binding of calcium to the intracellular organelles which increases the intracellular

calcium ion concentration (Carsten, 1974). Progesterone stimulates the binding of calcium to the intracellular organelles which decreases the intracellular calcium ion concentration and results in muscle relaxation.

Evidence for this is provided by Carsten (1979). Carsten separated a microsomal fraction from pregnant uteri and compared the ATP -.dependent calcium binding of this microsomal fraction in the presence and absence of progesterone (50µg/ml). Progesterone significantly increased the calcium binding from 9.7 to 11.7 nmoles of calcium/ng protein ($p < 0.01$). This confirms that in the presence of progesterone the binding of calcium to intracellular organelles is increased and the availability of calcium to the myosin-actimyosin reaction is reduced. Smooth muscle contraction is inhibited.

Progesterone also inhibits the prostaglandin- and oxytocin-induced calcium ion influx.

In a similar experiment, Carsten (1974), investigated calcium ion binding to the sarcoplasmic reticulum in the presence of progesterone and either oxytocin or prostaglandin $F_{2\alpha}$. Progesterone (50µg/ml) increased ATP - dependent binding of calcium when added to the incubation assay and both prostaglandin (0.01 to 0.1 µg/ml) and oxytocin (50µ IU/ml) reduced calcium binding. However, the addition of either prostaglandin $F_{2\alpha}$ or oxytocin to the incubation medium after preincubation with progesterone did not significantly alter the progesterone response. This indicates that progesterone is antagonistic to the action of both prostaglandin and

oxytocin (Carsten, 1974).

These findings show that an inhibitory effect of progesterone on uterine activity could be mediated through its effect on calcium ion flux.

THE EFFECT OF PROGESTERONE ON GAP JUNCTION FORMATION

Synchronised uterine contractions are essential for the expulsion of the fetus and are dependent on a simultaneous activation of all the myometrial cells. The two components essential for a co-ordinated response are the connective tissue matrix and the intercellular connections - gap junctions. Gap junctions are thought to be the site of low resistance pathways to current flow in excitable tissues including smooth muscle and have been demonstrated in the myometrium during active labour (Garfield, Sims and Kannan, 1978). Progesterone is thought to be one hormone controlling their formation (Garfield, Kannan and Daniel, 1980).

Human myometrial tissue at various stages of gestation has been quantitatively examined with the electron microscope for the presence of gap junctions (Garfield, Rabideau, Challis and Daniel, 1979). These authors obtained specimens from the myometrium of women undergoing caesarean section and inspected these with the electron microscope. Gap junctions were present in 12 of 22 women who had been in active labour, but in only 2 of 30 women prior to the onset of labour.

Garfield and co workers' (1979) conclusions from these studies were:

1. Gap junctions are present in human myometrium.
2. They may play a significant role in the termination of pregnancy.
3. The number increases as term approaches and the highest number is found in labour.
4. They may be a common pathway in all species for the onset of parturition as they are found in the rat and the sheep (Garfield et al,1979).
5. The absence of gap junctions is favourable for the maintenance of pregnancy.
6. The formation of gap junctions provides additional low resistance pathways for electrical transmission and is a prerequisite for normal labour.
7. The factors controlling the formation of gap junctions may control the onset of parturition.

The hypothesis that the steroid hormones and prostaglandins are involved in gap junction formation has been investigated in the rat (Garfield et al, 1980). Myometrial tissues from pregnant rats were incubated in tissue cultures. Progesterone, oestradiol-17β ,indomethacin and/or prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were added to the preparation. The tissues were then fixed and histologically examined for the number of gap junctions formed per tissue length. These studies demonstrated that prostaglandin, oestradiol and progesterone all have a controlling influence on gap junction formation. The number of gap junctions were significantly reduced by the addition of indomethacin to the culture confirming a role for prostaglandins. Oestradiol-17β or progesterone alone did not alter the number of gap junctions

but together they caused a significant reduction in the number from 16.3 to 9.0 per 1000 um of plasma membranes ($p < 0.025$) (Table 1.1).

Garfield and co-workers (1980) concluded that the steroid hormones and prostaglandins interact to modulate gap junction formation in the rat myometrium. They considered that progesterone in the presence of increased progesterone receptors (high oestrogen) would inhibit gap junction formation. The steroid hormones are thought to regulate the formation of gap junctions by controlling protein synthesis as demonstrated by an inhibitory effect of actinomycin D on their formation (Garfield, Merrett and Grover, 1980).

TABLE 1.1 Hormonal control of Gap Junction Formation.

Number of gap junctions per 1000um of plasma membrane. (mean ± S.E.M.		
Hormone environment	Control	Treated
Indomethacin	24.7 ± 2.4	7.7 ± 2.7 *
Oestradiol-17β (50 ng/ml)	16.3 ± 1.9	14.0 ± 3.2
Progesterone (150 ng/ml)	16.3 ± 1.9	15.9 ± 4.0
Oestradiol-17β and Progesterone	16.3 ± 1.9	9.0 ± 2.9 *

Garfield, Kannan & Daniel (1980)

*$P < 0.025$

EFFECT OF PROGESTERONE ON PROSTAGLANDIN SYNTHESIS AND RELEASE

There is strong evidence that prostaglandins are involved in the mechanisms of initiation of human parturition.

1. Exogenous prostaglandins of the 2 series will stimulate the human uterus to contract without demonstrable changes in the steroid hormone concentrations (Bygdeman and Hamberg, 1967).
2. Prostaglandins have been used successfully to induce labour (Karim, Trussell, Patel and Hillier, 1968).
3. The uterine sensitivity to prostaglandins increases as pregnancy progresses (Karim and Hillier, 1979).
4. Myometrial contractility can be suppressed by prostaglandin synthetase inhibitors such as indomethacin and acetyl salicylic acid (Lewis and Schulman, 1973).
5. Inhibitors of prostaglandin synthetase will arrest premature labour (Zuckerman, Reiss and Rubinstein, 1974).
6. The concentration of 13, 14 dihydro-15-keto-prostaglandin $F_{2\alpha}$ (PGFM) increases during labour in the amniotic fluid (Keirse, Mitchell and Turnbull,1977) and in the maternal plasma (Sellers, Mitchell, Anderson and Turnbull, 1981).

Knowledge of the factors controlling prostaglandin synthesis and release in man is increasing. In the sheep, which can be studied in more detail, there is evidence that prostaglandin release precedes labour and is controlled by the steroid hormone concentrations (Liggins, Grieves, Kendall and Knox, 1972). These authors demonstrated a good correlation between the rising levels of oestradiol-17β and $PGF_{2\alpha}$ prior to

labour. Administration of stilboestrol led to an increase in prostaglandin levels in the utero-ovarian vein and this oestrogen-stimulated release of prostaglandin was completely blocked (for 24 hours or more) by the simultaneous administration of progesterone (200 mg). These findings suggest that oestrogen and progesterone may control prostaglandin synthesis and/or release in the ewe.

In human pregnancy, evidence is accumulating that progesterone can inhibit prostaglandin synthesis (Gustavii, 1977; McDonald, Porter, Schwarz and Johnson; Abel and Baird, 1980; Wilson, Liggins, Aimer and Watkins,1986).

The biosynthetic pathway of the prostaglandin 2 series involves mainly the deacylation of glycerophospholipids by phospholipases A_2 and C to liberate free fatty acids. Arachidonic acid in its free form is then converted by prostaglandin synthetase to active prostaglandins. Free arachidonic acid does not accumulate in human tissues; rather, it is present in the esterified form in various glycerophospholipids. The glycerophospholipids of fetal membrane are specifically enriched with arachidonic acid, (Schwarz, Schultz and MacDonald, 1975). The source of the prostaglandins which appear in the maternal circulation and amniotic fluid is considered to be predominantly the fetal membranes and decidua and control over their synthesis to be mediated by phospholipase A_2 (MacDonald et al, 1978).

Evidence favouring phospholipases rather than cyclooxygenase as the rate limiting step in uterine prostaglandin synthesis is as follows:

1. Arachidonic acid precursors are readily available (18% of the fatty acid content of human chorioamnion is arachidonic acid (Schwarz et al, 1975).
2. Intra-amniotic administration of arachidonic acid to women in the mid trimester causes abortion (MacDonald et al, 1974).
3. Phospholipase A_2 has been found in human uterine tissues (Grieves and Liggins, 1976).
4. The rapid release of large quantities of prostaglandins into the amniotic fluid and maternal circulation following amniotomy is more consistent with activation of phospholipases than of cyclooxygenase (Sellers et al, 1981).
5. Wilson, Liggins, Aimer and Watkins (1986) have demonstrated that progesterone inhibits the release of arachadonic acid from stimulated perfused endometrial cells.

There is both direct and indirect evidence that progesterone regulates the activity of phospholipase A_2 . Gustavii (1977) proposed that the mechanism initiating uterine activity depended on the stability of lysosomes (Fig.1.1). Conditions leading to lysosomal breakdown and release of phospholipase A_2 would result in prostaglandin synthesis, whereas increased stability of the lysosomes would prevent prostaglandin synthesis and therefore inhibit uterine activity.

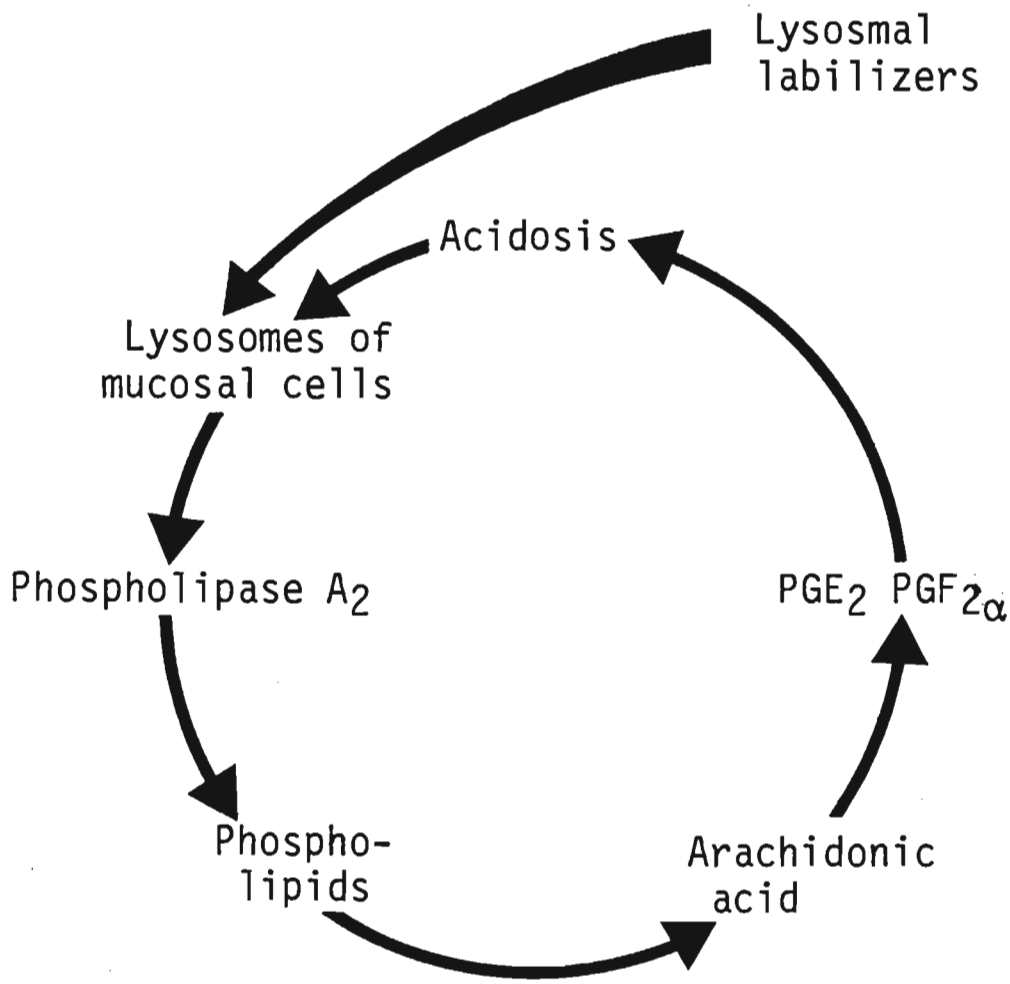

FIG 1.1 Hypothetical model of factors controlling uterine contractility (Gustavii, 1977).

Factors that may labilise lysosomes include:

1. Hypoxia and ischaemia which occur in the pregnant uterus during myometrial contractions (Brunk and Gustavii, 1973);
2. Osmotic stress from hyperosmolar and hypoosmolar solutions injected intra-amniotically (Brunk and Gustavii, 1973);
3. Prostaglandin $F_{2\alpha}$ increases lysosomal fragility by a direct action (Weiner and Kaley, 1972).

Decidual cells, in contrast to trophoblastic cells, are very sensitive to noxious stimuli. Decidual cells allowed to autolyse in vitro for one hour demonstrate a marked dispersion of lysosomal enzymes whereas trophoblastic cells still contain intact lysosomes after eight hours (Gustavii, 1972).

The effect of steroid hormones on the stability of endometrial lysosomes throughout the human menstrual cycle has been extensively investigated (Henzl, Smith and Boost, 1972). These investigators found that the number of lysosomes increased progressively throughout the menstrual cycle. They suggest that oestradiol-17β stimulates the formation of lysosomes and that the "withdrawal" of oestradiol-17β or progesterone is followed by the release of lysosomal enzymes. This pattern of lysosomal activity can be mimicked in castrated rabbits by treatment with oestradiol-17β and progesterone (Smith and Henzl, 1969). These studies suggest that the ovarian hormones during the menstrual cycle control the activity and number of lysosomes and that their decline at the end of the menstrual cycle leads to lysosomal break-down, activation of phospholipase A_2 and the release

of prostaglandin $F_{2\alpha}$.

Direct evidence for a link between steroid homone and prostaglandin systhesis has been provided by experiments with cultures of human endometrial tissue. Abel and Baird (1980) demonstrated that synthesis of $PGF_{2\alpha}$ was inhibited by 10^{-8}M progesterone. Skinner, Liggin, Wilson and Neale (1984) dispersed endometrial cells obtained from patients undergoing hysterectomy or dilatation and curettage for benign disease with collagenase and demonstrated that preiniubation with progesterone (10-8 M) inhibited the synthesis of $PGF_{2\alpha}$. This group later demonstrated that it was the release of arachidonic acid which was inhibited by progesterone (Wilson et al 1986).

The hypothesis that progesterone regulates the activity of phospholipase A_2 via a stabilising effect on lysosomal membranes (MacDonald et al, 1978) is supported by indirect evidence only.

1. There is a relationship between progesterone secretion by the corpus luteum during the luteal phase of the menstrual cycle and the maximal accumulation of lysosomes within the endometrial cells (Ferenczy and Richart, 1974).
2. The administration of progesterone at the end of a normal menstrual cycle prevents menstruation despite the decline of the function of the corpus luteum.
3. The administration of progesterone to the pregnant ewe prevents the oestrogen-induced rise in $PGF_{2\alpha}$ (Liggins et al, 1973).

4. ^{3}H-progesterone when added to homogenates of human chorioamnion is fixed to those subcellular organelles having the highest specific activity of lysosomal enzymes (MacDonald et al, 1978).

5. Progesterone stabilises erythrocyte membranes against hypotonic haemolysis (Seeman, 1966).

This evidence, although indirect, suggests that progesterone in the human endometrium and in fetal membranes may act by stabilising lysosomal enzymes and inhibiting phospholipase A_2 activity. Conversely, a decline in progesterone levels in the chorioamnion could result in increased phospholipase A_2 activity with conversion of glycerophospholipids to free arachidonic acid and prostaglandins.

EFFECT OF PROGESTERONE ON ELECTRICAL ACTIVITY OF THE MYOMETRIUM

The electrophysiology of the myometrium and in particular its hormonal control has been the subject of detailed reviews, (Carsten, 1968; Csapo, 1977; Currie, 1980). Progesterone is considered by some authors to have an effect on both the resting membrane potential and on the action potential of uterine muscle.

Claims for an effect of progesterone on the resting potential of the myometrium are controversial because of methodological problems. Some authors (Marshall, 1964; Csapo, 1961) suggest that myometrial cells in the presence of progesterone are hyperpolarised in comparison to an oestrogen dominated myometrium. Other authors, investigating the resting

potential of the rabbit myometrium, conclude that there is no difference betweeen oestrogen and progesterone dominated myometrium (Kao and Nishiyama, 1964). This conflict has not been resolved.

There is agreement however, that once the electrical activity of myometrial cells is brought to a functional level by oestrogen, treatment with progesterone has a characteristic effect on the action potential, (Marshall, 1964; Kuriyama and Csapo, 1961). The amplitude of the action potentials becomes irregular with a poor correlation between action potentials and mechanical activity. These findings have been supported by in-vitro work (Csapo, 1961). This author interprets this lack of synchronisation to mean that only small groups of cells are activated resulting in poor conduction between cell groups. Hence the synchronised contraction necessary for efficient uterine activity does not occur.

EFFECT OF PROGESTERONE ON MECHANICAL ACTIVITY OF THE MYOMETRIUM

Detailed studies of the hormonal regulation of myometrial activity were begun by Csapo (1956). He introduced an isometric technique to demonstrate both quantitative and qualitative differences in the behaviour of isolated uterine muscle strips in different hormonal environments. He concluded (Csapo, 1969) that there were four regulators of uterine activity:

- Uterine volume (Hippocrates, 46BC)
- Oxytocin (Dale, 1906)
- Oestrogen (Allen and Doisy, 1923)
- Progesterone (Corner and Allen, 1930)

Csapo obtained evidence that progesterone was the most important of these and proposed the progesterone block theory (Csapo, 1956). This theory states:

1. Isolated smooth muscle possesses inherent rhythmical contractile activity in the absence of stimulating or inhibiting agents.
2 Labour begins with the evolution of spontaneous contractility consequent upon the removal of inhibition i.e. 'progesterone withdrawal'.

This theory has been extensively investigated.
The contractile activity of the human uterus at term has been studied by intra-amniotic pressure recorders and external monitoring by external displacement transducers. These have become routine in combination with cardiography in assessing the fetal state during labour.

For ethical reasons, altering the hormonal environment is not possible in a term human pregnancy. However, the non-pregnant uterus readily lends itself to the study of the effects of progesterone. Progesterone levels are low in the follicular phase and are elevated in the luteal phase. Knaus (1926) demonstrated that the myometrial response to oxytocin which is present in the follicular phase of the menstrual cycle is absent in the luteal phase. He attributed this refractoriness to the action of progesterone on the

myometrium. Csapo and Sauvage (1968) have demonstrated a quantitative variation in uterine activity during the menstrual cycle which correlates with the progesterone concentration.

Further support for the 'progesterone block' theory has come from the work of Kerenyi et al, (1969). These authors measured the spontaneous uterine activity and the response to oxytocin (0.5 I.U) in both the follicular phase and luteal phase of the human menstrual cycle and in early pregnancy. There is less spontaneous uterine activity and less oxytocin-induced uterine activity in the luteal phase. Further the uterine activity present in the follicular phase can be suppressed by administration of progesterone (50 mg). In the early human pregnant uterus, there is minimal spontaneous uterine activity and the response to oxytocin is low, comparable to the luteal phase of the menstrual cycle. These authors concluded that progesterone suppresses uterine activity during the normal menstrual cycle and in early pregnancy.

It should be appreciated that the foregoing work was interpreted before the importance of prostaglandins was recognised; the extent to which apparent effects of progesterone directly on the myometrium were attributable, in fact, to inhibition of prostaglandin synthesis cannot be assessed.

EVIDENCE FOR A ROLE OF PROGESTERONE IN THE INITIATION OF PARTURITION

A. Animal Evidence

The rapid advances in our knowledge, over the last 25 years, of the control of uterine activity has resulted largely from studies in experimental animals, particularly sheep. The latter provides a useful preparation for studying pregnancy by techniques that are not possible in the human for ethical reasons nor in small animals for technical reasons. In the ewe, progesterone is considered to have an important role in the complex chain of hormonal events which precede parturition. Large portions of this chain have been explored and clarified but there remain unexplained facets. There is substantial evidence that maturation of the fetal hypothalmic-pituitary-adrenal axis during late pregnancy is responsible for the initiation of parturition in this species (Liggins et al, 1973; Thorburn and Challis, 1979). A prepartum surge of fetal cortisol modifies the maternal steroid environment and leads to the onset of labour. Fetal cortisol activates the placental enzymes 17α -hydroxylase, C17-20 lyase and aromatase (Anderson, Flint and Turnbull, 1975; Steele, Flint and Turnbull, 1976) (Fig 1.2). These enzymes are the rate-limiting steps for oestradiol-17β synthesis during late pregnancy. Their activation by fetal cortisol leads to increased conversion of progesterone to oestrogen which explains at least in part the prepartum decline in progesterone levels (Bassett et al, 1969) and the increase in oestradiol-17β levels (Challis, 1971). Hence the fetus via its pituitary-adrenal axis controls the level of

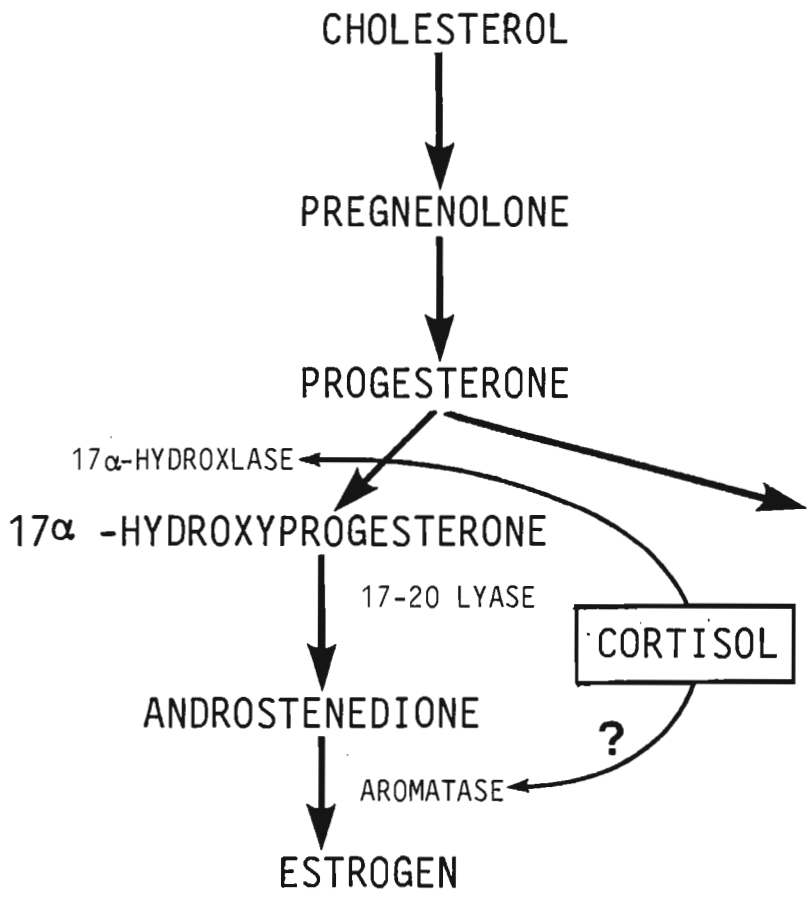

FIG 1.2 Pathway of biosynthesis of progesterone and oestrogen in ovine placenta, indicating the possible sites of action of fetal cortisol on 17α-hydroxylase, C17-20 lyase and aromatase. The Delta 5 pathway which may contribute to oestrogen biosynthesis is omitted (Liggins et al, 1977).

the maternal steroid hormones.

These steroid changes lead to increased prostaglandin production, progressive uterine activity, labour and delivery. The precise stimulus to increased prostaglandin production is less clear; it may be the raised concentration of oestrogen, the decline in the concentration of progesterone or their combination.

There is evidence that oestrogen is a major stimulus to the rise in $PGF_{2\alpha}$ levels at parturition.

1. There is a close temporal relationship between the rising concentrations of oestradiol-17β and $PGF_{2\alpha}$ (Currie, Wong, Cox and Thorburn, 1973).
2. Administration of oestrogen induces a rise in $PGF_{2\alpha}$ levels (Liggins et al, 1973).

There is also evidence that progesterone influences the concentration of $PGF_{2\alpha}$ although this is less certain.

1. Progesterone levels fall prior to parturition and precede the rise in concentration of $PGF_{2\alpha}$.
   Peripheral progesterone levels have been shown to fall prior to the onset of parturition in many species (the rabbit, Allen and Corner, 1929; the rat, Csapo and Wiest, 1969; the sheep, Bassett et al, 1969; the goat, Thorburn and Schneider, 1972).
2. Large doses of progesterone inhibit labour at term in the pregnant ewe.
   Administration of large doses of progesterone (100 mg/24hr) but not 80 mg/24hr prolongs pregnancy in some

cases (Bengtsson and Schofield, 1963). (This dose is far in excess of the physiological production rate).

3. The administration of progesterone prevents the dexamethasone-induced premature labour when administered in pharmacological but not physiological amounts.

   Liggins et al (1973), demonstrated that doses of progesterone below 100 mg/24hr did not delay the onset of dexamethasone-induced labour in the ewe nor influence the course of labour, although this dose was sufficient to maintain the peripheral concentration of progesterone within the normal prelabour range. A dose of 200 mg/24hr completely inhibited uterine activity and delivery did not occur.

4. Progesterone depresses the myometrial response to oxytocin.

   Liggins, Fairclough, Grieves, Forster and Knox (1977) found that after administration of a progestin (150 mg medroxyprogesterone acetate) a four fold increase in the rate of infusion of oxytocin was required to elevate the concentration of $PGF_{2\alpha}$ to the same degree as before treatment.

5. Large doses of progesterone will inhibit oestrogen-induced $PGF_{2\alpha}$ release.

   Oestrogen (stilboestrol 20 mg) induced a rise in the $PGF_{2\alpha}$ concentration in the utero-ovarian venous blood (Liggins et al, 1973); this change was completely inhibited by treatment with progesterone (200 mg/24hr).

6. Progesterone inhibits myometrial activity by a direct action independent of prostaglandins.

Lye and Porter (1978) monitored intrauterine pressure in the non-pregnant ewe before and after treatment with progesterone (50 mg sc/day).
They found a reduction in the frequency and amplitude of myometrial activity but were unable to abolish the responses to oxytocin (500 mIU) and prostaglandin $F_{2\alpha}$ (10 ug/min).

In some animal species (e.g. sheep) the fetus can control placental progesterone metabolism by activating placental 17α -hydroxylase. In other species (e.g. Man), placental 17α -hydroxylase is absent and the opportunity for the fetus to influence placental steroidogenesis by means of cortisol is not present.

Mammalian species can be classified according either to the source of progesterone in late pregnancy (placental or corpus luteum)or to the presence or absence of a cortisol-inducible placental 17α-hydroxylase (Liggins, 1981) (Table 1.2). The latter classification enables a number of predictions to be made concerning progesterone's role in the initiation of parturition for each species. It can be predicted that the presence of 17α -hydroxylase in placental-dependent species will be associated with marked hormonal changes at term (progesterone fall and oestrogen rise), with inducibility of preterm parturition by treatment with corticosteroids and oestrogens and with prolongation of pregnancy after progesterone treatment.

On the other hand, the absence of 17α -hydroxylase in the placenta, as in man, will be associated with a lack of marked

steroid hormone changes at term, non-inducibility of preterm parturition by treatment with corticosteroids and with a failure of progesterone treatment to prolong pregnancy.

TABLE 1.2 Classification of mammals according to the source of progesterone in late pregnancy and to the presence or absence of placental 17α -hydroxylase.

Species	17α -Hydroxylase in Placenta	Source of Progesterone
Sheep	Yes	Placenta
Cow	Yes	Placenta
Goat	Yes	Corpus luteum
Pig	Yes (?)	Corpus luteum (?)
Primate	No	Placenta
Guinea Pig	No	Placenta
Rat	No	Corpus luteum
Rabbit	No	Corpus luteum

Liggins, G.C. (1981)

HUMAN EVIDENCE

The endocrinology of parturition in man differs from other animals particularly in the part played by progesterone. The dramatic changes in circulating progesterone levels in many animals do not occur in women and the precise role of progesterone remains uncertain. Study of the physiology and endocrinology of parturition in women is difficult. Ethical

considerations prevent many experimental procedures which have clarified our knowledge of the initiation of parturition in animals; for this reason much of the evidence concerning the role of progesterone in the onset of human parturition remains circumstantial. This evidence has been gathered by:

1. Measuring serial progesterone levels in relation to uterine activity in;
   - threatened abortion,
   - premature labour,
   - and term delivery.
2. Analysing the effect of removal of progesterone by;
   - luteectomy,
   - and drug- induced inhibition of progesterone synthesis.
3. Studying the effect of progesterone administration in certain situations;
   - threatened abortion,
   - premature labour,
   - and in order to prolong pregnancy.

SERIAL PROGESTERONE LEVELS

Threatened Abortion

In the first trimester of human pregnancy there is a wide range of plasma progesterone levels (10-50 ng/ml; mean 25.5 ng/ml). Measurements of progesterone concentrations have a prognostic value in assessing patients with threatened abortion. Patients with threatened abortion in the first trimester whose pregnancies continue have significantly higher plasma levels of progesterone than those who

subsequently abort (Maraghy, Lamki, Pinkerton and Sheridan, 1978).

Premature Labour

A correlation between low plasma progesterone concentrations and premature labour has been reported (Csapo, Pohanka and Kaihola, 1973). In 11 patients in preterm labour (34-35 weeks gestation) the mean plasma progesterone concentration on admission was 100 ng/ml and fell to 71 ng/ml before delivery. (The mean progesterone concentration in a control group was 150 ng/ml). These authors concluded that progesterone has a role in maintaining uterine quiescence and that a fall in progesterone concentration led to premature labour. These findings have not been supported by other studies. There is substantial variation in plasma progesterone levels both between and within subjects (Challis, Workcwych and Patrick, 1981). These authors measured the plasma progesterone concentrations at 60 minute intervals throughout a 24 hour day in ten patients between 34 and 36 weeks gestation with a 'normal' pregnancy. The range was 327.9 to 507.5 nmol/l (103.1 to 159.4 ng/ml) - a variation of up to 49% from the daily mean. The author suggest that care should be exercised in the interpretation of progesterone values even if performed serially on one patient.

Prospective studies have failed to show a correlation between antenatal plasma progesterone levels and the incidence of preterm labour (Bell, 1983; Block et al, 1984). In the study by Bell, 29 patients comprising

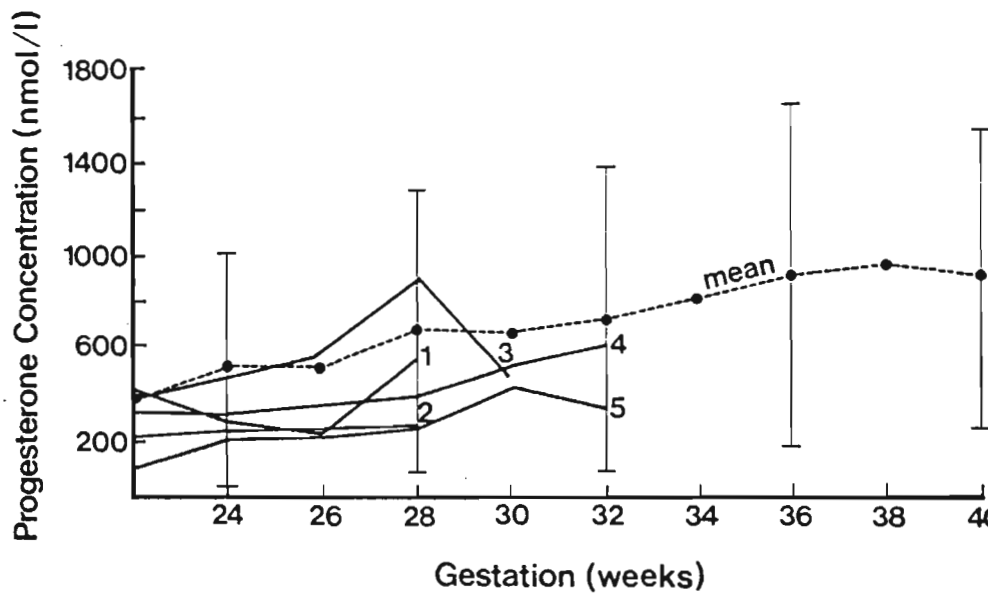

FIG 1.3 The concentration of progesterone in five high risk patients in preterm labour. The mean ± 2 S.D. in "normal" pregnancies is shown for comparison. (Bell, 1983).

a high risk group of 15 who had a history of preterm labour (five of this group delivered before 32 weeks gestation) and a control group of 14 who delivered at term, were studied. Venous blood was collected fortnightly from 20 weeks gestation and assayed for oestradiol-17β and progesterone. The progesterone levels in the high risk group were within two standard deviations of the control group and there was no significant difference between the trends in those of the high risk group who delivered prematurely and who delivered at term. Bell concluded that the steroid hormones do not play a role in the onset of human labour before term (Fig 1.3). Unfortunately, as in the study by Csapo and co-workers (1973), the subject numbers were small.

Term Labour

In human pregnancy, the synthesis of progesterone and oestrogen is controlled in an entirely different way from most other species studied. The fetal zone of the fetal adrenal is deficient in 3β -hydroxysteroid dehydrogenase (3β -HSD) and therefore secretes dehydroepiandrosterone sulphate (DHEAS) predominantly. The human placenta contains sulphatases and aromatase but no 17α -hydroxylase and 17,20 lyase and uses fetal DHEAS as a substrate for oestrogen synthesis rather than progesterone. Thus, in man there is no known mechanism for fetal cortisol to control progesterone metabolism. One would therefore, not expect serum progesterone levels to fall prior to parturition in the human.

There are two reports in the literature detailing a

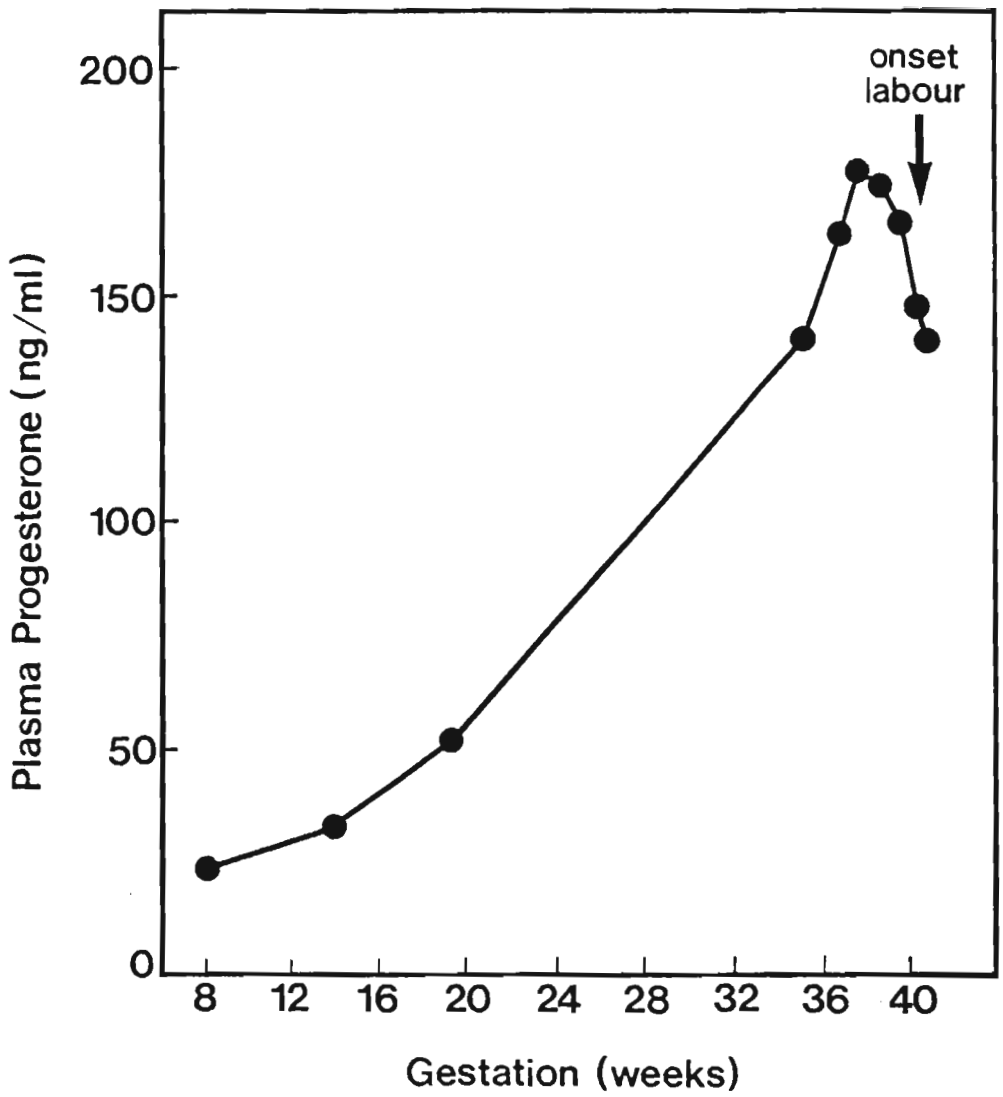

FIG 1.4 The concentration of progesterone during human pregnancy and spontaneous labour (Csapo A.I., Knobil E., Vandermolen H.J., Wiest H.J., 1971).

significant decline in progesterone levels prior to human parturition at term. The first, Csapo, Knobil, Vandermolen & Wiest (1971) studied 12 'normal' primigravidae. Venous samples were collected from approximately 33 weeks gestation; initially weekly, and latterly more frequently. These authors stated that near term pregnancy was characterised by a plateau in progesterone levels and clinical labour followed a slight decline in the serum progesterone concentrations (Fig 1.4).

The second study, Turnbull, Patten, Flint, Keirse, Anderson (1974) measured the concentration of progesterone serially in 33 'normal' primigravidae from the 20th week of pregnancy until spontaneous labour. They found a fall in peripheral plasma progesterone from a maximum at 36 weeks of 157 ± 7 ng/ml (499.3 ± 22 nmol/l) to 105 ± 11 ng/ml (332 ± 34.9 nmol/l) at term but there was no change in the last week of pregnancy. These authors plotted the progesterone and oestradiol-17β concentrations against time of spontaneous onset of labour rather than the gestation in weeks and showed a fall in progesterone and rise in oestradiol-17β levels as term approached (Fig 1.5). A fall in progesterone concentration occurred in only two thirds of the subjects examined. The remaining 11 patients had no significant change in plasma progesterone concentration prior to the initiation of labour.

The majority of similar studies have failed to demonstrate any significant fall in peripheral plasma progesterone concentrations before the onset of labour in the human

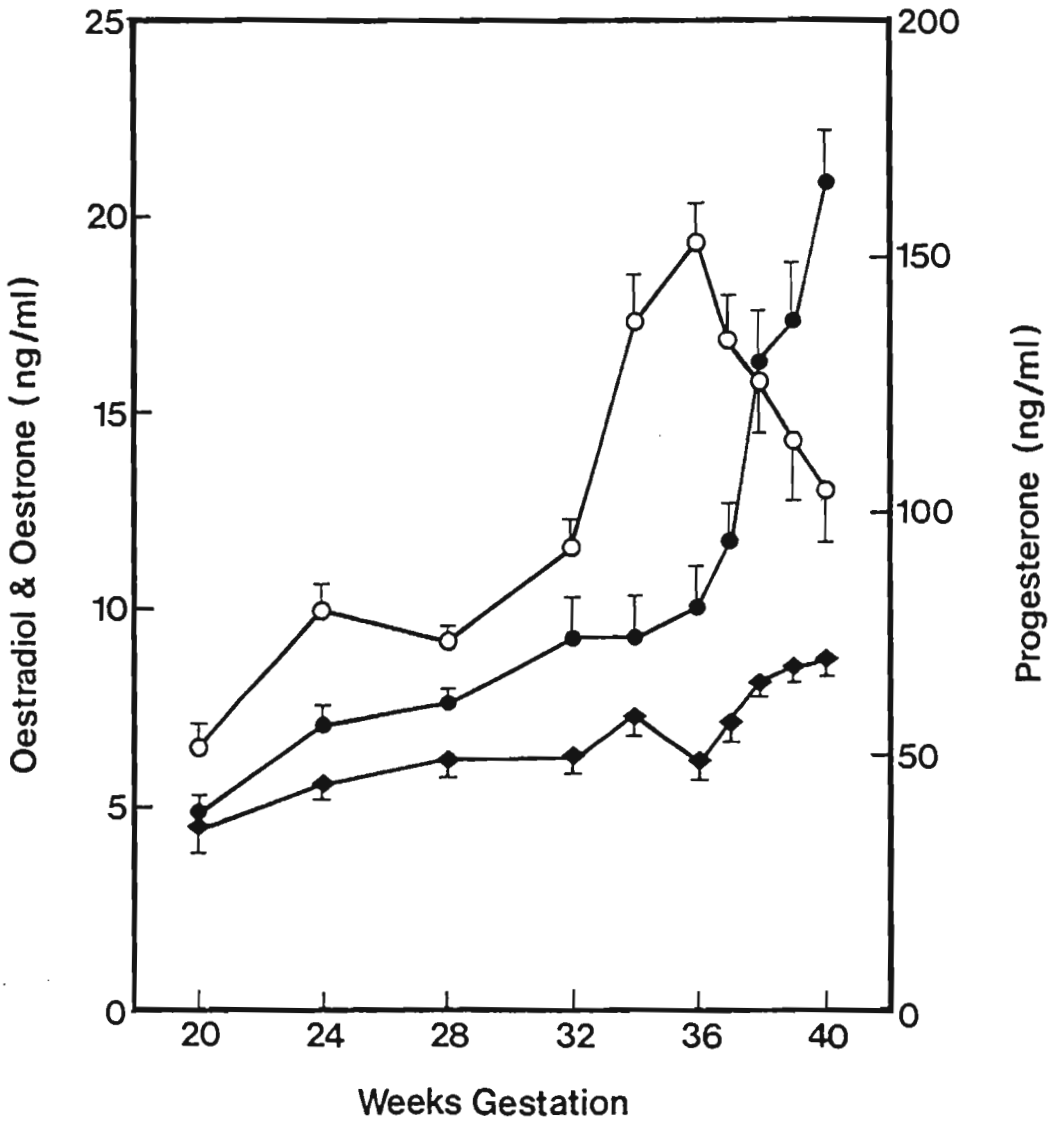

FIG 1.5 The concentration of progesterone (○——○), oestradiol-17β (●——●) and oestrone (◆——◆) (mean ± S.E.M.) measured serially in 33 primigravida women between the 20th week of pregnancy and the spontaneous onset of labour at term (Turnbull, A.C. Patten, P.T. Flint, A.P.K. Keirse, M.J.M.C. Jeremy, J.Y. Anderson, A.B.M, (1974).

(Yannone, McCurdy and Goldfein, 1968; Short and Eton, 1959; Llauro, Runnebaum and Zander, 1968; Block et al, 1954).

THE EFFECT OF LUTEECTOMY ON PROGESTERONE LEVELS

Animal Evidence

The importance of progesterone in reproduction was established by Corner in the 1920's. In animal studies he described the indispensibility of the corpus luteum and its hormone, progesterone, in the maintenance of pregnancy - "A hormone for gestation". The classical experimental design used in his study and in many to follow was a modification of that described to the German Gynaecological society in 1920 by Ludwig Fraenkel (Corner and Allen, 1930). Corner's experimental model was the rabbit. He surgically removed the corpus luteum prior to implantation (on the eighth day after mating). Removal of all or part of one ovary without disturbing the corpus luteum did not interfere with the pregnancy. However, if the corpus luteum was removed, pregnancy failed. Corner, in a second experiment, removed the corpus luteum then substituted for its function by administration of a crude extract from the corpus luteum - implantation was now successful and pregnancy resulted. This group of experiments demonstrated in this species the indispensibility of the corpus luteum and its secretory product, progesterone, for the establishment of pregnancy. Corner and Allen (1930) continued this work and the ultimate purification of the hormone and correct formula were announced a few years later in 1934.

Similar studies were performed in other species in which the ovary is the source of progesterone. Luteectomy leads to abortion and can be prevented by substitution therapy with progesterone in the rat and rabbit (Csapo, 1969). These observations led to a hypothesis postulating an all embracing role for progesterone in reproduction where not only was progesterone essential for the maintenance of pregnancy but also progesterone 'withdrawal' was considered an essential prerequisite to pregnancy termination. In all species progesterone was said to be the 'defence mechanism' of pregnancy.

Human Evidence

Although it was accepted that in various rodents progesterone has a role in pregnancy termination, it is not generally agreed that progesterone is important in the control of the initiation of parturition in women. There were three main disagreements with the theories of Csapo and Corner:

1. The development of progesterone assays failed to demonstrate a fall in peripheral plasma progesterone levels prior to labour in human pregnancy (Short and Eton, 1959).
2. Other authors, notably Caldeyro-Barcia (1961), considered that oxytocin was the hormone of importance in human pregnancy.
3. Oophorectomy in early human pregnancy was not invariably associate with abortion.

Csapo and co-workers (see Csapo and Pulkkinen, 1978 for review) performed a series of carefully controlled

experiments to clarify the role of progesterone in early human pregnancy. They studied 65 pregnant women who were being subjected to the combined surgical procedure of sterilisation and abortion. The trial consisting of laparotomy with tubal sterilisation and luteectomy on the first day; the patients were then observed in the ward for the following seven days when their pregnancies were terminated, or abortion completed, by dilatation and curettage. Blood samples were collected daily; plasma progesterone and oestradiol-17β were assayed. Uterine activity was assessed daily by two methods. A catheter was passed through the cervix each day into the extraamniotic 'space' to measure 'spontaneous' uterine activity. This was monitored for 30 minutes, then oxytocin (0.25 I.U.) was given intravenously and the intrauterine pressure monitoring continued for a further 30 minutes.

The subjects were divided into five groups based on the gestational age and treatment method (Table 1.3). This study showed a correlation between uterine activity and the degree of the progesterone decline. In group A (sterilisation without luteectomy) there was no change in the circulating serum progesterone concentration nor in the uterine pressure response to oxytocin. In group B (sterilisation and luteectomy) there was an immediate and progressive fall in serum progesterone concentration to levels below 5 ng/ml (15.9 nmol/l). This was accompanied by abortion on the third or fourth day at which time the serum progesterone level was less than 1 ng/ml (3.2 nmol/l). In group D (sterilisation and luteectomy at 60 days) there was a temporary decline in

serum progesterone levels but they did not fall below 10 ng/ml (31.8 nmol/l) and were returning towards pretreatment levels by the seventh day. This group showed no increase in uterine pressure response to oxytocin and the pregnancy continued. In group C (luteectomy and sterilisation at 50 days) the subjects showed a fall in the serum progesterone concentration but this did not fall below 5 ng/ml (15.9 nmol/l). There was some response to oxytocin as measured by pressure monitoring and this group was judged to have 'incipient' abortion but evacuation of products of conception was required on the seventh day (Fig 1.6).

TABLE 1.3 Design of trial to study the effects of luteectomy in early human pregnancy.

Group	Number of Subjects	Gestational age (days) *	Treatment
A	6	52	Sterilisation without luteectomy
B	33	47	Luteectomy and sterilisation
C	8	50	Luteectomy & sterilisation
D	10	60	Luteectomy & sterilisation
E	7	50	Luteectomy, sterilisation and progesterone replacement

Csapo & Pulkkinen, 1978

* Gestational age calculated as days after last menstrual period (cycle length 28 days).

