Studies on the Hormonal Events of Pregnancy, particularly in Relation to the Spontaneous Onset of Labour

A thesis submitted to the University of London

for the degree of

Doctor of Medicine

by

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Abstract

An increase in the plasma oestrogen:progesterone ratio precedes labour in sheep, and an increase in the saliva oestriol:progesterone ratio has recently been demonstrated in women prior to spontaneous term and preterm labour. Studies were undertaken to further investigate the hormonal changes of pregnancy, particularly in relation to the onset of labour, and ultrasound examinations were performed to determine whether fetal adrenal size correlates with maternal steroid hormone levels. Absorption of progesterone was also studied in pregnancy, with a view to the possible prevention of preterm labour in some women at a later date.

A rise in the saliva oestriol:progesterone ratio, prior to labour, was found in 68% of 28 normal women, and a ratio above the 90th centile for the gestation was found in 47% of the 17 women who went into idiopathic preterm labour. On serial monthly ultrasound examinations fetal adrenal size increased linearly; there was no correlation between adrenal and hormonal measurements at a given gestation. Adrenal size decreased rapidly during the first six weeks of neonatal life.

Maternal plasma oestrone, oestradiol, oestriol, progesterone, dehydroepiandrosterone sulphate, sex hormone binding globulin, human chorionic gonadotrophin, human placental lactogen and prolactin, and saliva oestrogen and progesterone levels were measured fortnightly from 20 weeks gestation in 20 normal women. Levels were comparable with previous studies; no interrelationships of significant importance were detected.

Hourly saliva cortisol levels were significantly increased in late pregnancy, but the diurnal variation was maintained. The increase in plasma and saliva cortisol levels was not caused by the increased corticosteroid binding globulin levels.

Thus an increased oestriol:progesterone ratio in the majority of women prior to term and idiopathic preterm labour was demonstrated, but it was concluded that neither saliva oestriol and progesterone, nor fetal adrenal ultrasound measurements, would be helpful in the prediction of preterm labour, in practice.

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Abbreviations and Symbols

ACTH	adrenocorticotrophic hormone
AVP	arginine vasopressin
^{o}C	degrees temperature in Centigrade
CBG	corticosteroid binding globulin
cpm	counts per minute
CRH	corticotrophin releasing hormone
DCC	dextran-coated charcoal
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulphate
E1	oestrone
E2	oestradiol
E3	oestriol
F	cortisol
g	grams
h	hours
hCG	human chorionic gonadotrophin
hPL	human placental lactogen
hMG	human menopausal gonadotrophin
IU	International Unit
IUGR	intrauterine growth retardation
L	litre
LSCS	lower segment cesarean section
mg	milligrams
MHz	megaHertz
ml	millilitre
MW	molecular weight
n	number (of subjects/values)
nmol	nanomoles
NVD	normal vaginal delivery
NSB	non-specific binding
P	progesterone
PBS	phosphate buffer solution
pg	picograms
PG	prostaglandin
$PGF_{2\alpha}$	prostaglandin $F_{2\alpha}$
PGE	prostaglandin E

PRL	prolactin
PROM	prolonged rupture of the membranes
rcf	relative centrifugal force
r_s	Spearman rank correlation coefficient
RIA	radioimmunoassay
SD	standard deviation
SHBG	sex hormone binding globulin
SROM	spontaneous rupture of the membranes
TS	transverse section
µg	micrograms
µl	microlitre
µmol	micromole
&	and
<	less than
>	more than
%	percentage

The work in this thesis is that of the candidate except for the following:

1) The ultrasound scans were performed by Miss Alison Thomas DCR DMU

2) The assays on plasma samples, the assays involving chromatography and some of the saliva assays were performed by Dr HHG McGarrigle PhD

Signed.....Elaine Scott.....

Acknowledgements

I would like to thank everybody who has helped me, one way or another, during my research period at UCH. I am deeply grateful to my supervisor, Miss Gillian Lachelin, for the valuable advice, friendly support and help which was always readily available; to Dr Tim McGarrigle, for introducing me to assay methodology as well as for much other teaching and advice, and for performing many of the assays in this thesis; to Miss Alison Thomas, whose expertise in ultrasonography was invaluable; to the midwives at UCH who helped in the recruitment of patients; and to the many patients and 'unsuspecting' colleagues who agreed to participate in these studies with no thought of personal gain. This thesis would not have been possible without their help.

Grateful thanks are also due to the various institutions which provided funding for this research:- the Florence and William Blair Bell Fellowship (1 year), the Special Trustees of University College Hospital (1 year), the Obstetric Department of University College, under the aegis of Professor D.V.I. Fairweather (7 months) and Roussel Pharmaceuticals (3 months).

I would particularly like to thank Mr A.C. Silverstone, who was not involved in the research, but who has shown unfailing interest as well as providing practical advice and assistance over the last 4 years. My gratitude and appreciation also go to my colleagues and friends Adjeidu Armar, Faye Iketubosin, Paul Serhal, Suzanna Arschavir, Sian Williams and Clare Wood all of whom have been especially tolerant and supportive. Finally, I owe a great deal to my brother, David, and to my parents for their enduring patience and love.

1 Introduction

The endocrinological events leading to the onset of parturition in the human are still incompletely understood. Previous work has demonstrated that there is a rise in saliva ('free') oestriol:progesterone ratios in women before the spontaneous onset of labour at term *(McGarrigle and Lachelin, 1984; Darné et al, 1987)*. An inappropriately early rise in the saliva oestriol:progesterone ratio for the gestation was noted in women who went into idiopathic preterm labour with intact membranes *(Darné et al, 1987)*.

The first study (Chapter 6) involved repeating the previous work in order to compare the results with previous findings. The intention was also to obtain serial collections of saliva, from 20 weeks gestation onwards, from women who would subsequently go into preterm labour, in order to determine whether saliva oestriol:progesterone ratios might be of use as a predictive test for preterm labour.

It has been felt for many years that fetal adrenal activity may be an important factor in relation to the onset of labour, and the finding of a surge in oestriol prior to the onset of labour was in keeping with this hypothesis, as it is the fetal adrenal which provides the precursors for the placental production of oestriol. In the second study (Chapter 7) the size and appearance of the fetal adrenal were monitored by serial ultrasound examination from 24 weeks gestation onwards, in a group of normal women from whom plasma and saliva samples were obtained at each visit. The aim was to determine whether there was any correlation between fetal adrenal size and maternal plasma and saliva oestriol and/or progesterone levels.

The fetal zone of the neonatal adrenal is known from histopathological studies to undergo marked involutional changes during the first weeks of neonatal life, and although some previous ultrasound studies of neonatal adrenal glands have been carried out, only one group has performed serial studies and then measuring only one adrenal parameter *(Hata et al, 1988).* The adrenal study was therefore extended to include serial ultrasound measurements of various adrenal parameters on healthy neonates from days 1 to 42 of extrauterine life.

If there is an inappropriate rise in 'free' oestriol:progesterone ratios in some women who go into preterm labour, it might be possible to treat these women with progesterone, using doses which would reverse the ratio and hopefully prevent preterm delivery. No previous studies have been reported on the absorption of progesterone in pregnancy, which is a state entailing many physiological changes which might influence the absorption and metabolism of progesterone. The third study (Chapter 8) involved the administration to pregnant women of single doses of progesterone, via the vaginal and oral routes, in order to monitor the resulting changes in plasma and saliva progesterone levels, and to determine the optimum route of administration in pregnancy.