In group E (sterilisation and luteectomy at 50 days plus progesterone replacement therapy) the serum progesterone

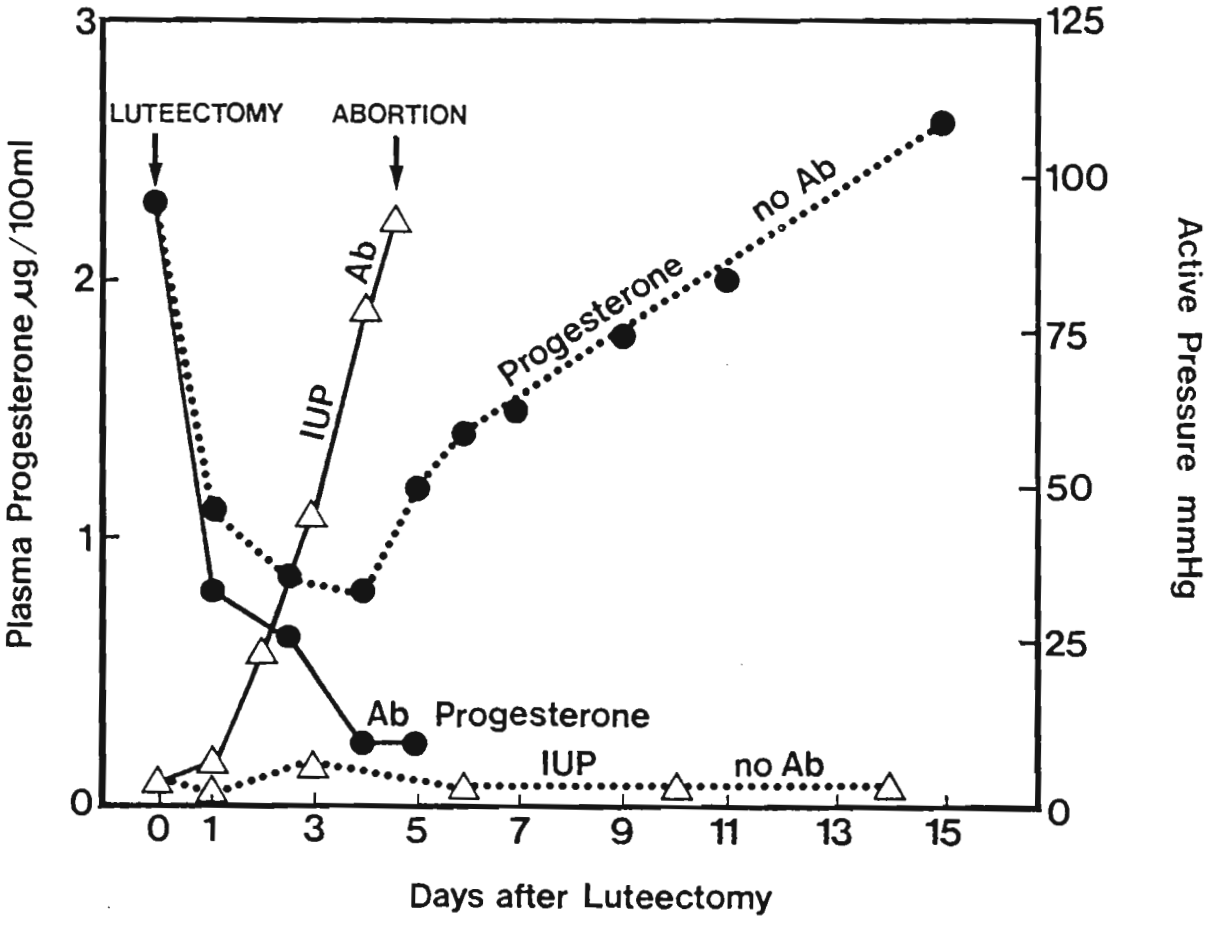

FIG 1.6 The effect of lutectomy on the concentration of progesterone and intrauterine pressure in women in early pregnancy. Subjects treated before 49 days of pregnancy, progesterone ( ) and intrauterine pressure ( ). Subjects treated after 49 days of pregnancy ,progesterone; ( ) and intrauterine pressure ( ) (Csapo and Pulkkinen, 1978).

level did not change from pre-luteectomy levels and there was no evidence of uterine activity nor response to oxytocin - the pregnancy continued.

These authors concluded:

1. There is a correlation between both spontaneous uterine activity, responsiveness to oxytocin and the serum progesterone concentration.
2. If the serum progesterone level falls below 5 ng/ml, abortion occurs in all cases.
3. Spontaneous uterine activity is measurably different from normal whenever the progesterone concentration falls below 10 ng/ml.
4. Luteectomy after 49± 2 days, although associated with a temporary decline in serum progesterone levels, does not significantly affect the serum progesterone concentrations nor lead to abortion.
5. The temporary decline in serum oestradiol levels which occurred in all groups was not related to outcome of pregnancy and therefore oestradiol is not essential in early pregnancy maintenance.
6. Progesterone is indispensible during early pregnancy and the corpus luteum is also indispensible as long as it is the major source of progesterone.

These experiments demonstrate that maintenance of pregnancy is dependent on the presence of a corpus luteum until 49 days when the placenta becomes capable of maintaining pregnancy in the absence of the corpus luteum.

This study of the effect of luteectomy can be criticised

because of the method used to assess uterine activity following luteectomy. Intrauterine pressure was measured by means of a microballoon inserted through the cervix into the uterus. Both the insertion of a catheter and the administration of oxytocin are known to stimulate uterine activity which could contribute to the factors leading to abortion after luteectomy. One cannot conclude from these data that luteectomy alone will necessarily lead to abortion in the absence of intrauterine manipulations.

ADMINISTRATION OF PROGESTERONE

Threatened and Recurrent Abortion

In the 1950's it was considered that faulty implantation could be associated with abortion and that a deficiency of a placental hormone might be an important and treatable factor in spontaneous abortion. A vogue developed for treating patients with threatened abortion or recurrent abortion with progesterone. Subsequently, it was conclusively demonstrated that progesterone does not prevent the progression of threatened abortion (Klopper and McNaughton, 1965).

Premature Labour

Many attempts have been made to prevent or arrest premature labour by treatment with progesterone. Initial trials (progesterone 200 mg/day) were not successful (Fuchs and Stakemann, 1960; Klopper and McNaughton, 1965). Recently Erny (1985) in a controlled trial treated 57 pregnant women in preterm labour with progesterone (400 mg micronised progesterone) or placebo. Placebo and/or bed rest had no

effect on plasma progesterone levels but was associated with decreased uterine activity in 42%. Oral progesterone increased plasma levels of progesterone by 50% and was associated with decreased uterine activity in 75% of cases. However, as the two groups were at different hospitals a comparison between entry criteria is not possible.

Progesterone to Prolong Human Pregnancy

A number of studies have demonstrated that progesterone (up to 200 mg intramuscularly twice daily) is unable to prolong pregnancy (Csapo et al, 1966).

LOCAL PROGESTERONE CONCENTRATION AND ITS CONTROL

Local 'Progesterone Block' Theory

The lack of a consistent fall in peripheral progesterone concentration prior to the onset of labour in women and the inability to inhibit established labour by systemic administration of progesterone led to the idea that progesterone diffusing from the placenta acts locally i.e. on the myometrium immediately underlying the placenta (Csapo, 1969). Thus the area of myometrium immediately under the placenta will be inhibited by progesterone to a greater degree whereas areas of the myometrium more distant from the placenta will have a lower progesterone concentration and be less inhibited.

In an attempt to prove this theory, Runnebaum and Zander (1971) isolated myometrial samples from the placental site and non-placental site at two points in gestation; 14 weeks and 40 weeks. At 14 weeks gestation the juxtaplacental

myometrium had a significantly higher progesterone concentration than the more distant site; 0.478 and 0.159 ug/g of tissue respectively ($p < 0.01$). In the term uterus there was no significant difference between sites. Inadequate precautions were taken in these studies to exclude the possibility that trapped venous blood containing widely differing concentrations of progesterone contributed to the differences found at 14 weeks.

There have been a number of other human studies attempting to reproduce the local effects of progesterone by injecting the hormone directly into the myometrium. Progesterone (in doses up to 500 mg) did not significantly affect myometrial activity (Wood, 1963).

Local Progesterone concentrations

It has been suggested that a possible explanation for the lack of detectable decline in progesterone levels in the human is simply because of the site chosen for sampling.

In the ewe, the progesterone concentration in the utero-ovarian vein is six times greater than peripheral levels. Changes in progesterone levels in the utero-ovarian vein provide a more sensitive index of placental progesterone secretion (assuming constant flow) than peripheral levels because of the buffering effect of protein binding in the peripheral circulation. For the same reason, changes in the local concentration of progesterone may not be detectable in the peripheral circulation. In an attempt to gain further support for this idea progesterone as well as other steroid hormones have been measured in various local tissues and

fluids.

In the amniotic fluid the progesterone concentration varies widely between individuals. It is low in late pregnancy and tends to decrease towards term (Turnbull, Anderson, Flint, Jeremy, Keirse and Mitchell 1977; Johansson and Jonasson, 1971 (Fig 1.7).Johansson and Jonasson considered that in early pregnancy amniotic fluid progesterone reflects serum levels and in late pregnancy the fall is a dilutional effect.

Concentrations of progesterone in the umbilical cord plasma have been measured in term infants to determine if there is any difference between patients delivered without a labour and those delivered after spontaneous labour (Maynard, Stein and Symonds, 1980). There was a difference in the two groups. Umbilical venous progesterone concentrations in infants delivered by caesarean section were much lower. These authors concluded that the placental progesterone concentration was a factor in the duration of labour - umbilical cord venous plasma progesterone was highest in infants delivered after prolonged labour and lowest in those delivered by elective caesarean section. However, the range of concentration in the umbilical cord plasma was vast (225 to 2132 ng/ml). Further, Shaxted, Heyes, Walker and Maynand (1982) measured the umbilical cord venous progesterone levels after both elective caesarean section and emergency caesarean section for fetal distress. They found the umbilical cord progesterone concentration was highest in those fetuses considered to be in jeopardy. There was no difference

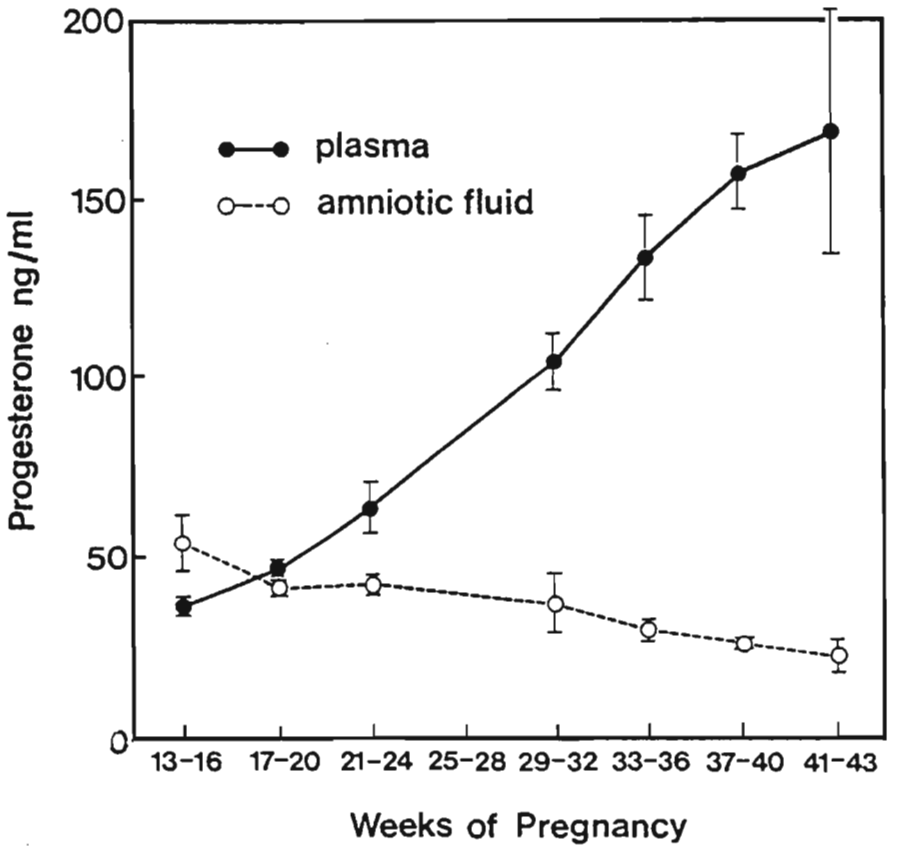

FIG 1.7 Levels of progesterone in plasma and amniotic fluid from the same patients. The value (mean ± S.E.M.) are grouped in four weeks periods (Johansson and Jonasson, 1971).

between cord progesterone levels obtained after elective caesarean section and after caesarean section in labour where the fetus was not considered to be in jeopardy. These authors concluded that progesterone is not involved in the onset of labour and that the fetus influenced progesterone production during parturition although they could offer no mechanism.

Other sites for serial sampling of placental venous blood are not possible for ethical reasons in the human but there have been studies in the Rhesus monkey (Thau, Lanmon and Brunson, 1976). In this species there is a decline in the progesterone concentration in uterine vein plasma with advancing gestation, while at the same time there is no fall in peripheral venous concentration. The fall in concentration of progesterone in the uterine vein probably represents increasing flow rates rather than falling production rates.

Control of Local Progesterone Concentrations

A decrease in the local progesterone concentration could be achieved either by altering local progesterone synthesis and metabolism or by inactivating progesterone by binding to local tissues. Neither would necessarily alter peripheral progesterone levels and could result in an increase in lysosomal phospholipase A_2 activity as detailed above.

Human placentae at term possesses 3 β-HSD activity and can use either DHEA or pregnenolone as a substrate (Koide and Torres, 1965). This provides a system with the potential for local

regulation of progesterone concentrations. Placental 3β -HSD activity was investigated by Torre, Breville, Tanguy et al, (1980). These authors obtained placentae after spontaneous vaginal delivery and elective caesarean section at term and determined the conversion rate of radio-labelled substrates. The ability of the placenta to transform pregnenolone to progesterone was found to be significantly higher after spontaneous vaginal delivery than after elective caesarean section ($p < 0.001$).

Conversion of progesterone to 20α -dihydroprogesterone (20α -DHP) by increased activity of 20α -hydroxysteroid dehydrogenase (20α -HSD) would also result in a fall in progesterone concentrations. 20α -HSD is active in human placentae (Diaz-Zagogo, Wiest and Ariasl, 1979). These authors in a study designed similarly to that described above demonstrated an increased conversion of radio-labelled progesterone to 20 α -DHP in placentae obtained after spontaneous vaginal delivery compared to those obtained after elective caesarean section ($P < 0.05$).

A binding substance which could act as an inhibitor of progesterone action has been found in human chorion and amnion (Schwarz, Milewich, Johnston, Porter and MacDonald, 1976). This substance was claimed to be different from cortisol binding globulin or cytosolic receptor (MacDonald et al 1978). Progesterone binding as measured by Schwarz et al, (1976) increased significantly after the 37th week of pregnancy and these authors attributed this change to increasing levels of this specific binding substance (Fig

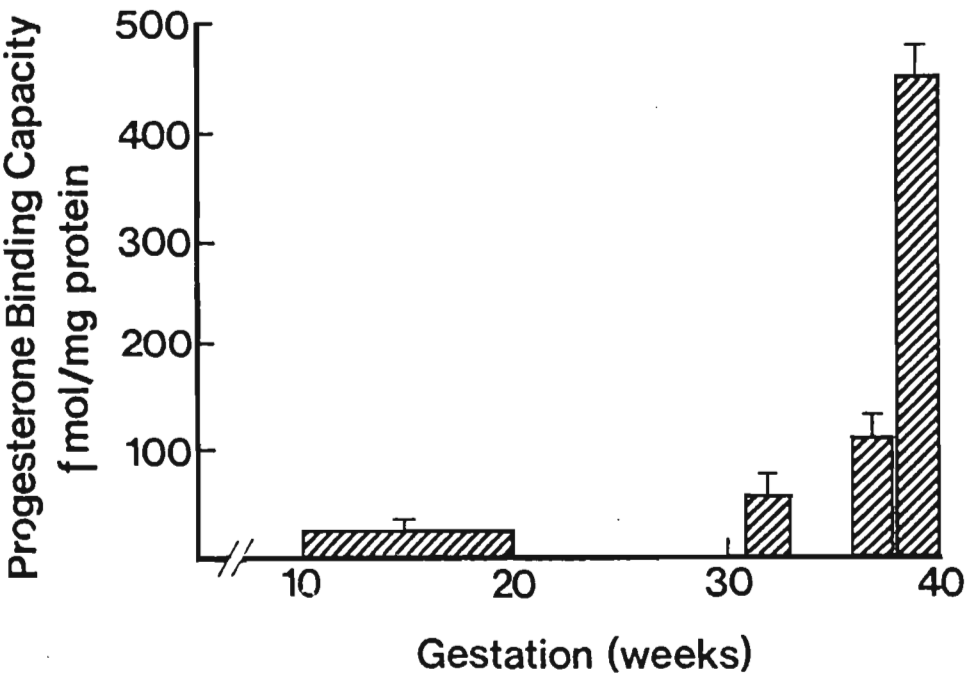

FIG 1.8 Progesterone-binding capacity of human fetal membranes throughout gestation. Height of each bar indicates mean value, line above each bar denotes S.E.M. (Schwarz, Milewich, Johnston, Porter and MacDonald, 1976).

1.8). The effect of increased binding capacity of progesterone might be to effectively 'withdraw' progesterone from the local tissues - without a change in peripheral concentrations.

PROGESTERONE SYNTHESIS AND MECHANISM OF ACTION

Cellular Effects

Regulation of protein synthesis in the target tissue is the principle action of any steroid hormone; the progesterone molecule must be transported to the target tissue via the blood stream and tissue fluids. It then penetrates the cell either by simple diffusion or facilitated diffusion and on entry is bound to a specific hormone receptor. This hormone-receptor complex is then transferred to the nucleus in an 'activated' form where it is bound to the cell genome. This results in increased RNA synthesis followed by increased protein synthesis. Regulation of protein synthesis is probably the primary mechanism by which progesterone regulates target tissue metabolism. However, there are undoubtedly other actions, for example by increasing lysosomal stability (see above), by increasing energy production and by modifying adenylcyclase activity and cyclic adenine monophosphate levels.

Progesterone Receptors

The effects of progesterone are modulated by binding either to plasma proteins or to cytosolic receptors. The plasma steroid binding proteins, corticosteroid binding globulin (CBG) and sex hormone binding globulin (SHBG), together with

other plasma proteins bind up to 90% of circulating progesterone in late pregnancy (Rosenthal et al, 1969). Only free (unbound) steroid is active. Any change in the plasma protein binding will alter the steroid available to the cell without effecting the total steroid concentration as measured by radioimmunoassay. SHBG and CBG rise during pregnancy (Tulchinsky & Chopra 1973; Towler et al 1976), as does the total progesterone concentration.

The early studies on progesterone receptors were performed in the chicken in which progesterone induces synthesis of a specific protein, avidin (O'Malley, 1967). The three characteristics of progesterone action are:

1. Large amounts are required to elicit a response.
2. Specific progesterone receptors are most abundant in tissues previously primed by oestrogen.
3. Oestrogen is necessary for a synergistic effect.

O'Malley (1967) injected radioactive progesterone into chickens and found accumulation in both the cytoplasm and in the nucleus of the oviduct indicating the presence of receptors at these sites. The cytoplasmic receptor was found to have a sedimentation co-efficient of 4.0 S with a striking affinity for progesterone alone. Oestrogen administration increases the concentration of progesterone receptors approximately 10-fold (Toft & O'Malley 1972).

In women, there is a physiological variation in the number of progesterone receptor sites in both the nucleus and cytosol during the menstrual cycle (Bayard, Kreitmann & Derache, 1978). The number of receptors is oestrogen

dependent (Horwitz and McGuire, 1978). As pregnancy advances the concentration of endometrial nuclear receptor sites increases significantly but the number in specimens obtained after spontaneous vaginal delivery was not significantly different from those obtained after elective caesarean section (Table 1.4).

Nuclear receptor sites are believed to be the obligatory intermediary of hormonal action. In the rat, where decreasing progesterone levels are involved in the induction of labour (Csapo, 1956) there is a decline in the number of nuclear receptors as term approaches (Vu Hai et al, 1978).

TABLE 1.4 Progesterone receptor sites per cell

Gestation	Total	Nuclear	Cytosolic
8 - 10 weeks	5247 ± 756	3455	1792
Elective LSCS at 38 weeks	11082 ± 8147*	10047	1035
Spontaneous term delivery	17348 ± 9832*	15932	1416

Kreitmann & Bayard, 1979.

* P = NS

As there is no decline in either progesterone levels or receptors numbers (in the myometrium) in women (Kreitmann and Bayard, 1979; Giannopoulos and Tulchinsky, 1979) it appears unlikely that the biological activity of progesterone in the human myometrium decreases prior to the onset of labour.

Progesterone Production

The human placenta at term produces 150 mg of progesterone per day and the maternal plasma levels reach 200 ng/ml. Throughout pregnancy the placental tissue content of progesterone appears to be constant, ranging from 2 to 5 ug/g of tissue (Runnebaum, Runnebaum, Stober, 1975). This large amount of progesterone in the human placenta is unusual, being approximately three times higher than in other species.

Steroidogenesis in women involves a complex interaction between the fetus and placenta (Diczfalusy, 1969). Cholesterol is the precursor for progesterone production, cholesterol side chain cleavage occurring in the mitochondria. The placenta does not possess the enzymes necessary to carry out the entire steps in the steroid production (Fig 1.2). Cholesterol derived from low density lipoproteins (LDL) is assimilated from the maternal circulation and is utilised for progesterone synthesis (Winkel et al, 1980). LDLs are taken from plasma, bound to specific high affinity plasma membrane receptors and lysosomal enzymes affect the hydrolysis of the protein compound giving free amnio acids. Hydrolysis of the cholesterol ester component of LDLs gives rise to free cholesterol and free fatty acids (Simpson, 1978). This is a rate limiting step in placental progesterone synthesis as it is dependent on the number of LDL receptors on the plasma membrane and also on the availability of cholesterol to the placenta i.e. the maternal-placental blood flow.

The next step is conversion of cholesterol to pregnenolone with the loss of the six carbon side chain. This mitochondrial reaction is catalysed by cholesterol side chain cleavage enzyme (CSCC). This enzyme is rate limiting in other steroid producing tissues controlled by trophic hormones e.g. ACTH and LH. However, no trophic stimulus has yet been identified in the placenta.

Pregnenolone is converted to progesterone by 3β -HSD and 4, 5-isomerase enzymes which are found in both the mitochondria and microsomes of human placenta and have an optimal pH of 7.2.

The idea that the fetus and the placenta are two components of an endocrine unit is based on the findings of a specific distribution of key steroid metabolising enzymes between the two (Diczfalusy, 1969). There is collaboration between the fetal adrenal, liver and placenta in oestrogen biosynthesis. The fetal adrenal, being deficient in 3β -HSD, produces large amounts of 5 steroids especially DHEA and DHEAS. The placenta possessing 3β-HSD, sulphatase and aromatase enzymes converts DHEA and DHEAS to oestrone and oestradiol-17β and 16(OH)-DHEAS to oestriol.

Control of Progesterone Production

Regulation of progesterone production by the placenta is thought to be autonomous. A number of regulatory mechanisms have been suggested:

1. Human chorionic gonadotrophin (hCG) may stimulate cholesterol metabolism. The addition of hCG to

trophoblastic cell cultures has been shown to have little effect on progesterone secretion and anti-hCG antibody does not inhibit progesterone production.

2. The availability of cholesterol to placental mitrochondria appears to be a rate limiting step for progesterone synthesis. Although the concentration of LDLS's in maternal plasma is high, both the concentration of LDL receptors on the trophoblast cell surface and placental blood flow determine the rate of progesterone production (Simpson, 1978).

3. The activity of cholesterol side chain cleavage enzyme in other steroid tissues is controlled by specific trophic hormones (ACTH or LH) and has a high activity in mitochondria from placental tissues.

4. A role for maternal endocrine glands has been investigated in animals. Removal of maternal pituitary, adrenals or ovaries (after 7 weeks) and the administration of ACTH, gonadotrophins or cortisol have no effect on progesterone production by the human placenta.

5. Since maternal serum progesterone levels are within normal limits in cases of fetal death or anencephaly, the fetus does not appear to play an important role in regulation of progesterone production by the placenta.

Evidence for a fetal contribution to placental progesterone synthesis has been provided by Challis et al, (1981). In a group of women treated with synthetic glucocorticoids they

found an 80% suppression of plasma cortisol, oestrone and oestradiol-17β; oestriol levels were reduced by 50% and progesterone levels were reduced by 20%. They concluded that the fall in progesterone levels was due to suppression of the fetal adrenal secretion of pregnenolone sulphate.

DHEA appears to be a potent competitive inhibitor of the conversion of pregnenolone to progesterone, suppressing 3β -HSD activity. Since DHEAS is dependent on fetal adrenal production (Siiteri, 1981) this inhibitory action offers the potential for fetal regulation of placental progesterone production.

The enzyme 3β-HSD is common to both the oestrogen and progesterone biosynthetic pathways. It is not known whether separate placental 3β -HSD's exist for C19 and C21 steroids. The evidence cited above suggests that a large increase in the production of cortisol and/or DHEAS by the fetal adrenal during the last few weeks of gestation could result in inhibition of placental 3β -HSD i.e. in human pregnancy, progesterone synthesis could be inhibited while oestrogen production continued (Fig 1.9).

INHIBITION OF PROGESTERONE SYNTHESIS

The development of synthetic steroids which inhibit 3β-HSD provides a new method of investigating the role of progesterone in parturition.

Epostane (2α,4α,5α,17β-4,5-epoxy-17-hydroxy-4, 17-dimethyl-3-oxoandrostane-2-carbonitrile) is a synthetic steroid which has been demonstrated in vitro to be a competitive

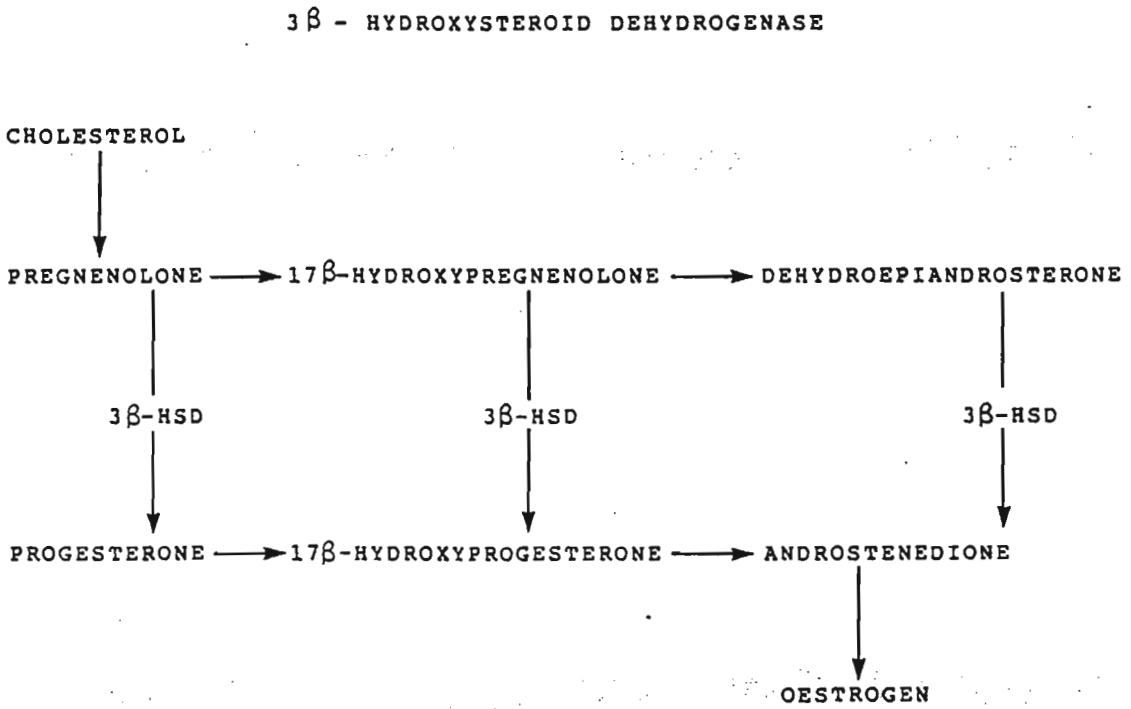

FIG 1.9 Involvement of 3β-hydroxysteroid dehydrogenase in the synthesis of progesterone and oestradiol in the ovary and placenta

inhibitor of 3β-HSD (Stirling Winthrop Research, Fig 1.10).

In the rat (Creange, Anzalone, Potts and Schane,1981) administration of Epostane (40 mg/kg) results in inhibition of 3 β-HSD and a fall in serum progesterone concentration to 20% of the pretreatment value.

A mitochondrial preparation from the human placenta has been used to demonstrate the effect of Epostane on progesterone synthesis from cholesterol. This preparation was used to study the effects of various concentrations of Epostane (0.1 to 100 mg) on 3 β-HSD activity. A significant dose-related inhibition of the conversion of pregnenolone to progesterone without any effect on the mitochondrial-cholesterol side chain cleavage enzyme system was found (Fig 1.11).

Tolerance studies in normal healthy male volunteers demonstrated no evidence of toxicity. An oral tolerance study of variable single oral doses up to 1600 mg excluded any effect on cortisol production, hepatic enzymes, bone marrow function or gastrointestinal tract (Sterling Winthrop Research, 1981).

The ability to inhibit placental and ovarian steroidogenesis during the menstrual cycle or in early pregnancy offers a new method of investigating the role of progesterone.

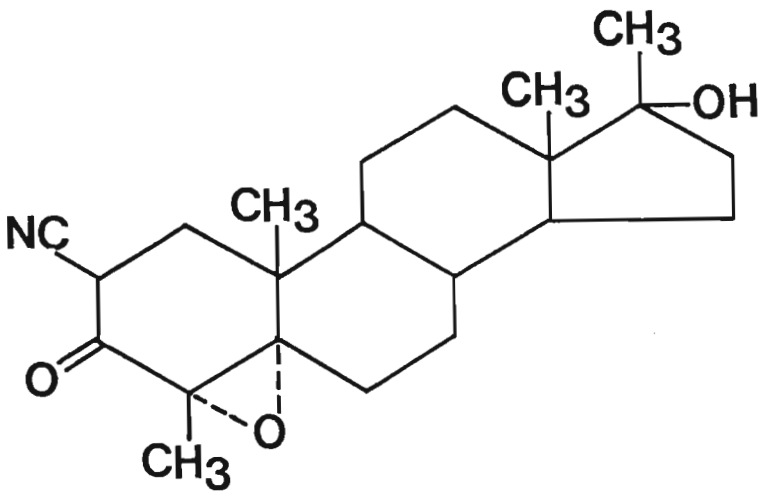

FIG 1.10 Epostane (WIN 32,729) a competitive inhibitor of 3β -hydroxysteroid dehydrogenase, as used in the studies described in this thesis.

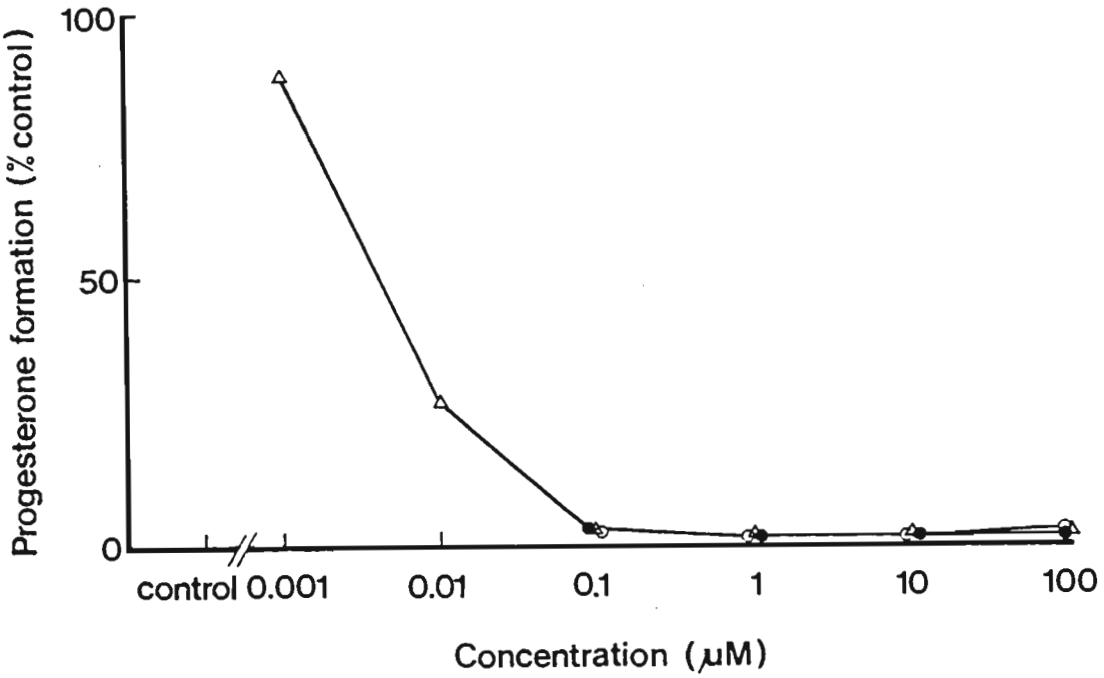

FIG 1.11 The effect of Epostane on 3 β-hydroxysteroid dehyrogenase activity in mitochondria from human placenta (Sterling Wintrop Research, 1982).

Epostane has been used in this thesis to investigate:

1 . The effect of inhibition of progesterone secretion on initiation of uterine activity in the ewe.

2. The effect of inhibition of progesterone secretion during the luteal phase in normally cyclic women.

3. The effect of inhibition of progesterone secretion on maintenance of early human pregnancy.

CHAPTER 2

LATE PREGNANT EWE

During the past three decades our understanding of the mechanisms controlling parturition and the involvement of the fetus in this process have advanced tremendously. This is largely due to chronic catheter studies in experimental animals. These studies have allowed the role of steroid hormones in the regulation of parturition in animals to be clarified. The experimental animal chosen for many of these studies was the domestic sheep. It was in this species that Liggins, Kennedy & Holm (1967) provided the first experimental evidence that the fetal lamb was involved in the initiation of parturition. It is now clear that maturation of the hypothalamic-pituitary-adrenal axis in the fetal lamb in late pregnancy links fetal maturation with parturition. The resulting fetal cortisol surge induces changes in the pattern of placental steroid metabolism in the ewe and results in labour and delivery. However, the expectation that this knowledge would lead to an understanding of the mechanisms of initiation of parturition in woman has not been fulfilled. There are distinct species differences, most importantly in the control of the metabolic pathways for progesterone. Despite this limitation the sheep provides a good model for testing hypotheses and mechanisms not possible in man.

Fetal Cortisol

The centrepiece of the mechanism initiating parturition in the ewe is a rapid increase in the secretion of cortisol by

the fetal adrenal gland. It is the activation of the fetal hypothalamic-pituitary-adrenal axis which initiates parturition.

During pregnancy basal levels of fetal cortisol are less than 15 nmol/l. Recent studies suggest that this cortisol is derived by placental transfer from the ewe (Hennessy, Coghlan, Hardy, Scoggens & Wintour, 1982). Two to three weeks before parturition the fetal adrenal begins to secrete cortisol and levels rise steadily until term (Magyar, Fridshal, Elsner et al., 1980) to reach approximately 300 nmol/l on the day of delivery (Bassett & Thorburn, 1969; Nathanielsz, Comline, Silver & Paisey, 1972) (Fig 2.1). Adrenalectomy or surgical removal or destruction of the pituitary prevents the cortisol surge and labour does not occur (Liggins, Fairclough, Greives, Kendall & Knox, 1973). Conversely, parturition can be induced by infusion of glucocorticoids into the fetus which results in premature delivery of viable lambs with precociously mature lungs (Liggins, 1968). Thus, cortisol secretion by the fetal adrenal is involved not only in parturition but also in maturational events within the fetus particularly maturation of the fetal lung. The sharp increase in the concentration of cortisol in fetal blood is due both to increased cortisol production (Bassett & Thorburn, 1969) and to an increase in the concentration of cortisol binding globulin (Fairclough & Liggins, 1975).

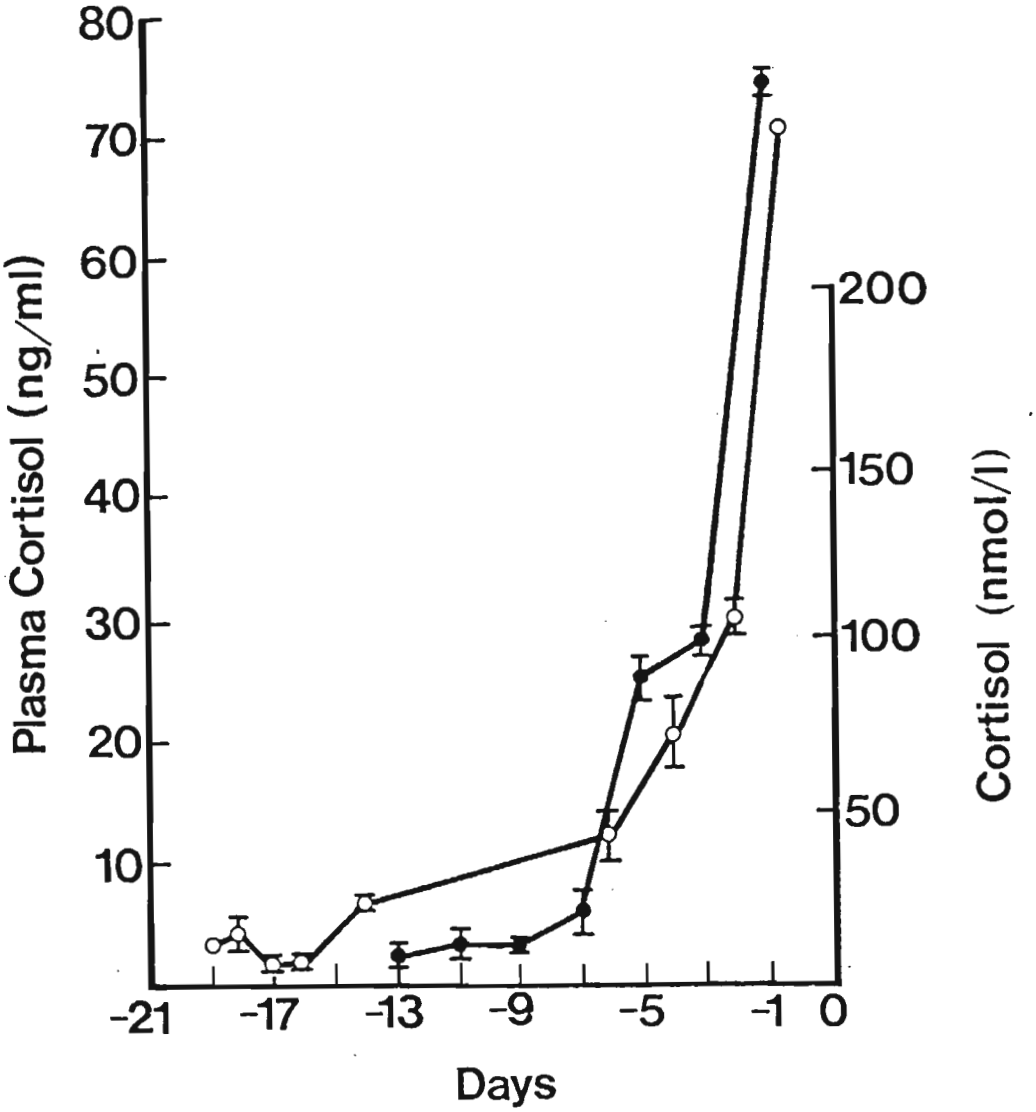

Fig 2.1 Plasma concentration of cortisol (ng/ml) in serial blood samples from chronically catheterised fetal lambs in the 21 days immediately prior to spontaneous parturition (Liggins, Fairclough, Grieves, Kendall, Knox 1973).

The mechanisms responsible for increased cortisol production by the fetal adrenal have yet to be identified. A rise in cortisol binding globulin can be stimulated by administration of ACTH to the fetus (Challis, Mitchell & Lye, 1985). ACTH infusion will initiate labour (Liggins, 1968) and pituitary ablation will prevent parturition; however an ACTH surge prior to spontaneous labour has not been demonstrated (Jones, Boddy, Robinson & Ratcliffe, 1977).

It is possible that an intra-adrenal mechanism may play an important regulatory role. In vivo studies demonstrate an increased fetal adrenal sensitivity late in gestation (Rose, Neis, Urbgan & Greiss, 1982). These authors administered a constant dose of ACTH to the fetus and measured the fetal cortisol response. The magnitude of the fetal cortisol response increased with advancing gestational age. Durand (1979) suggested that the increased adrenal response could be achieved by an increase in receptor number.

Placental Steroids

The sharp increase in the concentration of fetal cortisol modifies steroidogenesis in the maternal placental and leads to a decline in progesterone levels (Bassett, Oxborrow, Smith & Thorburn, 1969) and an increase in synthesis of oestradiol-17β (Challis, 1971). These endocrine changes precede labour and are similar to those obtained by

intrafetal administration of ACTH or glucocorticoid (Liggins et al, 1973). The mechanism of action of fetal cortisol involves activation of the placental enzymes responsible for the synthesis of oestrogen from C21 steroids. The immature placenta contains aromatase activity and can convert androstenedione to oestrogens but is denied a placental source of C19 precursors by the relative inactivity of 17α-hydroxylase (Fig 1.2). Fetal cortisol or exogenous glucocorticoids induce the activity of both 17 α -hydroxylase and C17-20 lyase and thus stimulate oestrogen synthesis in the placenta from C21 steroids (Anderson, Flint & Turnbull, 1975; Steele, Flint & Turnball, 1976). This converts the placenta from being primarily a progesterone secreting gland into an oestrogen secreting gland.

1. Oestrogen

During pregnancy the concentration of total unconjugated oestrogen increases as parturition approaches (Challis, 1971). The major maternal oestrogens are oestrone, oestradiol-17α and oestradiol-17β in a ratio of approximately 2:1:1 and the ratio of sulpho-conjugated to unconjugated oestrogens is 2:1. From approximately the 120th day of pregnancy maternal conjugated oestrogens rise steadily with a rapid rise over the last 48 hours (Fig 2.2).

2. Progesterone

Fig 2.2 Utero-ovarian venous plasma concentrations of progesterone ( ), oestrone sulphate ( ) and total conjugated oestrogens ( ) during delivery of an intact lamb (Flint etal, 1976).

Progesterone is derived almost entirely from the placenta during late gestation and rises steadily throughout pregnancy in the maternal plasma to reach a peak approximately one week prior to delivery (Fig. 2.2). Peripheral concentrations are approximately one-fifth of utero-ovarian levels. In spontaneous parturition the concentration begins to fall five days prior to parturition (Bassett,Oxborrow, Smith & Thorburn, 1969) from values in the peripheral blood of 22-35 nmol/l to 3 nmol/l on the day of delivery (Fig 2.2). Infusions of both ACTH and dexamethasone result in a fall in the concentration of progesterone in the maternal plasma.

These placental steroid changes have five main effects:

1. They increase prostaglandin production from the various intrauterine tissues (Louis, Parry, Robinson, Thorburn & Challis, 1977).
2. They facilitate oxytocin release from the maternal pituitary.
3. They increase myometrial and possibly endometrial oxytocin receptors (Thorburn & Challis, 1979).
4. They aid formation of gap junctions and thus synchronise the myometrial response (Garfield, Kannan & Daniel, 1980).
5. They enhance the calcium ion influx and thus allow the myometrial cell to contract (Carsten, 1979).

Prostaglandins

Prostaglandin $F_{2\alpha}$ levels in plasma are raised during parturition and have a stimulatory effect on uterine musculature. The stimulus to the release of prostaglandin is the alteration in the placental steroid levels secondary to the fetal cortisol surge.

The experimental evidence implicating oestrogens as an important link to prostaglandin production are:

1. The concentration of $PGF_{2\alpha}$ in the utero-ovarian vein increases in parallel to the rising oestrogen output which occurs in spontaneous labour and in premature labour induced by ACTH or dexamethasone (Liggins et al., 1973).
2. The administration of stilboestrol to the ewe induces a rise in prostaglandin concentration in the utero-ovarian vein (Liggins et al., 1973).
3. The administration of stilboestrol in the above experiment resulted in a 90% reduction in the myometrial threshold to oxytocin although progesterone levels were unchanged.
4. The oestrogen-induced $PGF_{2\alpha}$ release as measured in the utero-ovarian vein can be blocked by pharmacological amounts of intramusuclar progesterone (200 mg/day), but not by physiological amounts.
5. Parturition can be induced by administration of oestrogen near term (Hindson, Schofield & Turner, 1967).

The place of progesterone in the control of prostaglandin synthesis in the sheep is less clear. It is generally agreed that progesterone is indispensible for the maintenance of pregnancy and is involved in prostaglandin synthesis but the relative importance of elevated oestrogen production and reduced progesterone levels are uncertain. In order to clarify the role of progesterone in parturition, the following experiments with an inhibitor of progesterone synthesis were performed.

MATERIALS AND METHODS

Animals

The experiments were performed on seven ewes at known times after fertile mating. The sheep were a variety of breeds, Swaledale x Blue-faced Leicester (Mules) or Suffolk x Mule. Gestational age of fetuses were determined from tupping date, taken as the date of marking by an intact Pole Dorset ram. The gestational age and the number of fetuses were confirmed by radiography at approximately 90 days of pregnancy, when fetal bone age and crown rump length were assessed. Both single and twin pregnancies were studied.

Catheters

Polyvinyl chloride catheters of internal diameter 2.0mm and

external diameter 4.0mm were obtained from Portex Ltd., Hythe, Kent, UK for cannulation of the maternal jugular vein, carotid artery and amniotic cavity. The amniotic cavity catheter was perforated several times along the terminal 5cm of its length. For cannulation of the utero-ovarian vein, fetal jugular vein and carotid artery, catheters of internal diameter 1.0mm and external diameter 2.0mm were used.

Surgical Procedures

Operations were performed at 115 $\pm$ 2 days of gestation. Ewes were fasted for at least 24 hours before operation. General anaesthesia was induced by intravenous injection of 1g thiopentone sodium (Pentothal, Abbott Laboratories Ltd., Kent, UK) and maintained, following tracheal intubation, with 2-4% halothane in oxygen, administered using a closed circuit system. Carbon dioxide was absorbed with soda lime. Spontaneous respiration was always present during operation. Subsequent surgical procedures were performed in conditions of strict asepsis, essentially as described by Flint, Anderson, Patten and Turnbull (1974).

Maternal jugular venous and carotid arterial catheters were introduced through an incision in the neck and sutured into place after vessel dissection and exposure. Other catheters were inserted following a para-midline incision running from the level of the umbilicus to the mammary gland. Two utero-ovarian venous catheters were inserted into branchs of

the main vessel in the broad ligament and 10cm of catheter advanced into the vessel, to ensure sampling from the main uterine vein. Fetal jugular venous and carotid arterial catheters were inserted after exteriorising the fetal head and neck through an incision through the allantois, amnion, chorion and uterus. The vessels were exposed by blunt dissection following incision of the skin of the neck, and the catheters inserted for a distance of 7cm. The incision was closed in a single layer. The amniotic catheter was then sutured to the skin of the fetal neck. The uterine incision was closed with linen sutures, the edges of the closed incision being inverted and closed again, to prevent leakage of amniotic fluid.

Intra-abdominal catheters were exteriorised through a stab incision in the flank of the ewe, which was subsequently closed with a purse string suture. The main abdominal incision was closed in two layers, musculo-peritoneal and skin. Both incisions were treated with a polymyxin, bacitracin and neomycin aerosol mixture (Stuart Pharmaceuticals Ltd., Cheadle, Cheshire, UK). In ewes carrying twins, both twins were catheterised.

Post-Operative Care

At the time of operation, all ewes received 4ml Streptapen (Glaxovet Ltd., Greenford, Middlesex, UK), containing 250mg procaine penicillin and 250mg dihydrostreptomycin sulphate

per ml, by intra-muscular injection. Post-operatively, ewes received the same antibiotic for three days. In addition, all ewes received 3ml Crystapen (Glaxovet Ltd) containing 600mg benzylpenicillin given via the amniotic catheter daily for three days, the fetus received 600mg benzylpenicillin daily for three days.

All catheters were flushed immediately post-operatively with sterile 0.9% saline containing 250 units heparin per ml (Burgess Ltd., Princes Risborough, Bucks, UK). Catheters were then flushed daily for three days and once every two days thereafter. Ewes were kept in metabolism cages and fed 1kg dried grass pellets and 1kg ewe and lamb pencils per day, with water ad libitum.

Experimental Procedure

Ewes were allowed to recover for at least ten days after surgery before the experimental procedures were begun. All blood samples were collected into chilled plastic tubes containing acetylsalicyclic acid (5mg/ml whole blood) and ethylenediaminetetraacetic acid (EDTA) (70mg/ml whole blood). Tubes were centrifuged within 15 minutes of sample collection at 4^{o}C and 2000 rpm for 15 minutes. The plasma was stored frozen at -15^{o}C until assayed. Two samples (-30 min and 0 min) were taken before treatment and then sampling was continued until delivery. The first post treatment sample was taken at 30 minutes and subsequently at 1, 2, 4, 8, 12,

18, 24, 30, 34 hours. Measurements of pH and blood gas tensions were made using a Corning pH/blood gas analyser, model 165 (Corning Ltd, Halstead, Essex, UK). Haematocrit was determined using a micro-haematocrit centrifuge (Hawksley Ltd., London, UK).

Epostane (2α, 4α, 5α, 17β -4, 5-epoxy-17-hydroxy-4, 17-dimethyl-3-oxoandrostane-2-carbonitrile) in DMSO (1 ml) (dimethyl sulphoxide) a synthetic steroid which in vitro and in vivo in rats and rhesus monkeys inhibits 3 β -hydroxysteroid dehydrogenase (3 β -HSD) results in a decline in progesterone production was given intravenously via the maternal jugular vein as a slow infusion. A pilot study had demonstrated that 100mg Epostane was effective in inducing labour.