The controlling factors for the changing hormonal environment with gestational age are ill understood. The purpose of the fourth investigation (Chapter 9), which involved a serial study of a variety of steroid and protein hormones in plasma and saliva in the second and third trimesters of pregnancy, was to determine whether any interrelationships or controlling factors between the hormones could be detected. In no previous study have oestrone, oestradiol, oestriol, progesterone, dehydroepiandrosterone sulphate, human placental lactogen, ß-human chorionic gonadotrophin and

prolactin been measured serially and simultaneously in the same plasma samples, nor has an assessment of the correlation of these hormones with oestrogens and progesterone, measured in simultaneously collected saliva samples, been made.

Cortisol is known to have an important role in the onset of parturition in sheep, and although it seems likely that it may have an important part to play in human pregnancy and parturition, its precise role remains uncertain. It is known that cortisol levels are markedly raised in human pregnancy but the mechanisms leading to this rise are also uncertain. The aim of the final study (Chapter 10) was to attempt to further elucidate the mechanisms responsible for this increase by studying the diurnal variation of saliva cortisol, and alterations in plasma cortisol, progesterone, oestrogen and corticosteroid binding globulin levels in various groups of non-pregnant, pregnant and puerperal women.

All of the studies described in this thesis were approved by the local ethics committee.

2 Background Knowledge concerning the Onset of Parturition

The mechanisms controlling the spontaneous onset of labour are complex, and still require elucidation in the human. The reason that human parturition is incompletely understood is primarily because of the ethical and technical difficulties in research involving humans. However, the physiology of parturition in certain animals, such as the sheep, has been investigated in great detail, and provides a frame of reference with which to compare the process of parturition in women.

Parturition in the sheep

The onset of parturition in the ewe has been shown to be profoundly influenced by the fetus.

Binns et al *(1960)* described a syndrome of prolonged pregnancy in sheep carrying lambs congenitally deformed by a teratogenic agent, which they later proved to be contained in skunk cabbage (Veratrum californicum) eaten by the ewe in the first two weeks of pregnancy. The affected lambs had pituitary glands, but the neural connections were either missing or abnormal *(Kennedy, 1971)*. Subsequently, another syndrome of prolonged pregnancy in sheep from South West Africa was described by Basson et al (1969), who showed that the disorder 'Grootlamsiekte' was also induced by ingestion of a teratogenic agent - Salsola tuberculata. 'Grootlamsiekte' caused no signs of toxicosis in the ewes except for the abnormal gestation length and retarded udder development. The lambs, however, had anatomical abnormalities including hypophyseal, adrenal and thymic atrophy, and mild Leydig cell hypoplasia in the male lambs, with the female lambs having polycystic

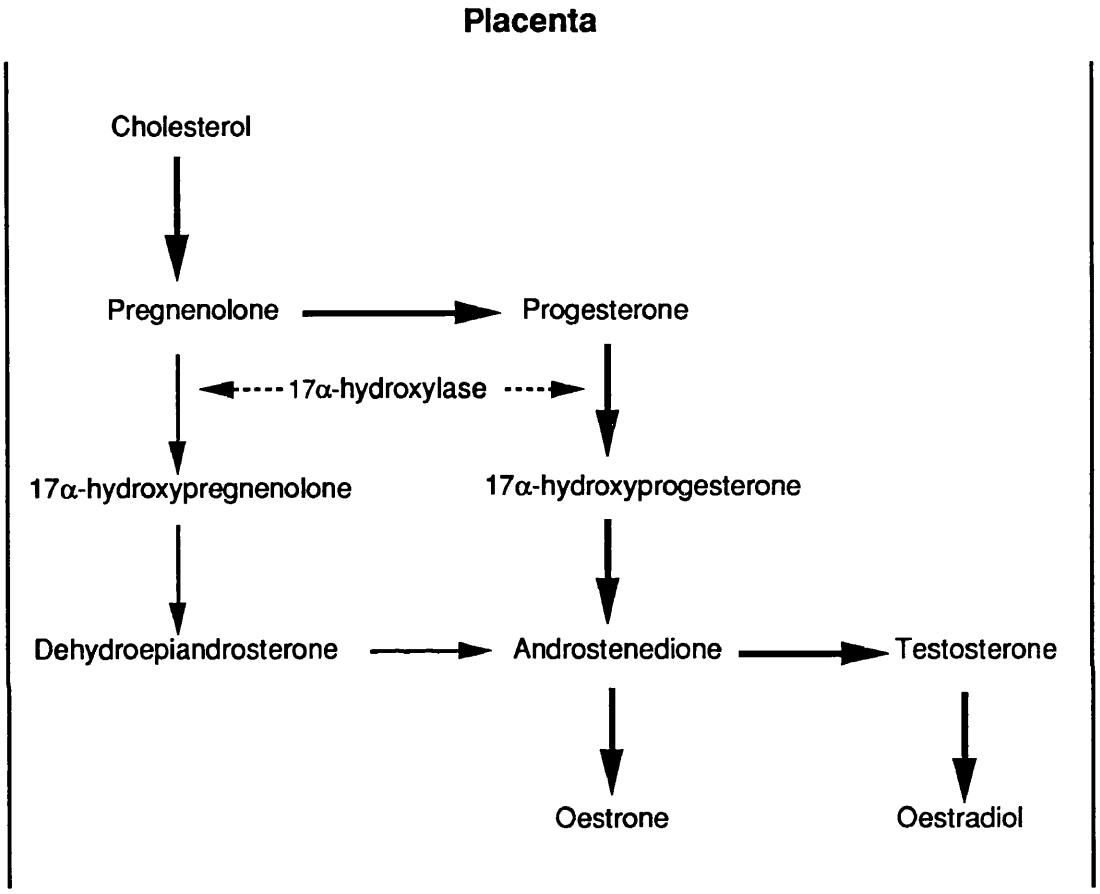
ovaries and hypertrophied female genitalia. Prolonged pregnancy occurred in the above syndromes only in the absence of a normal fetus ie., when both of twins or all three of triplets were affected. Thus, circumstantial evidence existed which implicated the fetus in the mechanisms controlling parturition.

Liggins et al *(1967)* showed that destruction of 70% or more of the fetal pituitary resulted in an indefinite prolongation of pregnancy in sheep. Spontaneous parturition at term (147 days) occurred in multiple pregnancies, unless all the fetuses were hypophysectomized. Section of the fetal pituitary stalk was also associated with prolonged gestation, providing the stalk section was carried out earlier than 116 days gestation. When the operation was carried out at 116 days gestation or later, the lambs tended to deliver preterm and were noted to have enlarged adrenals, suggesting that hypersecretion of ACTH had occurred *(Liggins et al, 1973).* Conversely, infusion of ACTH or cortisol into fetal lambs caused preterm delivery after a latent period of 4-7 days and 48-72 hours respectively *(Liggins, 1968).* Fetal infusions of mineralocorticoids failed to interrupt pregnancy whereas infusion of dexamethasone, (a synthetic glucocorticoid with no mineralocorticoid activity), was highly potent in inducing parturition *(Liggins, 1969).*

These experiments demonstrated that an intact fetal pituitary-adrenal axis was necessary for the spontaneous onset of parturition at term in the sheep, and that activation of this pathway could result in preterm labour.

Endocrine changes in the sheep have been studied in detail. It has been shown that parturition is preceded by an increase in the concentration of cortisol in fetal plasma *(Bassett and Thorburn, 1969).* This reflects an increase in the fetal adrenal secretion rate of cortisol *(Liggins et al, 1973).*

Fig. 2.1 Schematic representation of the steroid pathways in the sheep placenta.



The rise in cortisol secretion acts on the placenta to induce changes in placental steroid output *(Liggins et al, 1977).* Placental 17α-hydroxylase activity is increased causing an increased conversion of progesterone to 17α-hydroxyprogesterone, and hence an increase in placental oestrogen output *(Flint et al, 1975)*, (Fig. 2.1).

Liggins and Grieves *(1971)* were the first to suggest that prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) plays a role in the onset of labour in sheep. $PGF2\alpha$ levels are raised in maternal and fetal plasma during the 24 hours prior to parturition, and the stimulus to the myometrial production of $PGF_{2\alpha}$ was suggested to be the high levels of circulating unconjugated oestrogens *(Thorburn et al,1972).* Consistent with this hypothesis was the finding by Liggins et al *(1973)* that the myometrial concentration of $PGF_{2\alpha}$ rises after the administration of stilboestrol. Studies in nonpregnant sheep show that progesterone is necessary for prostaglandin synthetase activity and suggest that either subsequent progesterone withdrawal or an increase in oestrogen will enhance the release of $PGF_{2\alpha}$ *(Thorburn and Challis, 1979).* The complex action of progesterone on uterine prostaglandin (PG) was demonstrated in the guinea pig *(Blatchley and Poyser, 1974).* They showed that progesterone enhances the capacity for PG synthesis, but inhibits the $PGF_{2\alpha}$ release; whereas oestrogen stimulates PG synthesis particularly from tissue previously exposed to progesterone. Thus the marked increase in the oestrogen:progesterone ratio at term is a powerful stimulus to PG release.

Chronic aortic infusions of $PGF_{2\alpha}$ and the acute administration of high doses of $PGF_{2\alpha}$ will induce uterine contractions *(Liggins et al,1973; Mitchell et al,1976).* Greatest activity is induced when $PGF_{2\alpha}$ is given close to delivery. The cervix can synthesize prostaglandins and incubation of the ovine cervix is associated with release into the medium of substantial

quantities of prostanoids of which PGE_2 and PGI_2 predominate *(Ellwood et al, 1981)*. The parturient ovine cervix releases significantly greater quantities of PGE_2 and PGI_2 than the nonparturient cervix; and cervical vein levels of PGE_2 and PGI_2 increase sharply at the time when cervical ripening begins *(Ellwood et al, 1981)*. $PGF_{2\alpha}$ infused into the lumen of the cervix or PGE_2 infused into its arterial supply will result in local softening and dilatation *(Ellwood et al, 1981; Fitzpatrick, 1977)*. When sheep are treated with meclofenamic acid (a prostaglandin synthetase inhibitor), the rate of cervical ripening and dilatation is reduced, and delivery is frequently associated with cervical dystocia *(Mitchell and Flint,1978)*.

Longitudinal studies on sheep show no increase in basal maternal plasma oxytocin levels during pregnancy. Mean maternal oxytocin levels do not rise significantly until during the second stage of labour, *(Glatz et al, 1981)*. The smooth muscle of the ovine uterus is relatively insensitive to oxytocin until near term, when a fall occurs in the threshold of stimulation by oxytocin *(Hindson et al, 1969)*. Flint et al *(1975)* showed in the sheep that pressure of the fetal presenting part on the cervix and vagina activates a neurohumeral reflex (the Ferguson reflex), resulting in maternal secretion of oxytocin that not only stimulates uterine contractility, but also stimulates the release of $PGF_{2\alpha}$ from uterine tissues. This in turn stimulates uterine contractions and further oxytocin release, so that the process of parturition is accelerated *(Mitchell et al, 1975; Mitchell and Flint, 1978)*.

In summary, in the sheep model, it would seem that a rise in the oestrogen:progesterone ratio, occurring in response to an increase in fetal cortisol production, brings about an increase in prostaglandin release leading to the spontaneous onset of labour.

Parturition in subhuman primates

Studies on subhuman primates have offered the opportunity of investigating an animal model which, like the human, lacks placental 17α-hydroxylase, and therefore depends on an intact fetoplacental unit for oestrogen production. Oestrone (E1) and oestradiol (E2) have been shown to increase significantly in the maternal plasma of the rhesus monkey (Macaca mulatta) during the last 2 weeks of gestation *(Challis et al, 1977; Walsh et al, 1984)*. There is a parallel and significant increase in fetal E1 levels *(Walsh et al, 1984)*. Simultaneous measurements of dehydroepiandrosterone sulphate (DHEAS) showed a significant increase in fetal but not maternal levels, suggesting an increase in fetal adrenal activity before parturition. Placental oestrogen production in monkeys depends on adrenal androgen precursors *(Walsh et al, 1980)*, and it has been shown that there is no increase in maternal androgens during the last 30 days of gestation *(Challis et al, 1975)*, providing further evidence that the increase in fetal oestrone before labour is secondary to increased fetal adrenal activity and DHEAS production rate. The administration of dexamethasone will cause preterm labour in the sheep, but not in the rhesus monkey. Endocrine studies on rhesus monkeys following dexamethasone treatment showed a decrease in maternal plasma levels of E1, E2 and cortisol (F) and a concomitant fall in fetal plasma levels of E1 and F, *(Walsh et al, 1979)*. In the same study, it was demonstrated that fetal administration of ACTH, after maternal suppression with dexamethasone, restored both maternal and fetal E1 to its previous levels. As ACTH is not thought to cross the placenta *(Miyakawa et al, 1974)*, this finding reinforced the supposition of a role for the fetal adrenals in the prepartum oestrogen surge, and showed that ACTH is likely to be the main regulator of the fetal adrenals in the rhesus monkey. In the amniotic fluid of rhesus monkeys, a rise in the concentration of E1 precedes the increase in amniotic fluid prostaglandin F2α metabolite, prior to vaginal delivery after 134 days gestation *(Walsh et al, 1984)*.