During experiments, uterine activity was monitored by recording the intrauterine pressure changes detected by a transducer (Bell and Howell type 4-422-0001-1-B4MS) connected to the fluid filled amniotic catheter. Pressure changes were amplified and recorded using a six channel hot wire recorder (Devices Ltd, Kent, UK). The apparatus was calibrated to reach full scale deflection with a pressure change of 25mm Hg. In most cases, recordings were made continuously during the experiment.

A control study of three animals received the vehicle only

and were sampled in a similar manner.

Termination of Experiments

Ewes were killed either by exsanguination after being stunned by a captive bolt, or by lethal overdose of phenobarbitone sodium (Expiral, Abbot Laboratories Ltd, Queensborough, Kent, UK). The ewes were put down after delivery or when labour was well established in order to obtain tissue for histological examination. Immediately after death, midline laparotomy was performed and the fetus(es) removed. Viability of the lambs was assessed at this time.

Radioimmunoassay

Maternal levels of progesterone, oestrodiol-17β , 13, 14 dihydroprostaglandin $F_{2\alpha}$ (PGFM) and cortisol were measured in peripheral venous blood and in the utero-ovarian vein. Fetal levels of progesterone and cortisol were measured from either the fetal carotid artery or jugular vein. The specific radioimmunoassays are described in the appendix.

Statistical Analysis

The data was recorded and analysed using a CTL 8050 computer. The mean $\pm$ SEM was calculated at each time interval for each hormone. The first two pretreatment measurements, 30 minutes and immediately prior to treatment, were averaged to give a "pretreatment value". A paired Student's T test was performed to test for significant differences.

RESULTS

Controls

Injection of the vehicle (DMSO) alone had no effect on the plasma concentration of maternal or fetal PGFM, progesterone, oestrodial-17β or cortisol. Further, there was no evidence of uterine activity over the subsequent 24 hours.

Outcome

One of the seven ewes aborted on the third post-operative day (No. 372). The remaining six ewes were studied and received 100mg Epostane on day 131 ± 2. Uterine contractions were detectable within 4 to 6 hours after administration of Epostane. Three ewes delivered at 24.75, 33 and 33.5 hours after drug administration. The remaining three experiments were terminated when the animals were in established labour as determined by the intraamniotic pressure monitor trace and cervical assessment. One animal was in obstructed labour with a dead fetus at full dilatation at 33.75 hours; the other two were terminated at 36 hours and 39 hours at approximately 6cm dilatation (assessed at post mortem).

All fetuses maintained their arterial pO2 within the normal range during the first twelve hours of labour. The range was 13.6 - 27.6 psi. The pH range was from 7.24 to 7.39. One

fetus had a persistently low p02 but normal pH. (This fetus sucumbed with obstructed labour).

The three ewes which delivered vaginally delivered 4 live lambs (one set of twins).

The mean fetal weight was 3.97 ± 0.84kg and the mean weight of each adrenal was 0.28 ± 0.07gms.

Maternal Plasma Hormone Concentrations

Injection of vehicle alone (DMSO) had no effect on the levels of maternal hormones measured.

1. Progesterone:

The mean peripheral progesterone concentration prior to treatment was 73.36 ± 8.3 nmol/l in the uterine vein and 24.1± 4.42 nmol/l in the peripheral vein. These concentrations were similar to those previously described at this gestational age (130 days) (Bedford, Challis, Harrison & Heap, 1972).

In the uterine vein, the concentration of progesterone fell to 3.2 ± 1 nmol/l within 30 minutes of injection and remained below 3 nmol/l for eight hours (Fig. 2.3) ($p < 0.001$). The peripheral plasma concentration reflected this change (Fig 2.4). The rise in the concentration of progesterone in the

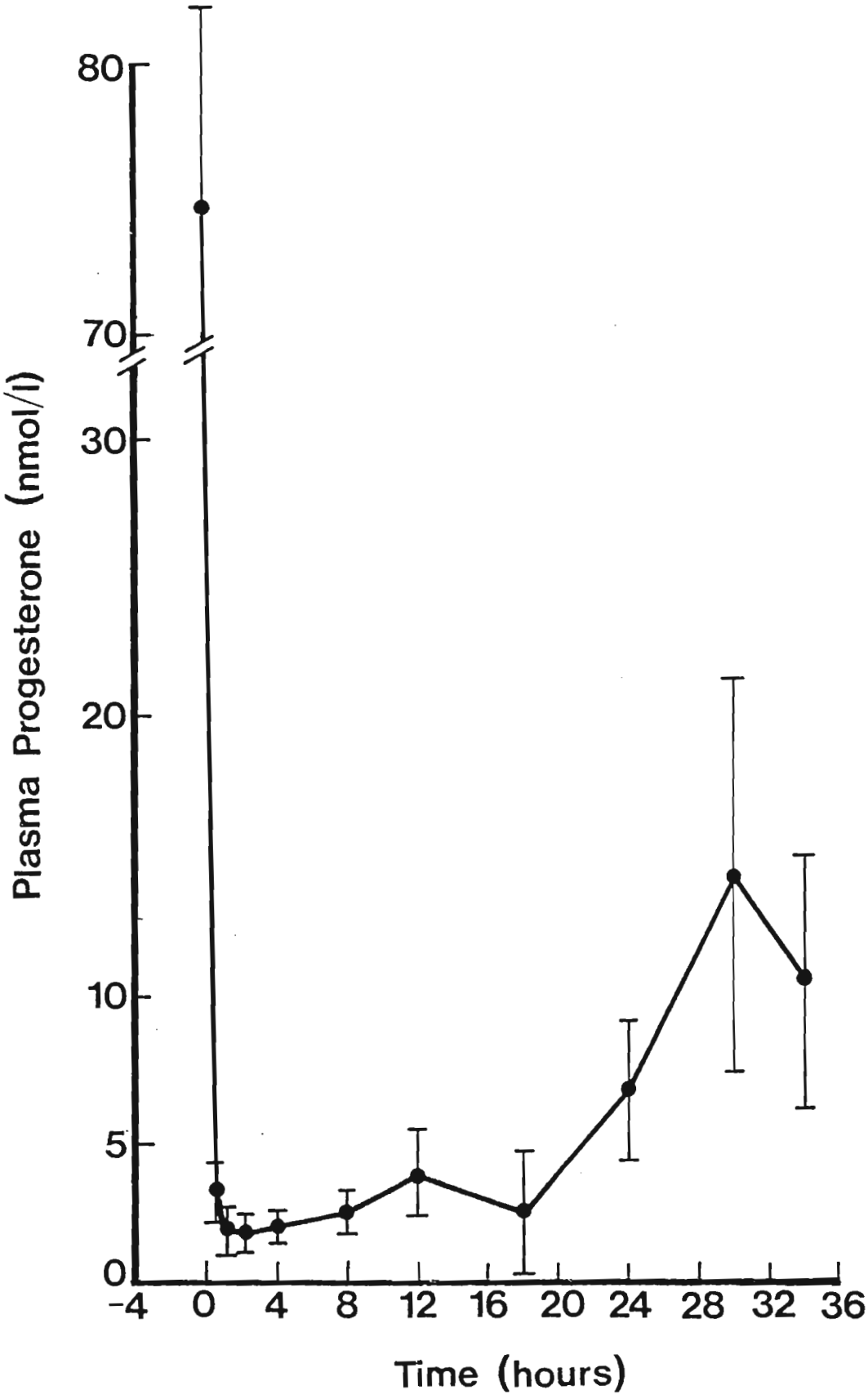

Fig 2.3 Serial concentrations of progesterone (nmol/l) in the uterine vein after Epostane 100mg (Mean ± S.E.M.).

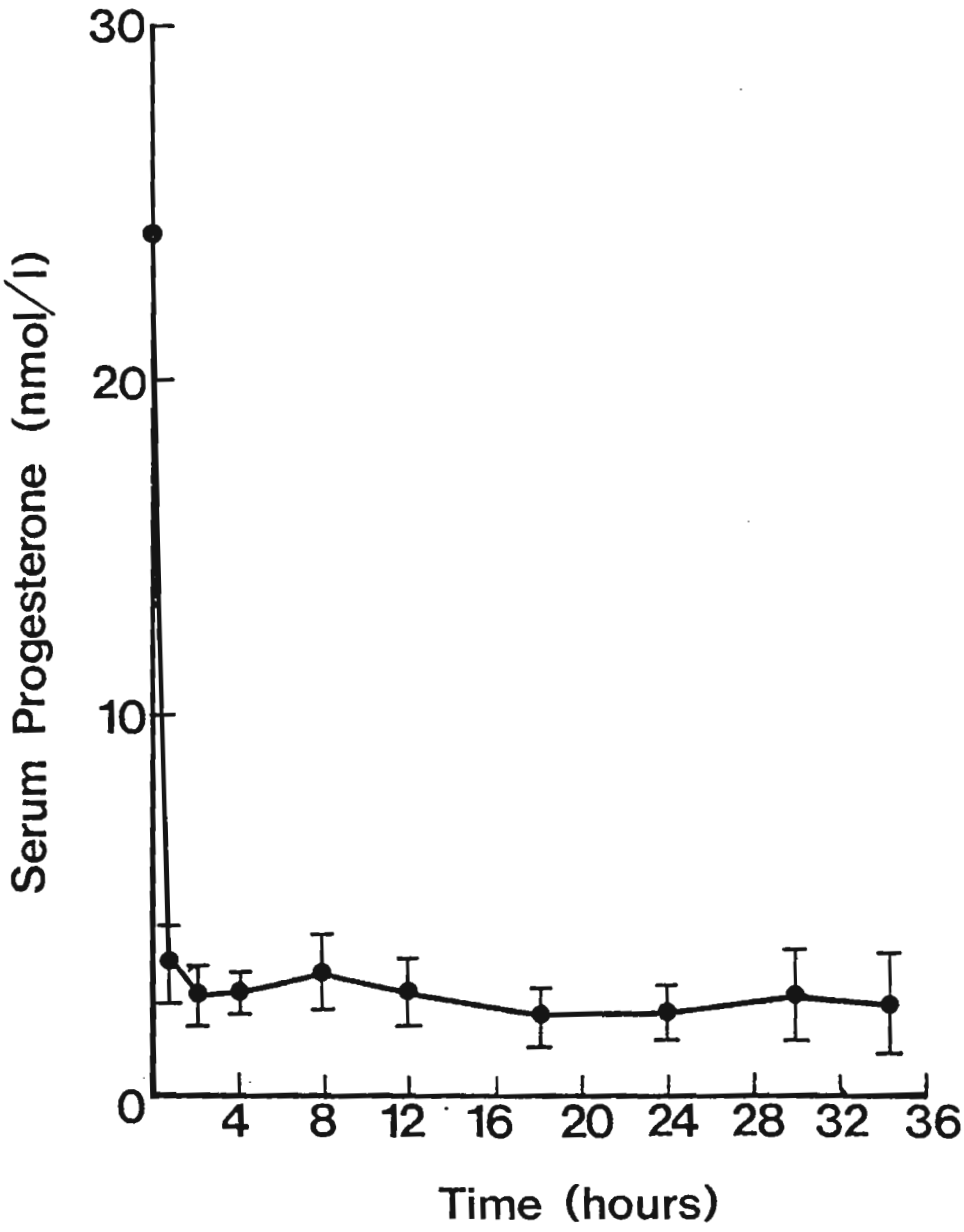

Fig 2.4 Serial concentrations of progesterone (nmol/l) in the peripheral blood after Epostane (100mg) (Mean ± S.E.M.).

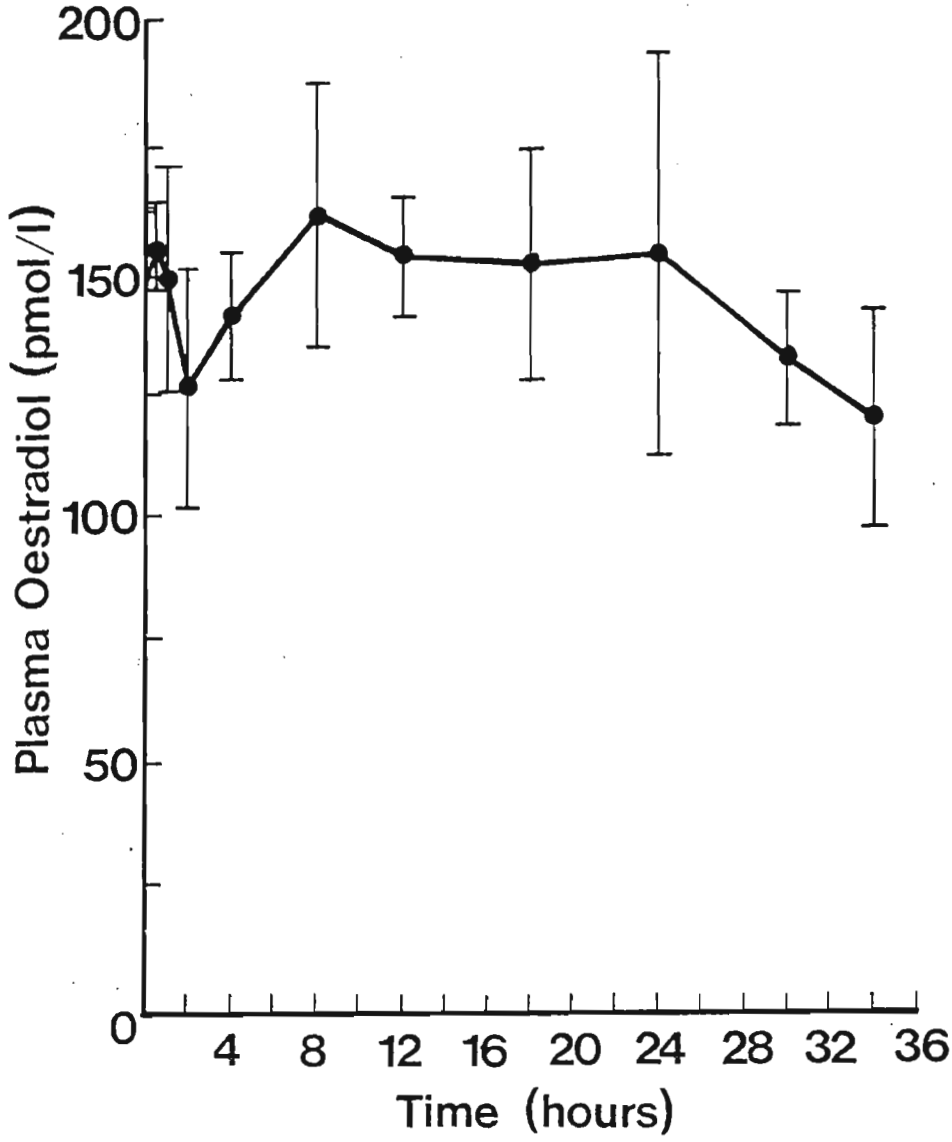

Fig 2.5 Serial concentration of oestradiol (pmol/l) in the uterine vein after Epostane (100mg) (Mean + S.E.M.).

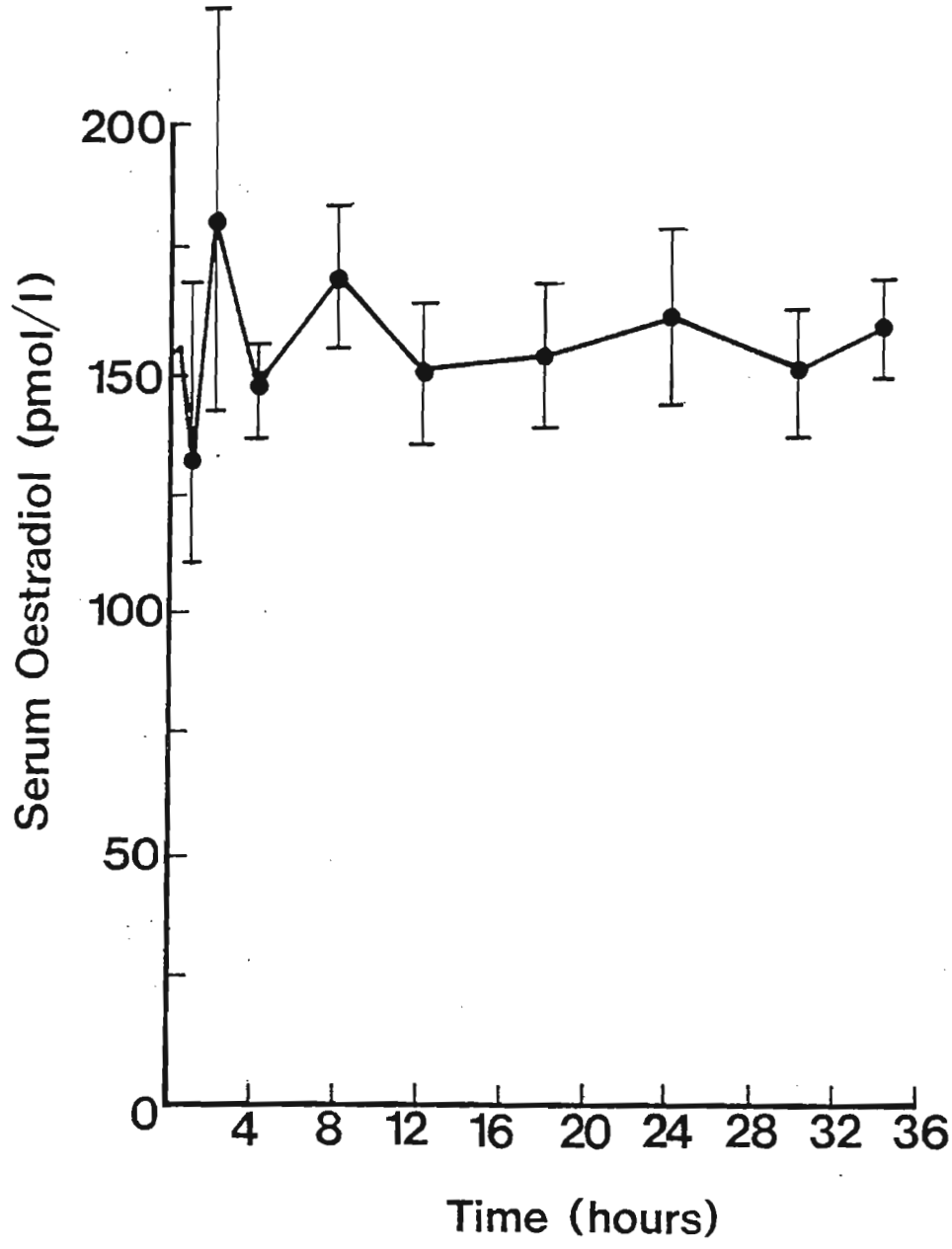

Fig 2.6 Serial concentration of oestradiol (pmol/l) in the peripheral blood after Epostane (100mg) (Mean + S.E.M.).

uterine vein which occurred immediately prior to delivery (14.85 ± 7.2 nmol/l) was not significant and was not detected in the peripheral plasma.

2. Oestradiol-17 β:

The mean pretreatment oestradiol-17β concentration was 154.8 ± 17.1 nmol/l (utero-ovarian vein) and 152.6 ± 14.7 nmol/l (peripheral vein). There were no significant change throughout the experiment from either site (Fig. 2.5 and 2.6) and the concentration remained significantly below that found in spontaneous labour (Anderson, Webb & Turnbull, 1981).

3. Cortisol:

Prior to the onset of labour the normal diurnal variation in the material peripheral cortisol levels was demonstrated. Maternal cortisol levels did not rise in parallel to fetal levels and were elevated in labour.

4. PGFM:

There was a sharp progressive rise in PGFM concentrations which was significantly different from the pretreatment value by 4 hours (in the uterine vein $p = 0.05$). In the utero-ovarian vein, PGFM rose from 4.80 ± 0.40 nmol/l (similar to concentrations previously described at the same gestational age; Mitchell, Flint, & Turnbull, 1976) to 34.0 ± 6.0 nmol/l at 30 hours (a 7-8 fold increase) (Fig 2.7). Similar values were reflected in the maternal peripheral

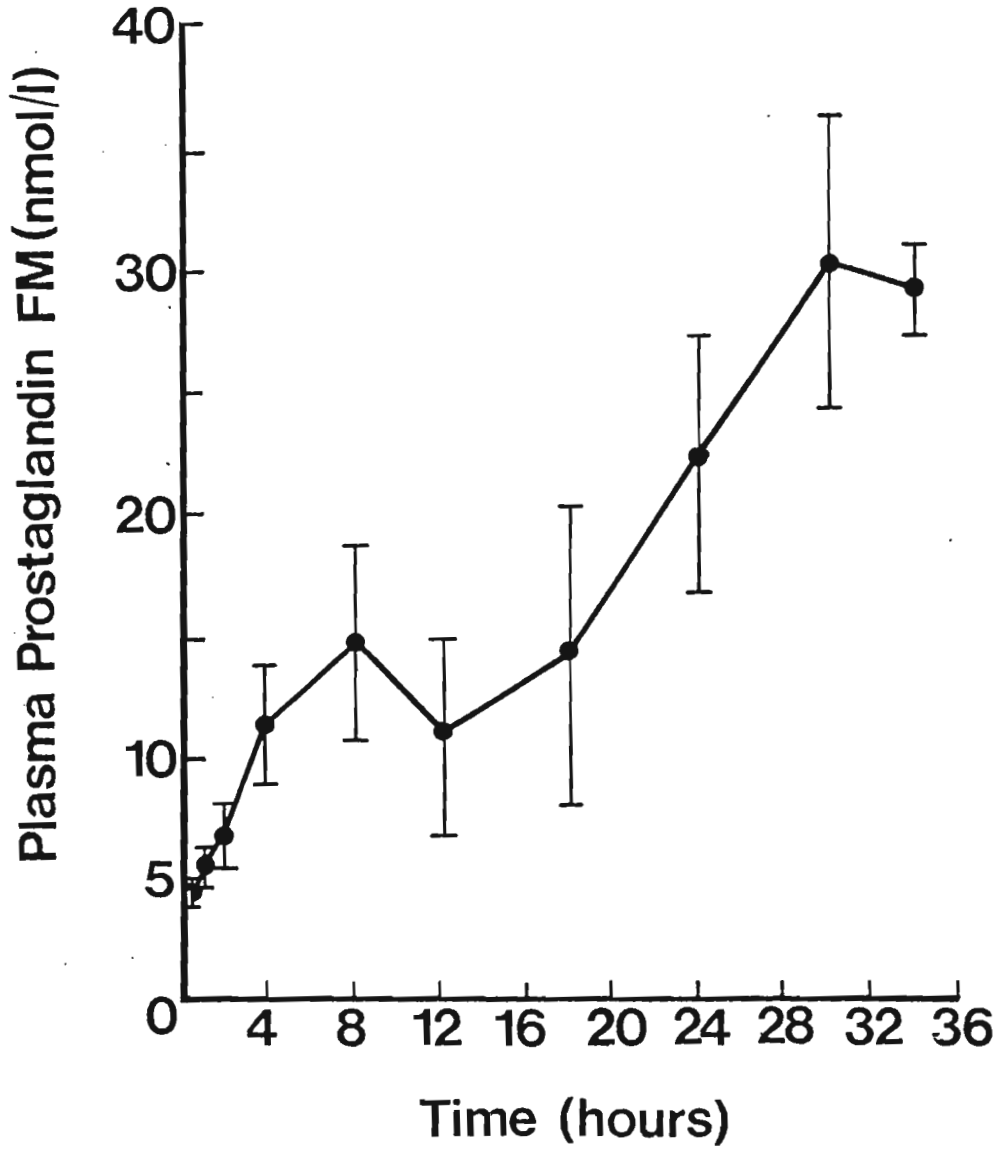

Fig 2.7 Serial concentrations of PGFM (nmol/l) in the utero-ovarian vein after Epostane (100mg) (Mean ± S.E.M.).

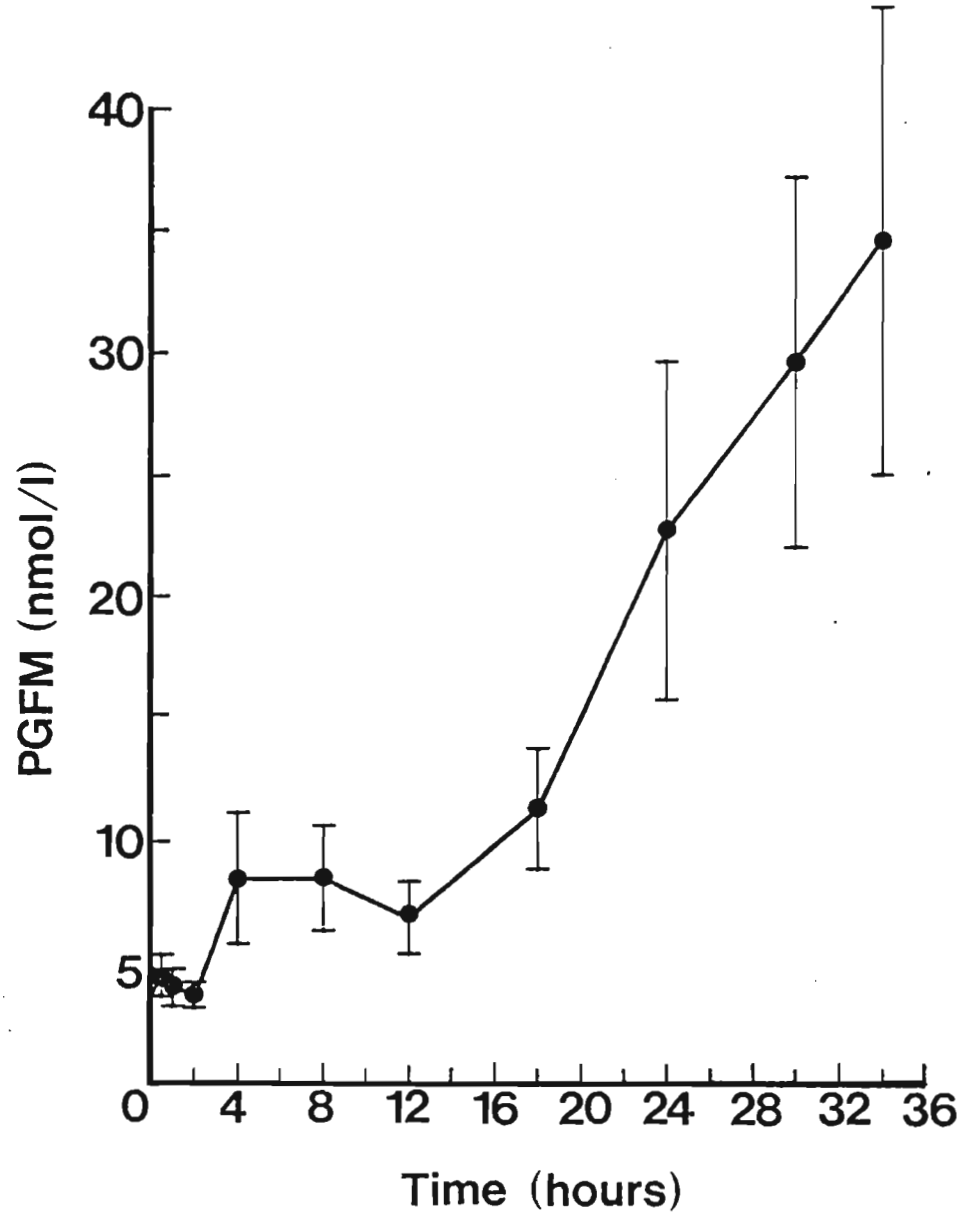

Fig 2.8 Serial concentrations of PGFM (nmol/l) in the peripheral blood after Epostane (100mg) (Mean ± S.E.M.).

circulation (Fig 2.8).

Fetal Plasma Hormone Concentration

1. Progesterone:

The fetal progesterone concentration prior to treatment was 2.8 ± 0.3 nmol/l and fell to levels not detectable by the assay on all subsequent samples.

2. Cortisol:

Fetal cortisol rose significantly throughout the study. There was an initial significant fall from the pretreatment value of 11.4 ± 2.5 nmol/l to 8.0 ± 2.5 nmol/l at one hour ($p = 0.02$). Then a progressive rise to 65.2 ± 21.5 nmol/l (a 5-fold increase) 30 hours after drug administration (Fig 2.9).

DISCUSSION

This study demonstrates 3 points;

1. As judged by the fall in the concentration of progesterone in utero-ovarian blood, Epostane is an effective inhibitor of placental 3 β -HSD in the ewe.
2. A fall in the production rate of progesterone alone is sufficient to stimulate prostaglandin production and to initiate the chain of events leading to parturition.
3. A rise in oestradiol-17β production is not an essential prerequisite for prostaglandin production and

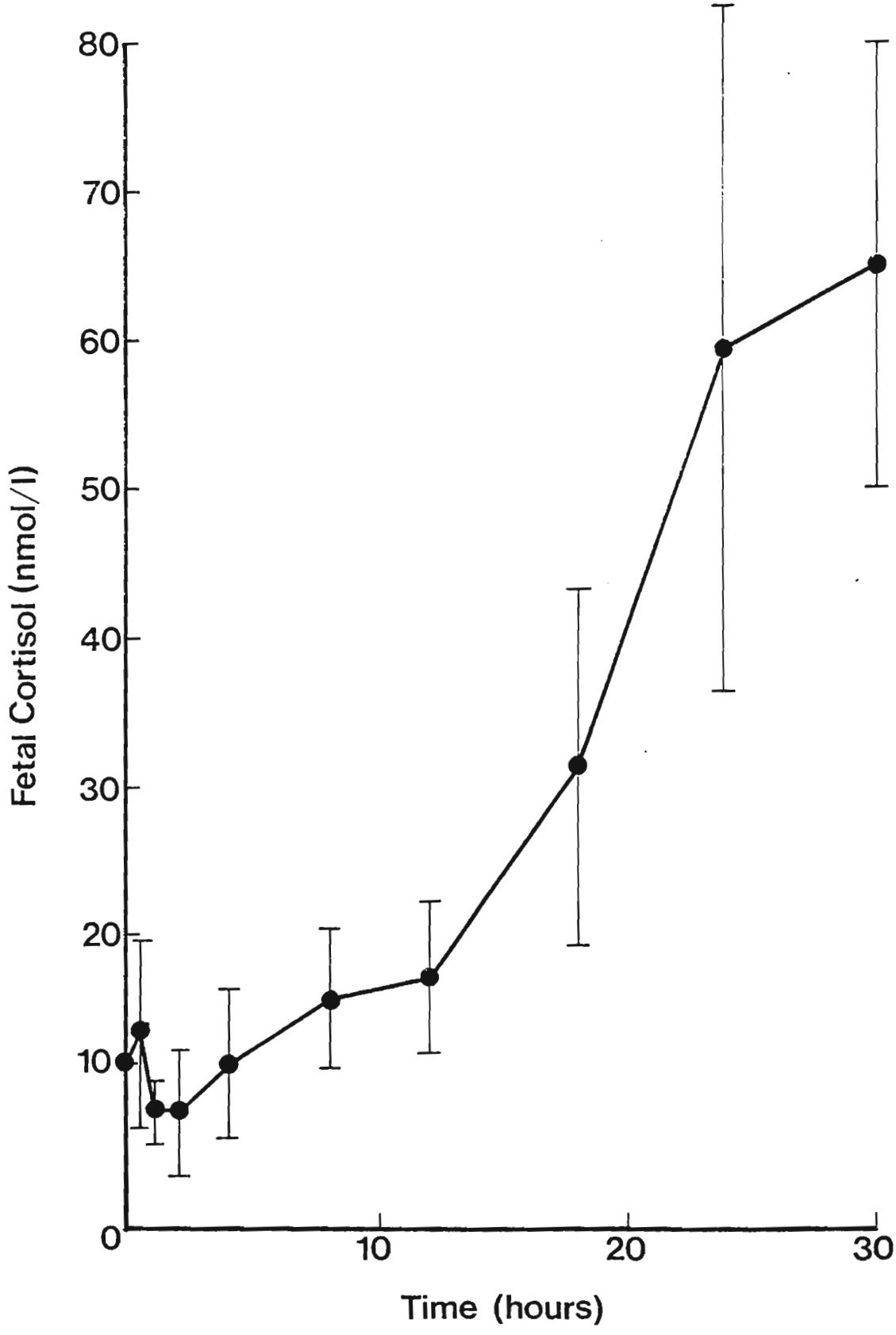

Fig. 2.9 The concentration of cortisol (nmol/l) in the fetal carotid artery after maternal administration of Epsotane (100mg) (Mean ± S.E.M.).

parturition.

Progesterone and Parturition

In the ewe the progesterone concentration in the peripheral plasma (Bassett et al 1969) and in the utero-ovarian vein plasma (Bedford et al., 1972) falls before spontaneous parturition at term. Similar changes precede labour in dexamethasone - or ACTH-induced premature parturition (Liggins, 1968). In these situations the fall in progesterone levels follows activation of the placental enzymes, 17 α -hydroxylase, 17-20 lyase and aromatase by the increasing fetal cortisol concentration (Anderson et al., 1975).

The enhanced metabolism of progesterone leads to a rise in oestradiol-17β production, and this combination of placental steroid hormone changes stimulates the increased prostaglandin production (Liggins, 1981). However the relative importance of elevated oestradiol-17β production and reduced progesterone levels is uncertain.

In the present study, a novel approach to this question was achieved in experiments in which the production rate of progesterone was depressed resulting in a fall in progesterone levels in the presence of stable oestradiol-17β production. The fall in progesterone levels

after administration of Epostane is rapid in onset, being apparent within 30 minutes to reach levels comparable to spontaneous parturition. Oestradiol-17β levels in the utero-ovarian vein blood did not change significantly.

The effect on $PGF_{2\alpha}$ production and release was measured by the increase in the concentration of its relatively stable metabolite, PGFM. The concentration of PGFM rose rapidly and preceeded recordable uterine contractions. The PGFM levels were significantly elevated from pretreatment levels at 4 hours. This study demonstrates that a fall in the production rate of progesterone alone is sufficient to stimulate $PGF_{2\alpha}$production and leads to parturition.

It is of interest to compare the results of the present study with similar studies (Taylor, Webb, Mitchell and Robinson, 1982; Mitchell, Brenneche, Kraemer and Webb, 1983; Jenkin & Thorburn, 1985). Jenkin and Thorburn (1985) administered variable doses of Trilostane to late pregnant ewes. Trilostane is a preferential inhibitor of adrenal steroidogenesis and required doses 5-10 times higher than Epostane for termination of pregnancies in the monkey (Creange, Anzalone, Potts & Schane, 1982). The fall in plasma concentrations of progesterone was dose-dependent and after 100 mg Trilostane intravenously only 2 of 7 ewes delivered. The difference between the studies may be due to the relative potency of the agents used.

The effect of inhibition of 3 -HSD on peripheral progesterone levels is more pronounced after Epostane than Trilostane. Although the initial decline is similar (2.05 $\pm$ 0.8 ng/ml & 1.39 $\pm$ 0.40 ng/ml after Epostane and Trilostane respectively (Taylor et al., 1982)) after 100mg Epostane peripheral progesterone levels remained below 1.0 ng/ml until delivery whereas after Trilostane at 30-36 hours levels had returned to 14.07 $\pm$ 4.17ng/ml. The effect on protaglandin levels, as determined by maternal levels of PGFM, were also more pronounced after Epostane (an 8 fold increase compared with a 4 fold increase after Trilostane).

The effects of Epostane are reversible since levels of progesterone in utero-ovarian venous blood were rising at 30 hours. At the time of delivery progesterone levels were rising as has been demonstrated in previous studies (Taylor et al., 1982). This suggests that the stimulus to $PGF_{2\alpha}$ production is either the decline in progesterone levels or the absolute concentration of progesterone, and that it is not essential to maintain depressed progesterone concentration once the chain of events leading to parturition has begun.

Progesterone is considered to have a dual effect on uterine activity, acting indirectly by inhibiting the synthesis or release of oxytocin (Roberts & Share, 1969) and

prostaglandins (Liggins, 1981) and acting directly by blocking the stimulatory effect of prostaglandins and oxytocin (Lye & Porter, 1978). These authors (Lye & Porter, 1978) showed that it took up to 200 hours to regain myometrial activity which had been directly suppressed by progesterone. However, the decline in progesterone levels in spontaneous labour (Bassett et al., 1969) and after Epostane is abrupt and is followed immediately by rising PGFM concentrations. This suggests that the dominant effect of the fall in the concentration of progesterone is on prostaglandin synthesis. Further progesterone levels in the uterine vein are returning towards pretreatment values at the time of delivery which suggests that it is not essential to maintain the lowered progesterone level once labour is established. Perhaps this is why uterine activity, either dexamethasone induced or spontaneous, cannot be suppressed by progesterone therapy (Liggins et al., 1973).

Oestrogen and Parturition

Evidence implicating oestrogens in the stimulation of the synthesis and release of prostaglandins is substantial. Exomgenous oestrogens can induce uterine activity in late pregnant sheep (Hindson, Schofield & Turner, 1967) and cause an increased concentration of $PGF_{2\alpha}$ in the utero-ovarian venous blood (Liggins et al, 1973). During spontaneous delivery there is a close temporal relationship between the

concentration of oestradiol-17β and the concentration of $PGF_{2\alpha}$ in the utero-ovarian venous blood (Liggins et al, 1973). However, in the present study there were two unexpected findings. Firstly the concentration of oestrogen was not suppressed by inhibition of 3β -HSD, despite a marked decline in progesterone concentration. Secondly, $PGF_{2\alpha}$ production and release occurred in the absence of rising oestrogen levels.

There are 3 possible explanations for the failure of Epostane to suppress oestrogen production in the ewe;

1. The inhibition of 3β -HSD by Epostane is incomplete. Epostane inhibits the conversion of pregnenolone to progesterone but not the conversion of dehydroepiandrosterone (DHEA) to androstenedione, or 17α -hydroxypregnenolone to 17α -hydroxyprogesterone.
2. Oestradiol-17β synthesis is spared at the expense of other oestrogens.
3. At this gestation (130 days) 17α hydroxylase activity is very slow and unable to metabolise the precursor.

In order to resolve this question, maternal androstenedione and oestrone sulphate levels were measured. Plasma androstenedione levels in the maternal utero-ovarian venous blood rose slightly from 0.39 nmol/l prior to treatment to peak at 0.48 nmol/l at 4 hours and then returned to pretreatment values. Oestrone sulphate was not affected. That is despite a major alteration in the concentrations of

pregnenolone and progesterone the levels of androstenedione, oestrone sulphate and oestradiol remain unchanged. This confirmes that the enzyme 17α hydroxylase is rate limiting for oestrogen synthesis in the ewe.

The present data suggests that an increase in oestrogen levels is not essential for prostaglandin secretion but is consistent with the suggestion that a fall in the progesterone/oestradiol ratio is the usual stimulus for prostaglandin secretion (Liggins, 1981). Both fetal cortisol and Epostane significantly alter the progesterone/oestradiol ratio, although by different mechanisms, and result in prostaglandin secretion.

Prostaglandin and parturition

Prostaglandins have a dual role in parturition in the ewe. There is an increasing myometrial responsiveness to prostaglandin during parturition and also uterine production of prostaglandins increase sharply.

Confirmation for their role is not only the progressive rise as labour advances but also the blocking affect of prostaglandin inhibitors (e.g. meclofenamic acid) on intrafetal dexamethasone-induced parturition (Mitchell and Flint, 1978).

In the present study, prostaglandin production rose steadily prior to recordable uterine activity and followed the decline in progesterone levels.

Fetal cortisol and parturition

Although the factors responsible for the fetal cortisol surge are unclear, its stimulating effect on the activity of placental 17 α -hydroxylase (Anderson et al., 1975) alters placental steroid metabolism and leads to prostaglandin release.

In the present study fetal cortisol was measured to determine the effect of placental steroid changes on the fetal adrenal. There was an initial fall in fetal cortisol levels followed by a progressive rise.

The initial fall in fetal cortisol levels could be; due to a drug-related action of Epostane which may cross the placenta and inhibit fetal adrenal steroidogeneis; subsequent to a reduction in availability of precursors for fetal cortisol production; or by a fall in maternal cortisol levels.

In the present study, Epostane was not assayed; it is not known if the drug crosses the placenta. However, if Epostane did effect adrenal steroidogenesis in the fetus directly it would be expected to have a longer duration of action than

found in this study, in keeping with the duration of effect on placental 3 β -HSD. The rapid decline in maternal progesterone levels in spontaneous labour is reflected in the fetus and there is some evidence that the fetal adrenal uses progesterone as a precursor for cortisol production (Anderson et al., 1975). Hence reduced precursor could account for the transitory fall in fetal cortisol production. Thirdly, maternal cortisol falls after drug administration from 35.7 nmol/l to 13.25 nmol/l at 1 hour and the fall in cortisol levels in the fetus may simply reflect this change.

In the present study fetal cortisol levels rose 8 hours after the administration of Epostane and reached levels similar to those which occur in spontaneous parturition. This increase may be due to either; a "placental factor" stimulating the fetal adrenal; the increased availability of precursors; a fetal hypothalamic-pituitary-adrenal axis response to the preceding fall in cortisol; or the "stress" of labour.

Jones et al., (1977) suggested that ACTH-like agents may be secreted by the placenta but there is no reason to believe that inhibition of 3 β -HSD would stimulate its secretion. Inhibition of 3 β -HSD in the human results in elevated levels of pregnenolone (Van der Spuey, Jones, Wright et al., 1983) which could serve as substrate for fetal adrenal steroidogenesis. Measurements of fetal androstenedione also showed a progressive rise (from 0.6 nmol/l to 1.35 nmol/l at

delivery) suggesting that fetal adrenal steroidogenesis is not suppressed by Epostane.

The progressive fetal cortisol rise is presumably due to the effect of $PGF_{2\alpha}$ and PGE on the fetal adrenal. Both of these prostaglandins have been shown to stimulate the production of fetal cortisol (Louis , Challis, Robinson & Thorburn, 1976; Liggins, Scroop, Haughey, 1982). In spontaneous parturition the rapid rise in fetal cortisol would stimulate a rise in placental oestradiol-17β. In this study this did not occur presumably due to the falling level of substrate.

CHAPTER 3

EARLY HUMAN PREGNANCY

The role of progesterone in the termination of human pregnancy remains uncertain (see ChapterI). In some laboratory animals progesterone has been shown to have a central role in parturition (Liggins et al., 1973; Thorburn and Challis, 1979). In others, as in man, the evidence is conflicting . In human pregnancy the peripheral progesterone levels do not change prior to parturition and a pathway by which the fetus could alter progesterone metabolism in the placenta has not been demonstrated. This evidence conflicts with the data of Csapo and Pulkkinen (1978). These authors showed that surgical removal of the corpus luteum performed prior to the 49th day of pregnancy led to a decline in peripheral progesterone levels and abortion. They concluded that progesterone is essential for the maintenance of early human pregnancy and suggested that progesterone was essential throughout pregnancy and that a decline in progesterone levels was an essential prerequisite to the initiation of human parturition. This conflict has not been resolved and the work of Csapo and Pulkkinen (1978) has not been confirmed.

One reason for the continuing doubt is ethical considerations which limit experimental designs in man; studies have been restricted to the nonpregnant woman, early human pregnancy prior to termination and, in late human

pregnancy, to either serial peripheral samples or to single samples from the amniotic fluid, umbilical cord blood or uterine vein at caesarean section.

The development of a competitive inhibitor of progesterone synthesis (Epostane) provides the opportunity to investigate the role of progesterone in early human pregnancy without the problems inherent in the study described by Csapo and Pulkkinen (1978). The possibility of teratogenic effects precludes the use of this compound in continuing pregnancies. Accordingly, the study was performed in a group of women awaiting therapeutic termination of pregnancy.

METHODS

Trial Design

Sixty-five healthy women who were awaiting therapeutic termination of pregnancy were studied between five and 18 weeks of pregnancy.

The study was performed in three sections:-

Trial 1. A single dose, double-blind trial of Epostane (100 mg or 50 mg) or placebo included 45 subjects who were subdivided into three gestational age groups (five to seven weeks, eight to 11 weeks and 12 to 18 weeks of pregnancy).

Trial 2. A multiple dose, double-blind, one day study of Epostane (300 mg and 400 mg) and placebo (n =5).

Trial 3. A multiple dose uncontrolled study of Epostane (1500mg, 100mg eight hourly for five days) (n =5).

Subjects

The women were interviewed immediately after approval for termination of pregnancy and the nature of the investigation explained. They were studied in the time between approval for termination and the procedure being performed.

Selection was based on the following criteria:

1. Reasonable certainty as to the gestational age as assessed by menstrual history and clinical examination and/or an ultrasound scan,
2. Good general health and not receiving any medication,
3. Weight less than 80 kg,
4. Normal full blood screen and biochemical profile (haemoglobin, haematological indices and blood film, serum urea, electrolytes, total protein, albumin, bilirubin, alkaline phosphatase and asparate serum transaminase),
5. No contraindication to the proposed treatment.

Radioimmunoassays

Levels of progesterone, oestradiol-17β and cortisol were measured in the peripheral venous serum by specific radioimmunoassay as described in appendix. The assays were validated for human serum and the methods did not differ significantly from that described in the appendix apart from sample dilution.

Statistical Methods

The data was coded and analysed using a CTL8050 computer. The mean ± S.E.M. was calculated for each subgroup at each time interval. The first two pretreatment measurements, 30 minutes prior to treatment and immediately prior to treatment, were averaged to give a "pretreatment" value. All subsequent results were expressed as a percentage of this value.

A SPSS (statistical packages for the social sciences) programme was used on an IBM 2980 computer to perform a two-way analysis of variance, the two variables being gestational age (5-7, 8-11, and 12-18 weeks of pregnancy) and drug dosage (placebo, 50 and 100 mg Epostane) for each hormone at each time interval. Whenever a significant difference was detected indicating at least one of the groups was different, an unpaired Student's test was performed to determine which groups were different.

A paired Student's test was performed on the haematological and biochemical data to detect treatment effects.

TRIAL 1

Single dose study Epostane 100mg, 50mg and Placebo.

Method

Forty-five women entered the study, 15 in each gestational age group (5-7, 8-11 and 12-18 weeks of pregnancy). The volunteers in each subgroup were randomly allocated by a

double-blind procedure to one of three treatment groups (placebo, 50 mg and 100 mg Epostane).

TABLE 3.1 The design of Trial 1 (Epostane in early pregnancy)

Dosage of Epostane (mg)	Gestational Age Groups (weeks from last menstrual period)		
	6-7 weeks	8-11 weeks	12-18 weeks
0	5*	5	5
50	5	5	5
100	5	5	5

* Number in each sub-group.

Subjects were admitted 24 hours prior to the planned termination of pregnancy. The weight, height and blood pressure of each subject was recorded and an intravenous catheter was inserted into a forearm vein for blood sampling. Two pretreatment samples were taken 30 minutes apart and the tablet administered with a glass of water immediately after the second. Subsequent samples were at 30, 60, 90, 120, 180, 240 and 360 minutes. The patients' blood pressure and pulse were recorded at the time of blood sampling. Twenty-four hours after the study each subject was questioned as to side effects from the medication and whether she had noticed abdominal pain or vaginal bleeding. A final blood sample was

taken for hormone analysis plus a haematological and biochemical screen to detect adverse drug effects. Termination of pregnancy was then performed by a method appropriate for gestational age.

Results

Clinical Effect

One patient reported vaginal bleeding six hours after treatment (she had received a placebo). All other subjects denied symptoms which could be attributed to treatment. There were no uterine activity, vaginal bleeding nor adverse drug effects.

Hormone Measurements

1. Serum Progesterone.

There was significant decline in the peripheral serum progesterone concentrations after a single tablet of Epostane (Table 3.2). The magnitude of this decline increased both with increasing drug dosage (Figs 3.1, 3.2, 3.3, 3.4) and with increasing gestational age (Fig 3.5). The mean pretreatment serum progesterone concentrations in each gestational age group were within the normal range for the laboratory and there was no significant differences between the treatment groups. The mean pretreatment value for the 5-7 week gestation group was 60.0 nmol/l, for the 8-11 week gestation group 70.2 nmol/l and for the 12-18 week gestation group 111.9 nmol/l.

There was a significant dose-related effect ($p < 0.001$). When all gestation groups were combined the magnitude of the decline in serum progesterone levels after Epostane increased with increasing drug dose (Fig 3.4).

TABLE 3.2 The concentration of progesterone (nmol/1) after a single tablet of Epostane (50 or 100 mg) or placebo in early pregnancy.

Gestational Age Group (weeks)	Dosage of Epostane (mg)	Initial Value (nmol/l)	Percentage Change at 4 hrs	Percentage Change at 24 hrs
5-7	0	59.8 ± 13.7	104.6 ± 27.2	84.6 ± 5.0
	50	57.9 ± 10.2	80.9 ± 6.5	97.3 ± 6.0
	100	74.4 ± 14.0	51.1 ± 10.5	75.1± 11.0
8-11	0	74.4 ± 15.6	101.6 ± 5.0	104.4 ± 5.2
	50	68.0 ± 5.0	**64.6 ± 3.8	**76.2 ± 6.9
	100	68.1 ± 12.1	**43.3 ± 5.7	**64.7 ± 4.8
12-18	0	99.5 ± 18.8	105.3 ± 16.9	123.0±12.1
	50	126.6 ± 1.6	**41.4 ± 2.1	**81.9 ± 2.9
	100	109.7 ± 4.8	**35.5 ± 4.1	*74.7 ± 9.6

Values represent mean ± S.E.M. (N = 5 at each dose)

Significant differences from placebo group are indicated (*P < 0.05, **P < 0.01).

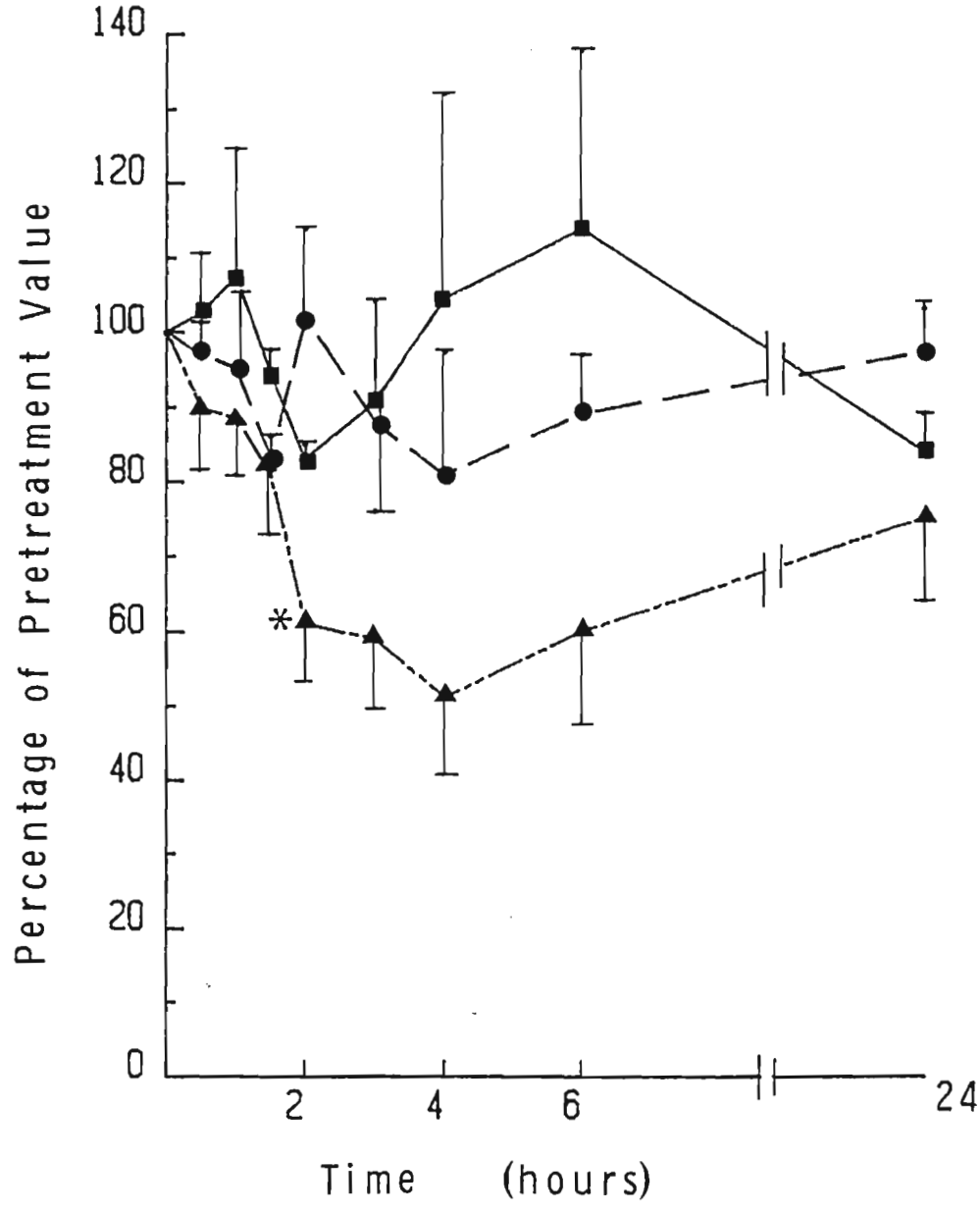

FIG 3.1 Percentage change in the concentration of progesterone (mean ± S.E.M.) at 5 - 7 weeks gestation after a single tablet of Epostane 50mg (●——●) and 100mg (▲——▲) or placebo (■——■). There was a significance difference from the placebo group (*$p < 0.05$).

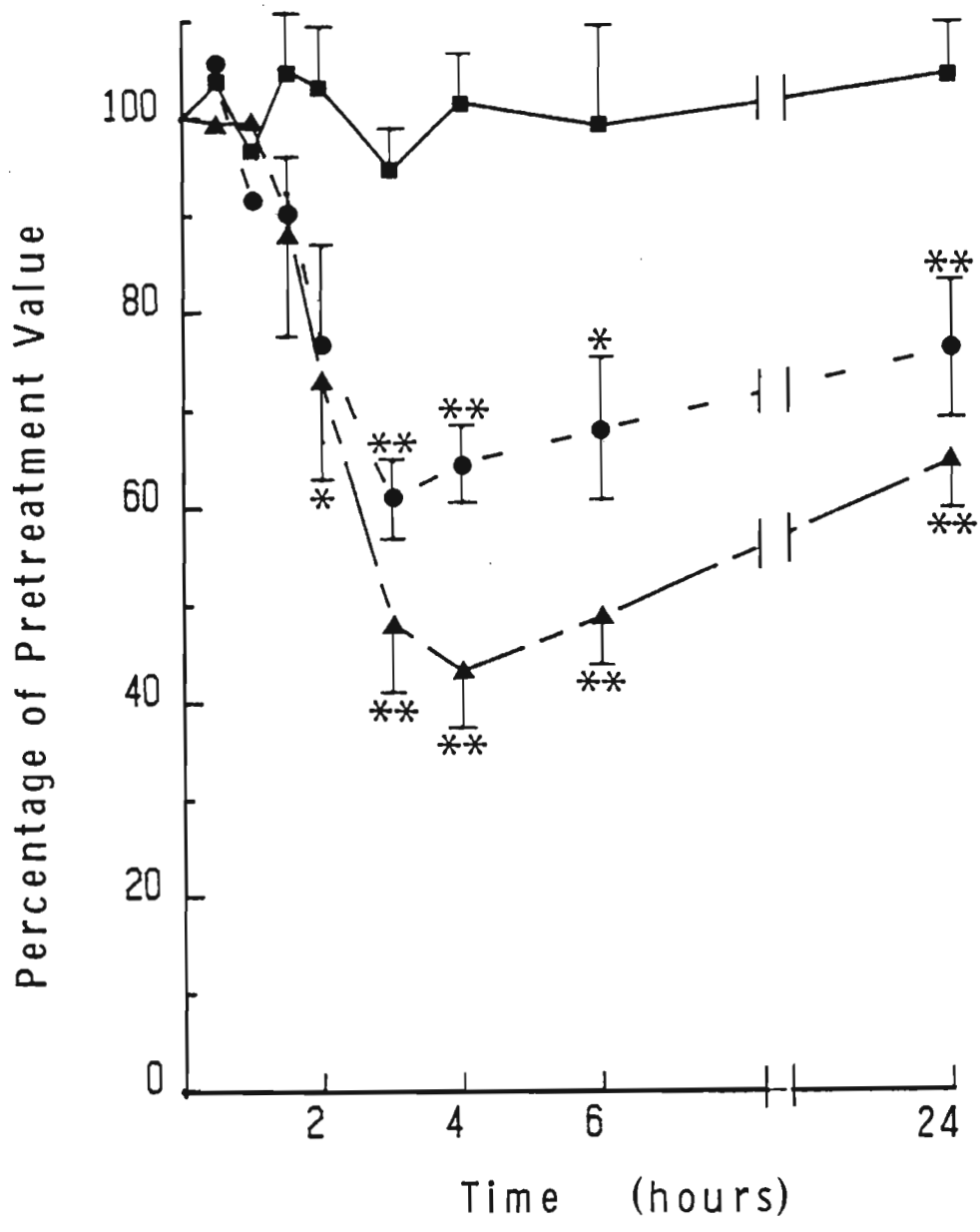

FIG 3.2 Percentage change from the mean of the control period in the concentration of progesterone (mean + S.E.M.) at 8 - 11 weeks gestation after a single tablet of Epostane 50mg (●——●) and 100mg (▲——▲) or placebo (■——■). There were significance differences between the treatment and placebo groups (* $p < 0.05$, ** $p < 0.01$).

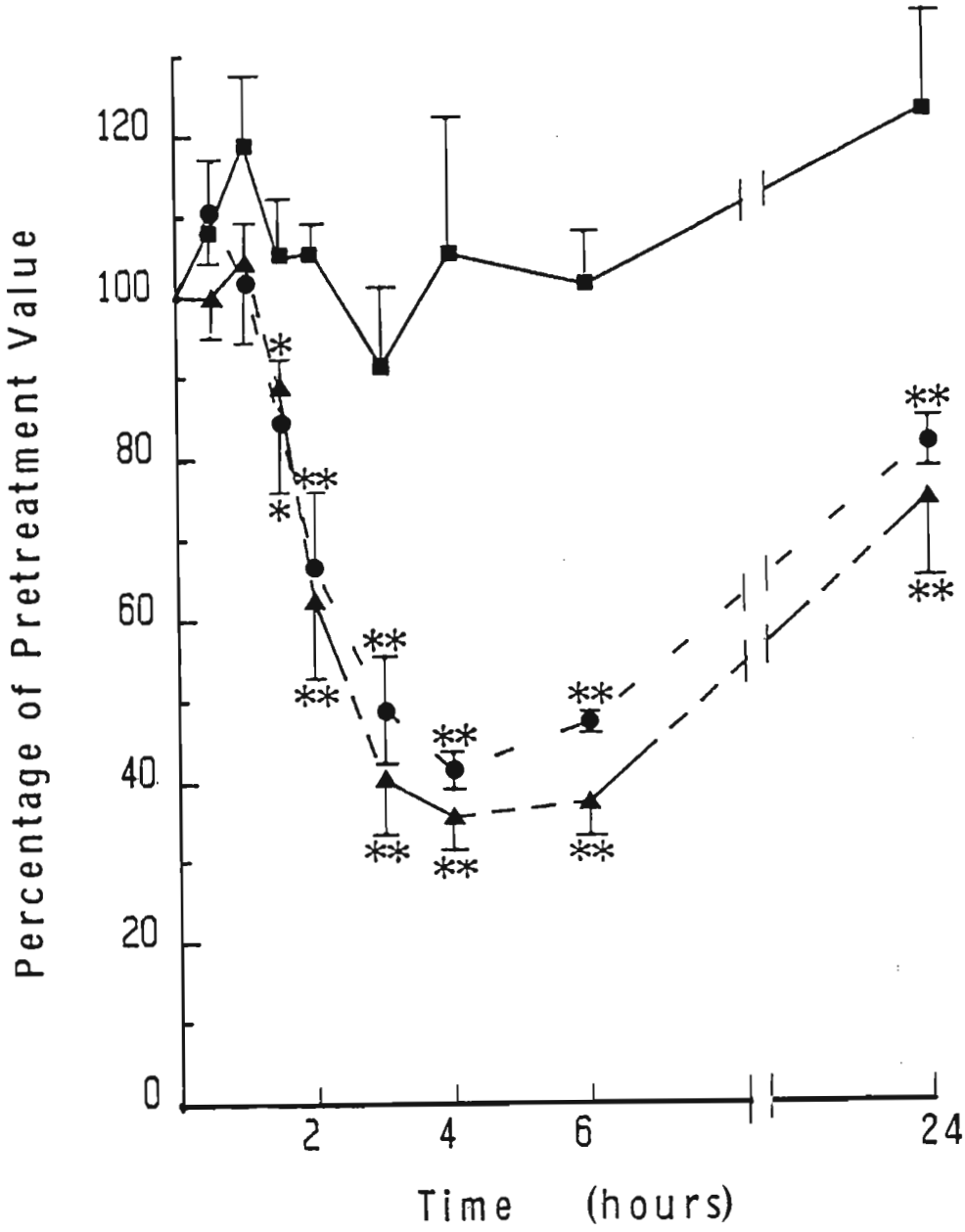

FIG 3.3 Percentage change from the mean of the control period in the concentration of progesterone (mean + S.E M.) at 12 -18 weeks gestation after a single tablet of Epostane 50mg (●——●) and 100mg (▲——▲) or placebo (■——■). There were significance differences from the placebo group (* $p < 0.05$, ** $p < 0.01$).

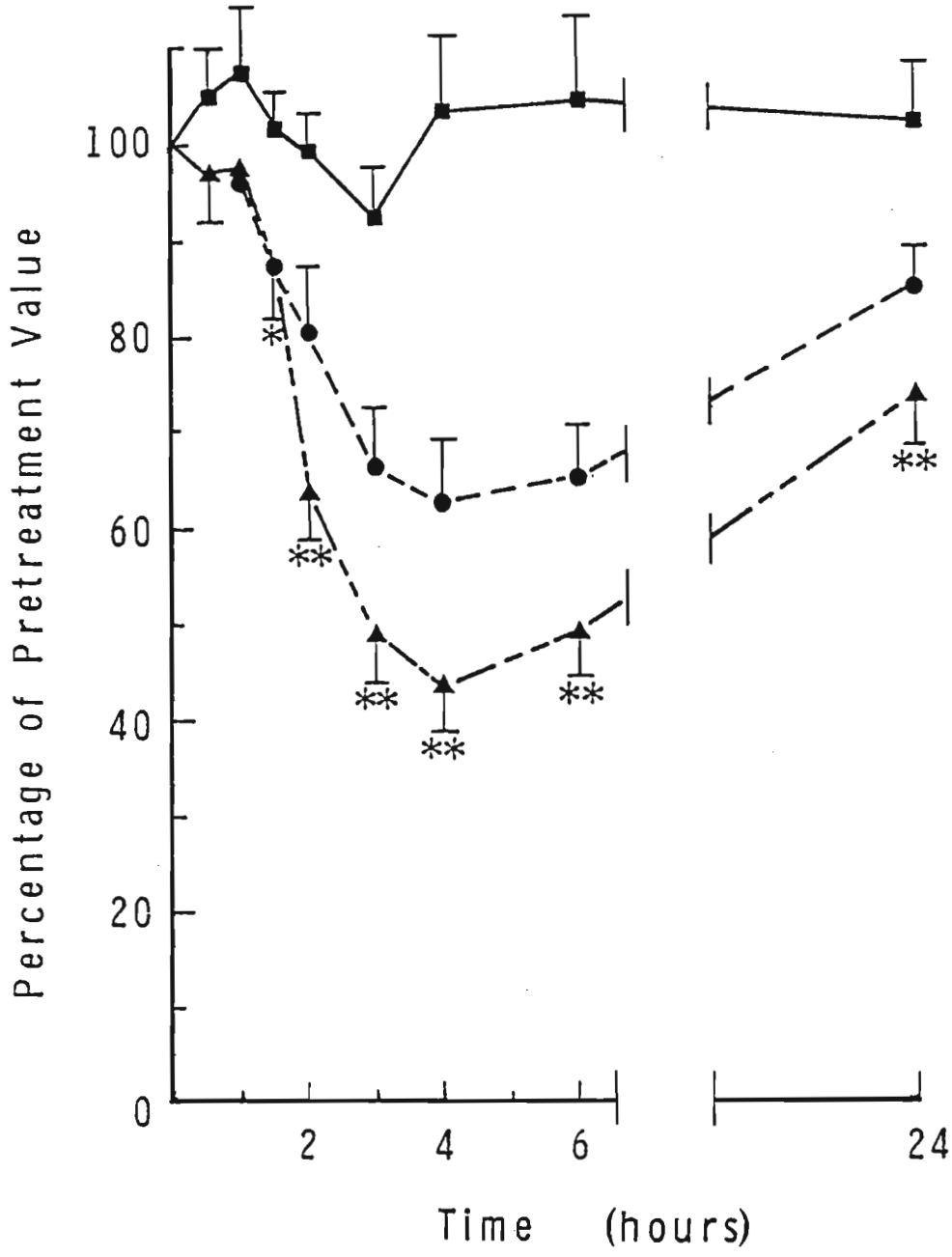

FIG 3.4 Percentage change from the mean of the control period in the concentration of progesterone (mean + S.E M.) after a single tablet of Epostane 50mg (●——●) and 100mg (▲——▲) or placebo (■——■). All gestational age groups combined n=15. There were significance drug-related effects (* $p < 0.05$, ** $p < 0.01$).

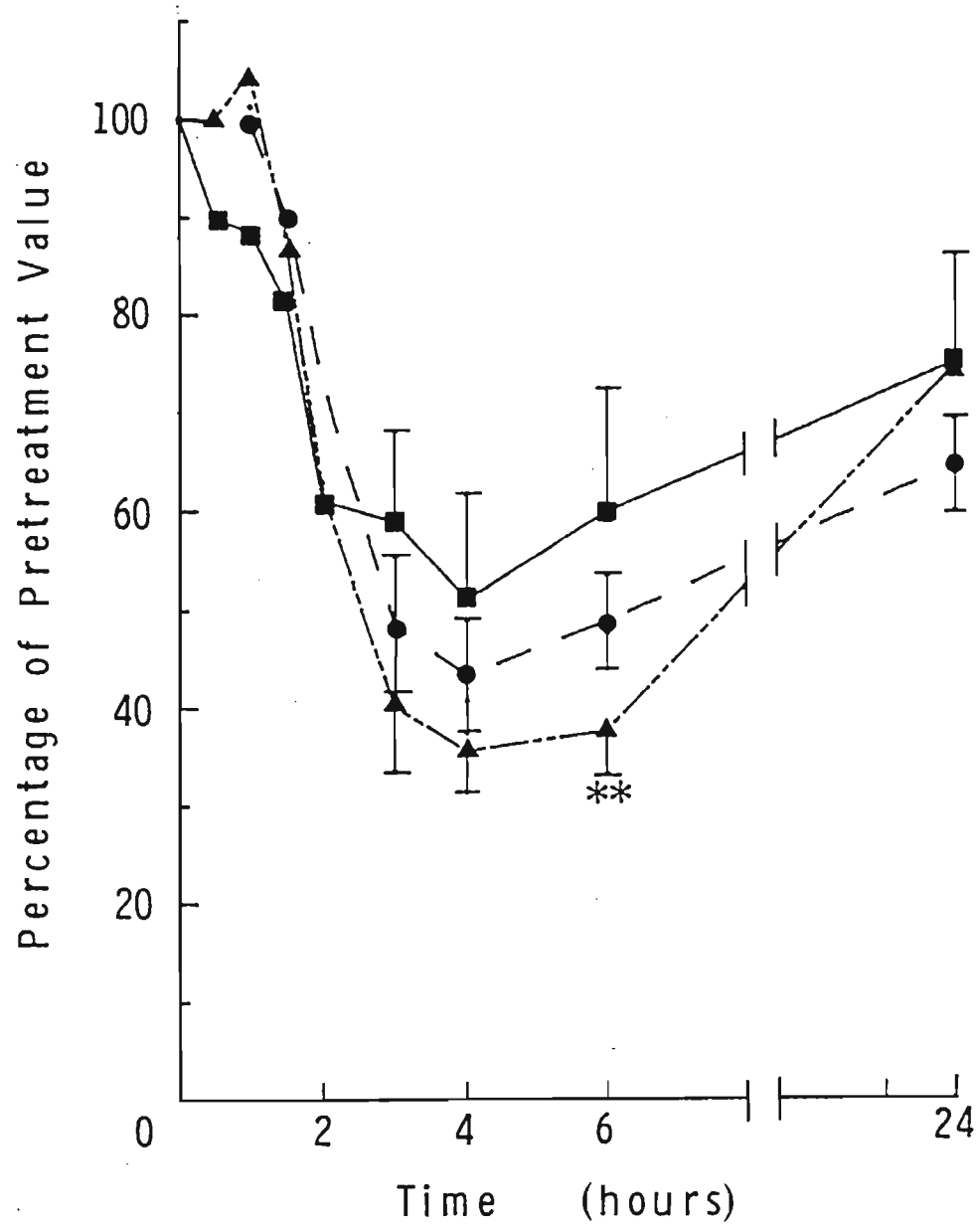

FIG 3.5 Percentage change in the concentration of progesterone (mean + S.E.M.) after Epostane (100mg) in the three gestational age groups. 5 -7 weeks (■——■), 8 - 11 weeks (●——●) and 12 - 18 weeks (▲——▲). There was a significant gestational age-related effect at 6 hours after treatment (* $p < 0.001$).

A significant gestational-age effect was also demonstrated. Fig 3.5 analyses those subjects who received 100 mg Epostane in each gestational age group. There was a significant difference between these groups six hours after table ingestion ($p < 0.001$).

In all subjects the effect on serum progesterone levels was temporary, the nadir was at four hours and the serum progesterone concentration was returning towards pretreatment levels by 24 hours.

2. Serum Oestradiol -17β .

There was a significant decline in serum oestradiol-17β levels after treatment with Epostane which followed the same time course as the fall in the concentration of serum progesterone (Table 3.3).

The pretreatment concentration of oestradiol-17β were within the normal range for this laboratory. The mean values were; 4.0 nmol/l at 5-7 weeks; 7.6 nmol/l at 9-11 weeks and 23.7 nmol/l at 12-18 weeks gestation (Table 3.3). There was no significant difference between the mean pretreatment concentration of serum oestradiol-17β in each treatment group.

The decline in serum oestradiol-17β levels was temporary, the nadir was at four hours and levels were returning towards pretreatment value at 24 hours (Fig 3.6, 3.7, 3.8).

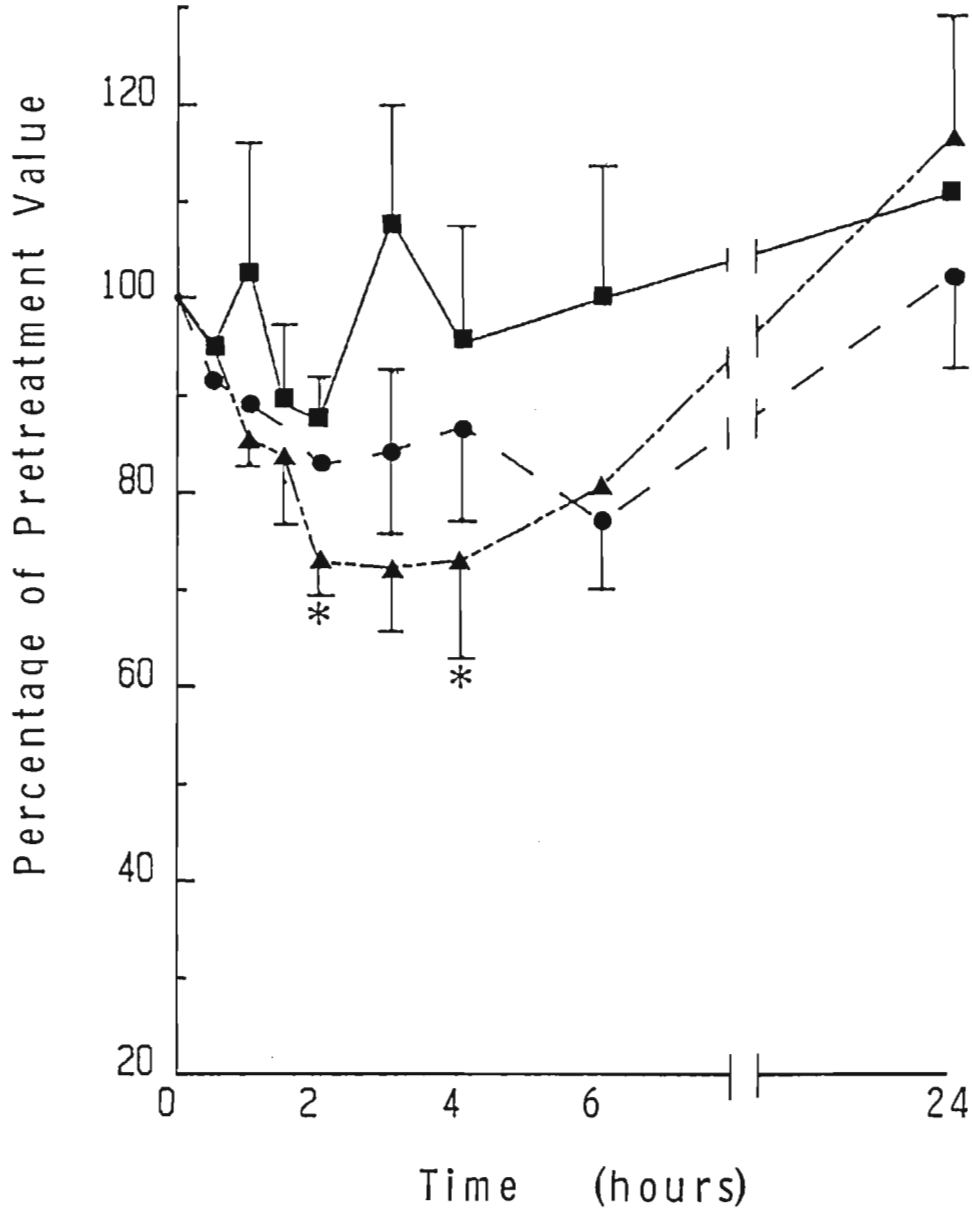

FIG 3.6 Percentage change in the concentration of oestradiol-17β (mean ± S.E.M.) at 5 - 7 weeks gestation after a single tablet of Epostane 50mg (●——●) and 100mg (▲——▲) or placebo (■——■). There was a significance difference from the placebo group (* $p < 0.05$).

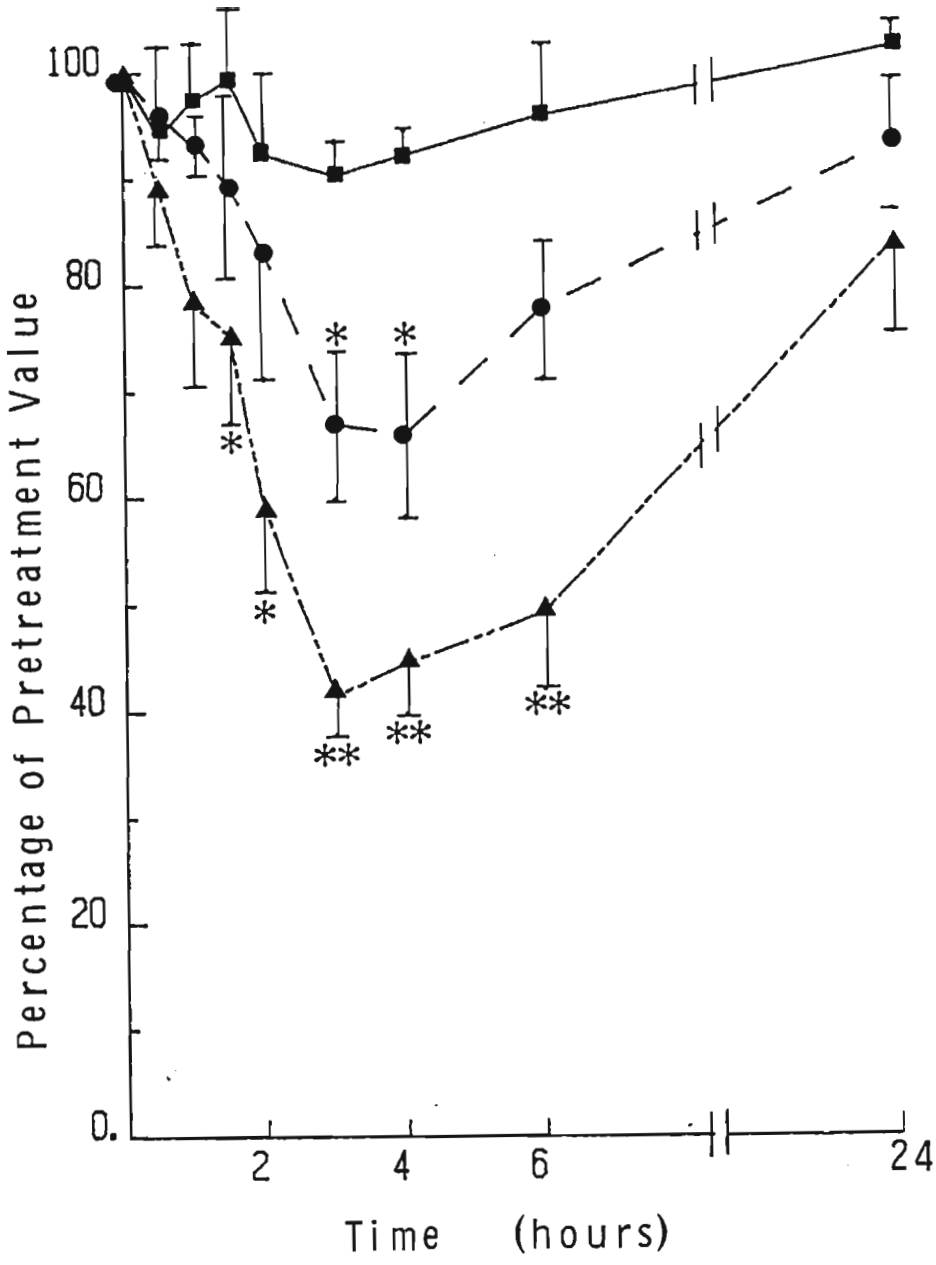

FIG 3.7 Percentage change in the concentration of oestradiol-17β (mean ± S.E.M.) at 8 - 11 weeks gestation after a single tablet of Epostane 50mg (●——●) and 100mg (▲——▲) or placebo (■——■). There were significance differences from the placebo group (* $p < 0.05$, ** $p < 0.01$).

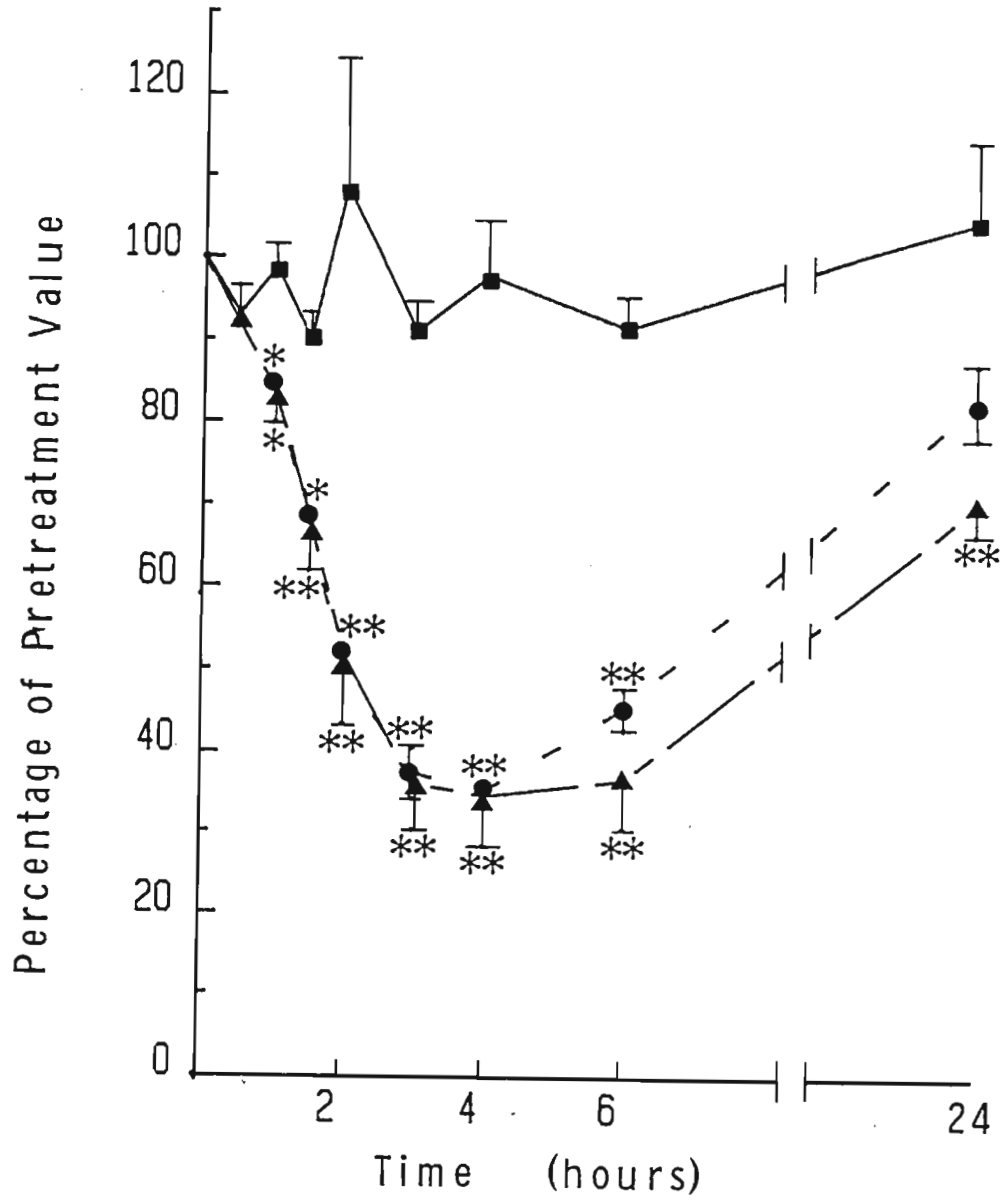

FIG 3.8 Percentage change in the concentration of oestradiol-17β (mean ± S.E.M.) at 12 - 18 weeks gestation after a single tablet of Epostane 50mg (●——●) and 100mg (▲——▲) or placebo (■——■). There were significance differences from the placebo group (* $p < 0.01$).

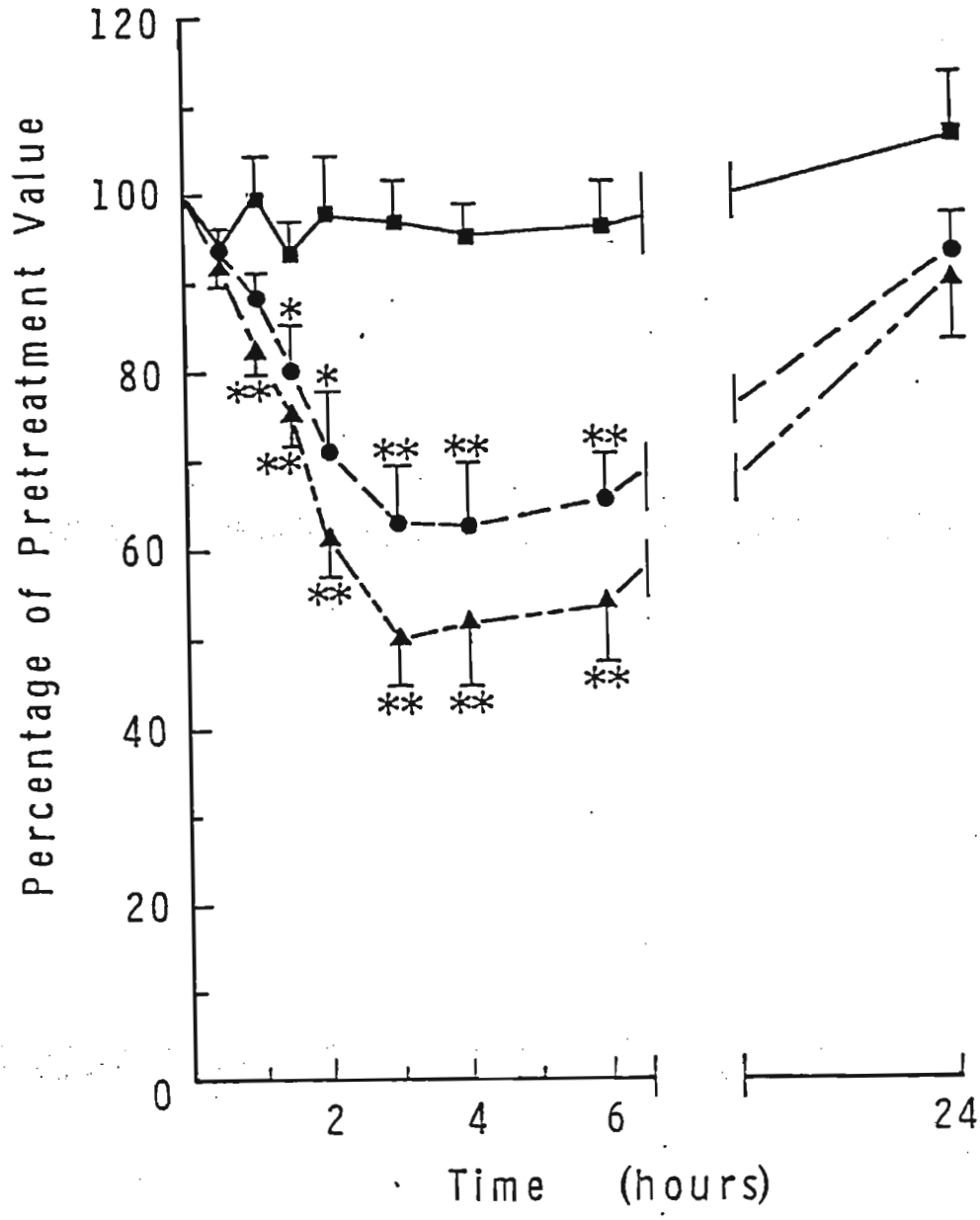

FIG 3.9 Percentage change in the concentration of oestradiol-17β (mean ± S.E.M.) after a single tablet of Epostane 50mg (●——●) and 100mg (▲——▲) or placebo (■——■). All gestational age groups combined, n=15. There were significance drug-related effects (*p < 0.05, ** p < 0.01).

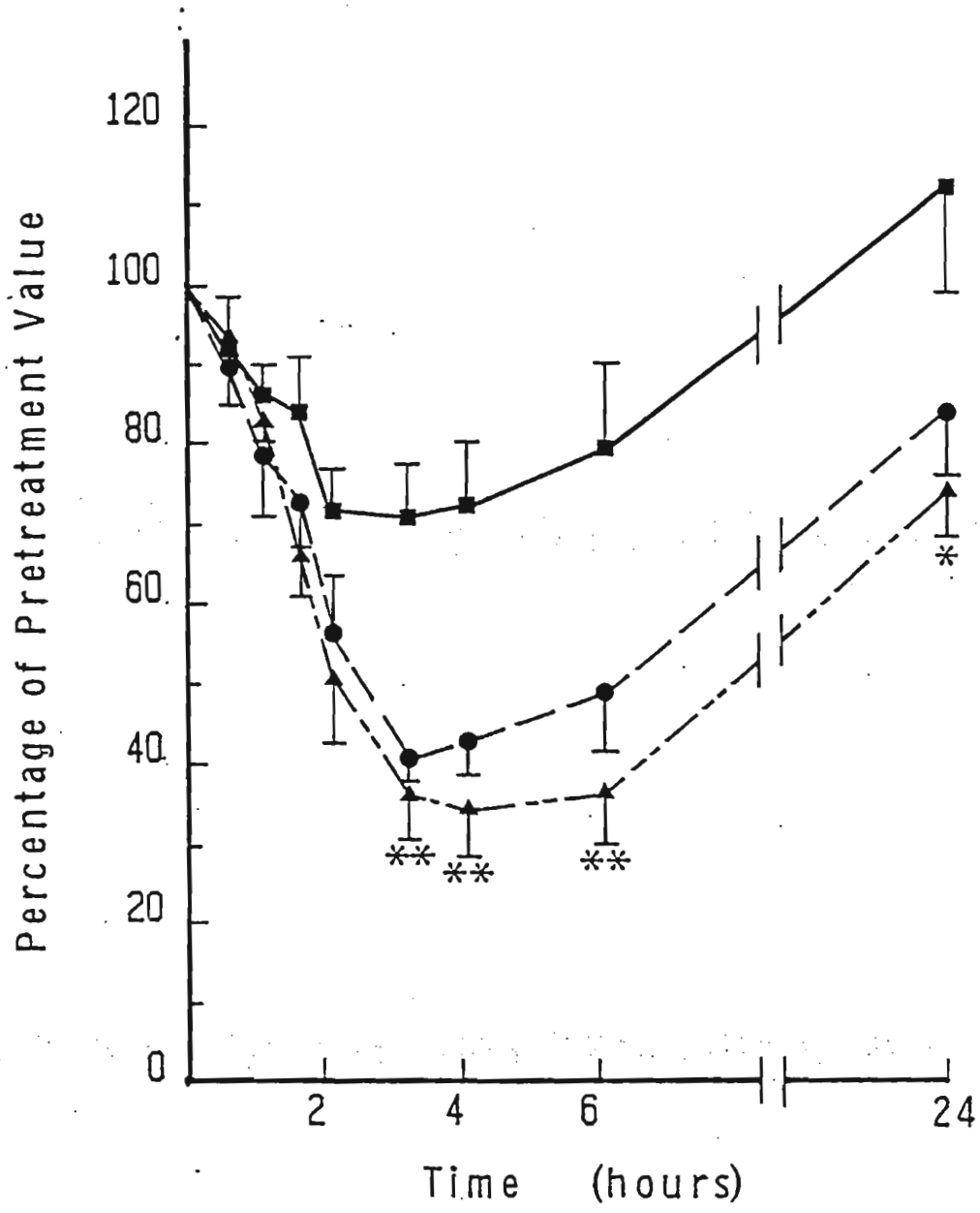

FIG 3.10 Percentage change in the concentration of oestradiol-17β (mean ± S.E.M.) after Epostane (100mg) in the three gestational age groups.

5 -7 weeks (■——■),

8 - 11 weeks (●——●) and 12 - 18 weeks

(▲——▲). There was a significant

gestational age-related effect (* $p < 0.05$, ** $p < 0.001$).

TABLE 3.3 The concentration of oestradiol -17β (nmol/l) after a single tablet of Epostane (50 or 100 mg) or placebo in early pregnancy.

Gestational Age Group (weeks)	Dosage of Epostane (mg)	Initial Value (nmol/l)	Percentage Change at 4 hrs	Percentage Change at 24 hrs
5-7	0	4.92 ± 1.08	95.4 ± 12.0	111.1 ± 18.1
	50	4.12 ± 0.48	86.8 ± 9.4	102.5 ± 10.1
	100	3.01 ± 0.73	*73.0 ± 9.9	116.8 ± 12.5
8-11	0	7.4 ± 1.8	92.2 ± 2.2	102.2 ± 2.3
	50	6.0 ± 1.7	*66.0 ± 7.7	93.2 ± 6.0
	100	9.4 ± 0.8	**44.5 ± 4.8	83.8 ± 8.3
12-18	0	23.1 ± 0.7	97.9 ± 6.9	104.8 ± 9.8
	50	24.7 ± 4.9	**35.3 ± 2.2	82.9 ± 4.4
	100	23.2 ± 5.0	**34.5 ± 6.1	70.1 ± 3.4

Values represent mean ± S.E.M. (N=5 at each dose)
Significant differences from the placebo group are indicated (*P < 0.05; **P < 0.01).

There was a significant dose-related effect. When the data from all subjects was combined, treatment with 50 mg and 100 mg of Epostane was shown to cause a decline in serum oestradiol-17β to 62.7 ± 6.8% and 51.6 ± 6.6% of the pretreatment value respectively ($p < 0.001$) (Fig 3.9). The decline in serum oestradiol-17β levels was also related to the gestational age. After 100 mg Epostane serum oestradiol-17β levels declined at four hours to 73.0 ± 9.9%, 44.5 ±

4.8% to 34.5 ± 6.1% of the pretreatment values in the three respective gestational age groups (Fig 3.10).

3. Serum Cortisol.

The mean serum cortisol level in the placebo group in each gestational age group followed the expected daily diurnal pattern (Fig 3.11, 3.12, 3.13). The concentration in the treated groups did not differ significantly from this curve. There was no significant drug- or gestation- related effect (Table 3.4).

TABLE 3.4 The concentration of cortisol (nmol/l) after a single tablet of Epostane (50 or 100 mg) or placebo in early pregnancy.

Gestational Age Group (weeks)	Dosage of Epostane (mg)	Initial Value (nmol/l)	Percentage Change at 4 hrs	Percentage Change at 24 hours
5-7	0	477.7 ± 73.5	65.9 ± 9.0	82.1 ± 17.3
	50	442.5 ± 34.6	71.0 ±14.3	120.2 ± 20.3
	100	687.0 ±113.1	43.1 ± 4.1	76.8 ± 13.4
8-11	0	445.7 ±107.0	75.2 ± 7.0	102.5 ± 11.0
	50	587.2 ±136.1	70.1 ±10.3	139.3 ± 30.1
	100	610.4 ±147.8	46.2 ± 7.6	108.4 ±16.0
12-18	0	516.1 ± 68.0	89.1 ± 10.2	80.8 ± 4.3
	50	853.7 ± 79.0	50.2 ± 4.9	80.5 ± 8.4
	100	514.6 ± 93.2	87.5 ± 16.7	102.0 ±10.5

Values represent mean ± S.E.M. (N=5 at each dose)

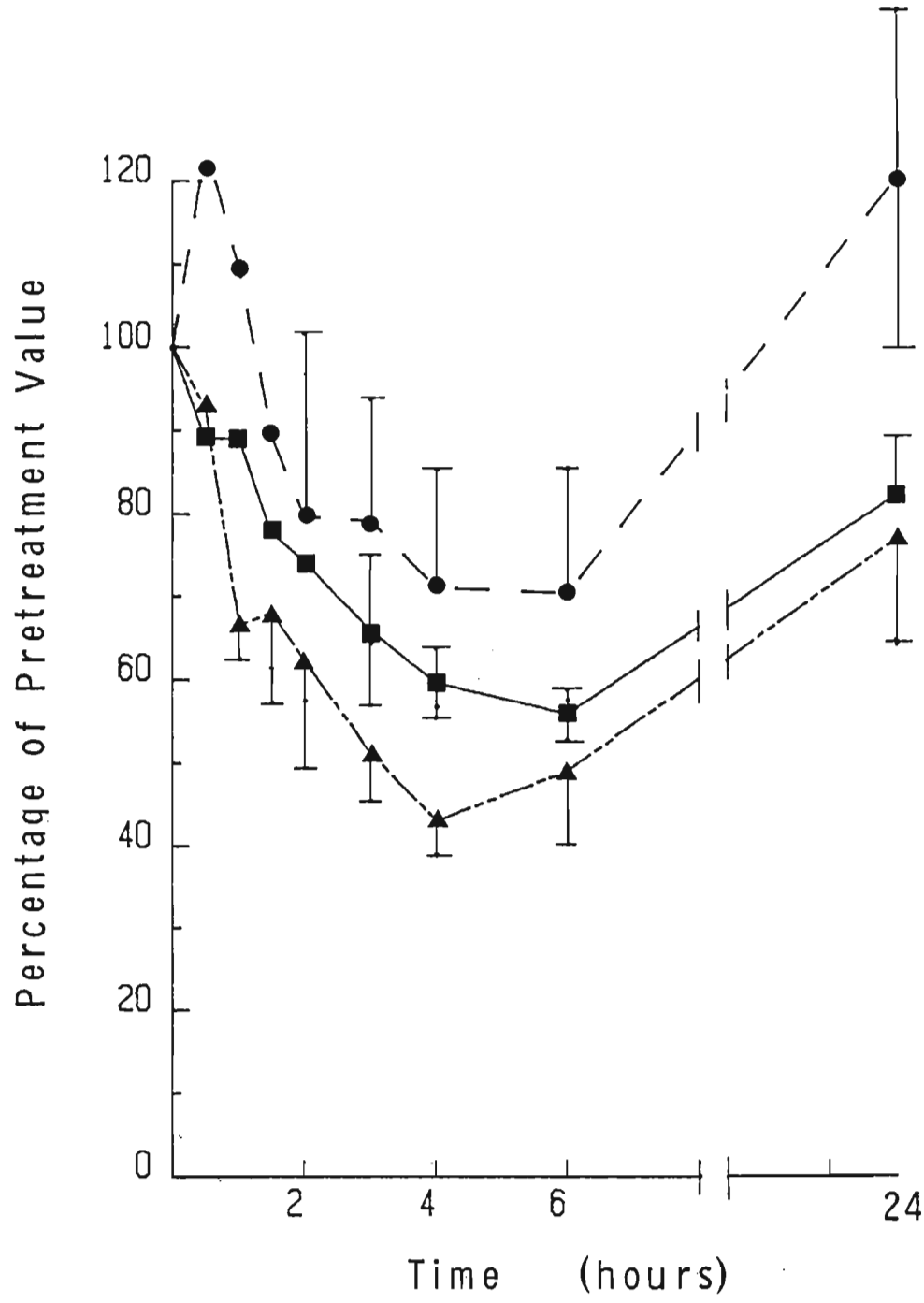

FIG 3.11 Percentage change in the concentration of cortisol (mean ± S.E.M.) at 5 - 7 weeks gestation after a single tablet of Epostane 50mg (●——●) and 100mg (▲——▲) or placebo (■——■). The treated groups did not differ significantly from the control.