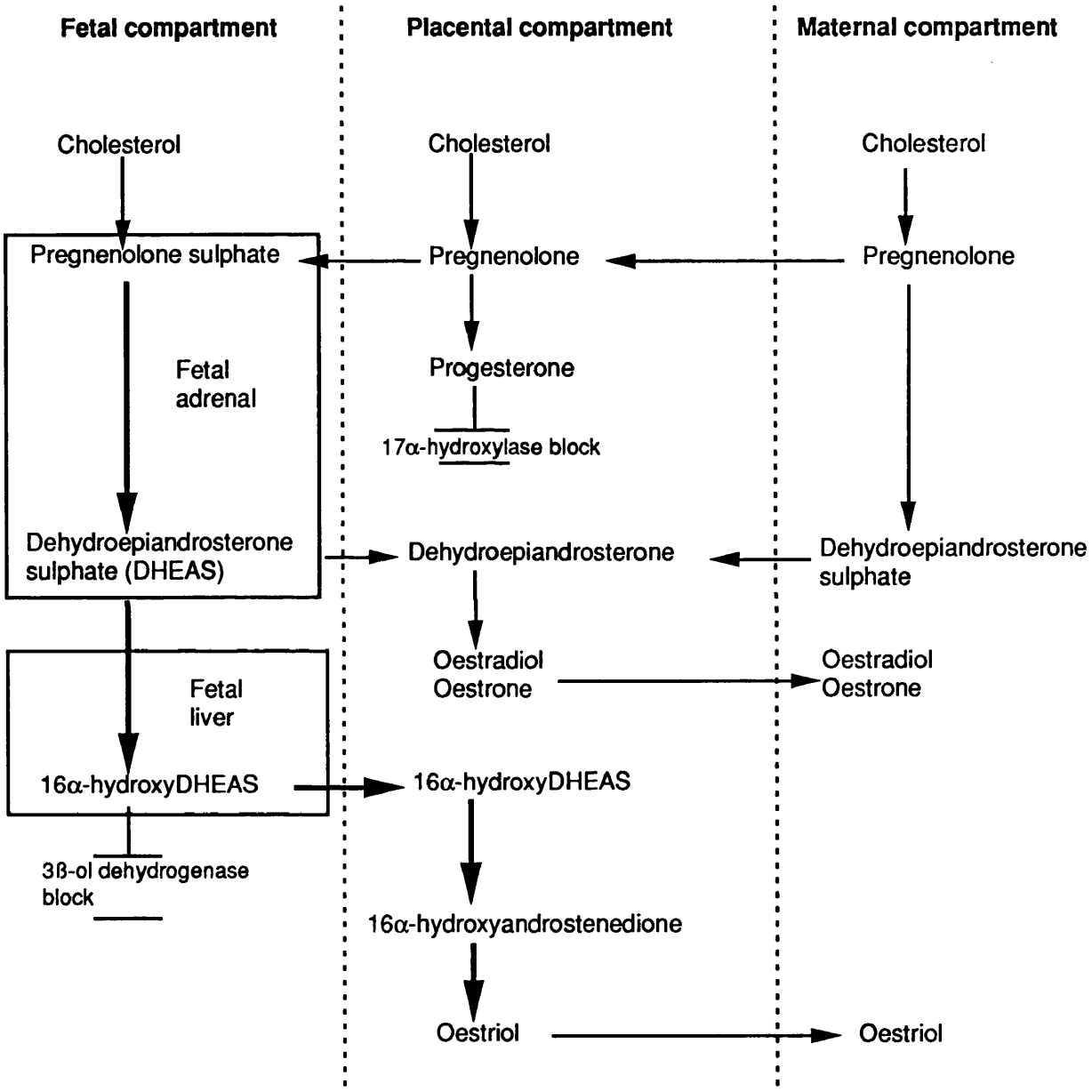
Parturition in the human

The main differences between parturition in the sheep and in the human arise from the fact that the human placenta lacks the enzyme 17α-hydroxylase, and therefore is unable to synthesize androgens or oestrogens without an extraplacental source of suitable substrate.

Diczfalusy *(1964)* found that, in humans, both the placenta and the fetus lack certain enzymes that are essential for the synthesis of steroids during pregnancy. He demonstrated that some enzymes, which are absent in the placenta, are present in the fetus (for example 16α-hydroxylase); also, some enzymes, which are not found in the fetus, are active in the placenta (for example 3ß-hydroxysteroid dehydrogenase). These findings led him to suggest the concept of a fetoplacental unit.

The precursor for steroid synthesis in the placenta is cholesterol derived from the maternal circulation *(Hellig et al, 1970, Simpson et al,1978)*, as the capacity for placental trophoblastic tissue to synthesize cholesterol *de novo* is limited *(Van Leusden and Villee, 1965)*. Cholesterol is converted via pregnenolone to progesterone (P), which is produced in large amounts - 250mg or more per day at term *(Simpson, 1983)*. The fetal contribution to progesterone production is thought to be nil or minimal, because the fetal adrenal gland is deficient in the 3ß-hydroxysteroid dehydrogenase Δ4-5isomerase enzyme complex *(Diczfalusy, 1974)*. Confirmatory evidence for this are the facts that progesterone production is not significantly reduced after fetal death providing the placenta continues to function *(Cassmer, 1959)*, and in anencephalic pregnancies maternal plasma P levels are comparable to those in women with normal pregnancies *(Chattoraj et al, 1976)*.

Fig. 2.2 Schematic representation of the steroid pathways in the human fetoplacental unit.



The fetal adrenal is capable of synthesizing cholesterol *de novo (Carr and Simpson, 1981)* and only approximately 20% of fetal cholesterol is maternal in origin *(Hellig et al, 1970)*. The fetal adrenal takes up LDL-cholesterol *(Carr et al, 1980)* and metabolizes it principally to dehydroepiandrosterone sulphate (DHEAS), which then undergoes 16α-hydroxylation in the fetal liver. This product is then further metabolised in the placenta by sulphatase and 3ß-hydroxysteroid dehydrogenase Δ4-5 isomerase enzymes to 16α–hydroxyandrostenedione, which is aromatised to form oestriol *(Diczfalusy, 1974)* (Fig. 2.2). Less than 1% of the DHEAS produced by the human fetal adrenal gland is derived from pregnenolone of non-fetal origin *(Carr et al, 1980)*. The placenta synthesizes oestrone and oestradiol from DHEAS via androstenedione and testosterone. The fetus and the mother contribute to placental DHEA in approximately equal amounts *(Siiteri and MacDonald, 1967)*.

Although the process of steroidogenesis differs in the sheep and the human, the idea that the fetus plays a role in the onset of parturition is common to both species.

Early studies described an increased incidence of prolonged gestation in women with anencephalic fetuses (a malformation associated with varying degrees of adrenal hypoplasia) *(Malpas,1933; Comerford,1965, Milic and Adamsons,1969)*. This was disputed to some extent by Honnebier and Swaab *(1973)* who found an increased proportion of both preterm and post term labour in women with anencephalic fetuses compared to women with normal fetuses, although the mean gestation at delivery was not significantly different in the two groups when only those pregnancies without hydramnios were considered. Another clinical syndrome, placental sulphatase deficiency, (which is associated with low oestriol levels due to an

** Very little is known about fetal ACTH levels in the human, although studies have suggested that they are similar to maternal levels both in the second trimester *(Economides et al, 1987)* and at term *(Allen et al, 1973; Winters et al, 1974).* However, minimal or no transplacental passage of ACTH is thought to occur *(Allen et al, 1973; Miyakava et al, 1974)*, and Economides et al *(1987)* found no correlation between maternal and fetal ACTH levels.

inability of the placenta to cleave steroid sulphates), is said to be marked by a lack of cervical ripening and difficulty with induction of labour particularly in primigravidae *(France et al, 1973)*, although again, this was subsequently disputed *(Bedin et al, 1980)*. The length of gestation in women carrying babies with congenital adrenal hyperplasia due to 21-hydroxylase deficiency is within the normal range *(Price et al, 1971)*. Anderson et al, *(1971)* noted that the mean fetal adrenal weight of infants who delivered preterm without any apparent reason was higher than the mean fetal adrenal weight of those delivered preterm due to antepartum haemorrhage.

The circumstantial evidence that the human fetal pituitary-adrenal axis has a role to play in the onset of parturition is less clear-cut than in the sheep model. As far as it is known, fetal cortisol can exert its effects on parturition only via activation of 17α-hydroxylase, and in the absence of this enzyme high fetal corticosteroid levels, produced by exogenous means, are not associated with preterm delivery *(Liggins and Howie, 1972; Anderson and Turnbull, 1973; Gennser et al, 1977)*.

Maternal cortisol levels are raised in pregnancy for reasons that are not completely clear; this is discussed further in Chapter 10. However, maternal cortisol is not thought to cross the placenta in any significant quantity, and most of it is converted by the placenta to its biologically relatively inactive metabolite, cortisone *(Murphy et al, 1974)*. There is conflicting data about the levels of ACTH in pregnancy (Chapter 10), but there is not thought to be transfer of ACTH from mother to fetus *(Simmer et al, 1974)*. ✳✳

It has not been possible to perform longitudinal studies to determine fetal cortisol levels, but the data available suggests that fetal cortisol is

** The mean gestational age was not stated for the spontaneous labour or the induced labour groups, and therefore it could be argued that the induced fetuses were less mature, and therefore had lower fetal cortisol levels.

present from 10-18 weeks gestation *(Murphy, 1973)* and that, after an initial fall in the second trimester, it rises with gestation with a final surge between 37+ and 41+ weeks gestation *(Murphy, 1982)*. Levels of fetal cortisol are higher at delivery following the spontaneous onset of labour than at vaginal delivery following induction, supporting the theory that increased activity of the fetal adrenal gland plays a role in the onset of parturition *(Murphy, 1973; Goldkrand et al, 1976)*. ** However, a role for fetal cortisol in the onset of labour has not been confirmed in women, and the other main secretory product of the fetal adrenal, 16α-hydroxy DHEAS, seems more likely to be of significance.

Following the findings of an increased oestrogen:progesterone ratio in sheep prior to parturition, a similar change was looked for in the human. Initially, it was thought that there was a fall in maternal plasma progesterone levels prior to human parturition *(Csapo et al, 1971)* with a rise in the oestradiol:progesterone ratio *(Turnbull et al, 1974)*. Since then, many studies have been performed looking at maternal plasma oestriol (E3), oestradiol (E2) and progesterone levels in the weeks prior to parturition at term, most of which have failed to substantiate any significant changes *(Tulchinsky et al, 1972; Shaaban and Klopper, 1973; Mathur et al, 1980; Laatikainen et al, 1980; Haartikainen-Sorri et al, 1981)*, although Batra et al *(1983)* found a significantly lower P:E2 ratio in women in labour compared to non-labouring women.

Similar studies have been carried out on women in preterm labour with conflicting results. Tamby Raja et al *(1974)* found an increased plasma E2:P ratio in women in preterm labour compared to normal women of comparable gestation at term. Another study noted a significant fall of the plasma P:E2 ratio in women with idiopathic preterm labour compared to

controls, even though both the P and the E2 tended to be lower than the control group *(Cousins et al, 1977)*. However, subsequent studies have not confirmed these findings *(Bell , 1983; Block et al, 1984)*.

All these reports concern the measurement of the total unconjugated hormones in plasma, with no distinction made between the non-protein bound or biologically active portion and the protein bound moiety. Therefore, further studies were undertaken to look specifically at the non-protein bound hormones in plasma. Yet again, no consistent changes could be found in either the E2:P ratio *(Anderson et al, 1985; Wilcox et al, 1985)*, or the E3:P ratio *(Moutsatsou and Oakey, 1986)*. However, these studies involved relatively infrequent sampling restricted to the last 2 to 7 weeks of pregnancy.

Thus, prior to the pilot study of McGarrigle and Lachelin *(1984)* and their subsequent work *(Darné et al, 1987)* on salivary steroid levels in pregnancy, (Chapter 6), very little evidence was available to support the idea of an increased oestrogen:progesterone ratio related to the onset of labour. However, although the matter remained unresolved, various studies were performed to assess the effect on the pregnant woman at term of oestradiol, DHEAS, and oestriol.

Pinto et al, *(1964,1965,1967)* performed the earliest experiments, which involved the intravenous infusion of oestradiol. They found an increase in uterine activity, increased responsiveness to oxytocin, and active ripening of the cervix, (demonstrated histologically as increased oedema, vascularity and vacuolisation of the basal epithelium). Also, E2 was an effective agent in hastening the onset of labour in women at term compared with controls; and if treatment with an oxytocic agent was required, the total

oxytocic dose given was smaller. Two further studies using oestradiol valerate extra-amniotically in a single dose, tended to confirm the positive effects of oestradiol on cervical ripening and ease of induction *(Gordon and Calder, 1977; Craft and Yovich, 1978).*

Other experiments have involved repeated intravenous injections of DHEAS, which leads to an increase in plasma and tissue E2 with no change in E3 or P. DHEAS was found to improve the Bishop score and myometrial susceptibility to oxytocin earlier than in women receiving placebo, and it was hypothesized that these actions were induced by the E2 formed from DHEAS *(Mochizuki and Tojo; 1980).*

A larger study involving the administration of extra-amniotic oestradiol, oestriol or plain gel via a balloon Foley catheter did not demonstrate any benefit of either oestrogen over the controls, in terms of improvement in Bishop score *(Thiery et al, 1979).* However, only a single dose of hormone was administered and the results were assessed after only 12 hours, and it is possible that different results might be obtained with treatment over a longer time period. When extra-amniotic oestriol gel was compared with $PGF_{2\alpha}$ gel, they were found to be equally effective in ripening the cervix, but oestriol was less likely to stimulate uterine activity *(Quinn et al, 1981).*

Whilst the endocrine evidence as regards oestrogen:progesterone ratios and the onset of labour remained controversial, the evidence from these clinical studies, in which the oestrogen:progesterone ratio was increased via exogenous means, tended to support the concept of an increasing ratio being involved in the onset of parturition. The known and opposing effects of oestrogen and progesterone on myometrial activity are

** Serum relaxin has been measured in a cross-sectional study during human pregnancy *(Maclennan et al, 1986)*. It was found that concentrations in the third trimester were lower than in early and mid pregnancy, but that levels rose again during labour. One hypothesis was that low relaxin levels might be found to precede some preterm labours, but this was not confirmed in the study by Bell et al *(1988)*, who found relaxin levels in women undergoing preterm labour to be mostly within the normal range. Purified porcine relaxin, administered vaginally or intracervically, in a dose of 1-2mg, has been shown to have a beneficial effect on cervical ripening *(Maclennan et al, 1980; Evans et al, 1983)*. No additional beneficial effect on cervical ripening was noted in a study where relaxin (2mg) was combined with oestradiol (10mg), although the authors acknowledged the small numbers of women studied *(MacLennan et al, 1981)*. It is not yet clear whether the effect of relaxin on cervical ripening is mediated systemically or by direct action at the site of local application *(Maclennan et al, 1986)*. Most of these studies support the suggestion that relaxin may play a role in facilitating cervical ripening and parturition at term.

discussed in Chapter 6, and would also accord with the theory. (Studies which have looked at the beneficial effects of progesterone in inhibiting preterm labour are discussed in Chapter 8.)

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An increase in the rate of prostaglandin biosynthesis by intrauterine tissues is important in the events leading to parturition in the human, as well as in the sheep. The pregnant human uterus is more sensitive to the effects of prostaglandins than the pregnant sheep uterus, in which increasing sensitivity to prostaglandin administration is seen only as parturition approaches. In human pregnancy, administration of prostaglandins can stimulate evacuation of the uterine contents at any stage *(Turnbull, 1983)*.

It was demonstrated that the concentration of prostaglandins in human decidual tissue obtained at 3-10 weeks gestation was lower than that measured in the endometrium at any stage in the normal menstrual cycle *(Maathuis and Kelly, 1978)*. The human conceptus therefore apparently interferes with endometrial synthesis of PGE and PGF soon after implantation, possibly by inhibiting the uterine tissue prostaglandin synthetase system. Since decidual prostaglandins are suppressed even when the pregnancy is ectopic, the inhibiting factor may act systemically rather than locally *(Abel et al,1980)*. The identity of prostaglandin inhibitors and the mechanism of action remains unsolved.