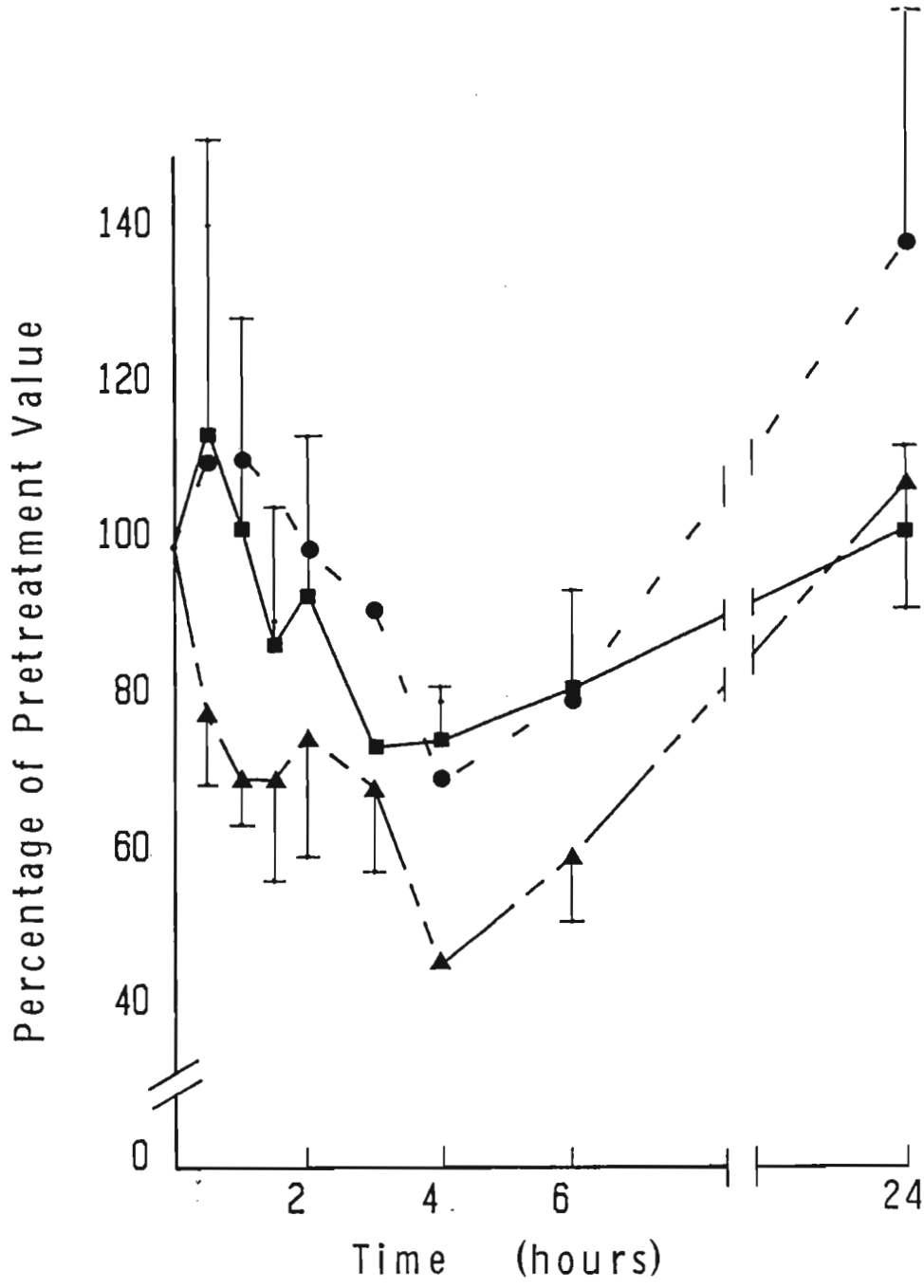

FIG 3.12 Percentage change in the concentration of cortisol (mean ± S.E.M.) at 8 - 11 weeks gestation after a single tablet of Epostane 50mg (●——●) and 100mg (▲——▲) or placebo (■——■). The treated groups did not differ significantly from the control.

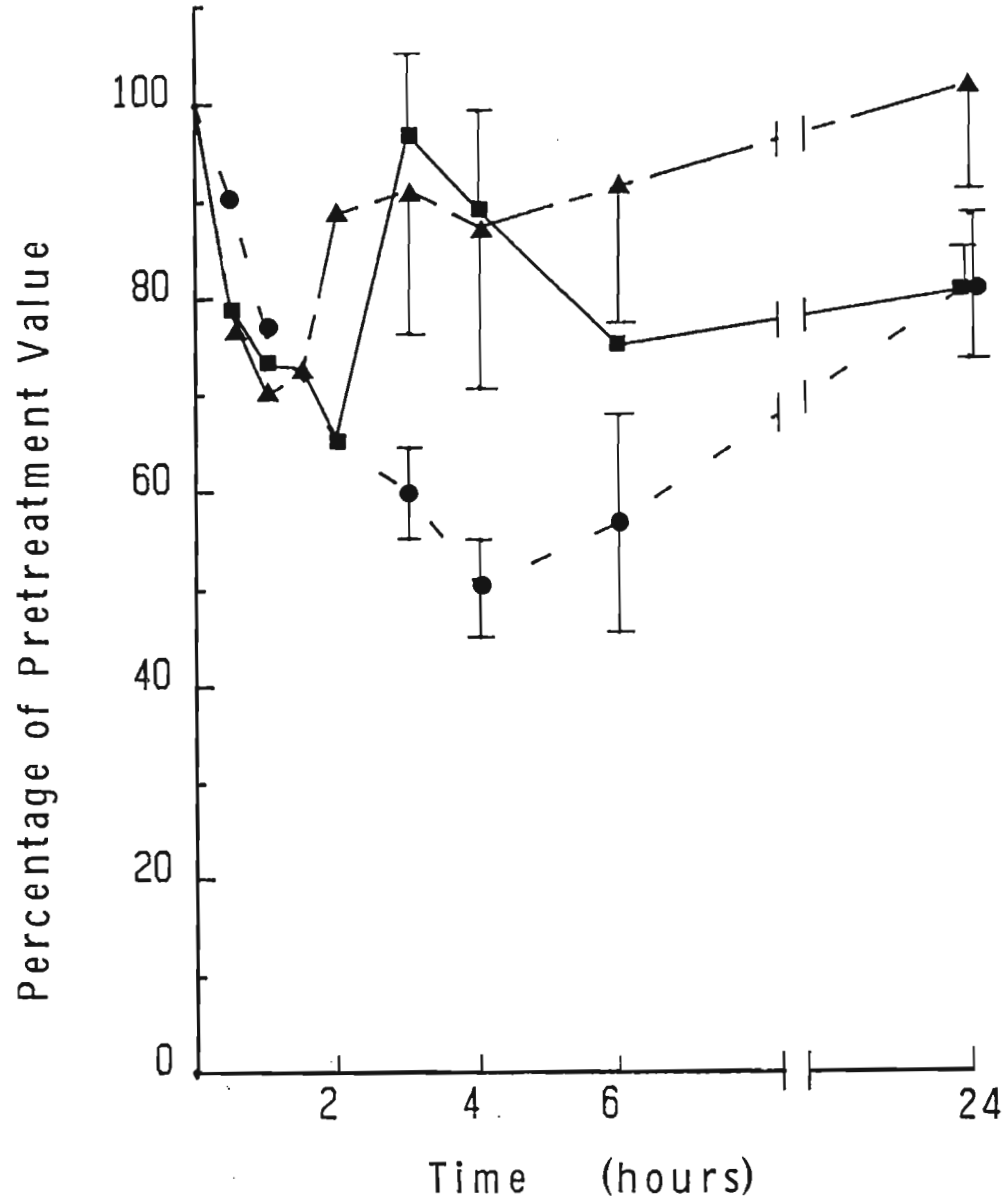

FIG 3.13 Percentage change in the concentration of cortisol (mean ± S.E.M.) at 12 - 18 weeks gestation after a single tablet of Epostane 50mg (●——●) and 100mg (▲——▲) or placebo (■——■). The treated groups did not differ significantly from the control.

Haematology and Biochemistry

There are no significant differences between the haematological and biochemical indices taken before and after treatment in any of the above groups.

TRIAL 2

Multiple dose study (Epostane 300mg, 400mg, and Placebo).

Method

Fifteen subjects between 12 and 18 weeks gestation were selected as described above. Ten entered a double-blind controlled trial of placebo and 400 mg Epostane (100 mg 6 hourly). Another five subjects (uncontrolled) received 300 mg Epostane (100 mg 8 hourly) to determine if there were any significant differences between 300 mg and 400 mg Epostane in a 24 hour period. Intrauterine pressure monitoring was performed in this subgroup.

The study design was similar to the first trial. All subjects were admitted 24 hours prior to planned termination of pregnancy. The first study (placebo and 400 mg Epostane) began at 1200 hours. Samples were taken three hourly with the tablet administered after alternate samples.

In the study using 300 mg Epostane (100 mg 8 hourly), treatment commenced at 1500 hours, blood samples were taken four hourly and tablets were administered after alternate samples. Before treatment began a No.12 Foley Catheter was

carefully inserted through the cervix to a depth of 10 cm. The procedure did not initiate bleeding or pain in any subject. The catheter balloon was filled with 10 ml of sterile water and the lumen filled with sterile normal saline and connected to a transducer (Bell & Howell, Type 4-422-001-10B4MS) which was held at the level of the cervix. Pressure changes were amplified and recorded using a six channel, hot wire recorder (Devices Ltd, Kent, United Kingdom) which had been previously calibrated to reach a full scale deflection with a pressure change of 100 mmHg. This pressure monitoring was performed for one hour at eight hourly intervals.

Results

Clinical Effect

Three of the five women treated with 400 mg Epostane (100 mg six hourly) reported mild, transient, 'period-like' pains. These began at 10, 19 and 19 1/2 hours respectively after treatment and continued for 1-2 hours. The pain was described as a mild cramp and was not severe. There was no bleeding and no change was detectable on examination of the cervix. The placebo group denied symptoms. There were no reported side effects.

In the trial of 300 mg Epostane (100 mg eight hourly) there were no symptoms nor recordable uterine activity in the first 15 hours. Three of the five treated women reported mild transient 'period-like' pains at 15, 16 and 18 hours respectively. These symptoms coincided with recordable

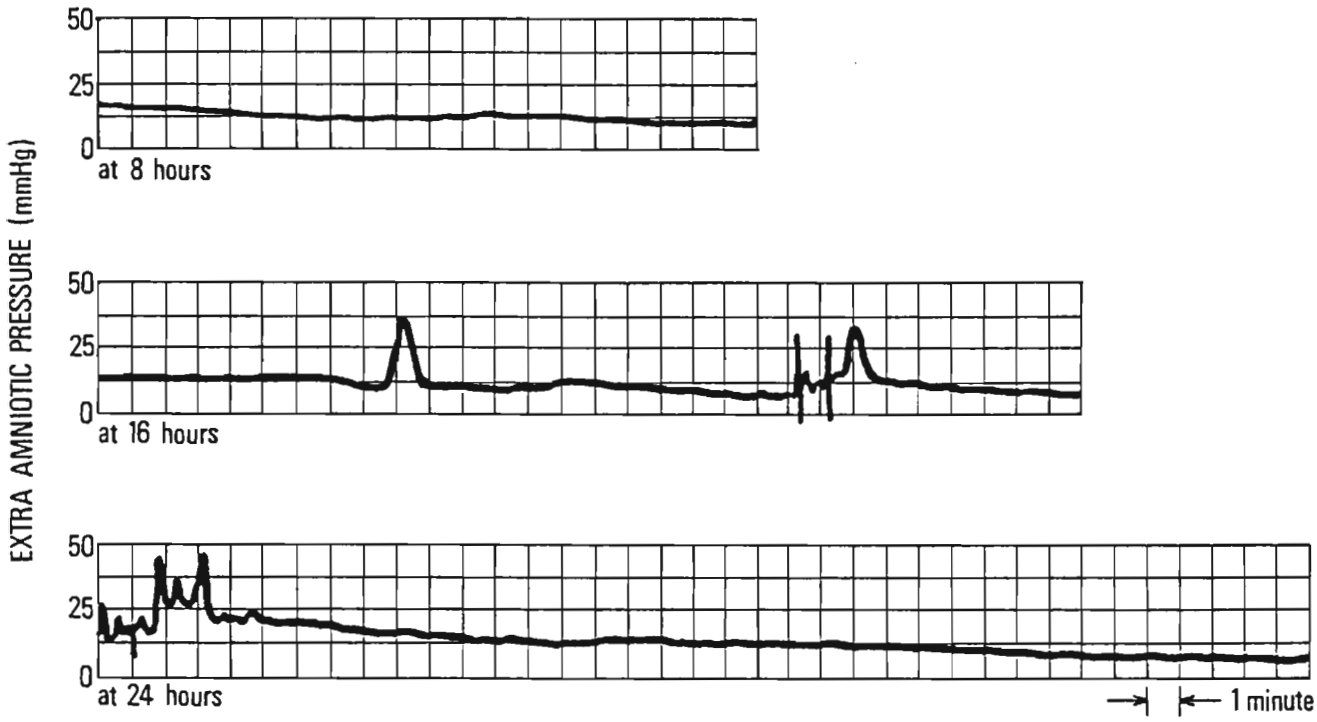

Fig.3.14 Extra-amniotic pressure changes (mm Hg) after Epostane (300mg) (Trial 2) recorded via a transcervical catheter on a Bell Howell transducer (Subject No.3). Three time intervals are demonstrated; 8, 16 and 24 hours after treatment commenced.

uterine activity (Fig 3.14). The two patients who denied symptoms had no recordable uterine activity.

Hormone Measurements

1. Serum Progesterone:

The concentration of serum progesterone fell significantly after multiple dose Epostane. In the trial of 400 mg Epostane (100 mg 6 hourly), the concentration of serum progesterone fell from 141.9 ± 14.3 nmol/l to a nadir of 17.2 ± 3.4 nmol/l at 21 hours after which represents a decline to 11.8% of the pretreatment value. In the placebo group, the concentration of serum progesterone did not change significantly (Fig 3.15). In both treatment groups, the serum progesterone level was below the critical value described by Csapo and Pulkkinen (1978) for at least six hours. There was no significant difference between the two treatment regimens. Multiple-dose treatment suppressed the serum progesterone level throughout the treatment.

2. Serum Oestradiol-17β .

There was a decline in the concentrations of serum oestradiol-17β after Epostane to values which were significantly different from the pretreatment value at all time intervals (Fig 3.16). The magnitude of the decline and its time course followed that of serum progesterone. In the placebo group, there was no significant variation from the pretreatment value of 11.1 ± 2.4 nmol/l throughout the 24 hour study period. After 300 mg Epostane the concentrations

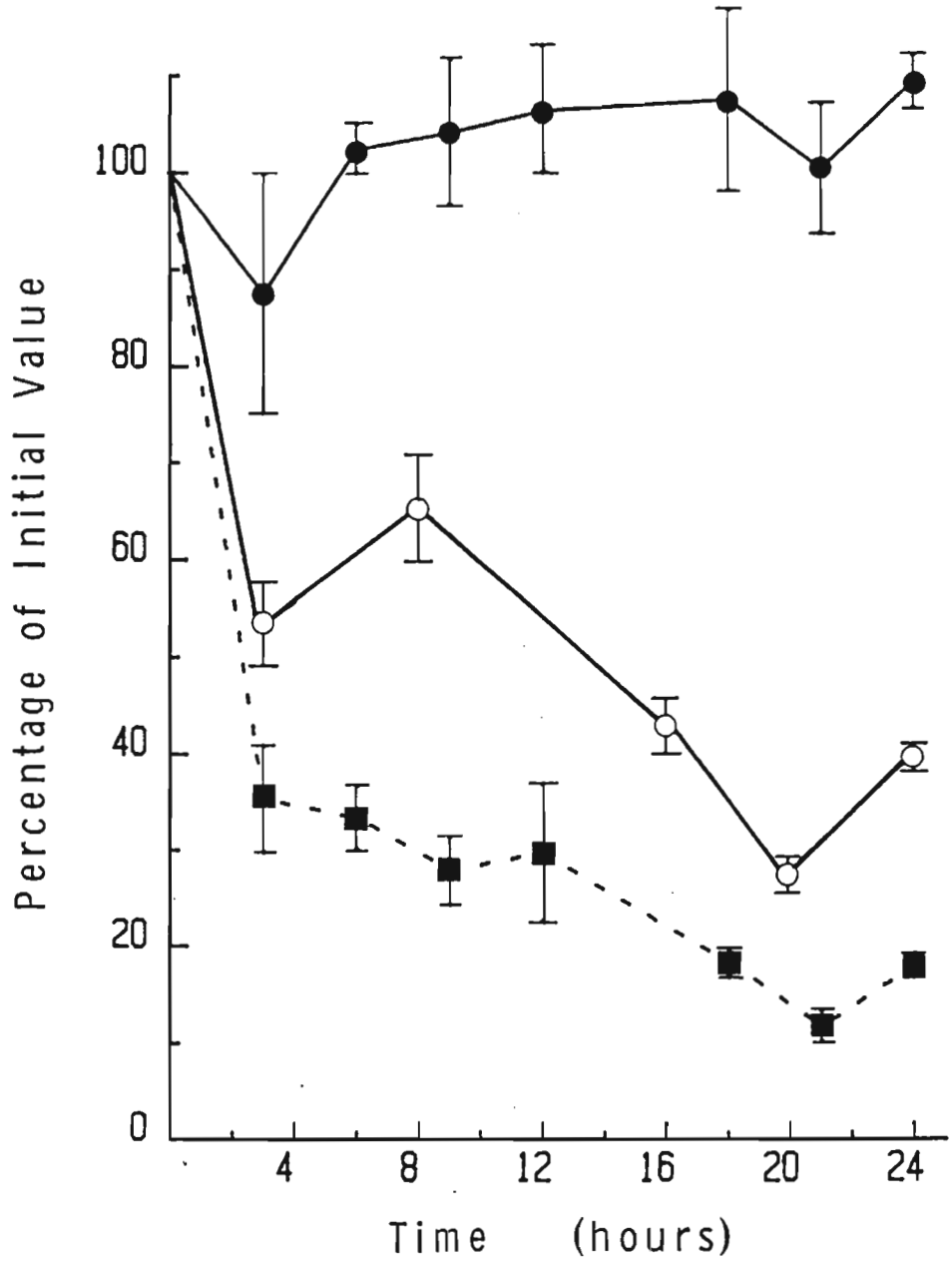

FIG 3.15 Percentage change in the concentration of progesterone (mean ± S.E.M.) at 12 - 18 weeks gestation after Epostane 300mg (○——○) and 400mg (■——■) or placebo (●——●). The treated groups differed significantly from the control group at all time intervals ($p < 0.01$). There was no difference between the treatment groups.

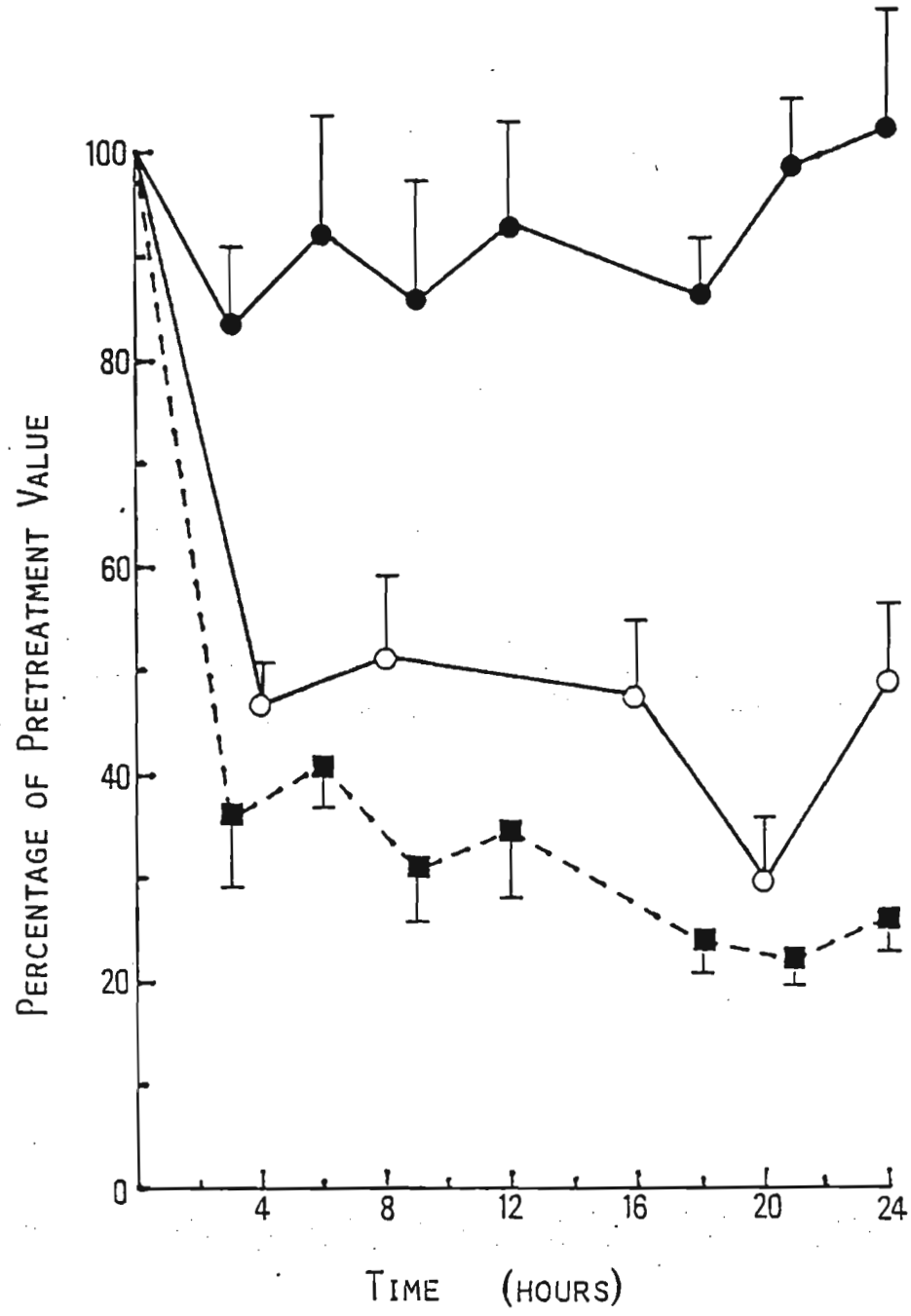

FIG 3.16 Percentage change in the concentration of oestradiol-17 β (mean ± S.E.M.) at 12 - 18 weeks gestation after Epostane 300mg (○——○) and 400mg (■——■) or placebo (●——●). The treated groups differed significantly from the control group at all time intervals ($p < 0.01$). There was no difference between the treatment groups.

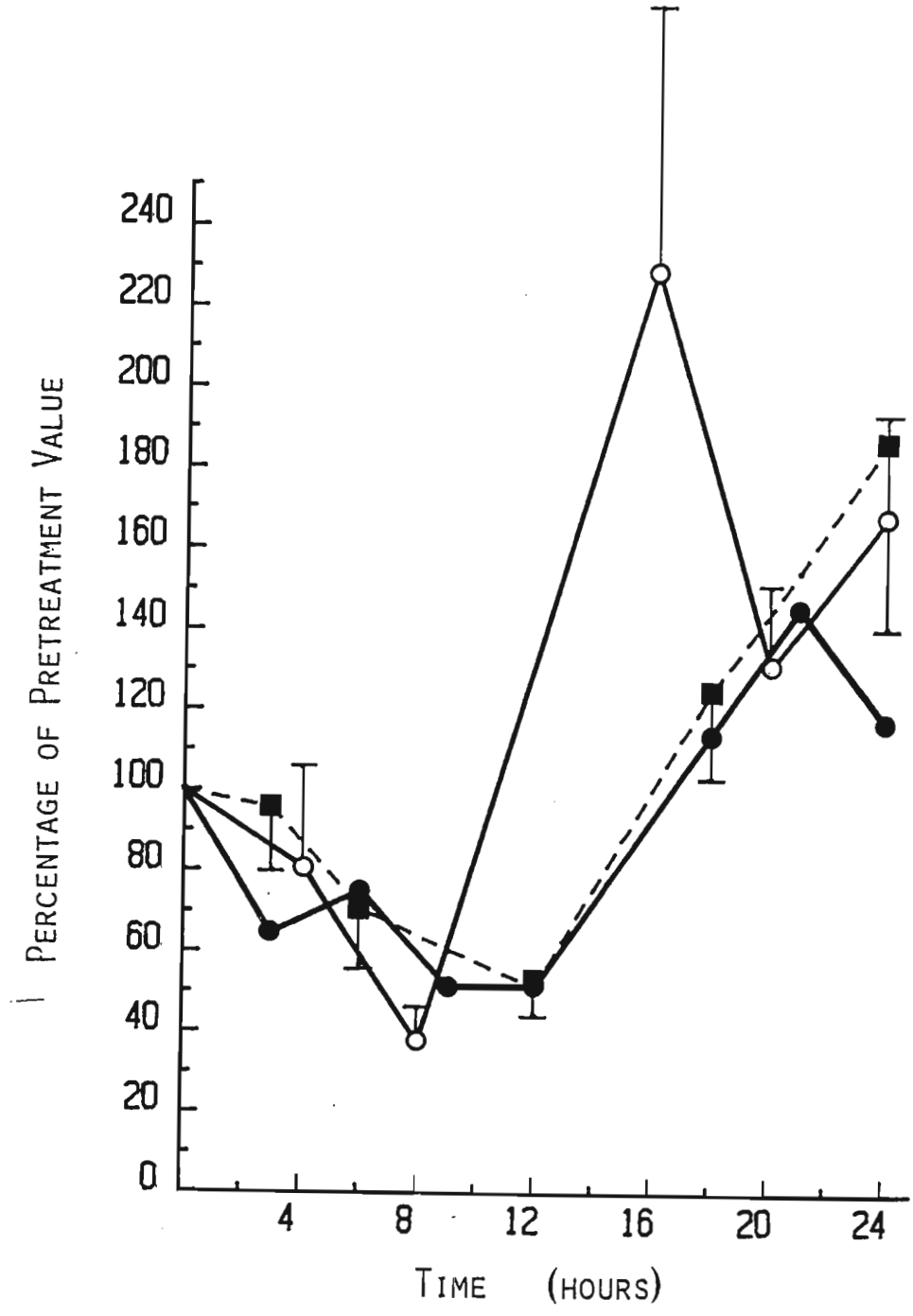

FIG 3.17 Percentage change in the concentration of cortisol (mean ± S.E.M.) at 12 - 18 weeks gestation after Epostane 300mg (○——○) and 400mg (■——■) or placebo (●——●). There was no significant difference between groups when values standardised for time of day.

of serum oestradiol-17β fell from 17.4 ± 3.4 nmol/l to 4.5 ± 0.5 nmol/l at 24 hours (a decline to 30% of the pretreatment value). After 400 mg Epostane levels fell from a pretreatment value of 15.4 ± 2.3 nmol/l to reach a nadir of 3.35 ± 0.63 nmol/l (a decline to 22% of the pretreatment value at 21 hours). There was no significant difference between the treatment groups.

3. Serum Cortisol.

The peripheral concentration of serum cortisol in the placebo group followed the expected daily diurnal variation (Fig 3.17). The concentration in the treated groups did not differ significantly from this curve if the curve produced after treatment with 300mg Epostane is displaced to the right to account for the change in sampling time.

Haematology and Biochemistry

There are no significant differences between the haematological and biochemical indices taken before and after treatment in any of the above groups.

TRIAL 3

Epostane 1500 mg (100 mg eight hourly for five days).

Method

Five volunteers were selected as described above. The trial was performed in the 7 days between approval for termination and surgical termination. All subjects were between 8-12 weeks gestation. There was no control group. Immediately

after selection a pretreatment blood sample was taken. The subjects returned daily and samples were taken by single venepucture from a forearm vein. Blood pressure and pulse were measured and subjects were questioned as to effects of medication. All samples were taken between 0800 and 1000 hours. The tablet was administered immediately after the second pretreatment sample and subsequent tablets were self administered eight hourly for five consecutive days. Subjects were informed that they could withdraw from the study at any time.

RESULTS

Clinical effect

Four of the five subjects had no symptoms. In particular, there were no side effects attributable to the drug and no evidence of uterine activity and/or vaginal bleeding. The fifth subject reported vaginal bleeding on the second day and at her request the pregnancy was terminated surgically. The cervical os was not dilated or effaced. Histological examination of the products of conception showed no differences from those obtained after termination of pregnancy at a similar gestation.

1. Serum Progesterone

There was a significant fall in serum progesterone levels after treatment with 1500 mg Epostane; the pretreatment value of 72.5 ± 12.1 nmol/l fell to reach a nadir of 13.1 ± 1.4 nmol/l (a fall to 18% of the pretreatment value) four

days after treatment ($p < 0.001$) (Table 3.5) (Fig 3.18). In the five subjects, the peripheral concentration of serum progesterone was below the critical value described by Csapo and Pulkkinen (1978) for four to five days.

There was not a major difference in the extent of decline in the level progesterone achieved with Epostane 1500 mg (100 mg 8 hourly for five days) over that achieved after a single 100 mg tablet. After Epostane 100 mg serum progesterone fell from 68.1 to 29.5 nmol/l a decline to 43 per cent of the pretreatment value. After Epostane 1500 mg the progesterone level declined from 72.5 to 13.1 nmol/l at 4 days a decline to 18 per cent of the pretreatment value. The major difference was in the duration of supression of serum progesterone concentration.

TABLE 3.5 The concentration of progesterone (nmol/l) in 5 pregnant subjects treated with 1500 mg Epostane (100 mg 8 hourly for five days.

Subject Number	Gestational Age (weeks)	Pretreatment Value	Serum Progesterone nmol/l Day 1	2	4	6	8
1	9	96.4	36.8	28.3	15.9	26.1	-
2	7	54.4	24.8	22.8	11.1	35.0	46.7
3	11	101.4	33.7	15.9	-	37.2	73.8
4	7	59.9	19.4	10.2	12.4	47.9	67.7
5	8	51.2	19.8	12.7	-	-	-**
Mean		72.5	26.9	17.9	13.1	35.3	62.7

**Withdrew from study after 2 completed days.

2. Serum Oestradiol-17β.

There was a significant decline in serum oestradiol-17β concentration after treatment with Epostane (100 mg 8 hourly for five days). The concentration of serum oestradiol-17β fell from a pretreatment level of 7.85 ± 0.26 nmol/l to 48% of the pretreatment value one day later (Fig 3.19).

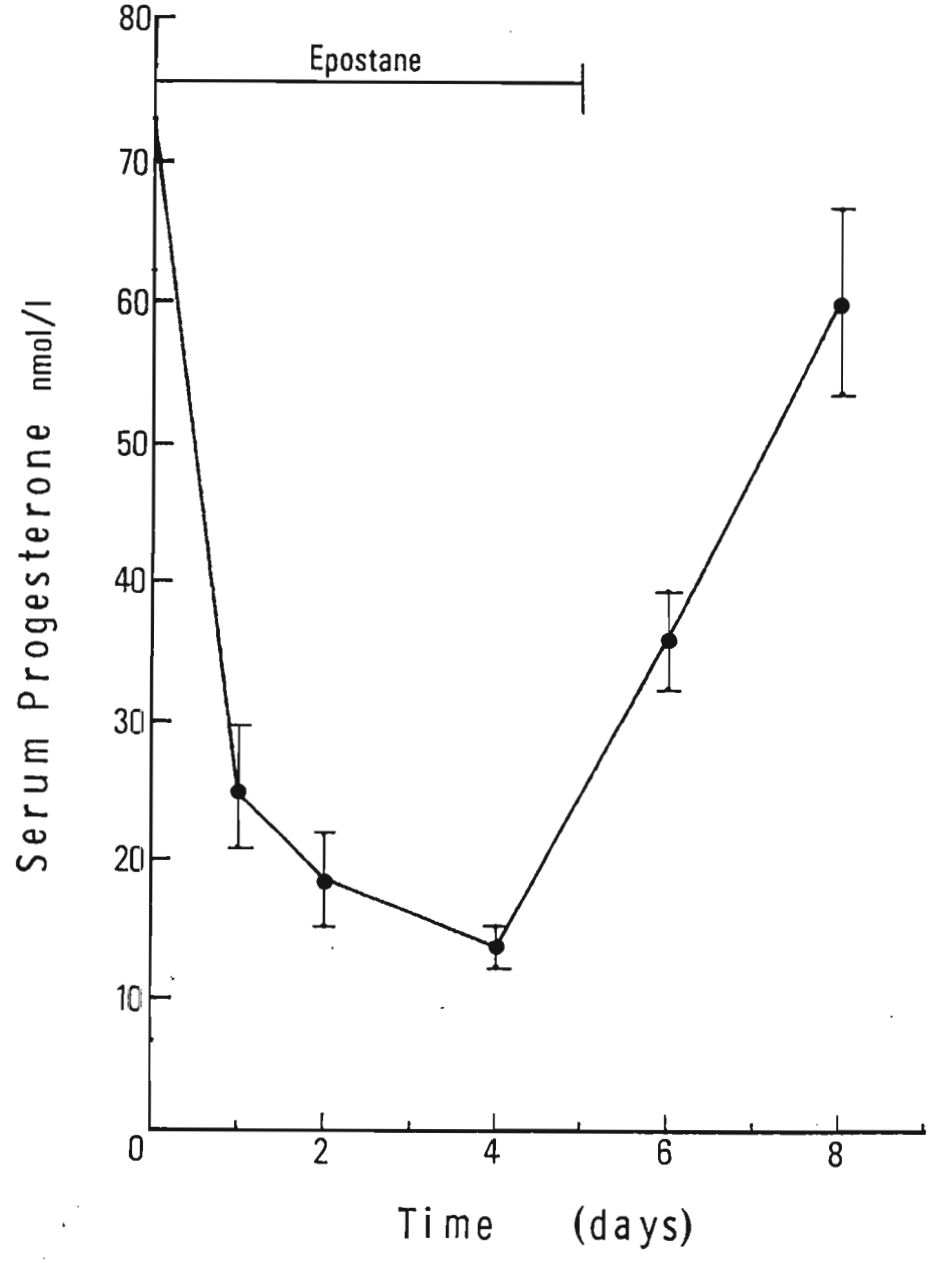

FIG 3.18 Changes in the concentration of progesterone (mean ± S.E.M.) at 8 - 11 weeks gestation after Epostane (100mg 8 hourly for 5 days).

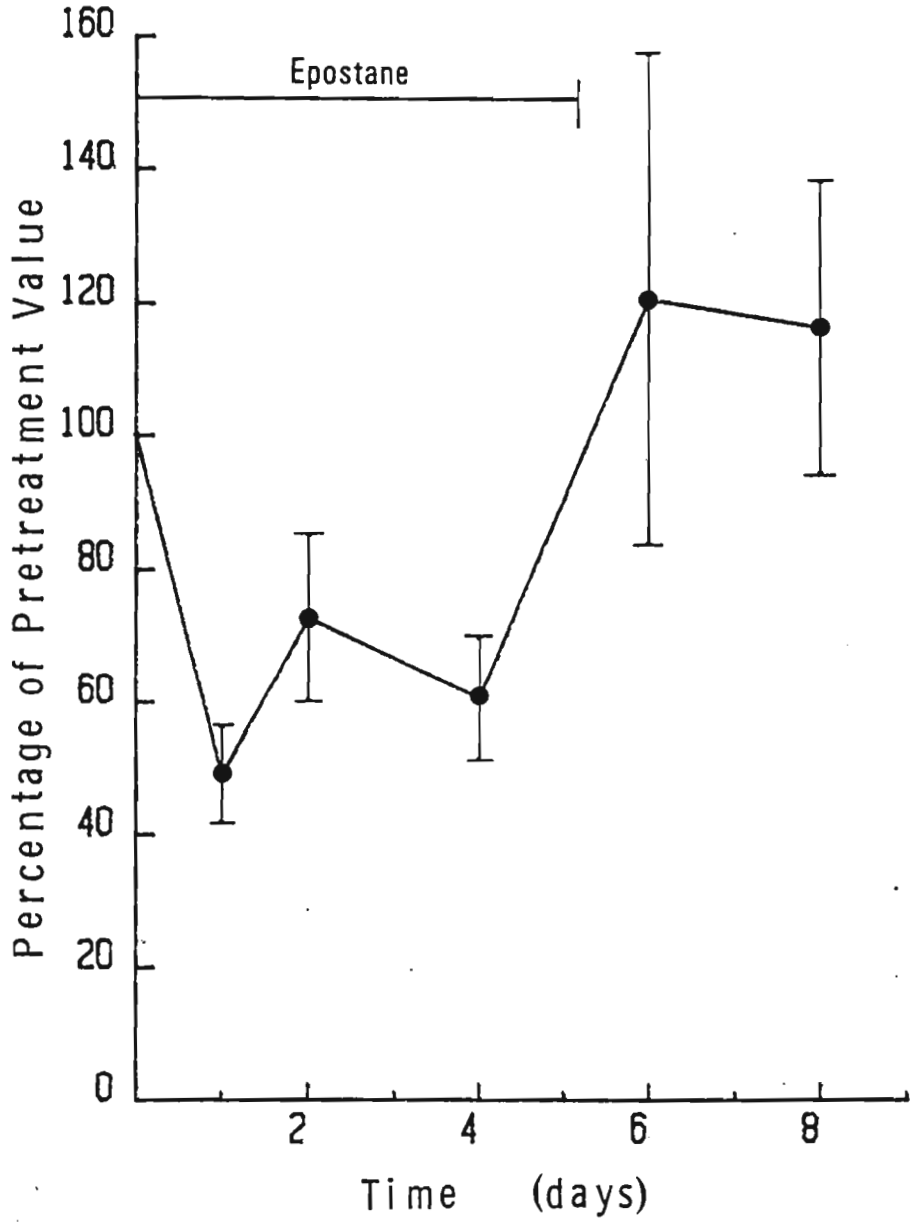

FIG 3.19 Percentage change in the concentration of oestradiol-17β (mean ± S.E.M.) at 8 - 11 weeks gestation after Epostane (100mg 8 hourly for 5 days).

Peripheral serum cortisol concentrations did not differ significantly from the pretreatment value throughout the study (Fig 3.20).

Haematology and Biochemistry

There was no signification change in the haematological or biochemical indices after treatment with 1500 mg Epostane.

DISCUSSION

This study demonstrates that competitive inhibition of 3β -HSD results in a significant decline in the concentration of serum progesterone. The decline was dose- and gestation-related and was not associated with side effects or clinical evidence of toxicity. However, despite suppression of progesterone production after 1500 mg Epostane to levels of 10 to 15% of the pretreatment value, the pregnancies continued.

Csapo & Pulkkinen (1978) showed that following luteectomy in early pregnancy the level of serum progesterone fell and abortion resulted. In their study, whenever the peripheral serum progesterone level fell below 10 mg/ml (31.8 nmol/l) uterine activity was present and at 4 ng/ml (12.7 nmol/l) abortion occurred.

Invitro experiments with Epostane (Rabe T, personal communication) demonstrated an inhibitory effect of Epostane on human placental 3β -HSD in tissue culture. Creange et al. (1981) in the rat and the rhesus monkey showed that treatment

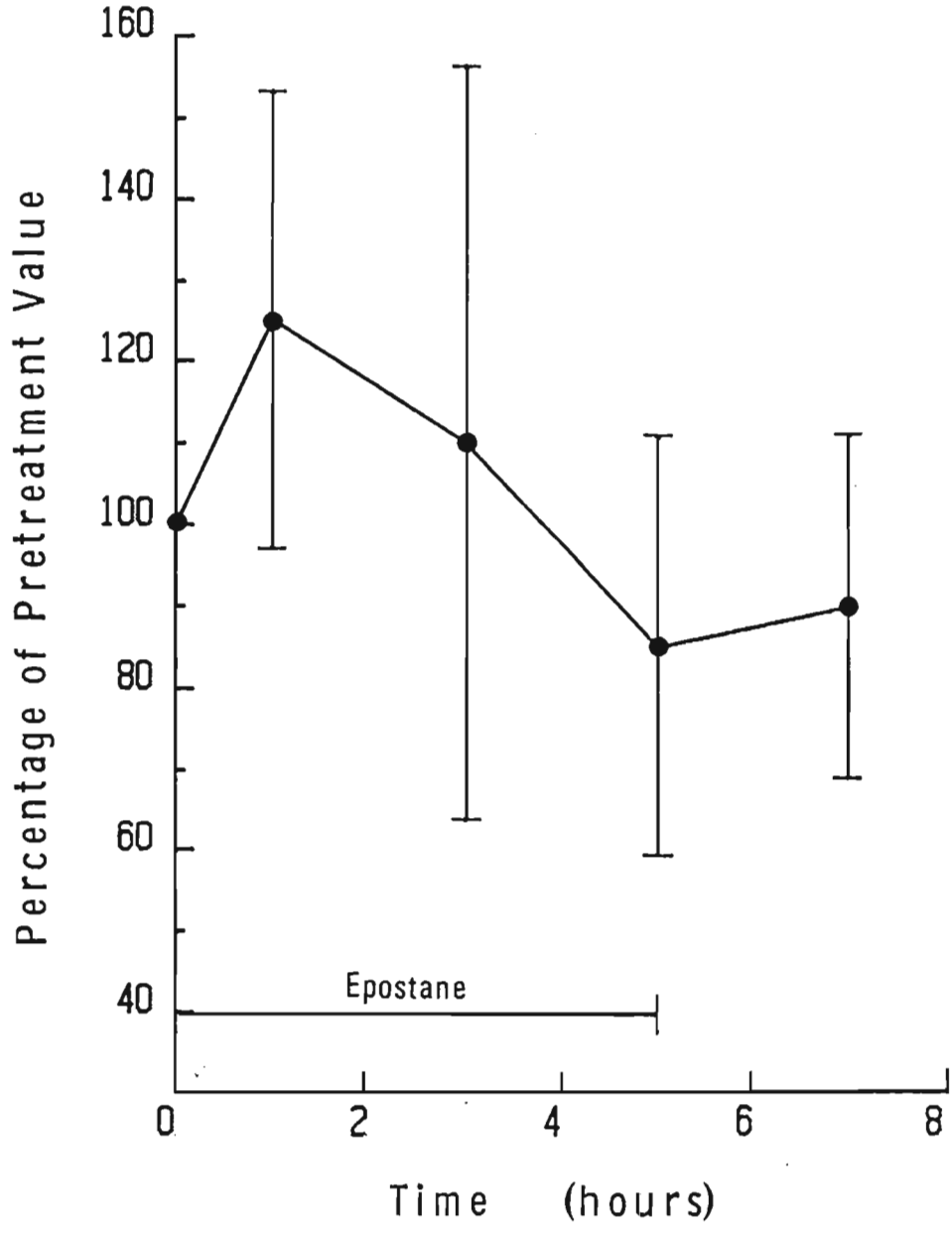

FIG 3.20 Percentage change in the concentration of cortisol (mean ± S.E.M.) at 8 - 11 weeks gestation after Epostane (100mg 8 hourly for 5 days).

with a single dose of Epostane resulted in a fall in serum progesterone levels and abortion occurred.

The initial single-dose trial demonstrated for the first time that Epostane is effective invivo as an inhibitor of 3β -HSD in the human. The magnitude and the duration of suppression of progesterone production is dose-related (Fig 3.4). Despite a fall to 38.9 nmol/l (35.5 ± 4.1% of the pretreatment value) after 100 mg Epostane in the 12-18 week gestation group there was no clinical effect. In order to ascertain whether the lack of clinical effect was due to insufficient degree or duration of suppression of progesterone production increased dosage of Epostane was given in further multiple dose trials. These trials demonstrated a prolonged suppression of progesterone concentration and further lowering of values at the nadir. Epostane (100 mg) at 8-12 weeks gestation caused a decline to 43% of the pretreatment value whereas Epostane (1500mg) caused a fall to 20% of the pretreatment value. The effects of Epostane 300mg and 400mg on progesterone levels were similar. For this reason, a dose of 100 mg eight hourly for five days was chosen in the final trial to determine if prolonged suppression of progesterone production would induce a clinical effect.

Epostane (1500 mg) caused a highly significant decline in the concentration of serum progesterone with prolonged suppression and achieved the aim of reducing the serum

progesterone concentration below the minimal values following luteectomy described by Csapo & Pulkkinen (1978). In the five treated subjects progesterone concentration was below this value (10 mg/ml, 31.8 nmol/l) for four to five days.

The degree of suppression of progesterone production was related to the duration of gestation which suggests that placental steroidogenesis is more susceptible to inhibition of 3β -HSD than ovarian steroidogenesis. In the study of the effects of a single dose of 100 mg Epostane in the 5-7 week group at which time the source of progesterone is predominantly ovarian the concentration of progesterone fell only to 51.1% of the pretreatment value but in in the 12-18 week group (predominantly placental production) the levels fell to 35.5 ± 4.2% ($p < 0.001$).

The fall in oestradiol-17β concentration was also dose- and gestation- related. In the 5-7 week group (ovarian production) 100 mg Epostane caused a fall to only 86.8 ± 9.4% of the pretreatment value ($p < 0.05$) whereas in the 12-18 week group (placental production), the fall was to 34.5 ± 6.1% of the pretreatment value ($p < 0.001$). There was an intermediate decline (44.5 ± 4.7%) in the intermediate group (8-12 week gestation). This indicates that placental oestradiol-17β production was inhibited by competitive inhibition of 3β-HSD by Epostane. Progesterone is synthesised entirely from pregnenolone via the pathway catalysed by the 3β -HSD enzyme. However, oestradiol-17β production can be

either via the delta 4 or 5 pathway. Although Epostane is known to inhibit the 3β -HSD enzyme involved in the conversion of pregnenolone to progesterone (Fig 1.11), it does not follow that it inhibits the conversion of 17-hydroxy-pregnenolone or DHEA to 17-hydroxyprogesterone or androstenedione respectively (Fig 1.12). There is some evidence that in the ovary the predominant pathway for oestradiol-17β synthesis is the delta 5 pathway (Ryan & Smith 1965).

In the 5-7 week group, oestradiol could be synthesised through pregnenolone, 17-hydroxypregnenolone, DHEA and androstenedione, bypassing progesterone. The placenta may lack the necessary delta 5 enzymes hence the inhibition of oestradiol-17β production at later gestational ages.

Serum cortisol concentration was not significantly affected despite a fall in serum progesterone levels to 35% of the pretreatment value in some groups. This indicates sparing of adrenal steroidogenesis which may be due to limited access of the drug or a different 3β -HSD enzyme in the adrenal.

The aim of this series of experiments was to assess the role of progesterone in the maintenance of pregnancy and the initiation of parturition. The development of a competitive inhibitor of 3β -HSD which had been shown previously invitro to inhibit progesterone production provided the ideal opportunity to reassess progesterone's role. It was not possible for ethical reasons to study the effect of Epostane in ongoing pregnancies and therefore the three groups

described above were selected. The work of Csapo (1972, 1978) has influenced attitudes to the role of progesterone in pregnancy maintenance and initiation of parturition. This author states that "progesterone is indispensible during early pregnancy and the corpus luteum is indispensible as long as it is a major source of progesterone". He infers from his studies that progesterone also is indispensible in late pregnancy, its "withdrawal" at term being a major factor in the initiation of parturition.

This trial of Epostane in early pregnancy showed that a fall in serum progesterone concentration similar to that achieved by luteectomy (Csapo and Pulkkinen, 1978) fails to cause abortion. (Fig 3.2). There are a number of possible explanations for this conflict:

1. The decline in serum progesterone concentrations achieved with Epostane was insufficent to initiate uterine activity: Luteectomy (Csapo and Pulkkinen, 1978) caused a fall in serum progesterone concentration from 37.8 7.6 to 10.5 2.2 nmol/l at 24 hours. Epostane 1500mg caused a fall from 72.5 12.1 nmol/l to 26.9 3.1 nmol/l 24 hours after tablet ingestion and maintained low values for 4-5 days.