Concentrations of prostaglandins in amniotic fluid are markedly higher at term before the onset of labour than earlier in gestation *(Keirse et al, 1974)*. At the onset of labour, there is a dramatic increase in the concentrations of arachidonic acid and prostaglandins *(Keirse and Turnbull, 1973; MacDonald et al, 1974; Keirse et al, 1974; Keirse et al, 1977; Mitchell et al, 1978; Mitchell et al, 1979)*. The amniotic fluid concentrations of

$PGF_{2\alpha}$ and PGE rise further during labour, correlating with increasing cervical dilatation *(Keirse and Turnbull, 1973; Keirse et al, 1974)* . Amniotomy, which is a procedure widely used in the induction of labour, causes an acute elevation of prostaglandin concentrations in amniotic fluid *(Mitchell et al, 1976)*, and a marked increase in concentration of PGFM in the maternal circulation *(Mitchell et al, 1977)*.

Thus, the evidence for the involvement of prostaglandins in human parturition is substantial. Furthermore, it may be that other products of arachidonic acid metabolism, formed by the lipoxygenase pathway, such as hydroxyeicosatetraenoic acids (HETE's) are also involved *(Mitchell et al, 1983)*. What is not yet clear is the mechanism by which prostaglandin production is increased at term. Factors in amniotic fluid which alter amnion prostaglandin production include epidermal growth factor, transforming growth factor α, interleukin-1, tumour necrosis factor, platelet activating factor, oxytocin, glucocorticoids, endothelins 1 and 2, and renin *(Mitchell and Lundin-Schiller, 1990)*. The modulation of prostaglandin production by oestrogen and progesterone has already been discussed. CRH and ACTH both stimulate the output of PGE2 and $PGF_{2\alpha}$ by human amnion cells from 13-15 weeks and term pregnancies, and by mixed placental, chorion and decidual cultures at term *(Challis et al, 1990)*.

Overall, the regulation of prostaglandin biosynthesis appears to result from a complex interplay of several stimulatory and inhibitory substances whose activities may be increased or decreased near term. A number of these substances are produced by the fetus and may play a role in the control of membrane rupture and uterine contractions at the time of parturition.

Conflicting results on oxytocin have been obtained in pregnancy, with oxytocin levels either rising gradually towards term *(Dawood et al, 1979; Sellers et al, 1981)* or remaining at low pre-pregnant levels until labour *(Leake et al, 1981)*. Levels are significantly raised in the late first stage and second stage of labour *(Gibbens and Chard, 1976; Leake et al, 1981)*. The source of circulating oxytocin found in maternal plasma is not clear *(Husslein, 1987)*. Fetal umbilical arterial concentrations have been shown to be higher than umbilical vein concentrations, indicating significant fetal production of oxytocin during labour *(Chard et al, 1971; Dawood et al, 1978; Sellers et al, 1981)*. Myometrial oxytocin receptor levels increase in late pregnancy to reach maximal levels in early labour *(Fuchs et al, 1982; Soloff, 1983)*. However, the consensus seems to be that oxytocin acts mainly in the promotion of increasing uterine activity in established labour, and in ensuring the efficiency of the final expulsive phase, rather than in the initiation of labour.

In summary, the function of the uterus changes dramatically at the onset of labour. Throughout pregnancy, the myometrium remains quiescent allowing fetal development, growth and maturation. As term approaches, changes occur which allow the onset of rhythmic, coordinated uterine contractions resulting in cervical dilatation and the expulsion of the fully developed fetus. Although much has been accomplished, more work is required before a complete understanding of the mechanisms involved can be attained.

3 Preterm Labour.... an Important Problem to Solve

Preterm labour is defined as labour occurring in a pregnancy before 37 completed weeks (259 days) gestation. The terms 'length of gestation' and 'weeks gestation' are used to mean the time since the first day of the last menstrual period in a regular cycle, or the time since fertilization plus two weeks. Preterm delivery is a very common problem, occurring in approximately 7% of all deliveries. Although many factors are known to be associated with preterm labour, there is no obvious cause in approximately 50% of women presenting in preterm labour *(Ritchie and McClure, 1982).*

In a recent study carried out in the Northern Region of England, idiopathic preterm labour, either spontaneous or following prolonged rupture of membranes, accounted for 43% of the singleton deliveries between 24-31 weeks gestation *(Wariyar et al, 1989).* Whilst there are many causes of preterm delivery, (for example antepartum haemorrhage, cervical incompetence, pregnancy-induced hypertension, fetal abnormality, rhesus isoimmunisation and maternal problems), it is still true to say that an effective, safe way of preventing or arresting idiopathic preterm labour would be the biggest single contribution that could be made to reduce perinatal morbidity and mortality.

In the Northern Region study, the prognosis for long term survival without disability among babies (24-31 weeks gestation) alive at the start of delivery was only 54% in singleton pregnancies complicated by idiopathic spontaneous preterm labour or preterm rupture of membranes, compared to 73% among singleton pregnancies complicated by pregnancy-induced hypertension or antepartum haemorrhage. Nearly a fifth (19%) of the

neonatal survivors after spontaneous preterm labour were severely disabled, as were 6% of the survivors whose delivery followed spontaneous rupture of membranes before labour. Gestation rather than birthweight was found to be the most powerful predictor of mortality and morbidity at birth in preterm babies. The mode of delivery had little discernible effect on mortality or morbidity among survivors once the obstetric factors precipitating delivery and the gestational age were taken into account. On average the surviving babies at 24-27 weeks gestation and at 28-31 weeks gestation required 27 days and 7 days of intensive care respectively *(Wariyar et al, 1989).*

An exercise in costing was recently carried out by the neonatal intensive care unit at University College Hospital, which is a regional referral centre for neonatal intensive care. The mean length of stay for the whole population of admissions was 20 (range 1-295) days, and the mean length of stay for babies of 26 weeks gestation was 63 (range 1-295) days. Thirty-three percent of the baby days were spent in intensive care, thirty-seven percent in high dependency and thirty percent in the special care unit. The rough estimates of total cost per baby (in 1990) were £506 per day, £306 per day and £182 per day for the intensive care, high dependency and special care units respectively. (These figures are rough guides only, and may have underestimated the cost by up to 20%.) Furthermore, these costs do not take into account the costs of the continuing care, which the children with residual severe disability will require for the rest of their lives.

Women who have had one or more preterm labours have a higher risk of preterm labour than the general population, but the majority of preterm labours occur in primiparous women. A successful predictive system is therefore very difficult to devise. Various scoring systems have been suggested *(Kaminski and Papiernik, 1974; Fedrich, 1976; Creasy et al,1980;*

Bouyer et al, 1986). The first two studies utilised known risk factors for preterm labour in the history such as low maternal age and weight, smoking, low social class, single parent, threatened abortion in current pregnancy, abortion in previous pregnancy, antepartum haemorrhage in previous pregnancy, perinatal loss in previous pregnancy and previous preterm delivery. Creasy et al *(1980)* used a scoring system which involved socioeconomic status, past history, daily habits and also details from the current pregnancy, with a reassignment of risk scores at 26-28 weeks gestation. However, even this detailed scoring system found only 39% of the primiparous preterm labours in the high risk group and 77% of multiparous preterm labours. A preterm birth prevention programme in which Creasy's scoring system was used, and combined with special antenatal care for those women at high risk, achieved a reduction in the preterm delivery rate from 6.75% to 2.43%. Only 17.5% of the high risk women developed preterm labour, demonstrating the need for a more discriminative test *(Herron et al, 1982).* Other preterm birth prevention programmes, which also used scoring systems combined with special antenatal care for high risk women, have failed to achieve any reduction in the preterm delivery rate or improvement in perinatal outlook *(Main et al, 1989; Goldenberg et al, 1990).*

Cervical examination has been put forward as another possible method of identifying high risk women for preterm delivery *(Stubbs et al, 1986).* A recent study found a significant increase in the prediction of preterm delivery by vaginal examination at 25-28 and 29-31 weeks gestation *(Blondel et al, 1990).* However, the improvement in preterm labour prediction was only slight and the authors concluded that it was not sufficient to enable recommendation of the practice without further assessment, particularly as others have suggested that weekly vaginal examinations beginning at 37 weeks gestation may be a contributory factor to rupture of

the membranes *(Lenihan, 1984)*, and that a rapid increase in circulating prostaglandin concentrations occurs after vaginal examination in late pregnancy *(Mitchell et al, 1977)*, and that the practice is associated with a potential risk of infection that is correlated with preterm birth *(Toth et al, 1988)*. Serial ultrasound examinations of the cervix do not carry these risks, and may be of some use in women in the second trimester of pregnancy to differentiate between a competent and an incompetent cervix *(Michaels et al,1986)*.