A lower initial concentration of serum progesterone in the luteectomy group reflected an earlier gestational age (7 2 weeks) compared with 9 1 week in the current study. The percentage decline to 27% and 36% respectively of initial values and the rate of decline that occurred within 24 hours are comparable (Fig 3.21). However, the lower serum

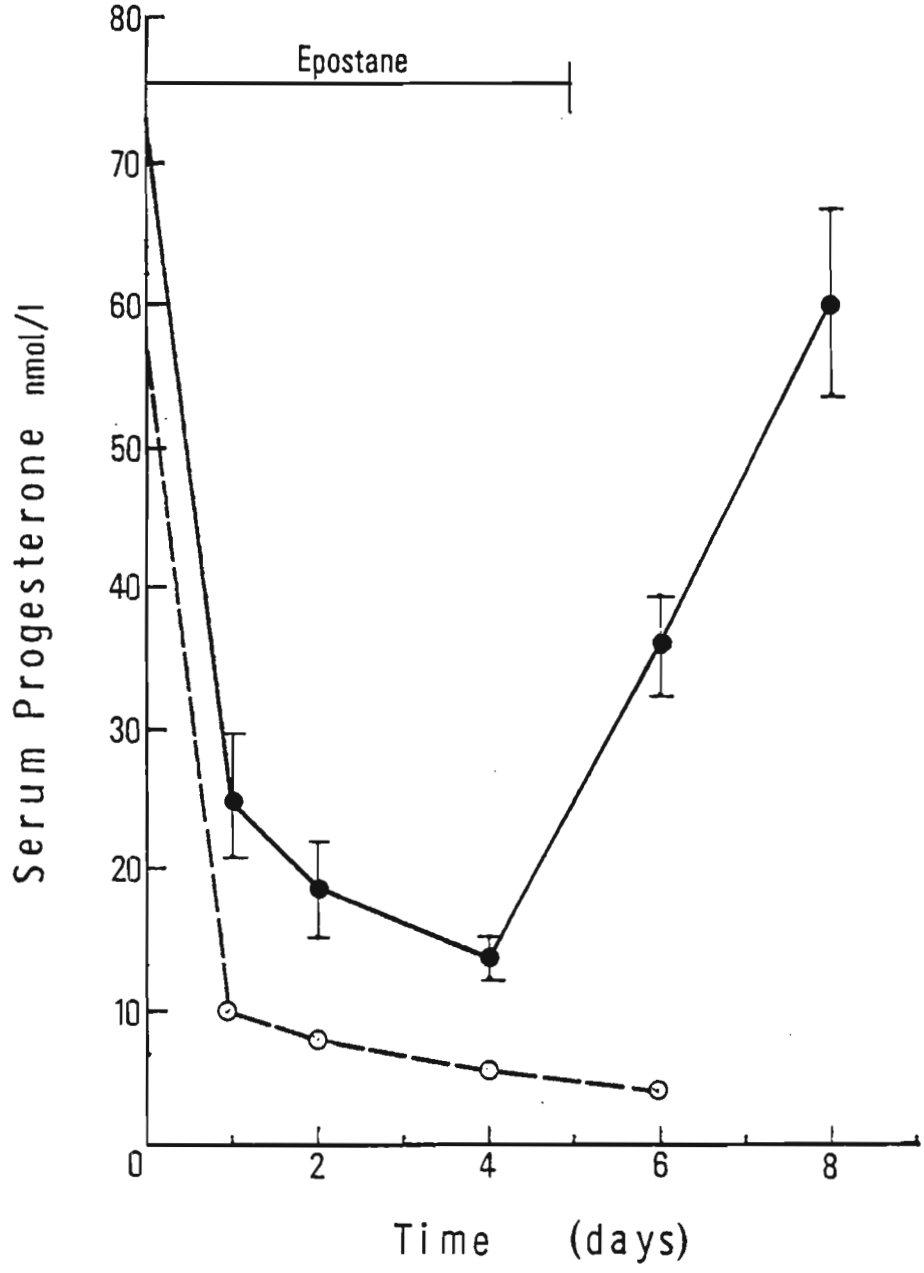

FIG 3.21 A comparison between the effects of Epostane (100mg 8 hourly for 5 days) (●——●) and luteectomy (○——○) on the concentration of progesterone (nmol/l) at 8 - 11 weeks gestation (Data of Csapo and Pulkinnen, 1978).

progesterone concentration after luteectomy (< 6 nmol/l) was not achieved after 1500 mg Epostane, (minimum 13.0 ± 1.4 nmol/l).

The suggested mechanism(s) of action of progesterone in maintaining uterine quiescence are either direct inhibition of uterine activity or inhibition of enzymes responsible for synthesis of prostaglandin and/or synthesis of other uterine stimulants. In the luteectomy study, abortion occurred whenever the serum progesterone concentration fell below 12.7 nmol/l and uterine activity was recorded whenever serum progesterone fell below 31.8 nmol/l (Csapo and Pulkkinen, 1978). Despite similar levels being achieved by inhibition of progesterone production with Epostane significant uterine activity did not occur. This demonstrates that neither a rapid change in progesterone levels, a fall to one third of pretreatment levels nor a moderate alteration in progesterone - oestrogen ratio will initiate uterine activity. A further decline in the concentration of pregesterone may initiate uterine activity, however as the alterations achieved in this study failed to do so it is unlikely that progesterone is directly involved in the initiation of uterine activity in the human.

2. Changing progesterone-oestrogen ratio:

The fall in serum progesterone concentration and the rise in serum oestradiol-17β concentration described in the ewe (Liggins et al, 1973) led to the suggestion that it is the

ratio of progesterone to oestrogen which is important in the control of human uterine activity. After luteectomy, both serum oestradiol-17 β and progesterone fall and abortion follows without a change in the progesterone-oestrogen ratio.

In the subjects treated with Epostane there is a variable effect on oestradiol-17β production at different gestations. In the early gestation group there was an alteration in the progesterone-oestrogen ratio which was not apparent in the later groups. After 1500 mg Epostane the serum progesterone levels fell to 19.3% of the pretreatment value and serum oestradiol-17β to 60.5% of the pretreatment value; despite this decrease in the progesterone-oestrogen ratio, uterine activity did not occur. Further experiments in which treatment with Epostane is combined with treatment with oestradiol-17β would be of interest.

3. The use of known uterine stimulants in the luteectomy study:

Csapo and Pulkkinen (1978) measured the effect of progesterone 'withdrawal' after luteectomy by the use of two uterine stimulants. They administered oxytocin (0.25 IU) daily and inserted an extra-amniotic catheter through the cervix for measurements of intrauterine pressure (A 60 min. record was made each day for seven to 10 days). Both are known uterine stimulants and can induce uterine activity It is conceivable that a lowering of the concentration of progesterone could change the uterus from a refractory organ to a reactive organ and that oxytocin and/or cervical trauma

could then lead to prostaglandin release, uterine activity and abortion.

CHAPTER 4

LUTEAL PHASE HUMAN MENSTRUAL CYCLE

Implantation, one of the critical steps in establishing pregnancy, requires the precise synchronisation of ovarian function with endometrial and embryonic development and depends on continued secretion of progesterone from the corpus luteum after ovulation has occurred. The corpus luteum together with its secretory product progesterone, has become a logical target for fertility control. Numerous agents including prostaglandins (Korda, Shutt, Smith, Shearman and Lyneham, 1975) and oestrogens (Gore, Caldwell and Speroff, 1973) have been tested for a disruptive effect on corpus luteal function. For various reasons these have had limited clinical use. A 3β -hydroxysteroid dehydrogenase (3β -HSD) inhibitor, Epostane, inhibits steroidogenesis in-vitro thereby causing a fall in the level of progesterone and has the potential to act as a luteolytic agent.

The pronounced changes in the genital tract during the menstrual cycle are controlled by the ovarian hormones oestrogen and progesterone. Following ovulation the Graafian follicle is transformed into a corpus luteum secreting predominantly progesterone which stimulates the full secretory activity of the endometrium in preparation for implantation. Progesterone is essential for both the development of the secretory endometrium and also its maintenance.

The normal ovulatory cycle is characterised by a progressive rise in the levels of progesterone from day 16 to day 20 of the cycle, a relatively stable plateau from day 20 to 24 and a decline from day 25 (Fig 4.1). Declining function of the

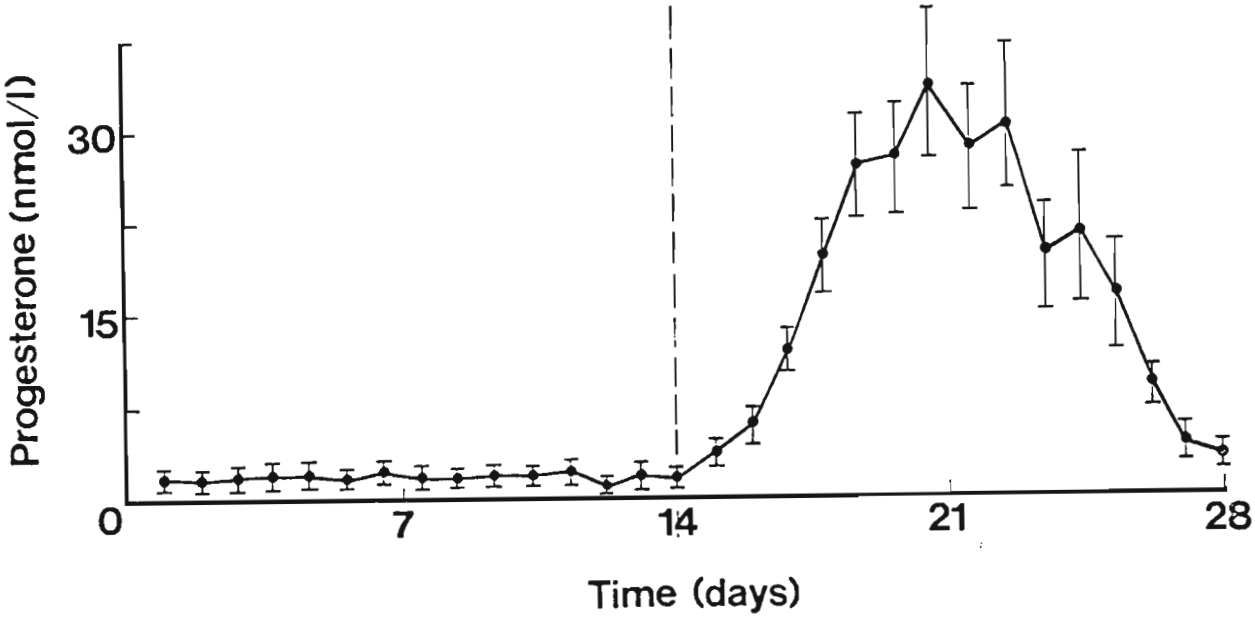

FIG 4.1 The concentration of progesterone throughout the menstrual cycle (Thorneycroft I H, Mishell D R, Stone S C (1971).

corpus luteal at the end of an ovulatory cycle leads to 'withdrawal' of progesterone 'support' to the endometrium; this results in shedding of the endometrium and menstruation. The effect on the endometrium of the decline in the level of progesterone is also demonstrated in the clinical situation when exogenous progesterone therapy is stopped such as after a cycle of combined oral contraceptive pill and after cyclical progesterone therapy for dysfunctional uterine bleeding.

Extensive investigation of the function of the corpus luteum and its susceptibility to external influences followed the American Department of Health proposals in 1969. These called on investigators:- "To examine the consequences of interrupted corpus luteal function in primates and humans at different periods in the reproductive process to help decide if means to inhibit corpus luteum function is a desirable contraceptive aim" (DHEW, 1969).

Csapo et al, (1972) conducted a series of experiments into the control of uterine activity by the corpus luteum. These authors investigated the effect of surgical removal of the corpus luteum in a group of pregnant women (cf Chapter 1) and also studied 32 non pregnant women. In the non-pregnant group luteectomy was performed at the time of abdominal sterilisation on day 21 of an ovulatory cycle. The non-pregnant subjects were divided into three groups;

1. Luteectomy without hormonal replacement (n=19);
2. Luteectomy plus progesterone replacement (100 mg daily for three days (n=8)

3. Luteectomy plus oestradiol-17β replacement (1mg daily for three days (n=14).

In subjects without hormone replacement (group 1), progesterone and oestradiol-17β levels fell rapidly and there was a progressive increase in the uterine pressure and in the response to oxytocin; menstruation followed at 38 ± 3 hours.

In the subjects receiving oestradiol-17β replacement (group 3), the serum oestradiol-17β level was maintained at pretreatment levels. The serum progesterone level fell rapidly, uterine pressure and the response to oxytocin increased similarly to the group without hormonal replacement; menstruation occurred at 43 ± 1 hours.

In those subjects who received progesterone replacement (group 2), the progesterone levels did not fall although the level of oestradiol-17β fell but there was no increase in either uterine pressure or response to oxytocin. Menstruation was delayed until 38 ± 10 hours after progesterone replacement therapy was discontinued. Csapo and coworkers concluded that the effect of luteectomy on uterine activity in the non-pregnant uterus is mediated by the decline in progesterone levels.

The concept of luteal phase contraception embraces not only those agents which are active prior to implantation but also those which interrupt pregnancy after implantation. The later were termed interceptive agents by Naqui and Warren (1971).

There have been three major areas of research into methods of disrupting implantation (Aitken and Harper, 1977):-

1. The disruption of luteal function by inhibiting the early luteotrophic activity of the blastocyst,
2. The disruption of luteal function by interfering with progesterone receptors in the endometrium and,
3. The disruption of luteal function by compounds acting directly on the corpus luteum.

The action of progesterone on the endometrium can be impaired either by interfering with progesterone receptors in the endometrium or by inhibiting the synthesis of progesterone from the corpus luteum or placenta.

The endometrium contains high affinity receptors for progesterone (Do and Leavitt, 1978) and their function can be altered by either a reduction in receptor number or by receptor blockade. The number of receptor sites is controlled by the ovarian sex steroids. Oestradiol-17β stimulates the production of progesterone receptors in the endometrium and elsewhere (Cidlowski and Muldoon, 1974). Progesterone both stimulates and inhibits its own receptor under various conditions. It initially induces the translocation of its own receptors to the nucleus and stimulates their production. However, these effects are temporary and followed by inhibition. This inhibition can be prevented by high levels of oestradiol-17β . Hence treatment with antioestrogens shortly after menstruation inhibits the oestrogen-induced synthesis of progesterone receptors, disturbs the postovulatory endometrium and disrupts

implantation (Major, Green and Heald, 1976). Blockade of progesterone receptors can be achieved by progesterone analogues which possess the ability to compete with endogenous progesterone for uterine binding sites but lack progestational activity. Progesterone analogues which irreversibly bind to the receptor molecule by the formation of covalent bonds were described by Warren (1973), but initial work suffered the disadvantage of having significant side effects. The antiprogesterone compound RU 486 has been used successfully to induce menstruation and abortion (Herrmann et al 1972). This drug does not have significant side effects but efficacy is variable.

The second method of disrupting implantation (assuming the work of Csapo et al, (1972) to be correct) is the use of compounds which act directly on the corpus luteum and interfer with the production of progesterone.

The natural luteolytic agent has yet to be described in man. In domestic and laboratory animals removal of the uterus will significantly extend the life of the corpus luteum (De Greef, Dullaart and Zeilmaker, 1976). This is thought to be due to the removal of a luteolytic factor, probably prostaglandin $PGF_{2\alpha}$ derived from the uterus (Behrman, Grinwich, Hichens and MacDonald, 1978). In man, neither hysterectomy (Beavis, Brown and Smith, 1969) nor systemic $PGF_{2\alpha}$ (Lemaire and Marsh, 1975) influence the function of the corpus luteum. Luteolysis does occur after injection of $PGF_{2\alpha}$ into the ovary (Korda et al, 1975) and when luteinised human granulosa cells are exposed in vitro to PGF (McNatty, Henderson and Sawer, 1975).

Oestrogenic compounds have been found to induce luteolysis in animals (Anderson, 1972) and oestrogen administered systemically may induce luteolysis in man (Gore, Caldwell and Speroff, 1973) although their mechanism of action is controversial.

Pharmacological agents which inhibit ovarian steroidogenesis has been the subject of considerable research (Aitken and Harper 1977). Theoretically, agents that disrupt the normal hormonal pattern of an ovulatory cycle could be used as interceptive agents. One such agent is an aromatase inhibitor which would prevent the aromatisation of the A ring and inhibit the synthesis of oestrogen (Brodie, Marsh, Wu, and Brodie, 1979). The absence of a preovulatory oestrogen surge would prevent release of luteinising hormone and ovulation would not occur.

A new approach is to inhibit the 3β -HSD enzyme system which is possible with the use of oral agents with minimal side effects (see chapter 1). Epostane shows the most promise and was used in the luteal phase of the human menstrual cycle in the work described in this chapter.

METHOD

Trial Design

Thirty-three healthy women who had been sterilised by laparoscropic clip occlusion or tubal diathermy volunteered to participate in the study. All had a regular menstrual cycle (length between 25 and 35 days) and were studied on day 20 ± 2 of their menstrual cycle.

This study was performed in three sections:-

Trial 1. A single dose double-blind study of Epostane (100 mg and 50 mg) and a placebo (n=15).

Trial 2. A 300 mg multiple dose double-blind study of Epostane (100 mg eight hourly for three doses) and placebo (n=10).

Trial 3. A 1500 mg multiple dose study of Epostane (100 mg eight hourly for five days) (n=8).

The placebo for all trials was vehicle alone.

Subjects

The women were interviewed the day after their sterilisation had been performed and the nature of the investigation explained. Volunteers were given a menstrual calender and (two to six months) later contacted by telephone to select a suitable study date.

Selections were based on the following criteria;

1. Regular menstrual cycle (25 - 35 days),
2. Previously sterilised (to avoid the possibility of any teratogenic effect),
3. Good general health and not receiving any medication,
4. Weight less than 80 kg.
5. Normal full blood screen (haemoglobin, white cell count and blood film), urea, electrolytes and liver function tests (total protein, albumin, bilirubin, alkaline, phosphatase and aspirate serum transminase),
6. No contraindication to steroid therapy.

Radioimmunoassay

The steroid hormone, progesterone, oestradiol-17β and cortisol, were measured as described in the appendix. Follicle Stimulating Hormone (FSH) was measured with a kit from the Department of Biochemical Endocrinology, Chelsea Hospital for Women (Fergusson and Loo, 1982).

Statistical Methods

The data was analysed as described in Chapter 3.

TRIAL 1

Single dose study (0, 50 and 100 mg Epostane).

Method

Fifteen women were studied and randomly allocated in a double-blind procedure to the three treatment groups (placebo, 50 mg and 100 mg Epostane).

The subjects were admitted to the hospital research ward for eight hours and an intravenous catheter was inserted into a forearm vein for sampling. Two pretreatment samples were taken 30 minutes apart and the drug administered with a glass of water immediately after the second sample. Subsequent samples were at 30, 60, 90, 120, 180, 240 and 360 minutes. The patients pulse and blood pressure were recorded at the time of blood sampling. After the sample taken at 360 minutes the catheter was removed and the patient returned home. Each subject returned 24 hours later for the final blood sample. At this time they were questioned as to side effects. Each subject recorded a menstrual calender for a further two months.

Results

Clinical Effect

There was no change in blood pressure, pulse, temperature or the expected menstrual pattern. All subjects denied side effects from the treatment.

Hormone Measurements

1. Serum Progesterone:

A single tablet of Epostane significantly lowered the concentration of progesterone in the peripheral blood in the luteal phase of the menstrual cycle (Table 4.1). The nadir occurred at four hours and was significantly different from the pretreatment level. Twenty-four hours after drug administration there was no significant difference between the groups (Fig 4.2).

TABLE 4.1 The concentration of progesterone (nmol/l) and percentage changes after a single tablet of Epostane (50mg or 100mg) or placebo in the luteal phase of the menstrual cycle.

Dose (mg)	n	Pretreatment	At 4 hrs	At 24 hrs
0	5	36.3 ± 5.4	29.4 ± 4.5	37.7 ± 3.8
		100%	80.9%	104.2%
50	5	31.5 ± 5.7	20.4 ± 4.1	23.5 ± 4.8
		100%	65.1% *	74.8%
100	5	23.9 ± 3.8	15.3 ± 2.5	22.4 ± 4.3
		100%	63.8% **	93.6%

Values represent mean ± S.E.M. Significant differences from the control group are indicated (* $P < 0.05$, ** $P < 0.01$).

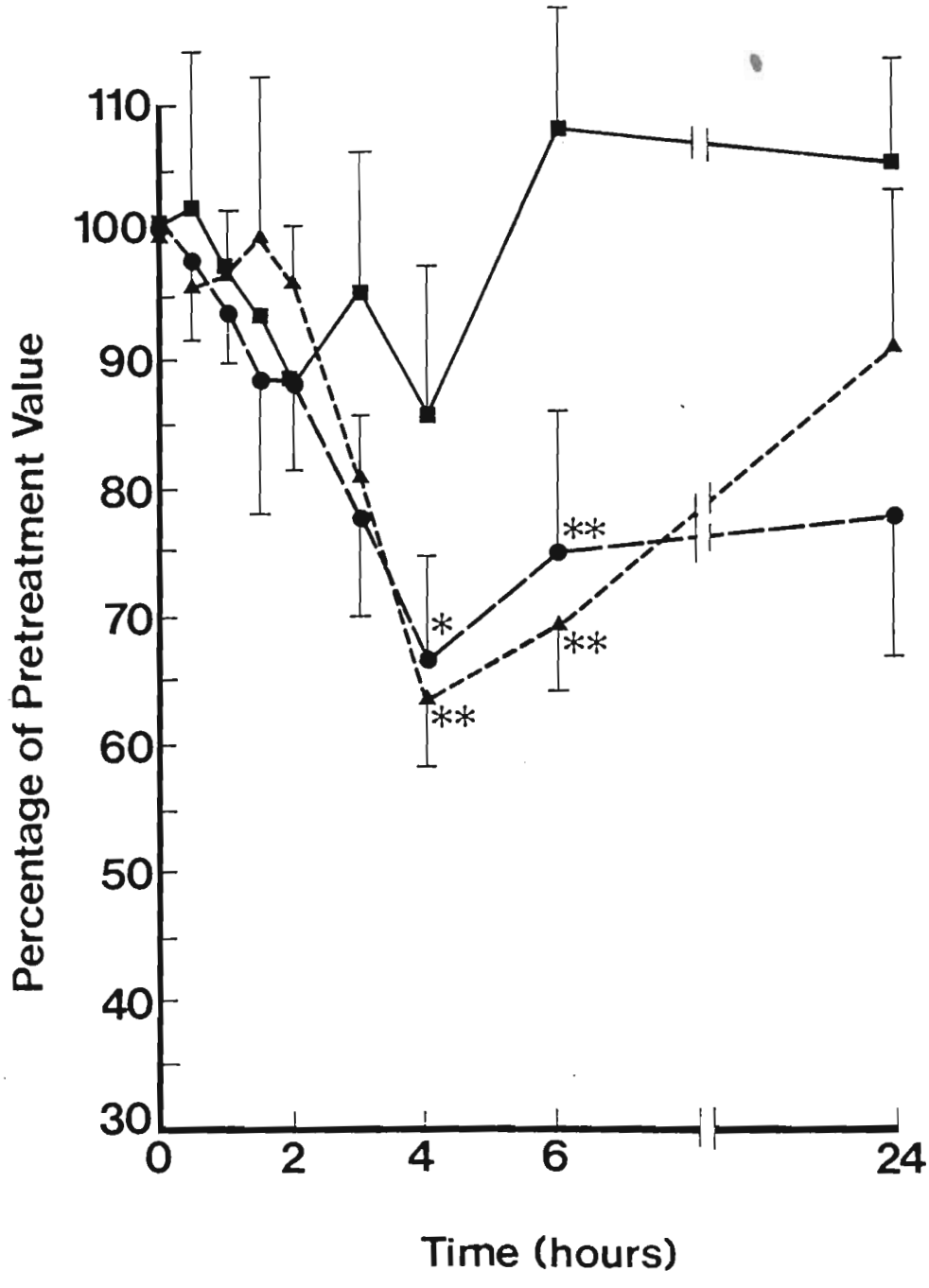

FIG 4.2 Percentage changes in the concentration of progesterone after Epostane 50mg (●——●) or 100mg (▲——▲) or placebo (■——■) in the luteal phase of the human menstrual cycle (mean ± S.E.M). Significant differences from the control group are indicated (* $p < 0.05$, ** $p < 0.01$).

2. Serum Oestradiol-17β

There was no significant change in the level of oestradiol-17β after treatment with Epostane (Fig 4.3). The mean pretreatment value of 0.89 nmol/l was within the laboratory's reference range for the luteal phase of ovulatory cycles (Table 4.2).

TABLE 4.2 The concentration of oestradiol-17β (nmol/l) and percentage changes after a single tablet of Epostane (50 or 100 mg) or placebo in the luteal phase of the menstrual cycle.

Dose (mg)	n	Pretreatment	At 4 hrs	At 24 hrs
0	5	0.63 ± 0.13	0.67 ± 0.11	0.70 ± 0.12
		100%	119%	155%
50	5	0.91 ± 0.17	0.68 ± 0.06	0.74 ± 0.09
		100%	80%	93%
100	5	0.72 ± 0.12	0.66 ± 0.16	0.67 ± 0.16
		100%	89%	90%

Values represent mean ± S.E.M.

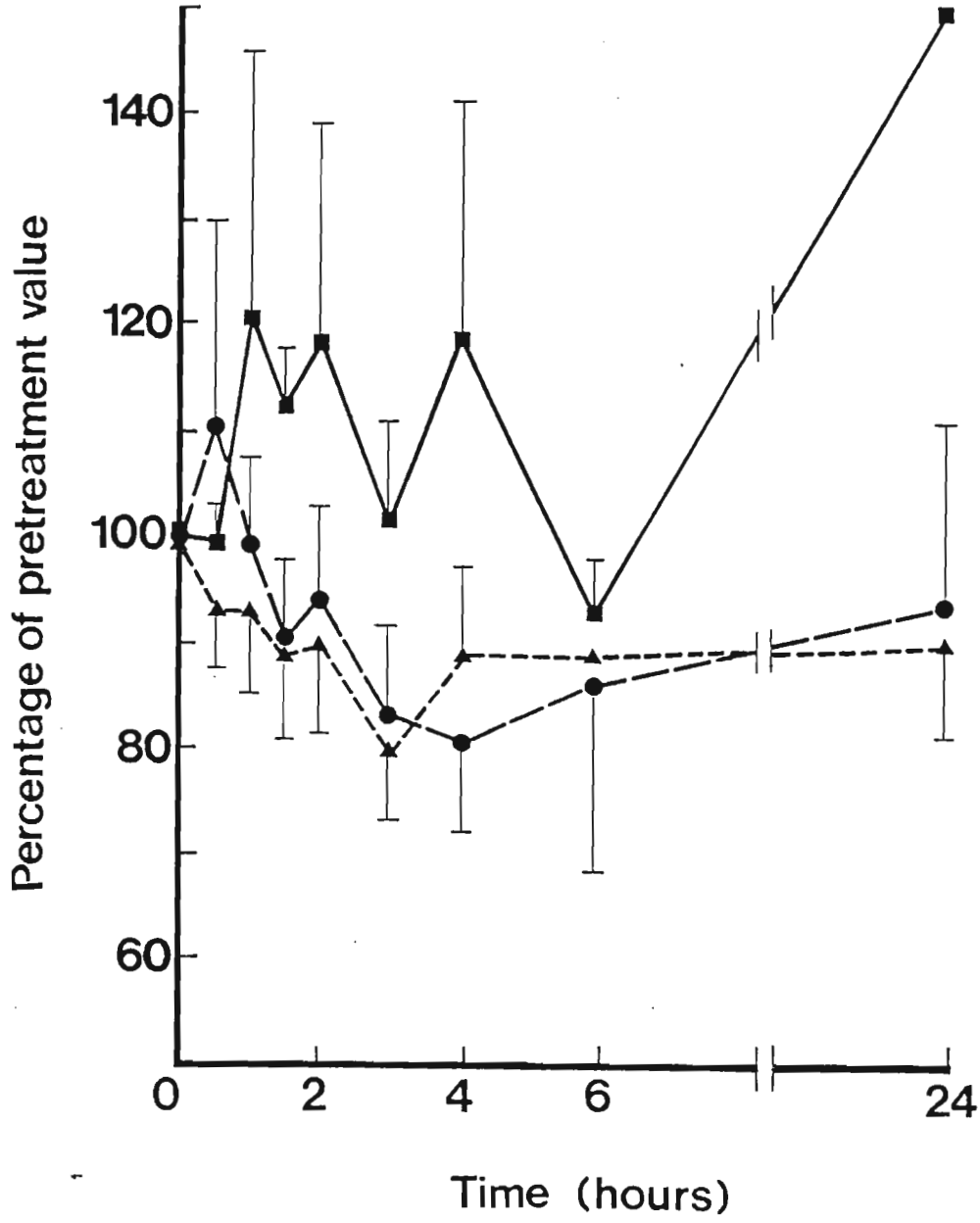

FIG 4.3 Percentage changes in the concentration of oestradiol-17β after Epostane 50mg (●——●) or 100mg (▲——▲) or placebo (■——■) in the luteal phase of the human menstrual cycle (mean + S.E.M). There were no significant differences from the control group.

3. Serum Cortisol.

The peripheral concentration of cortisol in the placebo group followed the expected daily diurnal pattern (Fig 4.4). The concentration in the treated groups did not differ significantly from this curve (Table 4.3).

TABLE 4.3 The concentration of cortisol (nmol/l) and percentage changes after a single tablet of Epostane (50 or 100 mg) or placebo in the luteal phase of the menstrual cycle.

Dose (mg)	n	Pretreatment	At 4 hrs	At 24 hrs
0	5	322.9 ± 40.2	271.8 ± 33.1	199.8 ± 43.2
		100%	91%	61%
50	5	429.8 ± 61.2	285.8 ± 38.6	323.4 ± 64.4
		100%	68%	74%
100	5	549.3 ± 113.0	276.9 ± 43.1	481.5 ± 92.5
		100%	60%	85%

Values represent mean ± S.E.M.

4. Serum Follicle Stimulating Hormone (FSH). There was no significant change in FSH concentration during the study (Fig 4.5).

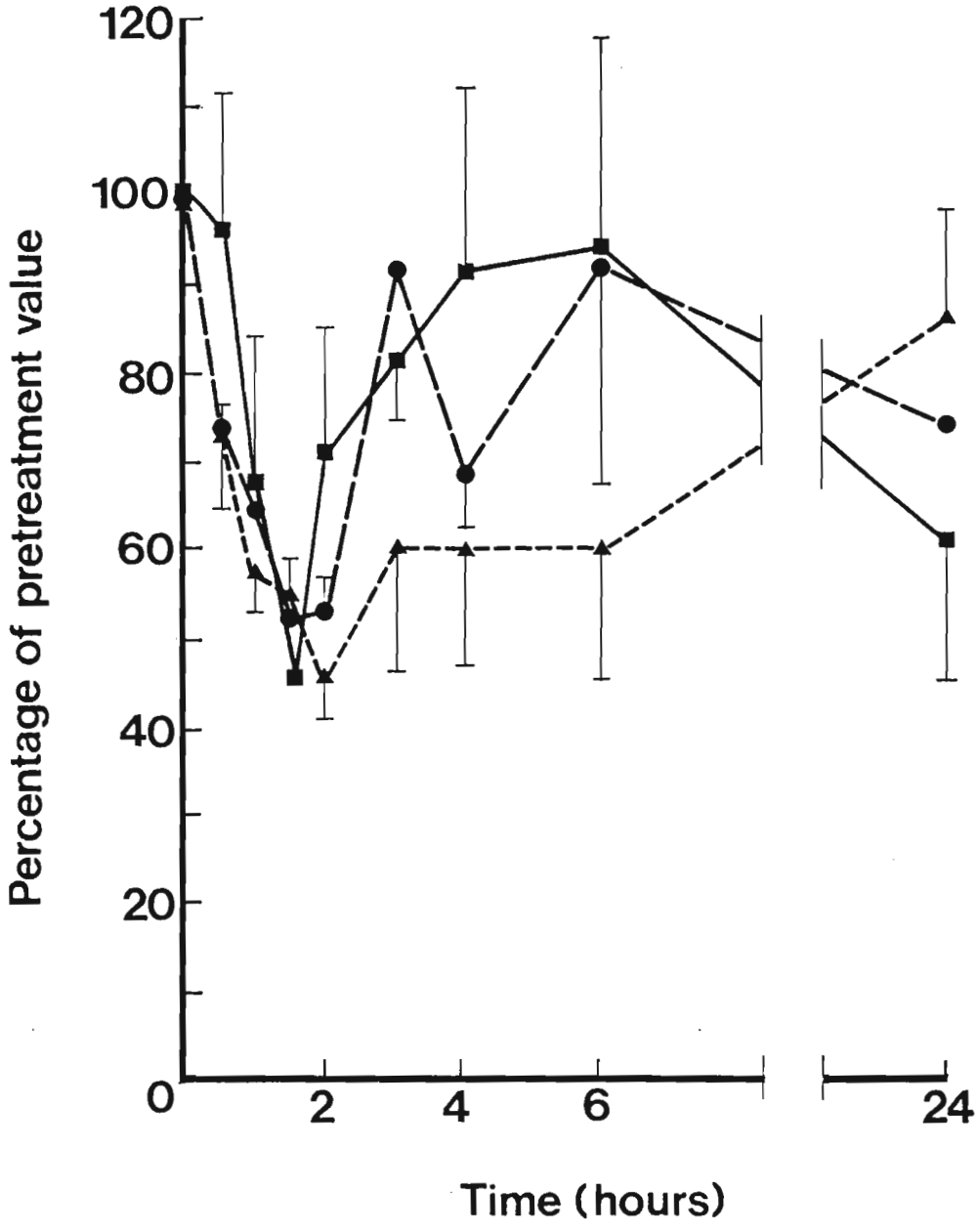

FIG 4.4 Percentage changes in the concentration of cortisol after Epostane 50mg (●——●) or 100mg (▲——▲) or placebo (■——■) in the luteal phase of the human menstrual cycle (mean ± S.E.M). There were no significant differences from the control group.

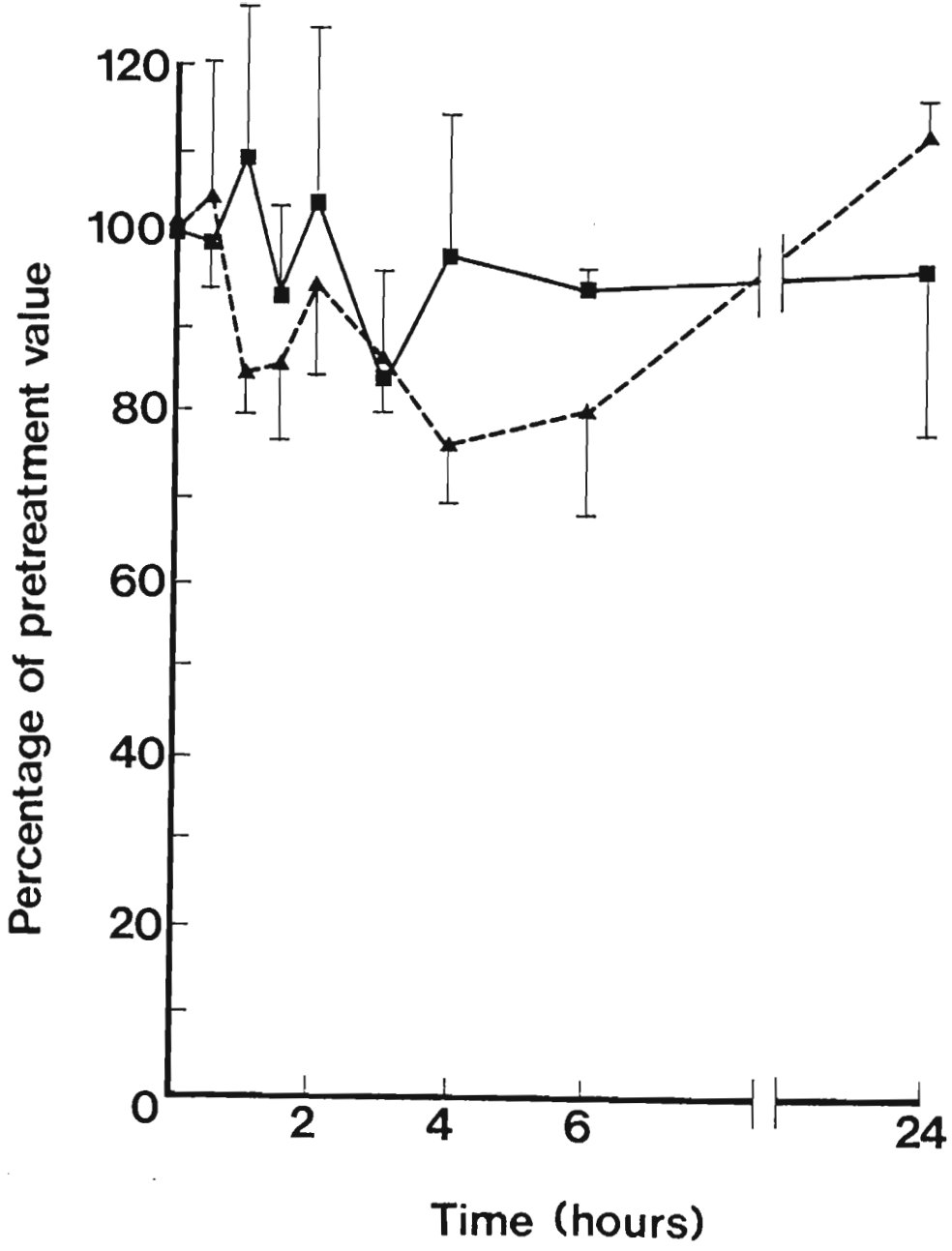

FIG 4.5 Percentage changes in the concentration of follicle stimulating hormone (FSH) after Epostane 100mg (▲——▲) or placebo (■——■) in the luteal phase of the human menstrual cycle (mean ± S.E.M). There were no signiciant differences from the control group.

Haematology and Biochemistry

Comparison of the pretreatment indices with those taken 24 hours after tablet ingestion showed no significant change.

TRIAL 2

Three hundred mg Epostane (100 mg eight hourly) or placebo for one day.

Method

This trial was designed to study the effect of further suppression of progesterone levels by an increased dose of Epostane.

Ten women were studied and randomly allocated in a double-blind procedure to two groups. Five received 100 mg Epostane eight hourly for three doses and five received a placebo.

The subjects presented daily between 0800 and 1000 hours for seven consecutive days. Treatment was administered on the second day immediately after the second pretreatment blood sample. Serum progesterone, oestradiol-17β and cortisol were measured in each sample.

At each visit the blood pressure and pulse were recorded and the patient was interrogated as to any side effects from the treatment. A final haematological and biochemical screen was performed two days after tablet ingestion.

Subjects were asked to provide a menstrual calender for the subsequent two months.

Results

Clinical Effects

There was no change in expected menstrual pattern and no symptoms that could be attributed to the drug.

Hormone Measurements

1. Serum Progesterone.

The serum progesterone level in the control group was 36.44 8.84 nmol/l prior to treatment and did not differ significantly for four consecutive days (Fig 4.6). The serum progesterone concentrations in the treatment group on the two pretreatment days were 32.21 ± 1.49 and 30.40 ± 3.05 nmol/l respectively. This fell immediately after treatment to 14.50 ± 2.61 nmol/l the next day ($p < 0.001$).

2. Serum Oestradiol-17β .

The serum oestradiol-17β level fell throughout the seven day period in both groups. There was no significant difference between the groups. In the control group the pretreatment serum oestradiol-17β level was 0.14 ± 0.04 nmol/l and 0.07 ± 0.02 nmol/l on the day after treatment. In the treatment group the comparable values were 0.09 ± 0.04 and 0.04 ± 0.04 nmol/l.

3. Serum Cortisol.

There was a slight decline in the serum cortisol levels in both groups during the study. This was not significant. In the placebo group the pretreatment value was 354.0 ± 15.0 nmol/l and the value 24 hours after treatment 290.0 ± 36.0 nmol/l. In the treatment group comparable values were 337.0 ± 45.0 nmol/l and 287.0 ± 5.0 nmol/l.

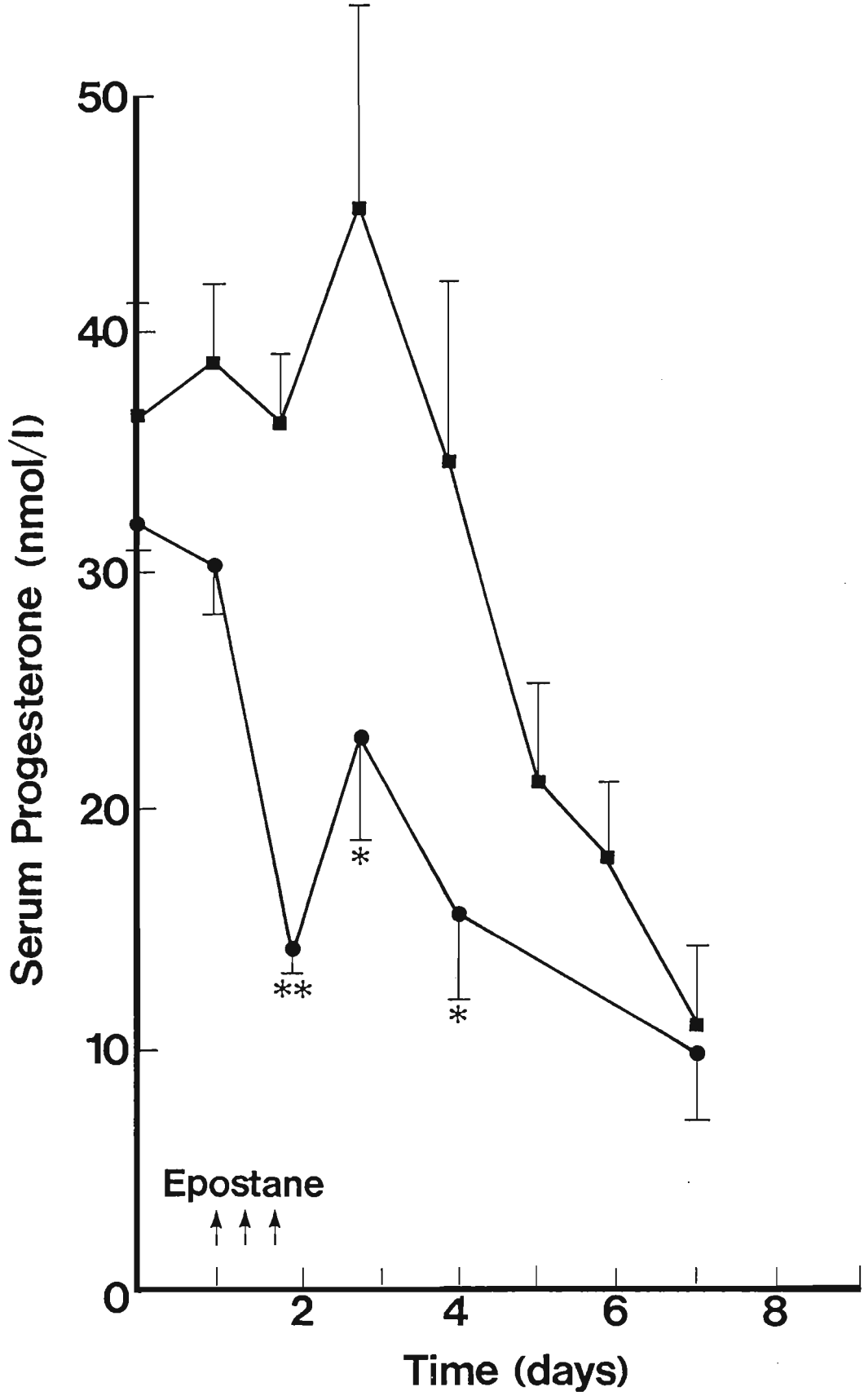

FIG 4.6 Changes in the concentration of progesterone (nmol/l) after Epostane (100mg 8 hourly for one day) (●——●) or placebo (■——■) in the luteal phase of the human menstrual cycle (mean ± S.E.M). Significant differences are indicated (* $p < 0.05$, ** $p < 0.01$).

Haematology and Biochemistry

Comparison of the pretreatment indicies with those taken 24 hours after tablet ingestion showed no significant change.

TRIAL 3

Fifteen hundred mg of Epostane (100 mg eight hourly) for five days.

Method

Eight women were selected and studied from day 20 ± 2 of their menstrual cycle. The selection and study protocol were as described previously. There was no placebo group. The subjects received 100 mg Epostane eight hourly for five consecutive days i.e. the second to seventh day of sampling.

Results

Clinical Effects

There was a significant shortening of the menstrual cycle ($p < 0.04$). Five of the eight women menstruated prior to the expected date. Ovulation was not confirmed in two of the remaining three subjects (Table 4.4). The early menstruation was described as 'normal' by the subjects and subsequent menstruation occurred at the expected time. The shortening of the menstrual cycle by four days in the six subjects who ovulated was highly significant ($p< 0.01$).

TABLE 4.4 Clinical effects of Epostane (100 mg 8 hourly for 5 days) in the luteal phase of the menstrual cycle.

Subject Number	Initial Progesterone (nmol/l)	Day Treatment Commenced	Day of Menstruation Expected	Observed
1	14.6	20	30	32*
2	36.6	19	28	25
3	79.2	21	30	24
4	12.1	18	27	27*
5	25.4	20	28	25
6	19.7	20	29	29
7	57.9	20	29	24
8	41.9	21	28	24

* Possible anovular cycles (France , 1981).

Hormone Measurements

1. Serum Progesterone.

There was a significant decline in the serum progesterone concentration on the first day of treatment ($p < 0.02$). The pretreatment progesterone level of 35.9 ± 2.91 nmol/l fell to 13.9 ± 0.5 nmol/l and was maintained below 10 nmol/l throughout the treatment period (Table 4.5, Fig 4.7).

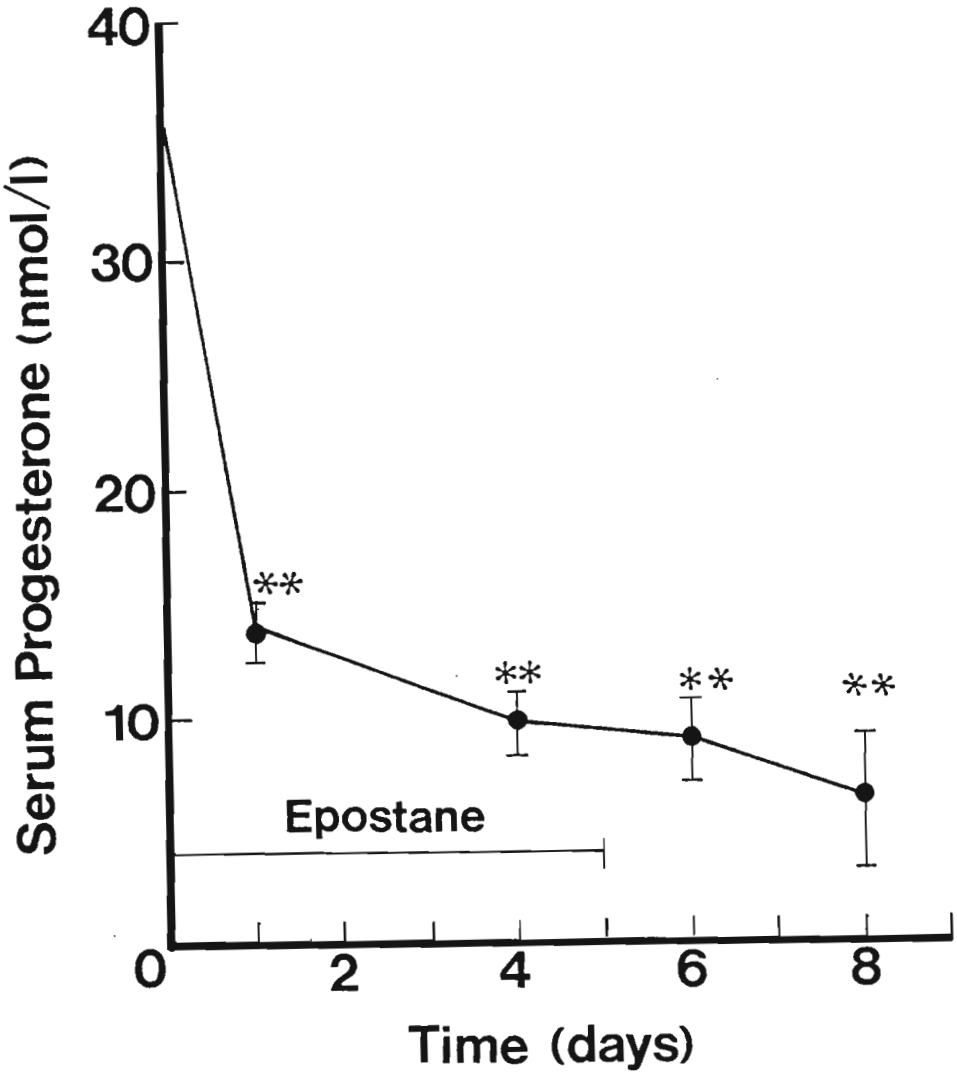

FIG 4.7 Changes in the concentration of progesterone (nmol/l) after Epostane (100mg 8 hourly for 5 days) in the luteal phase of the human menstrual cycle (mean ± S.E.M). Significant differences from the pretreatment value are indicated (* $p < 0.05$, ** $p < 0.01$).

TABLE 4.5 The concentration of progesterone (nmol/l) in the luteal phase of the menstrual cycle after Epostane 100 mg 8 hourly for 5 days.