Another difficulty is the diagnosis of preterm labour. At least 50% of women apparently in established preterm labour find that their contractions subside spontaneously and the pregnancy continues. The presence or absence of fetal breathing movements as demonstrated by real-time ultrasound has been shown to be helpful for women with intact membranes, to determine who will go on to delivery within 48 hours *(Castle and Turnbull, 1983)*, but this is a test which is only of value once the women are apparently in labour.

Ambulatory tocodynamometry was suggested as a possible tool in the early detection of preterm labour, when it was found that women who developed preterm labour had a significantly higher baseline contraction frequency, and developed an approximately twofold increase in uterine activity during the last day before admission and treatment for preterm labour, *(Katz et al, 1986)*.

A biochemical marker could clearly be very valuable in the prediction and prevention of preterm labour, particularly if a change in levels of the marker occurred some time before the actual onset of labour. Possible suggestions have included the measurement of prostaglandin metabolite

levels *(Fuchs et al, 1982; Weitz et al, 1986)* and urinary thromboxane excretion *(Noort et al, 1987)*. Another possible marker is major basic protein (a primary constituent protein of the eosinophil granule), which has been shown to rise 4 weeks prior to the spontaneous onset of labour at term, with a more acute rise having been noted in women at the time of preterm labour *(Coulam et al, 1987)*.

Certainly, no definitive predictive test for the onset of preterm labour has yet been found. It is probable that prevention of preterm delivery would be easier if treatment were started before labour was established. However, at present, medical efforts rest mainly on attempting to treat patients once the diagnosis of preterm labour has been made. Unfortunately, there is no ideal, hazard-free tocolytic agent available at present, and treatment does not always prevent delivery. One of the agents in most widespread use is ritodrine hydrochloride (a ß-adrenergic agonist). A recent national study in the United States demonstrated that the use of ritodrine (in as many as 40-50% of the preterm labours resulting in low birth weight infants) had had minimal, if any, impact on the incidence of low birth weight *(Leveno et al, 1990)*.

Preterm labour is an expensive problem both in financial terms to the provider of health care for the child, and in emotional terms for the parents. A sensitive, specific predictive test, and subsequent provision of effective treatment are required before the problem of preterm labour can be solved.

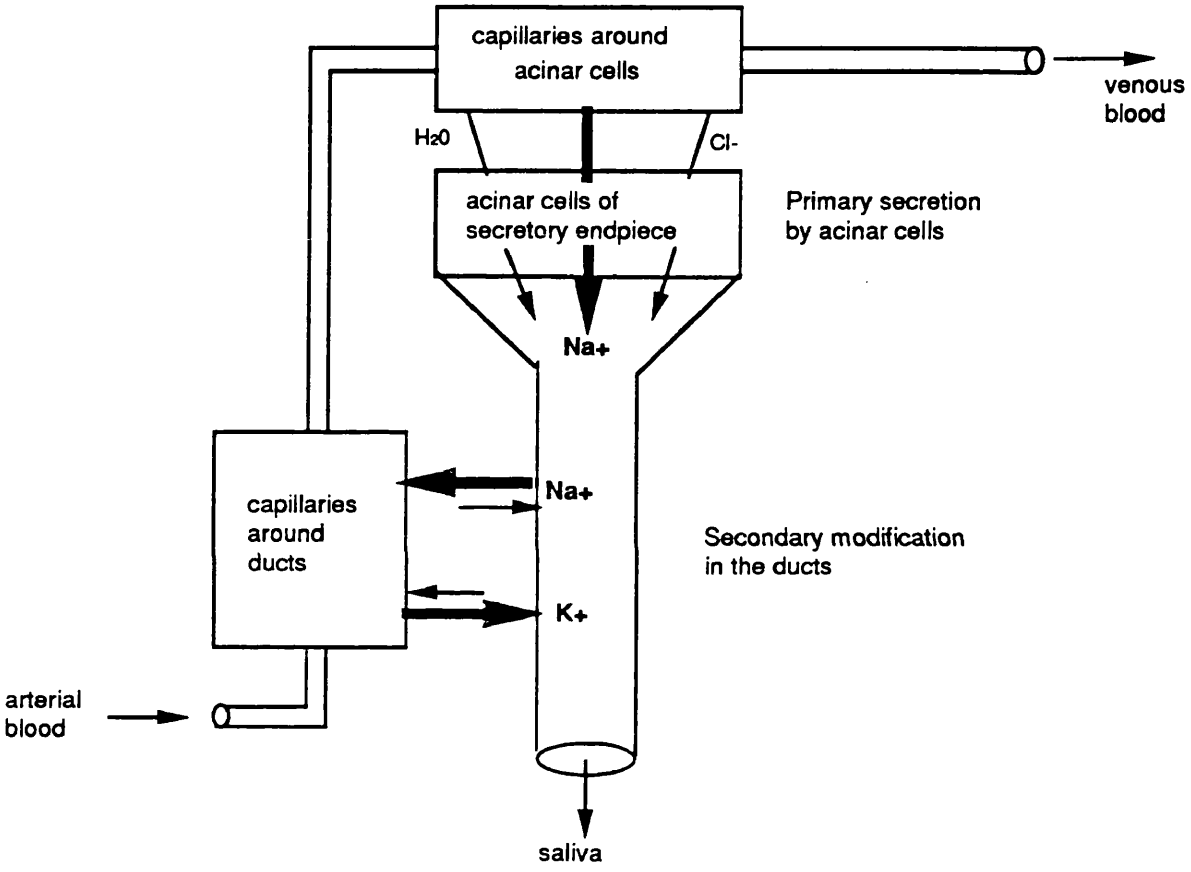
4 Mechanism of Saliva Formation

Saliva is formed by an active, energy-consuming process (Fig. 4.1). The acinar cells, which form the secretory endpiece of the salivary gland, actively pump sodium ions from the blood into the salivary gland endpiece. An osmotic pressure gradient is created which causes water to flow from blood to saliva through the tight junctions between the acinar cells. The primary secretion leaving the endpiece is thought to be approximately isotonic with plasma. As the saliva moves down the ductal system of the gland, the cells lining the ducts pump the sodium back into the blood; however, there is very little transfer of water across the ductal membrane, so that the resulting saliva is hypotonic *(Vining and McGinley, 1984).*

Although small molecules such as glycerol (molecular weight [MW] =92) may also pass through the tight junctions with the water, larger molecules such as sucrose (MW=342) are largely excluded. It is thought that there is a molecular mass cut-off at about 100-300 *(Vining et al, 1983).* Therefore, if steroids (MW 270-400) entered saliva only by this ultrafiltration route, salivary concentrations would be 1% or less of plasma unbound concentrations. This is true for steroid conjugates eg. dehydroepiandrosterone sulphate (DHEAS), which are poorly represented in saliva. However, unconjugated steroids are lipophilic and can pass through the lipid-rich membranes of acinar cells and so enter the saliva (intracellular diffusion). The concentrations of neutral steroids in saliva reflect those in the plasma free fraction, providing no other complicating factors are involved.