Subject Number	Pretreatment Value	Days 1	4	6	8
1	14.6*	10.8	12.4	13.1	17.5
2	36.6	11.1	7.0	10.8	1.3
3	79.2	18.1	7.3	-	-
4	12.1*	-	11.8	9.9	7.0
5	25.4	14.9	4.8	6.0	1.3
6	19.7	16.2	11.8	16.9	11.4
7	57.9	16.9	15.3	9.5	4.1
8	41.9	9.2	6.4	1.6	1.3
Mean	35.9	13.9**	9.8	8.9	6.3

** P <0.02

* Possible anovular cycles (France, 1981)

2 . Serum Oestradiol-17β.

There was no significant change in the serum oestradiol-17β concentrations throughout the seven day study (Fig 4.8). The pretreatment mean was 0.157 ± 0.017 nmol/l.

3. Serum Cortisol.

There was a steady and gradual decline in serum cortisol concentration throughout the seven day study. The pretreatment value of 349.7 ± 38.8 nmol/l fell to a level of 256.8 ± 36.5 at the time of final collection (Fig 4.9). There was a significant difference from the pretreatment

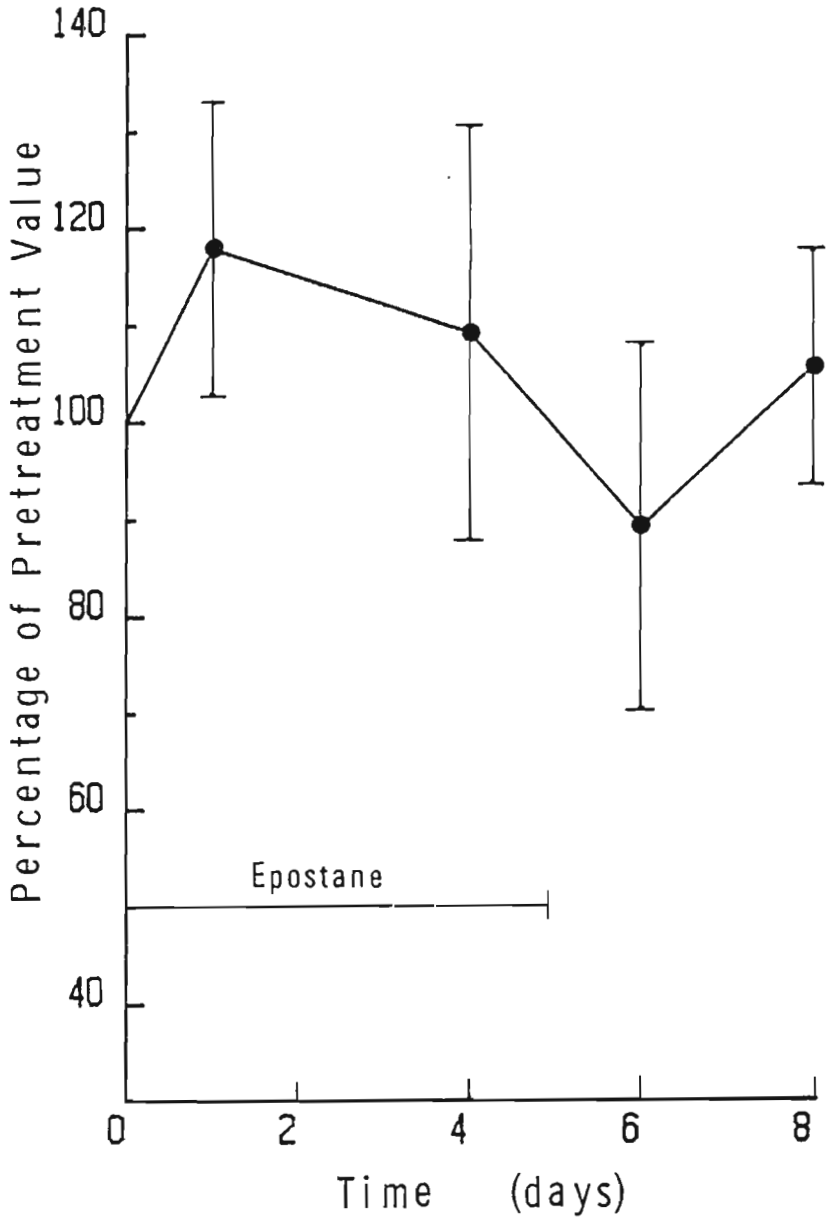

FIG 4.8 Changes in the concentration of oestradiol-17β (nmol/l) after Epostane (100mg 8 hourly for 5 days) in the luteal phase of the human menstrual cycle (mean ± S.E.M). There were no significant differences from the pretreatment value.

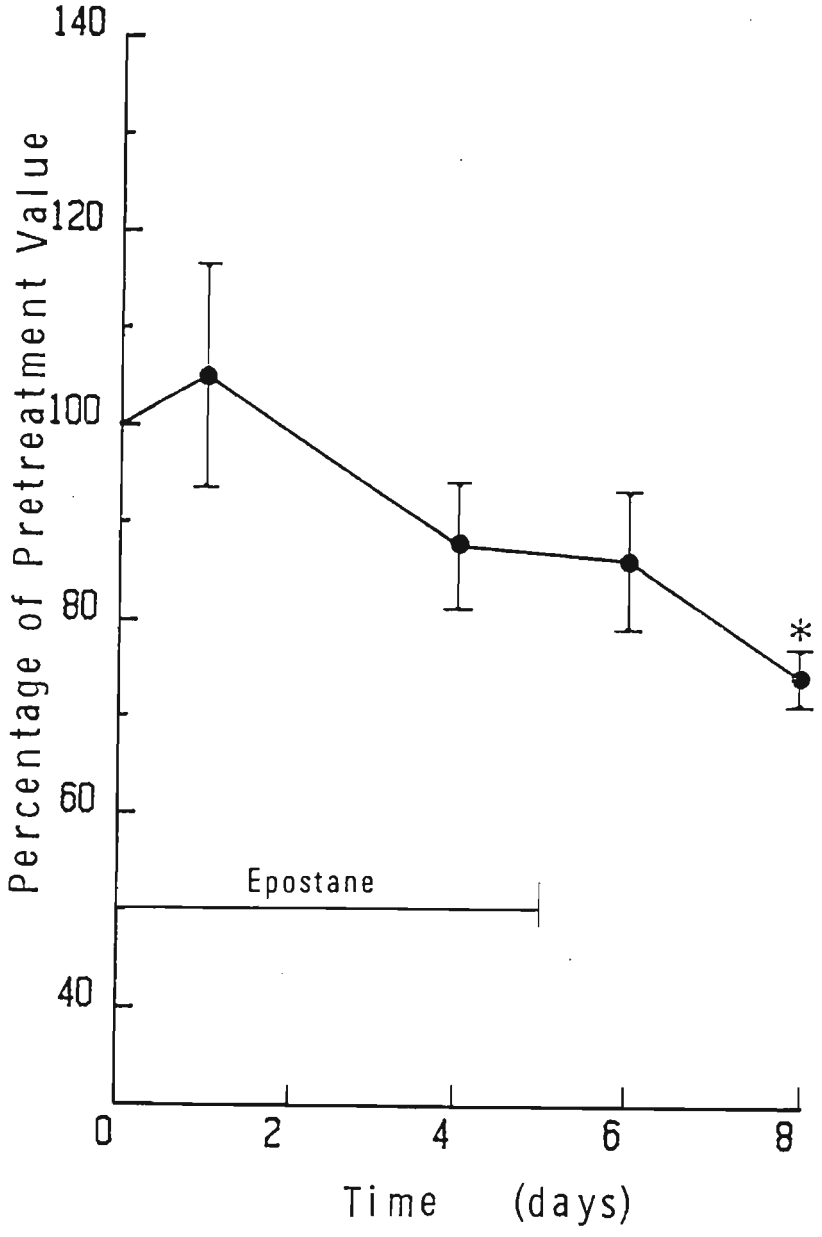

FIG 4.9 Changes in the concentration of serum cortisol (nmol/l) after Epsotane (100mg 8 hourly for 5 days) in the luteal phase of the human menstrual cycle (mean ± S.E.M). Significant differences from the pretreatment value are indicated (* $p < 0.05$).

value in the treatment group after eight days, ($p < 0.05$), but not after five treatment days.

DISCUSSION

This study demonstrates that competitive inhibition of 3β -HSD by Epostane leads to decline in progesterone levels and results in early menstruation. The inhibition of 3β -HSD is temporary and is achieved by oral medication without any adverse effects. Epostane has potential as a method of fertility control.

Progesterone is an obligatory requirement for implantation. Progesterone secretion during the luteal phase of an ovulatory cycle prepares the endometrium for implantation. Once fertilisation has occurred continued secretion is essential for successful implantation and maintenance of pregnancy during the first seven weeks (Csapo, 1977).

A precise balance of hormonal changes may be essential for the initiation of pregnancy. It has been suggested that minor aberrations may lead to spontaneous abortion (Edmonds, Lindsay, Miller, Williamson and Wood, 1982) and that the disturbance of the hormone balance could be used as a method of contraception (DHEW 1969). In the study performed by Edmonds et al., (1982) measurements of human chorionic gonadatrophin (hCG) were used to investigate early pregnancy loss or implantation failure. These authors studied 207 menstrual cycles in a group of women attempting to conceive;

198 were ovulatory; hCG was detected in 118; but only 57 pregnancies (48.7%) resulted and of these, six aborted. These authors concluded that although an abnormal conceptus could lead to abortion without menstrual delay other explanations such as 'hormonal imbalance' could be a factor.

Extensive investigations to detect the luteolytic agent in woman plus the administration of 'known' luteolytic agents has not yet provided a pharmacological agent suitable for human fertility control. One possible target for fertility control is the corpus luteum. Csapo (1977) confirmed the dependence of the secretory endometrium on progesterone by showing that the decline in progesterone levels acheived by luteectomy led to menstruation 38 ± 3 hours after the procedure was performed. Inhibition of ovarian steroidogenesis by a competitive inhibitor of 3β -HSD is a possible approach to fertility control. Espostane as demonstrated in this study, has that potential.

The ideal interceptive agent would inhibit progesterone synthesis for the duration of one menstrual cycle. It should be free of side effects and only require a single oral dose.

Trial I (single dose study) demonstrated that Epostane is an effective inhibitor of progesterone synthesis in the ovary and that the effect of a single tablet is temporary. The nadir was at four hours and the concentration of progesterone was returning to pretreatment levels at 24

hours (Fig 4.2) which suggests that the half-life of Epostane is approximately six hours. A dose regimen of one tablet eight hourly was selected in order to achieve prolonged suppression of progesterone production.

The degree of inhibition of progesterone synthesis was proportional to the pretreatment value. The higher the pretreatment value, the greater the percentage decline. Those patients with low pretreatment serum progesterone values (10 to 25 nmol/l) often did not have a significant change. One criticism of the trial is the selection of patients that was based on menstrual history rather than confirmed ovulation in the studied cycle . The availability of serum or urinary LH assays would allow improved selection; the subjects could collect early morning urine samples from day 12 to day 18 of each cycle and analysis could detect ovulation. Inclusion in this study would follow only if the LH surge was detected. This would also allow correct timing of treatment in relation to the day of ovulation.

Trial 2 (one day multiple - dose study) achieved a decline in the concentration of progesterone from 31.1 ± 1.8 nmol/l to 14.5 nmol/l 24 hours after treatment a decline to 46.3 ± 0.8% of the pretreatment value. This was achieved with a dose of 300 mg in 24 hours.

This fall was temporary, the level of progesterone returning towards pretreatment at 48 hours indicating that inhibition of 3β -HSD by Epostane is not irreversible. No clinical

effects were reported.

The temporary nature of the fall suggested that prolonged drug administration would be required to achieve a clinical effect. Further studies were performed with a similar dosage but prolonged administration.

Prolonged suppression of progesterone was achieved in Trial 3 (100 mg Epostane eight hourly for five days) where the serum progesterone level fell to 39.4% of the pretreatment value and was maintained at that level. Early menstruation occurred in five of the eight treated subjects.

The eight subjects can be subdivided into two groups. Those in whom ovulation was confirmed (subjects 2,3,5,6,7 and 8) and those in whom ovulation was questionable (subjects 1 and 4) (France, 1981). Accepting the criticism that one or two consecutive serum progesterone samples cannot clearly indicate ovulation or anovulation, a value of 15 nmol/l is usually taken as confirmation of ovulation (France 1981). The group in whom ovulation was confirmed had a decline in serum progesterone levels from 43.45 nmol/l to 14.4 nmol/l (33.1% of the pretreatment value) and menstruated five days after treatment began. The 'anovulatory' group had a decline in serum progresterone levels from 13.3 nmol/l to 11.3 nmol/l ie to 84.6% of the pretreatment value and menstruated 10 days after treatment began. In the 'anovular' group the serum progesterone concentration never rose above 15.0 nmol/l and the cycle was prolonged which may

be due either to anovulation or to inhibition of progesterone production by Epostane before the luteal phase plateau; irrespective of the reason, all eight subjects would not have had a conceptual cycle.

There are insufficient numbers in this current study to answer the question of how progesterone controls the stability of the endometrium. Is it the rate of fall of progresterone which leads to menstruation or is it a particular level which must be reached before menstruation can occur?

Inhibition of 3β -HSD was temporary. One possible effect of temporary inhibition of ovarian function was that once the drug was stopped normal ovarian function would continue. Menstruation would be merely delayed and conception may occur. Serum oestradiol-17β levels were not affected by Epostane despite the significant fall in serum progesterone levels. This may be due to the ovary metabolising pregnenolone via the delta 5 pathway which is thought to be dominant in the ovary (Ryan and Smith, 1965) (Fig 1.10).

In summary, Epostane effectively induced early menstruation in this study and could be used as a method of fertility control but has two major drawbacks. The first is the duration of administration. Further study is needed to explore sustained release capsules which could administer the drug without the need for repetitive tablet ingestion. The second and more difficult drawback is the reduction in the

cycle length to 24 days as has occurred in this study. This would be unacceptable both to the patient and may have long term consequences as far as endometrial development is concerned.

Epostane may have a place as a post coital agent or at other times in the menstrual cycle. A trial of Epostane immediately following a missed period is currently in progress.

CHAPTER 5

DISCUSSION

The aim of the work described in this thesis is to elucidate the role of progesterone in the control of uterine activity. A competitive inhibitor of the synthesis of progesterone, Epostane, was used in three in vivo situations, the luteal phase of the human menstrual cycle, early human pregnancy and late ovine pregnancy. In each of these experimental models, a significant decline in the level of progesterone in the peripheral circulation was achieved and the effect on uterine activity observed.

In the luteal phase of the human menstrual cycle the role of progesterone in 'maintaining' the endometrium was confirmed. A drug-induced decline in the level of progesterone on day 21 ± 2 of the menstrual cycle lead to menstruation. A substained reduction in the level of progesterone for 4-5 days was required to initiate menstruation. On the other hand in early human pregnancy a drug induced decline in the concentration of progesterone was not accompanied by uterine activity despite a sustained fall which was comparable to that achieved in the luteal phase of the menstrual cycle. The extent of this decline in the concentration of progesterone far exceeded the day to day variations observed in normal pregnancy at term. This observation adds confirmation to the generally accepted view that progesterone is not directly involved in human parturition. In the third experimental model a drug-induced decline in the level of progesterone in late gestation led to a marked rise in the level of prostaglandins and increased uterine activity which lead to parturition. Labour and delivery were not

associated with significant alteration in the level of oestradiol. This study clearly demonstrates that in ovine pregnancy progesterone is directly involved in the synthesis of prostaglandins and initiation of uterine activity.

The physiology and endocrinology of human parturition have been difficult to investigate. Ethical considerations and the inaccessability of the human fetus preclude the use of techniques so successfully exploited in animal studies. 'Animal models' have been used extensively for experimental purposes with the hope that the mechanism(s) discovered may be applicable to human pregnancy. The role of progesterone in the control of uterine activity has been extensively investigated in this manner.

Investigations into the endocrine events leading to parturition have changed direction in recent years. In the past, emphasis was on measurements of the concentration of the steroid hormones and prostanoids in maternal and fetal circulations, in spontaneous and induced labour. These studies led to our current understanding of the role of the steroid hormones in the initiation of parturition in experimental animals (Chapter 1). In the pregnant ewe the temporal relationship between the decline in the concentration of progesterone (Bassett et al, 1969), the rapid increase in the level of oestrogen (Challis, 1971) and the rapid prepartum rise in the concentration of prostaglandins (Liggins and Grieves, 1971) is well established. As it became clear that control of the synthesis of prostaglandins is a critical factor in the

initiation of human parturition, emphasis shifted to changes in hormone levels in uterine tissues and interactions between the fetus, the fetal membranes and the mother. For example, in vitro studies have demonstrated that the steroid hormones alter prostaglandin synthesis in human endometrial cell cultures (Abel and Baird, 1980; Wilson, Liggins, Aimer and Watkins, 1986). However, it remains unclear if changes in the concentration of the steroid hormones influence the synthesis of prostaglandins in vivo.

In this thesis, a new approach to the investigation of the role of progesterone in the control of uterine activity is described. The availability of a specific inhibitor of the synthesis of progesterone provided a method which enabled the investigator to induce a fall in the level of progesterone in the circulation. This allowed the testing of three hypotheses:

1. A reduction in the circulating level of progesterone in the luteal phase of the menstrual cycle will lead to menstruation.

2. A fall in the circulating level of progesterone in early human pregnancy will lead to abortion.

3. A fall in the circulating level of progesterone in the late pregnant ewe will initiate parturition.

HYPOTHESIS 1. "A reduction in the circulating level of progesterone in the luteal phase of the menstrual cycle will lead to menstruation."

The temporal relationship of the declining levels of progesterone and the onset of menstruation is well established (Landgren et al, 1977). Progesterone declines from a mid-luteal level of 54 nmol/l to 4.8 nmol/l at the onset of menstrual spotting and 2.4 nmol/l at 'full' menstrual bleeding (Thorneycroft et al, 1971). There is both a rapid decline and a sustained reduction in the concentration of progesterone before menstruation occurs. Administration of progesterone will delay menstruation and the normal menstrual cycle can be mimicked by the cyclical administration of oestrogen and progesterone.

Csapo and Pulkkinen (1978) confirmed the dependence of the endometrium on secretions from the corpus luteum. In their study in which the corpus luteum was surgically removed on day 21-23 of a menstrual cycle, the level of progesterone fell immediately to reach 32 percent of the pretreatment value 3 hours after luteectomy. Twenty four hours after surgery the level of progesterone had fallen to 13.5 percent of the pretreatment value. The level of oestradiol also fell and menstruation occurred 38 ± 3 hours after luteectomy. In a similar study in which the circulating level of progesterone was maintained, by intramuscular injections of progesterone after luteectomy, menstruation did not occur until 48 hours after discontinuation of exogenous progesterone therapy.

Limiting the availiabilliity of progesterone at the cellular level in the endometrium also, as expected, leads to menstruation. A receptor for progesterone has been characterised (Grody, Shrader and O'Malley, 1982) and shown to be a dimer, each component of which can separately bind progesterone. Progesterone receptor site concentration on endometrial cells increases during the follicular phase of the menstrual cycle in response to oestrogen (Grody et al, 1982). The number of receptors decline during the luteal phase. Recently, a 19-nortestosterone derivative (RU 486) which binds to the progesterone receptor and is dissociated slowly has been used to study the action of progesterone (Grananis, et al 1985). This agent lacks progestational activity itself but in blocking the access of progesterone to its receptor reduces the biological activity of progesterone. The administration of RU 486 during the luteal phase of the menstrual cycle can lead to menstruation (Herrmann et al, 1982).

Progesterone inhibits synthesis of prostaglandins from both proliferative and secretory human endometrium in vitro (Abel and Baird, 1980; Wilson et al, 1986). Consequently, the fall in progesterone secretion at the end of the menstrual cycle releases prostaglandins from this inhibition and large amounts of $PGF_{2\alpha}$ and PGE are found in menstrual blood (Pickles et al, 1965). Administration of prostaglandins during the luteal phase induces premature menstruation (Eliasson and Posse, 1960).

Markee's experiments (1940) with auto transplantation of endometrium into the anterior chamber of the eye of the rhesus monkey provided information on the effect of hormonal changes on the endometrium. Markee considered that the endometrium was not shed if the concentration of progesterone fell gradually but thought that abrupt and local changes in the levels of progesterone were required to initiate menstruation. However, it is not possible to ascertain from Markee's paper whether the critical factor in the initiation of menstruation was the rate of decline or the final concentration of progesterone reached.

Markee demonstrated that menstruation is preceded by a period of rapid regression of the endometrium caused by vasoconstriction of the spiral arteries leading to coiling and vascular stasis with injury to the endometrium. These changes could be mimicked by substitution and later withdrawal of synthetic oestrogen and progesterone. Markee attributed the vasoconstriction to a potent local vasaconstrictor (now considered to be one or more of the prostanoids). Attempts to reproduce the work of Markee have to date been unsuccessful (Belle and Schweppe, 1979).

In the present work a non-invasive method using a 3β -HSD inhibitor was used to reduce the ovarian production of progesterone. A single dose of 100mg of Epostane on day 21 of the menstrual cycle resulted in a fall in the concentration of progesterone to 61% of the pretreatment value within 24 hours, but menstruation did not occur. The effect of Epostane on the 3β-HSD enzyme system has been previously

demonstrated by Van der Spuy (1983) who showed that Epostane induced a rise in the level of pregnenolone and a concomitant decline in the level of progesterone.

The absence of a significant decline in peripheral levels of oestradiol after Epostane allowed investigation of the effect of a decline in progesterone levels alone on the luteal phase endometrium (in contrast to the study of Csapo and Pulkkinen, 1978).

In a second experiment (300mg Epostane), the level of progesterone fell rapidly from the mean treatment value of 32.2 ± 1.5nmol/l to 14.5 ± 2.6nmol/l at 24 hours. Despite the rapidity of this decline, menstruation did not occur.

In a later experiment, in which each subject received 1500mg of Epostane, (100mg eight hourly for five days) premature menstruation occurred in 5 of 8 women. The pretreatment value of 35.9 ± 2.9 nmol/l fell to 13.9 ± 0.5nmol/l at 24 hours (38 percent of the pretreatment value) without any clinical effect. Continued treatment led to a further decline which was sustained and remained below 10nmol/l from the 2nd to the 5th day of treatment. Menstruation occurred after five days of treatment. The concentration of oestradiol-17β did not decline in any experimental subject.

These experiments demonstrate that a decline in the level of oestradiol is not required to initiate menstruation. Menstruation occurred 38 ± 3 hours after luteectomy in the investigations of Csapo and Pulkkinen (1978), whereas 5 days

of treatment with Epostane were required in the present study. This difference may be attributable to lack of an effect of Epostane on oestradiol or to a greater decline in the level of progesterone achieved by luteectomy. This question could be addressed by investigating the effect of a larger dose of Epostane on the time to induce menstruation.

The view of Markee, that menstruation is initiated by a rapid decline in the level of progesterone, was not supported by the above experiments. A rapid decline in the level of progesterone to 38 percent of the pretreatment value in 24 hours did not lead to menstruation.

This study suggests that a sustained reduction in the level of progesterone rather than a rapid alteration in tissue levels is necessary to trigger menstruation.

HYPOTHESIS TWO. "A fall in the circulating level of progesterone in early human pregnancy will lead to abortion."

The role of progesterone in the control of uterine activity in human pregnancy remains controversial. It is generally agreed that a decline in the concentration of progesterone, which is demonstrable in many mammalian species before the onset of labour, does not occur in human pregnancy. Therefore a single hypothesis encompassing the dramatic changes in hormone concentration prior to parturition in all species is not possible.

In some species, such as the sheep and the goat, fetal cortisol activates the placental enzymes responsible for the conversion of progesterone to oestrogens. The level of progesterone declines while that of oestradiol rises. These changes in the steroid hormones stimulate the synthesis of prostaglandins. In the human placenta one of the enzymes, 17β hydroxylase, is absent (Ainsworth, Daenera and Ryan 1969). The human placenta cannot convert progesterone or pregnenolone to oestrogen and a pathway by which the human fetus can influence prostaglandin synthesis has yet to be described.

Evidence supports the view that a common pathway exists once prostaglandin synthesis has occurred. Nevertheless an increased in the synthesis of prostaglandins before parturition has yet to be demonstrated in women.

The evidence supporting a role for prostanoids in parturition is extensive. Prostanoids from the uterus and its contents

probably have multiple actions on the smooth muscle cells of the myometrium. Prostaglandins appear to mediate the effect of the steroid hormones on gap junction formation (Garfield et al, 1980), promote calcium ion influx (Carsten, 1974) and result in intracellular accumulation of cyclic AMP.

Throughout pregnancy the myometrium is both quiescent and unresponsive to stimulants. This state has classically been attributed to progesterone. Evidence supporting a role for progesterone in the control of uterine activity in pregnant women includes findings from the study of the effects of luteectomy (Csapo and Pulkkinen, 1978), the reduction in myometrial responsiveness to oxytocin induced by progesterone (Kerenyi, et al, 1969) and invitro studies of perfused decidual cells in which progesterone inhibited the release of arachidonic acid (Wilson et al, (1986).

The studies in early pregnancy described in this thesis demonstrate that despite a significant fall in the peripheral concentration of progesterone uterine activity was not induced. In the first experiment, a single dose of Epostane, (50mg or 100mg) effected a significant and rapid decline in peripheral level of progesterone for up to 24 hours after drug ingestion. The concentration of progesterone fell to 35.5 ± 4.1% (n=10) of the pretreatment value. A similar fall in serum oestradiol-17β levels was also recorded. In the second experiment (Epostane 300 and 400mg) a similar decline in the levels of progesterone and oestradiol occurred. In both of these studies no clinical effects were observed despite the magnitude and rapidity of the decline in progesterone levels.

However, in both trials the concentration of progesterone returned rapidly to pretreatment levels. The duration of the decline in concentration of progesterone from baseline values was less than 24 hours. The results show that an abrupt concomitant fall in progesterone and oestradiol levels does not cause significant uterine activity.

In order to answer the question of whether a sustained reduction in the concentration of progesterone was a prerequisite for the initiation of uterine activity a third experiment was performed. Epostane (1500mg) (100mg eight hourly for 5 days) was administered. There was a rapid decline in progesterone levels (to 20% of the pretreatment level) which was maintained for the five days of treatment. No subject reported uterine contractions. Uterine activity (assumed from slight vaginal bleeding) possibly occurred in one patient.

The results of this experiment are in conflict with that of Csapo and Pulkkinen (1978) who, as noted above, showed that abortion occurred after the decline in progesterone levels achieved by luteectomy. There are several possible explanations for this apparent discrepancy.

(i) The fall in the level of progesterone induced by Epostane may have been insufficient to induce uterine activity despite the magnitude and duration of the decline achieved (a fall to 20% of the pretreatment value for 5 days). It is unlikely that the rate of change in progesterone levels is important in the control of uterine activity as this study demonstrated a rapid decline without any clinical

effect. On the other hand, the absolute level of progesterone achieved may be important. Subsequent studies by others, with increased dosage of Epostane have achieved a further decline in the level of progesterone and uterine activity resulted (Webster, Gillmer and Phelps unpublished). However as Epostane did induce a substantial fall in the concentration of progesterone and as there is no consistant fall in the peripheral concentration of progesterone in spontaneous parturition, this method of inducing parturition is unlikely to be physiological.

RU486, a potent antiprogesterone steroid which acts on the receptor has been shown to interrupt early pregnancy (Herrmann et al, 1982). The efficacy of RU486 is related to the length of gestation, not the dose. In pregnancies of less than seven weeks gestation, RU486 has been shown to induce abortion in 33 of 42 women (Kovac et al, 1984), but in later pregnancy the drug is less effective. However, later in pregnancy (16 weeks gestation), RU486 in combination with low dose prostaglandin achieved complete abortion in all 16 treated subjects (Bygdeman and Swahn, 1985). RU486 appears more effective than Epostane in inducing abortion which is probably due to a more complete inhibition of the biological effects of progesterone. An alternative explanation is that Epostane also lowers oestradiol levels, so that the oestrogen to progesterone ratio is not altered. Further studies using larger doses of Epostane together with exogenous oestradiol to maintain oestrogen levels could resolve this question.

(11) As peripheral concentrations of progesterone do not

decline prior to parturition in pregnant women at term, it has been suggested that local hormonal changes in the uterine tissues themselves are important in modulating the synthesis of prostaglandins. In the present experiment Epostane achieved a significant decline in placental production of progesterone as reflected in the decline in the peripheral concentration of progesterone. A decline in the concentration of progesterone in the local tissue presumably also resulted. Nevertheless, uterine activity was not induced.

(111) It has been suggested that the ratio of oestrogen to progesterone is a factor in the control of uterine activity. In the latter experiments the levels of both progesterone and oestradiol fell, hence the ratio of oestradiol to progesterone remained relatively unchanged by the administration of Epostane. This may explain the absence of an effect on uterine activity. Experiments where Epostane and oestradiol were administered together could achieve a decline in the level of progesterone without an alteration in the level of oestradiol and may lead to uterine activity.

(1V) The different result achieved by Csapo and Pulkkinen (1978) may be due to the methods they used to measure uterine activity. These investigators measured intrauterine pressure with an extra ovular balloon inserted through the cervix. In addition, as part of their investigation, they assessed uterine responsiveness daily by intramuscular injections of oxytocin (0.25miu). These procedures may act as uterine stimulants though they do not usually induce progressive

uterine activity.

(V) An alternative suggestion is that the threshold concentration of progesterone required to inhibit phospholipase A activity is low. Below this level the synthesis of prostaglandins occurs - whereas above this level, synthesis is completely inhibited. The fall in the concentration of progesterone in response to the administration of Epostane may not have been sufficient to reach this threshold.

These investigations described above confirm that progesterone is important for maintenance of human pregnancy.

However, as the significant decline in progesterone levels induced by Epostane did not induce uterine activity, progesterone cannot alone control uterine activity.

The effect of Epostane on the peripheral level of oestradiol in human pregnancy was found to be dependent on the period of gestation. In the early gestational age group (5-7 week of pregnancy), where the ovary is the site of steroidogenesis, a decline in the level of oestradiol did not accompany the fall in the level of progesterone. In the later gestational age group (12-18 weeks of gestation), where the placenta is the site of steroidogenesis, there was a marked decline in both the level of oestrogen and progesterone. In the intermediate gestational age group there was an intermediate effect on the level of oestradiol. Synthesis of oestradiol in the human placenta is from fetal precursors via dehydroepiandrosterone sulphate (DHEAS) and presumably is

blocked by inhibition of placental 3β-HSD. The lack of a significant effect of Epostane on oestradiol synthesis in the ovary is consistant with the view that 3β-HSD is not rate limiting in the pathway to oestradiol in the ovary.

Although this study was performed in early pregnancy and results can not always be extrapolated to term pregnancy, a decline in the local concentration of progesterone did not induce uterine activity. A similar decline would be expected in term pregnancy after the use of Epostane. If this occurred without inducing uterine activity it would further support the hypothesis that progesterone is not involved in parturition in human pregnancy at term.

HYPOTHESIS THREE. "A fall in the circulating level of progesterone in the pregnant ewe will initiate parturition."

Although our understanding of the complex process of parturition in the pregnant ewe has advanced in the last 25 years, there remain aspects which require clarification. One critical step is the mechanism which initiates and controls the massive release of prostaglandins from the uterus which in turn is responsible for the initiation of parturition.

It is well established in ovine pregnancy that activation of the fetal pituitary - adrenal axis triggers parturition (Liggins et al, 1973). The resultant fetal cortisol surge activates the placental enzymes involved in the conversion of progesterone to oestrogen (Flint et al, 1975) and results in a decline in the concentration of progesterone together with a rise in the concentration of oestrogen in the maternal circulation.

It is widely accepted that myometrial contractions are both initiated and maintained by the uterine secretion of prostaglandins. The evidence for this is comprehensive and has been extensively reviewed (Liggins, 1981; Mitchell, 1981). However, it remains unclear whether the synthesis of prostaglandins is inhibited during pregnancy and this inhibition is removed prior to parturition or whether synthesis is stimulated immediately prior to parturition.

In the pregnant ewe the accepted hypothesis is that the increase in the oestrogen-progesterone ratio stimulates

prostaglandin synthesis. The major drive to $PGF_{2\alpha}$ synthesis is considered to be the increasing oestrogen concentration (Chapter 1). Progesterone is not usually considered to have a dominant role but there is conflicting evidence. High doses of exogenous oestrogens are required to initiate labour, which in some animals is abnormal (Liggins et al, 1977). Furthermore, in certain experimental situations delivery can follow the intrafetal administration of glucocorticoids without an increase in the maternal concentration of oestrogen (Kendall et al, 1977).

It is clear that progesterone has an important role in the pregnant ewe in maintaining uterine quiescence. The studies of Csapo (1956) on uterine muscle strips, of Carsten (1974) on the effect of progesterone on intracelluler calcium levels and of Garfield et al (1979) on gap junction formation describe physiological effects of progesterone which would support the suggestion that progesterone is the hormone responsible for inhibiting uterine activity during pregnancy. Lye and Porter (1978) demonstrated that progesterone has a direct action on uterine activity and that progesterone abolished the uterine reactivity to both oxytocin and prostaglandin $F_{2\alpha}$.

The only evidence against the hypothesis that progesterone inhibits uterine activity during pregnancy is the failure of physiological amounts of exogenous progesterone to inhibit labour (Bengtsson and Schofield, 1963; Liggins et al, 1973). Uterine activity could be blocked only by pharmacological doses of progesterone (200mg/day). Why were such large doses

required? There are two possible explanations. Progesterone is thought to inhibit uterine activity both by a direct action on the smooth muscle and by an indirect action mediated by modulating the synthesis of prostaglandins. At the site of synthesis of prostaglandins in the uterine epithelium, the concentration of progesterone is probably very high relative to the plasma level, since it is secreted by the adjacent trophoblastic cell. A dose of exogenous progesterone sufficient to prevent the normal prepartum fall in the peripheral concentration of progesterone may also prevent a fall in the concentration of progesterone in the myometrium but is unlikely to be sufficient to maintain the concentration in the tissues that are the site of synthesis of prostaglandins.

Hence if it is the oestrogen - progesterone ratio which controls the synthesis of prostaglandin the prepartum ratio may not be restored in the tissues by exogenous progesterone.

An alternative explanation is that once the decline in progesterone levels has triggered prostaglandin secretion, alteration in progesterone levels cannot prevent prostaglandin secretion. Evidence from this study supports this view. Progesterone levels in the uterine vein were found to be increasing 24 hours after Epostane administration. At which time prostaglandin levels were increasing and labour was progressing.

The availiability of a specific inhibitor of progesterone synthesis allowed the study of the effect of a drug-induced

decline in the concentration of progesterone without an alteration in oestrogen levels. Intravenous administration of Epostane to a pregnant ewe on day 131 of gestation led to a rapid decline in the maternal concentration of progesterone. There was no significant alteration in the concentration of oestradiol-17β in either the peripheral or uterine vein and levels remained significantly below that found in spontaneous labour. The concentration of PGFM level rose rapidly and labour was established within 6 hours. These results confirm that in this species a major reduction in the level of progesterone without an alteration in the concentration of oestradiol will lead to prostaglandin synthesis and parturition. A lesser reduction in the concentration of progesterone after inibition of 3β -HSD by Trilostane (Jenkin and Thorburn, 1985) did not consistently lead to delivery. This suggests that the concentration of progesterone or the oestrogen to progesterone ratio must fall to a specific threshold level for the initiation of parturition.

Further clarification of the role of oestradiol-17β could be forthcoming from studies where oestradiol-17β was given simultaneously with Epostane. Such studies could determine whether the resultant increase in the oestrogen - progesterone ratio would enhance the release of prostaglandins and further shorten the time interval to delivery.

The work in this thesis is consistant with the view that progesterone is involved in the inhibition of prostaglandin

synthesis during pregnancy in the ewe.

GENERAL COMMENTS

For experimental purposes investigating the role of progesterone in the control of uterine activity, Epostane remains a better tool than RU486 because its effect on the secretion of progesterone can be monitored by measuring the plasma concentration. The effects of RU486 , on the other hand, can not be monitored since plasma concentrations of progesterone are unaffected and the receptor site interactions cannot usually be determined in vivo.

The design of the studies have a number of deficiencies. In the experiments in the luteal phase of human menstrual cycle ovulation was not confirmed prior to the study. It was assumed that women with regular menstruation would ovulate consistently. This did not occur with all subjects. Future studies should confirm the time of ovulation in the cycle by determining the time of the LH surge. The final experiment did not include a control group and only 8 women were studied, in only five was ovulation confirmed by an elevated luteal phase progesterone level. However the findings were consistent in this small group.

The initial single dose human placebo control trials were time consuming but necessary because of the use of a potent inhibitor of steridogenesis with possible adverse effects on adrenal steroidgenesis. For this reason a study with an

incremental dosage increase was performed with close monitoring of the patient and her cortisol levels. A possible adverse fetal effect precluded studies of the effect of inhibition of the synthesis of progesterone at the time of major interest - the third trimester.

A control group was not used in the study of the effect of Epostane in ovine pregnancy because of lack of a sufficient number of ewes. Three ewes did act as their own controls receiving vehicle only in the interval between surgery and experiment.

The use of Epostane in this thesis clearly demonstrated the variations in pregnenolone metabolism in the three models studied. The effect of Epostane on the concentration of oestradiol-17β in the above three experiments depends on enzyme activity in the pathway of pregnenolone metabolism. It was expected that inhibition of the 3β-HSD enzyme system would lead to a decline in the concentration of both progesterone and oestrogen in the three experimental models studied. This only occurred in the in human pregnancy experiments.

During the luteal phase of the menstrual cycle, where Epostane (100mg eight hourly) reduced the concentration of progesterone from 35.9 to 8.9 nmol/l after five days of treatment (Fig 4.7, Table 4.5), the concentration of oestradiol in the peripheral blood remained unchanged (Fig 4.8). This observation is consistent with the view that in the human ovary, the enzyme aromatase is rate limiting with

regard to ovarian oestrogen production. With only a small conversion of androstenedione to oestrone / oestradiol, a reduction in availability of substrate under these experimental conditions of 75%, presumably would not significantly alter the rate of synthesis and hence the peripheral concentration of oestradiol remains unchanged.

In the pregnant ewe, where Epostane (100mg IVI) reduced the concentration of progesterone from 73 nmol/l to 3.2 nmol/l (Fig.2.3), the concentration of oestradiol in both the uteroovarian and peripheral circulation was not altered significantly (Fig 2.5 and 2.6). In ovine pregnancy, apart from the last 48 hours of gestation, when the activity of the placental enzyme 17α hydroxylase increases, placental oestrogen production occurs at a very slow rate (Heap, Galil, Harrison, Jenkin and Perry, 1977). Placental production of progesterone on the other hand, proceeds at a relatively high rate. The principal reason for the low level of oestrogen synthesis appears to be lack of formation of aromatase substrate, androstenedione (Liggins et al,1977 ; France et al,1988) due to absence of placental 17α - hydroxylase activity. It is not unexpected, therefore, that Epostane would produce a significant fall in the concentration of progesterone without a detectable fall in the level of oestradiol.

In human pregnancy at 12 to 18 weeks gestation, Epostane (100mg eight hourly) led to a significant reduction in the peripheral plasma concentration of both progesterone and oestradiol from 141.9 to 17.4 nmol/l and 17.2 to 4.5 nmol/l

respectively (Fig 3.15 and 3.16). The human placenta is rich in steroid sulphatase and 3β-HSD but lacks 17α hydrozylase. Progesterone is an end product of metabolism of pregnenolone derived largely from maternal cholesterol, while other oestrogens are formed predominantly from fetal precursors DHEAS and 16 OH DHEAS (Casey, MacDonald and Simpson,1981). In contrast to the human ovary and the ovine placenta, as confirmed by this study, Epostane should depress the synthesis of both progesterone and oestrogen in the human placenta through inhibition of 3β HSD. The concommitant fall in oestradiol concentration with that of progesterone, in this experiment, reflects the inhibition of both the conversion of dehydroepiandrostenedione to androstenedione and pregnenolone to progesterone.

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PUBLICATIONS

Inhibition of 3β-Hydroxysteroid Dehydrogenase (HSD) Activity in First- and Second-Trimester Human Pregnancy and the Luteal Phase Using Epostane

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Inhibition of 3β-hydroxysteroid dehydrogenase (3β-HSD) activity in first- and second-trimester human pregnancy and the luteal phase using Epostane*

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The effects of a competitive inhibitor of 3β-hydroxysteroid dehydrogenase (3β-HSD) (Epostane, Sterling Winthrop, Guildford, England) on serum progesterone (P), estradiol (E_2), and cortisol have been studied in three groups of pregnant women awaiting termination of pregnancy (5 to 8 weeks, 8 to 12 weeks, and 12 to 18 weeks of pregnancy) and 15 women in the luteal phase of the menstrual cycle. A single-dose randomized double-blind study was performed, each woman receiving a placebo, 50 mg of Epostane, or 100 mg of Epostane. In the pregnant group, there was a significant decline in the serum P concentration after both 50 mg and 100 mg of Epostane. The percentage fall increased with both drug dosage and advancing gestation. A similar fall in serum E_2 was observed. Both of these effects were temporary. In the luteal phase group, a significant decline in serum P was observed after 100 mg of Epostane, but the serum E_2 was not significantly different from the pretreatment concentration. Serum cortisol did not differ significantly from control values. These findings suggest that Epostane is an effective inhibitor of placental and ovarian 3β-HSD, which may have a role as an interceptive agent. Fertil Steril 42:875, 1984

Progesterone (P) is required both for the maintenance of a secretory endometrium during the menstrual cycle and for the continuation of human pregnancy.

Removal of the corpus luteum prior to the luteoplacental shift, at about 49 days, results in a dramatic fall in peripheral serum P and leads to uterine activity and abortion. Uterine activity, however, only occurred in the studies of Csapo and Pulkkinen[1] when the plasma P concentration fell below 10 ng/ml (31.8 nmol/l) and abortion when the P concentration was < 4 ng/ml (12.7 nmol/l). Administration of 100 mg P by twice-daily intramuscular injection prevented these changes.

The corpus luteum and the placenta are possible sites for pharmacologic intervention. Drugs that inhibit ovarian steroidogenesis in the luteal phase should prevent implantation, and early abortion should occur if ovarian and placental P production is reduced to the critical levels reported by Csapo and Pulkkinen.[1]

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An orally effective 3β-hydroxysteroid dehydrogenase (3β-HSD) inhibitor, Epostane (Sterling Winthrop, Guildford, England) (4,5-epoxy-17-hydroxy-4,17-dimethyl-3-oxo-androstate-2-carbontrile), has recently been synthesized. This is a 17-alkylated steroid molecule which has been shown to competitively inhibit the 3β-HSD 4,5-isomerase system, both in vitro and in vivo. The compound, which is nonandrogenic, does not have any intrinsic hormonal activity and has been found to inhibit P synthesis in the monkey at a dose that does not affect adrenal corticosteroid production.[2] The molecule has been shown to be free from toxicity in both acute studies on mice, rats, rabbits, and male volunteers and in chronic studies on rats and monkeys over 6 months.[3]

The pharmacologic and endocrine profile of this drug therefore suggests that it might be used to inhibit P synthesis from the corpus luteum in the luteal phase of the menstrual cycle and from the placenta and corpus luteum in pregnancy.

This study describes the effects of Epostane on serum P, estradiol (E_2), and cortisol in women during the luteal phase of the ovulatory cycle and in early pregnancy.

MATERIALS AND METHODS

SUBJECTS

Two groups of healthy women gave written informed consent to the study: 45 women who were between 5 and 18 weeks of pregnancy, and 15 women in the luteal phase of their menstrual cycle.

The pregnant group was subdivided into three groups of 15 women according to their period of gestation (5 to 8, 8 to 12, and 12 to 18 weeks of pregnancy). The patients had a history of regular menstrual cycles and were certain of the date of their last menstrual period, which agreed with the clinical assessment in all cases. The luteal phase group comprised 15 healthy women who had previously been sterilized and had a regular menstrual cycle. Women with a significant medical history, taking medication, or with a contraindication to steroid therapy were not recruited.

The Hospital Ethics Committee approved this study.

STUDY DESIGN

The subjects were randomly allocated by a double-blind procedure to one of three treatment regimens. Each patient received a single tablet of a placebo, 50 mg of Epostane, or 100 mg of Epostane. The duration of the study was 24 hours.

Each patient was examined to exclude any medical disorder. A complete blood count and a biochemical profile including urea, electrolytes, and liver function tests (total protein, albumin, bilirubin, alkaline phosphatase, and aspartate transaminase) were taken the day before the study. The pregnant group was studied in the 24-hour period immediately prior to termination. The luteal phase group was studied between days 18 and 24 of their menstrual cycle. The weight, height, blood pressure, and pulse of each subject were recorded.

The study was performed with the subject semirecumbent. Blood was collected with an indwelling venous catheter in a forearm vein. A pretreatment blood sample was taken for serum P, cortisol, and E_2 estimation. In the luteal phase, follicle-stimulating hormone (FSH) was also measured. Cannula patency was maintained by flushing with 1 to 3 ml of sodium citrate after each sample was taken.

Approximately 30 minutes later a second blood sample was taken, and the tablet was administered. Further samples were then taken 30, 60, 90, 120, 180, 240, and 360 minutes later. The patient's blood pressure and pulse were recorded at the time of blood sampling.

Twenty-four hours after tablet ingestion the patients were questioned to determine whether the tablets had caused any side effects. A final blood sample was taken at this time to measure the above hormone concentrations and to obtain a complete blood count, liver function tests, and to measure the urea and electrolyte concentrations.

The pregnant subjects had an abortion, as planned, later that day. The women in the luteal phase group were asked to inform us of the date of their next menstrual period.

ANTISERA AND CHEMICALS

Antisera were raised in goats against P-11-bovine serum albumin and E_2-17-bovine serum albumin, and the cortisol antiserum was raised in rabbits. The steroids, cortisol, P, and E_2, were obtained from Sigma Chemicals, London, England. The substances (1α,2α-^{3}H) P (1.96 TBq/mmol), (4^{14}C) cortisol (2 GBq/mmol), and (2,3,6,7-^{3}H) E_2-17β (3.99 TBq/mmol) were obtained from the Radiochemical Centre, Amersham, England.

The chemicals for the buffer solutions, Fisofluor 2 (a toluene-based scintillation fluid) and the light petroleum (bp 40 to 60°C), were obtained from Fison Scientific Apparatus, Loughborough, England. Diethyl ether was obtained from Mallinckrodt Chemical Works, St. Louis, MO, through Camlab Ltd., Cambridge, England. The phosphate buffer and the dextran-coated charcoal were prepared as described previously.[4]

RADIOIMMUNOASSAY

Progesterone

Serum P levels were assayed as described from this laboratory by Flint et al.[4] The intraassay and interassay coefficients of variation were 8.1% (n = 10) and 10.1% (n = 21), respectively (at 20 ng/ml).

Estradiol

E_2 was assayed in a manner similar to that for P, with the exception that diethyl ether was used for extraction. The intraassay and interassay coefficients of variation were 8.9% (n = 10) and 9.9% (n = 10), respectively (at 1 ng/ml).

Cortisol

The cortisol assay was based on that described by Lopez Bernal et al.[5] The intraassay coefficient of variation was 9.6% at 750 nmol/l (n = 8). The interassay coefficient of variation was 13.7% at 750 nmol/l (n = 18).

Follicle-Stimulating Hormone

This assay was performed with an FSH/luteinizing hormone kit purchased from Chelsea Women's Hospital, London, England.[6]

STATISTICAL METHODS

The mean (± standard error of the mean [SEM]) for each subgroup at each time point during the study was calculated. The first two pretreatment measurements were averaged to give a "pretreatment value." All subsequent results were expressed as a percentage of this value.

The results of the various treatment regimens and gestational age groups were compared using the Statistical Package for the Social Sciences. The analysis of variance subprogram was used to test for significant differences between the means

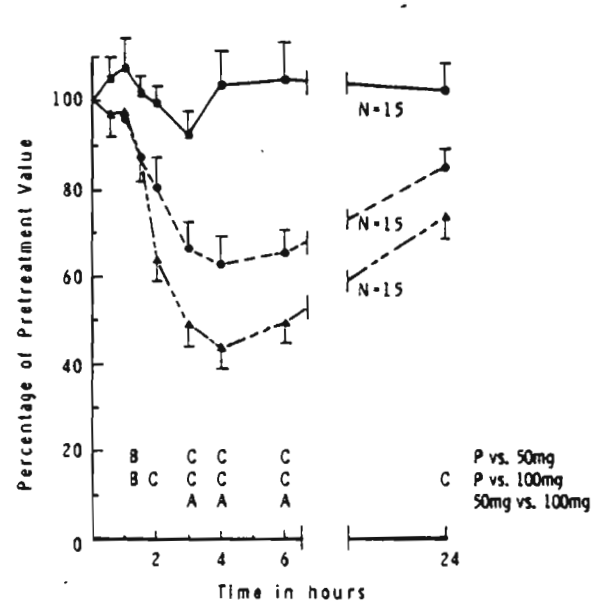

Figure 1
Changes in the peripheral concentration of serum P (mean ± SEM) in 45 pregnant women after administration of a placebo (■– – –■), 50 mg of Epostane (●– – –●), or 100 mg of Epostane (▲– – –▲). The pretreatment values were 24.5 ± 4.94, 26.36 ± 2.44, and 26.45 ± 3.33 ng/ml, respectively. This figure combines the data from all three gestation groups, 5 to 8, 8 to 12, and 12 to 18 weeks of pregnancy. There were significant differences between the three groups as indicated: *A*, $P < 0.05$; *B*, $P < 0.01$; *C*, $P < 0.001$.

in each cell. Whenever the analysis of variance program showed a significant difference between groups, Student's t-test was performed to determine where the difference lay.

RESULTS

EARLY PREGNANCY GROUP

Serum Progesterone

Administration of a single tablet of Epostane lowered the mean peripheral serum P concentration in all the treated groups (Fig. 1). The nadir was at 4 hours and was significantly greater with 100 mg than with 50 mg of Epostane (P = 0.05). There was no change in peripheral serum P concentration in the placebo group. The decline in the serum P concentration was significantly greater in the second trimester than in the first (Fig. 2) (P = 0.004). Twenty-four hours after drug administration the mean peripheral serum P concentrations in the treated groups were still below the pretreatment value.

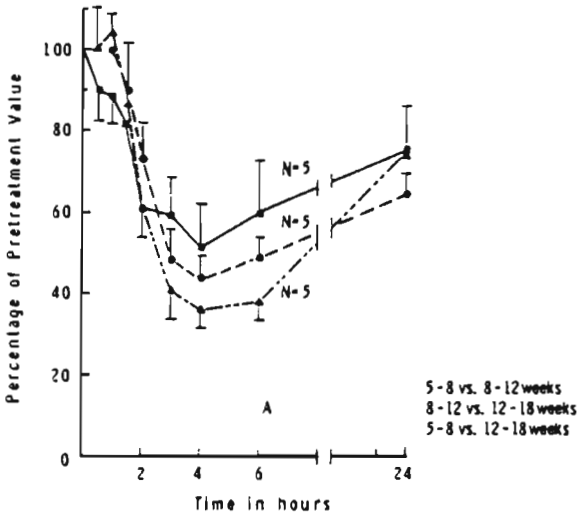

Figure 2
Changes in the peripheral concentration of serum P (mean ± SEM) after administration of 100 mg of Epostane to five pregnant women in each of the three gestation groups: 5 to 8 weeks (■– – –■), 8 to 12 weeks (●– – –●), and 12 to 18 weeks (▲– – –▲) of pregnancy. The pretreatment values were 23.4 ± 4.4, 21.4 ± 2.8, and 34.5 ± 2.8 ng/ml, respectively. There was a significant difference between the groups as indicated: *A*, $P < 0.05$.

Serum Estradiol

The peripheral serum E_2 fall after a single dose of Epostane paralleled that of serum P (Fig. 3). A significant decline was observed at 1 hour in all treated groups ($P = 0.003$). The effect was maximal at 4 hours, and the serum E_2 concentration had nearly returned to the pretreatment value by 24 hours. The decline in serum E_2 concentration also increased with drug dosage and advancing pregnancy (Fig. 4).

Serum Cortisol

The peripheral serum cortisol concentrations in the placebo group followed the expected daily diurnal pattern (Fig. 5). The concentration in the treated groups did not differ significantly from this curve. There was no significant drug or gestation effect.

LUTEAL PHASE GROUP

Serum Progesterone

Administration of a single tablet of Epostane also lowered the peripheral serum P concentration significantly in the menstrual cycle (Fig. 6). The maximum fall from the pretreatment value was at 4 hours when the circulating serum P concentration after 100 mg of Epostane was significantly below the pretreatment value ($P = 0.006$). There was no significant difference between the groups 24 hours after drug administration.

Serum Estradiol

There was no significant change in the peripheral serum E_2 concentrations throughout the study.

Serum Cortisol

The peripheral serum cortisol concentrations in the placebo group followed the expected daily diurnal pattern. The concentration in the treated groups did not differ significantly from this curve. There was no significant drug effect.

Serum Follicle-Stimulating Hormone

There were no significant changes in the serum FSH concentrations during the study.

CLINICAL EFFECT

Only one patient reported symptoms within the 24-hour study period. She had received a placebo

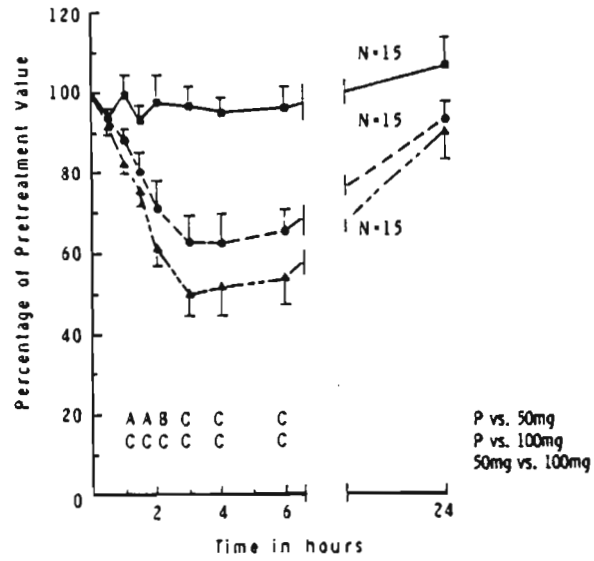

Figure 3
Changes in the peripheral concentration of serum E_2 (mean ± SEM) in 45 pregnant women after administration of a placebo (■– – –■), 50 mg of Epostane (●– – –●), or 100 mg of Epostane (▲– – –▲). The pretreatment values were 8.49 ± 1.2, 11.6 ± 2.4, and 11.39 ± 2.2 nmol/l, respectively. This figure combines the data from all three gestation groups, 5 to 8, 8 to 12, and 12 to 18 weeks of pregnancy. There were significant differences between the three groups as indicated: *A*, $P < 0.05$; *B*, $P < 0.01$; *C*, $P < 0.001$.

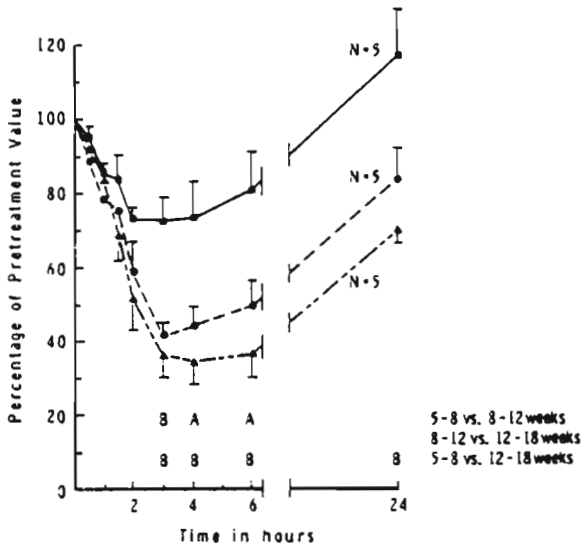

Figure 4
Changes in the peripheral concentration of serum E_2 (mean ± SEM) after administration of 100 mg of Epostane to five pregnant women in each of the three gestation groups: 5 to 8 weeks (■– – –■), 8 to 12 weeks (●– – –●), and 12 to 18 weeks (▲– – –▲) of pregnancy. The pretreatment values were 3.00 ± 0.74, 9.44 ± 0.78, and 23.19 ± 4.19 nmol/l, respectively. There were significant differences between the groups as indicated: *A*, $P < 0.05$; *B*, $P < 0.01$.

tablet. There were no significant changes in blood pressure, pulse, or temperature during the study.

In the treated pregnant group, there were no symptoms which could be attributed to uterine activity, and there was no vaginal bleeding nor any abdominal pain. In the luteal phase group, menstruation occurred at the expected time, and the subsequent menstrual cycle was also of normal length.

HEMATOLOGY AND BIOCHEMISTRY

Comparison of the pretreatment hematologic and biochemical indices with those 24 hours after tablet ingestion showed no significant change.

DISCUSSION

Administration of a 3β-HSD inhibitor, Epostane, to women in early pregnancy and in the luteal phase of the menstrual cycle resulted in a significant fall in the concentration of circulating P. Preferential inhibition of ovarian and placental hormone production occurred, with no apparent effect on adrenal steroidogenesis. The drug appears to specifically inhibit the conversion of pregnenolone to P as demonstrated in animals and in some women.[7] It has also been shown to produce a rise in the Δ-5 precursor, dehydroepiandrosterone, when given at the higher dosage levels.[7]

The magnitude of the decline in P concentration was related to the duration of pregnancy at the time of the study. At 5 to 8 weeks of pregnancy, when the corpus luteum is providing most of the circulating P,[1] the fall in the P concentration was significantly less than at 12 to 18 weeks' gestation, after the luteoplacental shift has occurred. This may indicate that the placenta is more susceptible to 3β-HSD inhibition than the ovary. The maximal P fall in the luteal phase group was to 63.5% of the pretreatment value, but at 12 to 18 weeks of pregnancy the same dose produced a fall to 35.5% of the pretreatment value.

A single dose of Epostane induced only temporary inhibition of ovarian and placental steroidogenesis, and in all the treated groups the P concentration was returning to pretreatment values by 24 hours. This demonstrates, first, that the block is temporary, a critical factor if this drug is to have a clinical use, and, second, that a multiple dose regimen will be needed to produce any clinical effects.

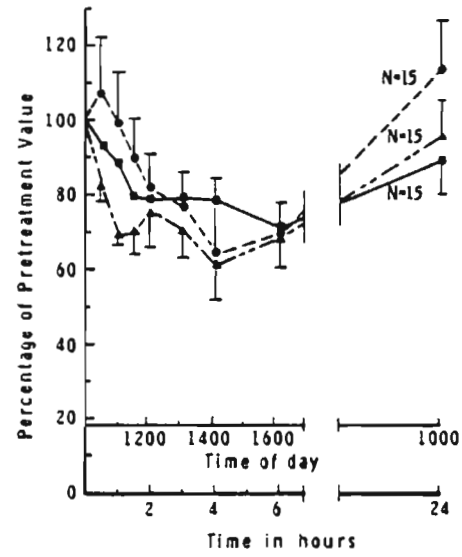

Figure 5
Changes in the peripheral concentration of serum cortisol (mean ± SEM) in 45 pregnant women after administration of a placebo (■– – –■), 50 mg of Epostane (●– – –●), or 100 mg of Epostane (▲– – –▲). The pretreatment values were 479.3 ± 83.2, 627.6 ± 82.6, and 604.0 ± 117.3 nmol/l, respectively. This figure combines the data from all three gestation groups. There were no significant differences between the three groups.

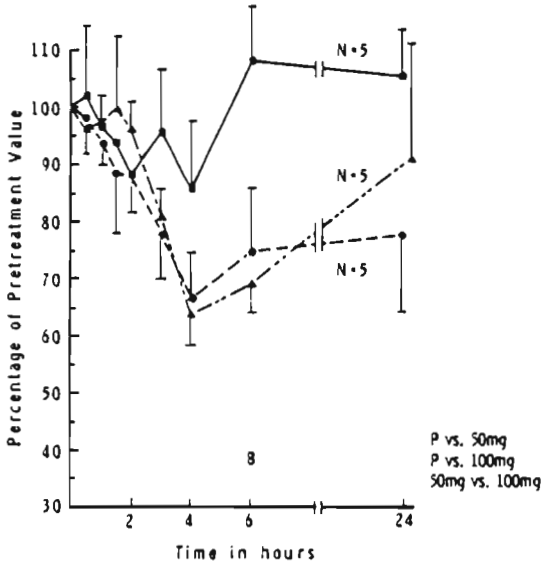

Figure 6
Changes in the peripheral concentration of serum P (mean ± SEM) after administration of a placebo (■– – –■), 50 mg of Epostane (●– – –●), or 100 mg of Epostane (▲– – –▲) to 15 women in the luteal phase of the menstrual cycle. The pretreatment values were 11.4 ± 1.7, 9.9 ± 1.7, and 7.5 ± 1.1 ng/ml, respectively. There was a significant difference between the groups as indicated: *B*, $P < 0.01$.

The drug caused a fall in the E_2 concentration in the pregnant women but not in those studied during the luteal phase of the menstrual cycle. In the pregnant group this decline was significant after 1 hour and reached a nadir at 4 hours.

The circulating cortisol concentrations in the control groups followed the expected diurnal pattern. The cortisol concentration in the treated groups did not differ significantly from the placebo groups, which suggests a minor effect, if any, on the adrenal. This is at variance with the findings of Van der Spuy et al.[7] In a similar study of pregnant women between 8 and 12 weeks they found significant early inhibition of adrenal 3β-HSD with a 100-mg single dose of Epostane between 4 and 6 hours. This was based on comparison of cortisol/dehydroepiandrosterone ratios between control and treated groups. The cortisol levels of their control group did not, however, follow the normal diurnal pattern as observed in this study, and this may explain this observation.

Androgenic 17-alkylated steroids have been incriminated in the etiology of hepatic hyperplasia, peliosis, and liver tumors.[3] Epostane is a 17-alkylated steroid, but it is nonoandrogenic, and in this study there were no significant drug-related changes in hepatic proteins, enzymes, or plasma bilirubin concentrations.

No clinical effects or side effects were noted in any of the women who received Epostane. Although the serum P concentration fell below the critical level for myometrial activity demonstrated by Csapo and Pulkkinen[1] of 31.8 nmol/l (10 ng/ml) for 2 to 3 hours in the group that received 100 mg Epostane, no patient was aware of uterine contractions. Presumably the decline in the mean circulating P concentration was of insufficient duration. A further reduction in P concentration would be expected after multiple doses of the drug; and if sufficient suppression of the concentration of P can be achieved, Epostane may prove to be an effective early abortifacient.

In the luteal phase group, there was no change in the date of expected menstruation despite a transient fall in the circulating P concentration. The subsequent menstrual cycle was also of expected length, indicating that there was no permanent enzyme block. Although the mean P fall was only to 63.8% of the pretreatment value, a multiple dose regimen may further reduce the circulating concentration sufficiently to induce menstruation. Inhibition of the ovarian 3β-HSD enzyme could thus provide a role for Epostane as an interceptive agent.

The sensitivity of the 3β-HSD enzyme system to Epostane in late pregnancy has yet to be determined. There was an increased effect with advancing pregnancy, which suggests that the compound may also induce uterine activity in late pregnancy.

In conclusion, Epostane is an effective inhibitor of ovarian and placental steroidogenesis without any short-term side effects or deleterious effects on adrenal steroidogenesis or hepatic function. Its ability to modify ovarian and particularly placental steroidogenesis suggests that it may have unique potential as an interceptive agent or abortifacient. In addition, if the drug is shown to be effective in late pregnancy, it may help to elucidate the role of P in human parturition.

Acknowledgments. We are grateful for the willing participation of the patients and for the cooperation of the nursing staff of the Oxford hospitals. The laboratory radioimmunoassays would not have been possible without the expertise of Drs. Lyn Harrison, Linda Glover, and Fiona Daniels. The P and E_2 antisera were the gift of Dr. B. J. A. Furr, I.C.I. Pharmaceuticals Division, Macclesfield, England. The cortisol antiserum was donated by Dr. Andreas Lopez Bernal, from this depart-

ment. We are also grateful to Professor Alexander C. Turnbull for his support and encouragement.

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Prolonged inhibition of placental and ovarian 3β-hydroxysteroid dehydrogenase during pregnancy and the luteal phase of the menstrual cycle

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Summary

The effects of a competitive inhibitor of 3β-hydroxysteroid dehydrogenase (3β-HSD), Epostane (Sterling Winthrop) on serum progesterone, oestradiol and cortisol have been studied in twenty women who were awaiting termination of pregnancy and thirteen women in the luteal phase (days 19–21) of their menstrual cycle. In the pregnant women there was a highly significant decline in the mean serum progesterone concentration to 13·0 nmol/litre (19 per cent of the pretreatment value) after 4 days of treatment. A similar fall in serum oestradiol was observed. Progressive uterine activity did not occur in association with these changes. In the luteal phase study there was also a significant decline in serum progesterone to 13 per cent of the pretreatment value and this was accompanied by uterine bleeding in 6 of 8 patients. The mean serum oestradiol concentration did not change. This treatment did not produce any adverse side effects and the mean serum cortisol concentrations in both studies did not differ from those of the controls. Epostane is an effective inhibitor of placental and ovarian 3β-HSD, and may have an important clinical role as an interceptive agent.

PROGESTERONE is considered to be indispensable for the maintenance of a secretory endometrium during the menstrual cycle and for the continuation of human pregnancy. At the end of the menstrual cycle a decline in progesterone production by the corpus luteum occurs and this induces menstruation. Csapo and Pulkkinen (1978) reported that removal of the corpus luteum before the luteo-placental shift at about 49 days' gestation resulted in a dramatic fall in serum progesterone concentration producing progressive uterine activity and abortion. Uterine contractions only occurred when the serum progesterone concentration fell to 32 nmol/litre (10 ng/ml) and abortion when circulating progesterone was less than 13 nmol/litre (4 ng/ml).

Progesterone synthesis from either the corpus luteum or the placenta is a possible site for pharmacological intervention. Drugs which inhibit ovarian steroidogenesis should disrupt hormone production in the luteal phase and prevent implantation. Similarly in early pregnancy inhibition of ovarian and placental steroidogenesis should confirm Csapo's theory and result in a critical degree of progesterone 'withdrawal' and subsequent abortion.

In separate work by Pattison *et al.* we studied the effect of a single dose of a new oral 3β-hydrooxysteroid dehydrogenase inhibitor, Epostane (4,5-epoxy-17-hydroxy-4,17-dimethyl-3-oxo androstane-2-carbonitrile, Sterling Winthrop) on ovarian and placental steroidogenesis. This significantly reduced the serum progesterone concentration in both the luteal phase of the menstrual cycle and in early pregnancy. There was a similar fall in oestradiol concentration in pregnancy but not in the luteal phase. There was no effect on cortisol production nor any adverse side effects.

Although 100 mg of Epostane induced a fall in circulating progesterone to 63·5 per cent of the pretreatment value in the luteal phase and to 43·3 per cent of the pretreatment value in the pregnant subjects, this effect was only maintained for 1–2 hours and there were no clinical effects. In the present study multiple doses of Epostane were used to prolong the progesterone suppression and enable further study of Csapo's theory of 'progesterone block'.

* Deceased 11 February 1983.

MATERIALS AND METHODS

Subjects and study design

Two groups of healthy women gave written informed consent to the study. Twenty women were between 5 and 18 weeks of pregnancy, and thirteen in the luteal phase of the menstrual cycle.

Fifteen of the pregnant subjects were studied for 1 day and five for 7 days. The fifteen women in the 1 day study were between 12 and 18 weeks of pregnancy and were studied during the 24 hours before termination of pregnancy. Ten were randomly allocated by a double blind procedure to one of two treatment regimes: each patient received either a placebo, or 100 mg of Epostane 6-hourly for 24 hours. The remaining five subjects received 100 mg of Epostane 8-hourly. Intrauterine pressure monitoring was performed in this group.

The five women in the 7 day study were 8–12 weeks pregnant and received 100 mg of Epostane 8-hourly for 5 days beginning 1 week before planned termination of pregnancy. All subjects had a history of regular menstrual cycles and were certain of the date of their last menstrual period. The size of the uterus was compatible with the calculated gestational age in all cases.

The luteal phase group comprised thirteen healthy women, who had previously been sterilised. All had a regular menstrual cycle. The study was commenced between days 19 and 21 of the cycle and continued until the subsequent menstrual period. Five received a placebo and eight 100 mg of Epostane 8-hourly for 5 days.

Women with a significant medical history, taking medication or with a contraindication to steroid therapy, were not recruited.

The Hospital Ethics Committee approved this study.

Study procedure and methods

Each patient was examined to exclude any medical disorder. A complete blood count and a biochemical profile, including blood urea, electrolytes and liver function tests (total protein, albumin, bilirubin, alkaline phosphatase and aspartate serum transaminase), were made on the day before the study. The weight, height, blood pressure and pulse of each subject were recorded.

Blood sampling in the 1 day studies was *via* an indwelling venous catheter in a forearm vein and in the 7 day studies by repeated venepuncture. A pretreatment blood sample was taken for serum progesterone, cortisol and 17β-oestradiol measurement. Cannula patency was maintained by flushing with 1–3 ml of sodium citrate 0·1 per cent B.P. after each sample.

In the 24 hour study a second pretreatment sample was taken approximately 30 minutes later and the tablet was administered. Further samples were then taken every 3 hours. Blood samples were taken in the morning at the same time in each subject during the 7 day study. Serum progesterone, oestradiol and cortisol were measured in all blood samples. The patient's blood pressure and pulse were recorded at the time of blood sampling.

Uterine pressure monitoring was performed in the five pregnant women who received 100 mg of Epostane 8-hourly for 1 day. A Foley catheter was inserted into the uterus at the beginning of the study and remained *in situ* throughout. A 30 minute pressure tracing was performed before treatment and every 8 hours thereafter.

On completion of the study the patients were questioned to determine whether the tablets had had any side effects. A final blood sample was taken at this time to measure the concentrations of the hormones and to obtain a complete blood count, liver function tests and urea and electrolyte estimations.

The pregnant subjects subsequently had an abortion as planned. The women in the luteal phase were asked to inform us of the date of their next menstrual period.

The materials used in the radio-immunoassays are being described elsewhere by Pattison *et al.* Serum progesterone and oestradiol levels were assayed as described from this laboratory by Flint *et al.* (1974). Serum cortisol levels were assayed according to the technique described by Lopez Bernal *et al.* (1982), also from this laboratory.

Statistical methods

Within each subgroup the mean $\pm$ s.e. for each time point during the study was calculated. The first two pretreatment measurements were averaged to give a 'pretreatment value'. All subsequent results were expressed as a percentage of this value.

The results of the various treatment regimens were compared by Student's *t* test.

RESULTS

Early pregnancy

Twenty-four hour study at 12–18 weeks of pregnancy

Serum progesterone. Treatment with Epostane, 100 mg 6-hourly, lowered the progesterone concentration to 11·8 $\pm$ 1·6 per cent (s.e.) of the pretreatment value 21 hours after drug administration. The circulating concentrations of the hormone were below the critical value described

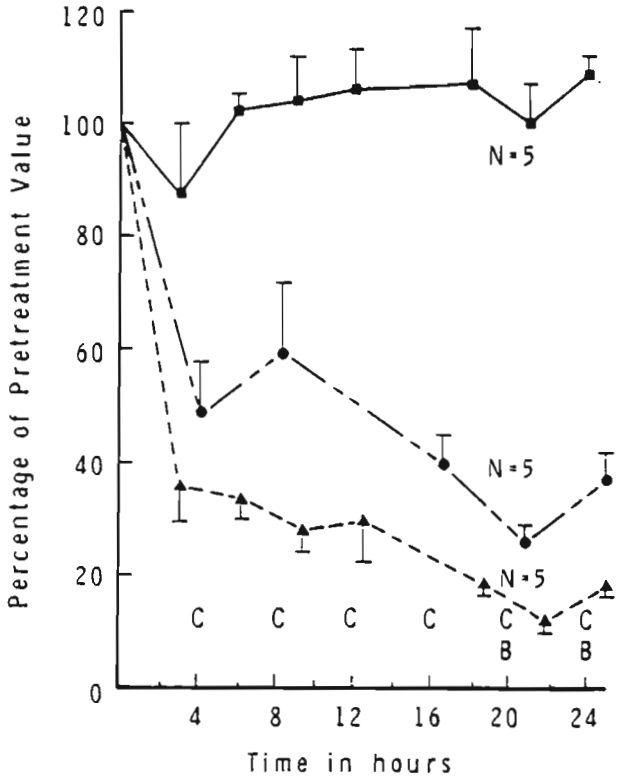

Figure 1. Changes in the peripheral concentration of serum progesterone in fifteen pregnant women (means ± s.e.) between 12 and 18 weeks of pregnancy after administration of a placebo (■) or 100 mg of Epostane 8-hourly (●) or 6-hourly (▲). The pretreatment values were 94·4 ± 9·2, 84·6 ± 12·7 and 141·8 ± 14·3 nmol/litre respectively. Differences between groups: B = $P < 0{\cdot}01$, C = $P < 0{\cdot}001$.

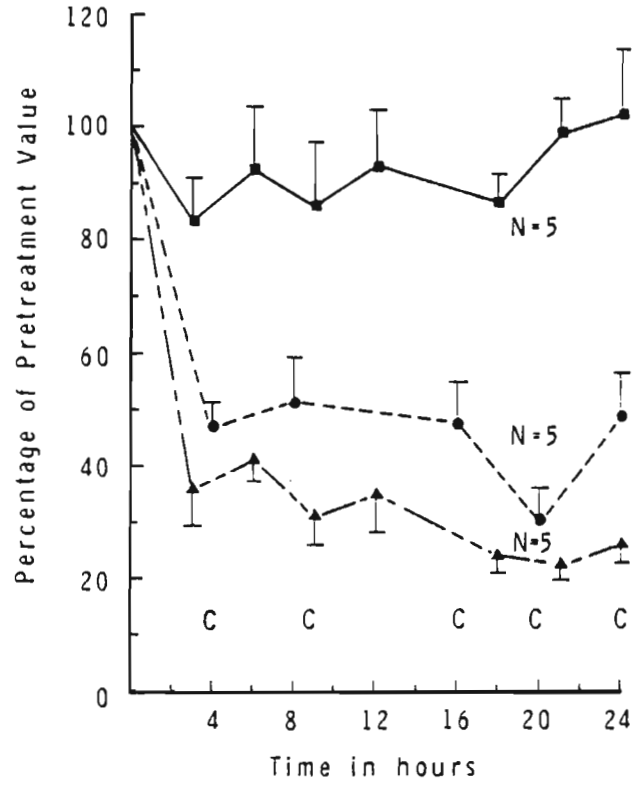

Figure 2. Changes in the peripheral concentration of serum oestradiol in fifteen pregnant women (means ± s.e.) between 12 and 18 weeks of pregnancy after administration of a placebo (■) or 100 mg of Epostane 8-hourly (●) or 6-hourly (▲). The pretreatment values were 11·1 ± 2·4, 17·4 ± 3·4 and 15·4 ± 2·3 nmol/litre respectively. Differences between groups: C = $P < 0{\cdot}001$.

by Csapo as necessary to produce progressive uterine activity for at least 6 hours (Figure 1). The subjects who received 100 mg Epostane 6-hourly showed lower plasma progesterone concentrations at 21 and 24 hours after treatment than those who received 100 mg 8-hourly ($P < 0{\cdot}002$).

Serum oestradiol. The fall in the mean peripheral serum oestradiol concentration after Epostane paralleled that of serum progesterone (Figure 2). There was no difference between the 6- and 8-hourly treatment groups.

Serum cortisol. Peripheral serum cortisol concentrations in the placebo group followed the expected daily diurnal pattern and those of the treated groups did not differ from this curve (Figure 3).

Clinical effects. Three of the five women treated with Epostane, 100 mg 6-hourly, reported mild temporary 'period like' pains. These began at 10 hours after treatment in one patient and 19 hours after treatment in two patients. The pain which continued for an hour or two was not severe and there was no vaginal bleeding. Clinical examination showed no cervical changes.

Three of the five women treated with Epostane, 100 mg 8-hourly, also reported mild temporary 'period like' pains. These began at 15, 16 and 18 hours after treatment and were associated with significant increases in intra-uterine pressure. The two patients who had no symptoms did not have recordable uterine contractions.

Five day study at 8 to 12 weeks of pregnancy

Serum progesterone. Treatment with Epostane, 100 mg 8-hourly, lowered the peripheral serum progesterone concentration from 72·5 ± 10·8 to 13·0 ± 1·6 nmol/litre at 4 days, a fall to 19 per cent of the pretreatment value (Figure 4). Four days after discontinuing the drug the mean serum progesterone concentration had nearly returned to the pretreatment value. In these five patients the progesterone concentration fell to the critical value described by Csapo as necessary to produce spontaneous abortion for between 4 and 5 days.

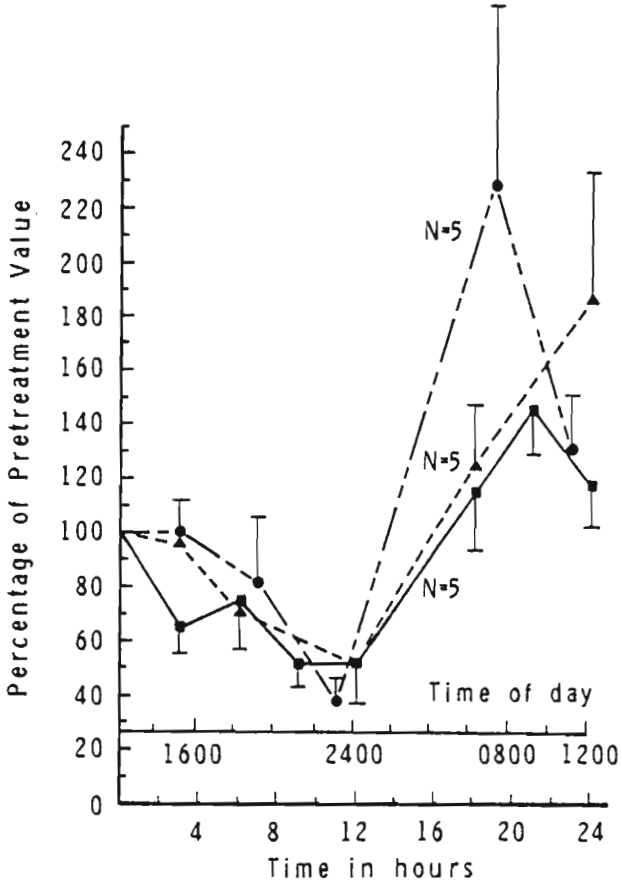

Figure 3. Changes in the peripheral concentration of serum cortisol in fifteen pregnant women (means ± s.e.) between 12 and 18 weeks of pregnancy after administration of a placebo (■) or 100 mg of Epostane 8-hourly (●) or 6-hourly (▲). The pretreatment values were 528 ± 61, 336 ± 44 and 521 ± 99 nmol/litre respectively. There were no significant differences between the three groups.

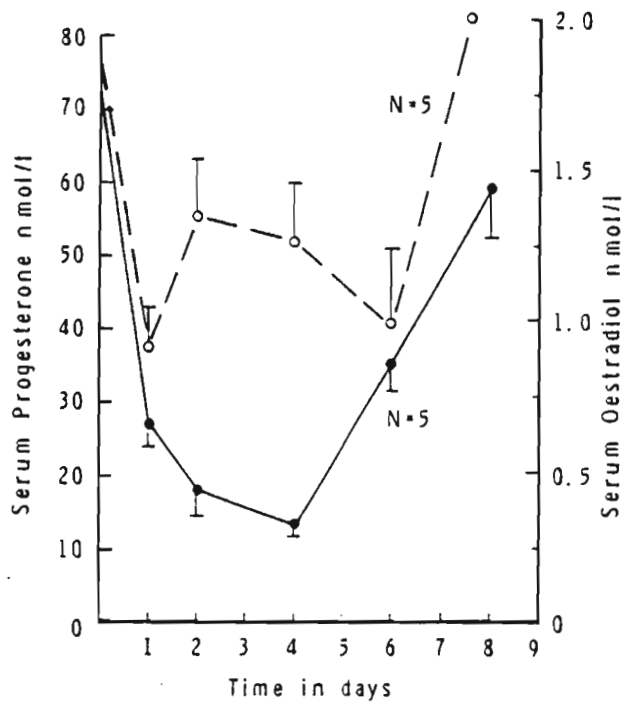

Figure 4. Changes in the peripheral concentration of serum progesterone (●) and oestradiol (○) (means ± s.e.) in five pregnant women between 8 and 12 weeks of pregnancy after administration of 100 mg of Epostane 8-hourly for 5 days.

Serum oestradiol. The peripheral serum oestradiol concentration also fell after Epostane treatment but the magnitude of the fall was smaller than that observed for progesterone (49 against 19 per cent) (Figure 4).

Serum cortisol. The peripheral serum cortisol concentrations did not differ from the pretreatment values throughout the study.

Clinical effects. Four of the five patients reported vaginal bleeding, two on the second and two on the third days of treatment. One patient requested admission because of vaginal bleeding associated with abdominal pain. The other three patients experienced painless 'spotting' only. The fifth patient did not have any symptoms.

Luteal phase study

Serum progesterone. The peripheral serum progesterone concentration fell significantly after treatment with Epostane, 100 mg 8-hourly, and did not recover before the subsequent menstrual bleed (Figure 5).

Serum oestradiol. There was no change in the peripheral serum oestradiol concentrations throughout the study.

Serum cortisol. The peripheral serum cortisol concentrations did not differ from the pretreatment value during the 5 treatment days.

Table I. Effect of Epostane, 100 mg 8-hourly, on serum progesterone and cycle length in 8 women in the luteal phase

Day of menstrual cycle	*Serum progesterone (nmol/litre)*		*Number of days menstrual cycle 'shortened'*
	Before treatment	*Day 2 of treatment*	
19	36·6	11·1	3
21	79·2	18·1	6
21	25·4	14·9	3
20	57·9	16·9	5
21	41·9	9·2	4
21	36·9	21·3	0
21	23·2	19·7	1
21	38·2	31·8	0
		Mean	2·75

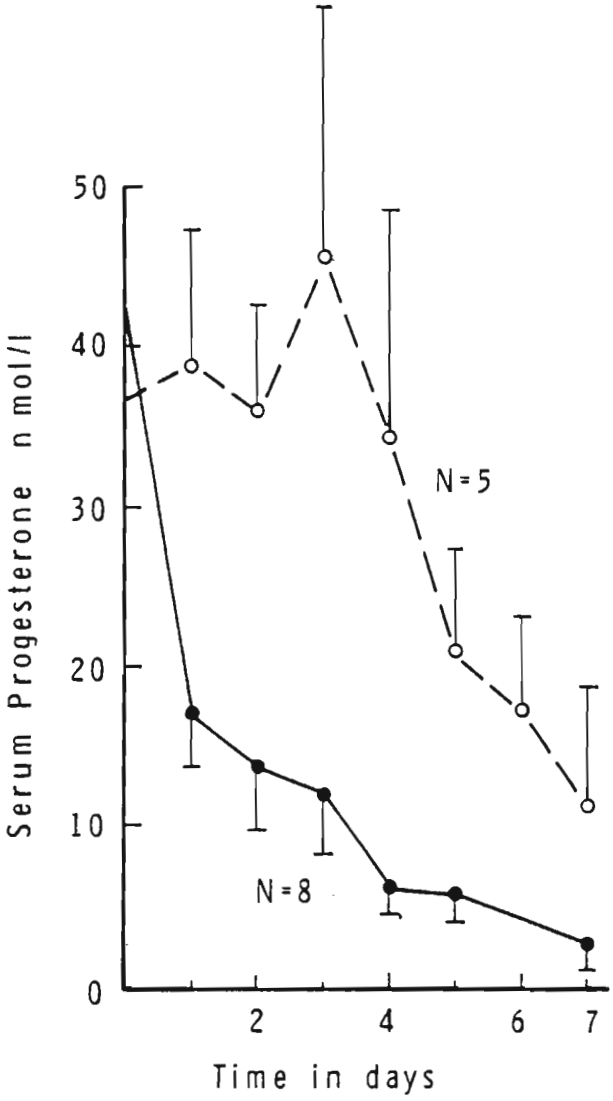

Figure 5. Changes in the peripheral concentration of serum progesterone (means ± s.e.) in thirteen women in the luteal phase of the menstrual cycle after administration of a placebo (○) or 100 mg of Epostane 8-hourly for 5 days (●).

Clinical effects. Six of the eight women bled before the expected date of menstruation. These 'menstrual bleeds' were described as normal by the patients. The remaining two women menstruated at the expected day. The menstrual cycle of this group of women was shortened by 2·75 days on average (Table I).

Haematology and biochemistry

Comparison of the pretreatment indices with those taken 24 hours after tablet ingestion showed no change.

DISCUSSION

The ability of Epostane, a competitive inhibitor of 3β-hydroxysteroid-dehydrogenase (3β-HSD), to reduce progesterone concentrations in women to those observed following luteectomy has been demonstrated in this study. The decline in progesterone concentration was accompanied by clinical effects both in early pregnancy and in the luteal phase of the menstrual cycle, but was not associated with any adverse side effect. In addition, serum cortisol concentrations remained in the normal range.

The effect of Epostane on serum oestradiol appears to be dependent on the site of oestrogen synthesis. In the luteal phase study serum oestradiol did not change significantly throughout treatment. In the 24 hour study between 12 and 18 weeks of pregnancy (placental production), serum oestradiol fell in parallel with serum progesterone. On the other hand, however, in the 5 day study between 8 and 12 weeks of pregnancy there was an intermediate effect with a slight fall in serum oestradiol suggesting that the ovary (corpus luteum) was still contributing to steroid production at this stage.

Inhibition of ovarian 3β-HSD in the luteal phase of an ovulatory cycle did cause a significant decline in serum progesterone concentration. This was associated with significant shortening of the menstrual cycle without any deleterious effects. Epostane thus appears to be an effective luteolytic agent when administered on day 20 of an ovulatory cycle. This study reaffirms the dependence of the endometrium on circulating progesterone concentration and suggests a possible role for Epostane as an interceptive postcoital agent.

Csapo and Pulkkinen (1978) reported the dependence of early pregnancy on progesterone production from the corpus luteum. They showed that progressive uterine activity occurred following surgical luteectomy when 'progesterone withdrawal' to less than 32 nmol/litre was achieved and that abortion occurred below 13 nmol/litre. Although the serum progesterone concentrations achieved in this study were comparable to those following luteectomy progressive uterine activity did not occur as expected.

In the initial 24 hour study a decline in the mean progesterone concentration to 17 nmol/litre was associated with mild temporary uterine activity in six of the ten treated women.

Prolonged reduction in peripheral progesterone concentrations was achieved using a dose of 100 mg of Epostane 8-hourly for 5 days. Despite the fact that the serum progesterone concentrations remained below the critical level of 32 nmol/litre described by Csapo for 4–5 days, only one patient reported significant vaginal bleeding or uterine activity. Assuming our progesterone

assay and that used by Csapo and co-workers produce comparable results these findings suggest either that 'progesterone withdrawal' alone does not induce progressive uterine activity in early pregnancy or that the critical level of progesterone concentration is closer to the level at which abortion occurs (13 nmol/litre). Work is continuing with larger daily doses of Epostane given for up to 10 days to investigate this.

One possible explanation for the failure of 'progesterone withdrawal' alone to induce uterine activity could be the use by Csapo of uterine stimulants to 'measure the degree of uterine activity'. This involved a combination of intra-uterine pressure monitoring with an extra-ovular balloon plus daily intramuscular oxytocin injections (0·25 m i.u.). Both are uterine stimulants but by themselves do not usually induce progressive uterine activity. Although the present study does not refute Csapo's theory that 'progesterone withdrawal' converts the uterus from a refractive to a reactive organ, it does cast doubt on this hypothesis. Studies are currently in progress to clarify the issue.

Acknowledgements

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APPENDIX

Antisera and Chemicals

Antisera raised in goats against progesterone-11-bovine serum albumin (BSA) and oestradiol-17β were the gift of Dr B J A Furr, ICI Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire. The cortisol antisera was raised in rabbits and kindly donated by Dr A Lopez Bernal, Nuffield Department of Obstetrics and Gynaecology. The PGFM assay used an antiserum donated by KT Kirton (Upjohn Co. Kalamazoo, USA).

The steriods; cortisol, progesterone and oestradiol were obtained from Sigma Chemicals, London. Prostaglandins were the gift of Dr J E Pike (Upjohn Co.; Kalamazoo). (1α, 2α - ^{3}H progesterone (1.96 Tbq/mmol), (4 C) cortisol (2GBq/mmol), (2,3,6,7- ^{3}H) oestradiol-17β (3.99 TBq/mmol) and (5,6,7,8,9,11,12(n)- ^{3}H) 13,14-dihydro-15-keto-prostaglandin in $F_{2\alpha}$ (2.96 Tbq/mmol) were obtained from the Radiochemical Centre, Amersham, Bucks. Fisfluor 2 (Toluene bases scintillation fluid) was purchased from Fisons. The chemicals for the buffer solutions and the light petroleum (bp.40° C - 80° C) were obtained from Fison Scientific Apparatus, Longborough, Leics. Diethyl ether was obtained from Mallinckrodt Chemical Works, St Louis, Missouri, USA through Camlab Ltd, Cambridge. The phosphate buffer (pH 7.4) for the progesterone, cortisol and oestradiol assays consisted of 5.4g $Na_2H_2PO_4 \cdot 2H_2O$, 35.8g $Na_2HPO4.12H_2O$ 9.0g NaCl, 1.0g NaNs and 1.0g gelatin made up to one litre with double distilled water. Dextran coated charcoal consisted of 6.3g charcoal, 0.63g dextran-75 and 100ml of phosphate buffer. This was diluted 1 in 10 before use.

Radioimmunoassays

Concentrations of progesterone, oestradiol-17β , cortisol, PGFM (a major and relatively stable metabolite of $PGF_{2\alpha}$) were measured in maternal peripheral and utero-ovarian venous plasma by specific radioimmunoassay. Progesterone, cortisol and PGFM were assayed in fetal venous plasma.

Progesterone:

Serum progesterone levels were assayed essentially as described from this laboratory by Flint et al (1974). The assay involved an extraction step with petroleum ether before radioimmunoassay in buffer.

Serum samples (50ul) previously stored at -15°C, were extraced once with 5ml petroleum ether by shaking for 30 minutes on a rotamix and after centrifugation at 1000 rpm for five minutes at 4°C. It was frozen over dry ice. The organic upper phase, was decanted and the solvent evaportaed under air at 40°C. The residue was taken up in 1.2-2.0 ml phosphate buffer, mixed and incubated at 37°C for 30 minutes and mixed again.

Duplicates (500ul) were taken for assay. 100ul of diluted antibody solution was added and mixed. 100ul of diluted label, approximately 11,000cpm (^3H) progesterone was added, mixed and incubated overnight at 4°C. The following morning the free and bound steroids were separated by the addition of 1.0ml of the dextran-coated charcoal suspension. After standing on ice for 15 minutes the dextran-coated

charcoal was removed by centifugation at 2000rpm at 4°C for 10 minutes and the supernatant, containing the free radioactivity, taken for measurement. This was added to vials containing 10mls of scintillation fluid. Radioactivity was counted in a Beckman LS 3133 T or LS 7000 liquid scintillation counter. Results were calculated using a logit-log transformation of B/Bo using a microcomputor (Intertek "Superbrain").

The following controls were included in each assay:

1. Serum samples with known amounts of progesterone (20ng/ml), were extracted and assayed in order to calculate the interassay and intrassay coefficient of variation.

2. Standard quantities of progesterone in buffer (0-500pg) which were not extracted were assayed to produce a standard curve.

3. A no "hormone" blank (1000cpm (^{3}H) progesterone was included to determine the maxium binding (Bo).

4. An antibody blank was assayed to measure the non specific binding (NSB) of radioactivity of the system.

5. Reagent blanks (100ul water blanks) were extracted to ensure there was no false elevation of levels due to non specific factors.

6. 10ul of diluted label, approximately 1000cpm (^{3}H) progesterone, in 500ul of serum was extracted in triplicate to calculate the percentage recovery from the extraction progress.

7. And the total counts for the assay were counted from 100ul of diluted (^{3}H) progesterone in buffer (45ul in 15ml dilution), for the recovery 10ul was used (10ul in 5ml dilution).

Using this method the intra-assay coefficient of variation was 8.1% at 20ng/ml (N = 10). The interassay coefficient of variation was 10.1% at 20ng/ml (N = 21). The reagent blanks (extracted from 100ul water) were not measurable. In the absence of added sample or standard 37.3 ± 4.5% (n = 10) (^{3}H) progesterone was bound. The percentage recovery from the extraction process was 83.4 ± 4.4% (n = 10). The sensitivity of the assay, defined as the mass of progesterone required to produce a response of two standard deviations from the zero point on the standard curve was 19 pg/tube. In specificity studies, cross reactivities, calculated as described by Abraham (1969) were: less than 1% for androstenedione, oestradiol-17a, Epostane, testosterone, cortisol, 17α -dihydroxyprogesterone, 20α -hydroxyprogesterone; less than 10%, for pregnan-3-ol-20-one; and less than 15%, for 5 -pregnane-3, 20-dione pregnenolone.

Oestradiol-17β :

Oestradiol was assayed in a similar manner to progesterone. Serum samples (500 ul) were taken, extracted with diethyl ether and the residue resuspended in 1.2ml phosphate buffer. 500ul duplicates were assayed.

The intra-assay coefficient of variation was 8.9% at 1mg/ml (n = 10) and the inter-assay coefficient was 9.93% at 1 ng/ml (n = 10). Reagent blanks were not measurable, initial binding of the antibody was 41.3 ±

4.7% (n = 10), the recovery was 85.6 ± 5.8% (n = 10) and the sensitivity was 6.0 pg/tube.

Cortisol:

The cortisol assay was performed by S. Phelps using the method described by Lopez-Bernal et al (1982) from this laboratory. It involved incubation at 60°C to denature the transcortin prior to assay.

Serum samples 50 ul were mixed with 1.95ml double distilled water and incubated at 60°C for 30 minutes. Duplicates (50 ul) of the heat treated samples were made up to 500ul with phosphate buffer and assayed directly.

The coefficients of variation at 750 nmol/l (n = 14) were; intra-assay 9.6%, inter-assay 13.7%. Bo was 43.9% and the sensitivity 15 pg/tube.

PGFM:

The assay for PGFM (13, 14 dehydro-15-oxo-prostaglandin $F_{2\alpha}$) was originally described by Mitchell, Flint and Turnbull (1976) who validated it for ovine plasma samples.

Plasma samples stored at - 15°C were thawed and 1.0ml aliquots acidified with 100ul 2.0M citric acid and extracted by shaking with cycle hexane/ethyl acetate (5ml) for 45 minutes at room temperature in siliconised glass tubes.

After centifugation at 1000 rpm for 5 minutes at 4°C the aqueous (lower) layer was frozen on solid carbon dioxide and the organic (upper) layer evaporated to dryness with nitrogen at 37°C and the extract resuspended in 1.0ml of assay buffer containing 1M-K_2HPO_4 (80 ml/l), 1M KH_2PO_4 (20 ml/l), 0.9% NaCL (900 ml/l), gelatin (1g/l) and NaN_2 (1g/l), pH 7.4. Recoveries were estimated by the addition of approximately 2000 cpm [^{3}H] PGFM to charcoal stripped bland sheep plasma samples which were subsequently extracted with the assay. Standards containing 0-5000 pg/ml PGFM plasma were also extracted for estimation of the inter-assay coefficient of variation.

100ul aliquots of the reconstituted buffer sample were assayed in duplicate for PGFM. Assays were carried out in plastic tubes, samples being assayed at two dilutions (1:1 and 1:4) the antisera was used at a final dilution of 1:15,000 (v:v) along with approximately 7,000 cpm [^{3}H] PGFM. Antiserum and label were diluted in buffer and added to the assay tubes in 100ul aliquots. Tubes were then vortex-mixed and incubated at 4°C overnight. Bound and free PGFM were separated by addition of a suspension of dextran coated charcoal containing 5g activated charcoal and 0.05g dextran (grade C, mw 60,000-90,000) per 500ml assay buffer; the suspension was chilled and added on ice in 1.0ml aliquots to each tube. Tubes were then vortex mixed and stored a 4°C for 15 minutes after addition of charcoal to the first tube. Charcoal was then sedimented by centrifugation at 2500rpm for 10 min at 4°C and the supernatant tipped into vials containing 10ml scintillation fluid. Radioactivity and results were performed as above.

The initial binding of the antibody and label was 43.0 ± 8.1% (mean 1 SD, n = 16). Cross-reactivity data for this antibody have been published previously (Cornette, Harrison & Kirton, 1974) and confirmed in our laboratory. Cross-reactivity with the primary prostaglandins PGE_1 PDE_2 and $PGF_{2\alpha}$ was <0.5%. A significant cross-reactivity of the antibody with the 15-oxo-PGF metabolite was obtained (8%), but the cross-reactivity with 13, 14-dihydro-$PGF_{2\alpha}$ was <0.7%.

The inter-assay coefficient of variation, determined from a 250pg/ml PGFM plasma standard included in duplicate in each assay, was 11.1%; the mean ± SD measured concentration of PGFM being 262.1 ± 5.3) pg/ml (n = 16). Extraction recoveries were 85.2 ± 5.3)%. Results were corrected for recovery. The mean sensitivity of the assay, defined as the mass PGFM detected 2 SD from the lowest point on the standard curve, was 7.5 pg/tube.