One suggested complicating factor is the possibility of metabolism of steroids within the acinar cells themselves. For example, there is circumstantial evidence of the presence of an oxidoreductase enzyme

Fig. 4.1 Diagrammatic representation of the electrolyte and water movement in the parotid gland.



** The relationship between saliva steroid levels and plasma unbound unconjugated levels has been confirmed for the following steroids.

Progesterone:	Poteczin et al, 1981; Wang and Knyba, 1985; Darné, 1987
Oestriol:	Vining et al, 1983; McGarrigle and Lachelin, 1983
Oestradiol:	McGarrigle and Lachelin, 1983
Oestrone:	McGarrigle and Lachelin, 1983
Cortisol:	Umeda et al, 1981; Peters et al, 1982; Walker et al, 1982; Vining et al, 1983
Testosterone:	Smith et al, 1979
Aldosterone:	Few et al, 1986

converting cortisol to cortisone in human salivary glands *(Greaves and West, 1963)*. However, this enzyme appears to have little influence on the relationship between plasma free and salivary cortisol, probably because the rapid transfer of cortisol from blood to saliva compensates for that lost from further metabolism *(Riad-Fahmy et al, 1987)*.

Another possible complicating factor is the salivary flow rate. Vining investigated the effect of flow rate on saliva cortisol and DHEAS concentrations. A high flow rate was produced by the use of citric acid crystals. The DHEAS concentration was markedly lower at the higher flow rate, but the cortisol concentrations were not significantly different. The saliva flow rate/salivary concentration relationships were not investigated for other steroids (such as progesterone), but as their diffusion rates are faster than that of cortisol, their salivary concentrations should not be affected by flow rate *(Vining et al,1983)*.

Contamination of saliva by gingival crevicular fluid can also be a source of error in salivary measurements, (as the gingival membrane appears to be very 'leaky' compared to the salivary gland, particularly in subjects suffering from gingivitis), but this appears to be a significant problem only when the plasma:saliva ratio of the steroid being measured is more than 1000:1 *(Vining et al, 1983)*.

In spite of these potential drawbacks, most studies have shown that saliva steroid levels provide a good reflection of the unbound unconjugated ('free' or biologically active) plasma steroid concentrations.** Saliva sampling has the added advantage of being a non-invasive test. It can be produced and stored at home if necessary, and enables multiple sampling to be carried out relatively easily.

5 Assay Materials and Methods

Origin of materials

Steroids

Chromatographically pure oestrone, oestradiol, oestriol, progesterone, cortisol, and dehydroepiandrosterone were all obtained from Steraloids Ltd, Croydon. Stock solutions of 1mg/ml in ethanol of each of these steroids were stored at -40°C in a deep freeze. All standards were prepared from these stock solutions by dilution with ethanol. Tritium labelled steroids were purchased from Amersham International, Amersham, Bucks. The specific activities are shown in Table 5.1.

Table 5.1 Specific activities of various tritium labelled steroids.

Tritium labelled steroids	Specific activity
(2,4,6,7-^{3}H) oestrone	80-100 Ci/mmol
(2,4,6,7-^{3}H) oestradiol	100-110 Ci/mmol
(2,4,6,9-^{3}H) oestriol	80-100 Ci/mmol
(1,2,6,7-^{3}H) progesterone	80-110 Ci/mmol
(1,2,6,7-^{3}H) cortisol	80-105 Ci/mmol
dehydro (1,2,6,7-^{3}H) epiandrosterone	60-100 Ci/mmol

Antisera

Rabbit anti-oestrone-6-(-0-carboxymethyl)-oxime-bovine serum albumin, rabbit anti-oestradiol-6-(-0-carboxymethyl)-oxime-bovine serum albumin, rabbit anti-oestriol-6-(-0-carboxymethyl)-oxime-bovine serum albumin, rabbit anti-cortisol-6-(-0-carboxymethyl)-oxime-bovine serum albumin, and rabbit anti-dehydroepiandrosterone-17-(-0-carboxymethyl)-oxime-bovine serum albumin were all purchased from Steranti Research Ltd, St Albans, Herts. Sheep anti-progesterone-11α-hemisuccinyl-bovine serum albumin was

purchased from Bioanalysis, Cardiff. They were all supplied freeze-dried and were reconstituted in a phosphate buffer solution and stored at -40°C in 100μl aliquots until required.

Kits

The sex hormone binding globulin [^{125}I] immunoradiometric assay kit was purchased from Farmos Group Ltd, SF-90460 Oulunsalo, Finland. The human chorionic gonadotrophin double antibody [^{125}I] radioimmunoassay quantitative kit, human placental lactogen and prolactin solid phase radioimmunoassays were all purchased from Diagnostic Products [UK] Ltd., Abingdon, Oxfordshire.

Chemicals

All inorganic chemicals were of Analar (Analytical Reagent) grade and were purchased from Fisons Ltd, Loughborough.

Solvents

All solvents were of Analar grade. Diethyl ether (peroxide free), benzene, methanol and ethanol were obtained from Fisons Ltd and were re-distilled just before use.

Water

All water used in the preparation of buffers as well as in the final stages of the washing of glassware was de-ionised just before use.

Pipettes

E-mil 'Goldline' glass pipettes were used for the pipetting of all standard solutions. 'Finn' pipettes with disposable plastic tips were used for the pipetting of all other plasma and saliva samples and aqueous solutions.

Glassware and plastic containers

Glass extraction tubes (1cm x 13cm) and glass reaction tubes (1cm x 10cm) were all reusable. After an assay the tubes were rinsed and soaked in 1% 'Decon 75' for at least an hour. They were then rinsed and immersed in 1% hydrochloric acid, and subsequently washed with de-ionised water. The tubes were finally heated at 350°C for 1 hour. Chromatography was carried out in glass columns 7.2cm long x 0.6cm internal diameter. These were constricted at one end and plugged with glass wool. The columns used for chromatography were washed and dried as described above before use. Plastic universal containers were purchased from Sterilin Ltd, Richmond; polystyrene vials (44mm x 11mm) for storage of plasma and saliva samples were purchased from Hughes and Hughes, Romford. Essex. Polyetheylene vials purchased from Packard, 9731 BK Groningen, Holland, were used both to store some of the saliva samples and as scintillation vials for counting.

Scintillation fluid

'Ultima' Gold liquid scintillation cocktail was purchased from Packard, 9731 BK Groningen, Holland.

Liquid Scintillation Counting

A Packard Tri-Carb (4000 series) Liquid Scintillation counter with a counting efficiency of 63% was used. Samples were mixed with 2.5ml scintillation fluid and counted in disposable 8ml vials. Counting continued until 10,000 counts accumulated per sample, or for 10 minutes, whichever occurred first.

Computations

All radioimmunoassay calculations were done on a Research Machines 3802 micro-computer. All statistical calculations were performed on an Apple Macintosh SE computer using the statistics packages Multistat version 1.01, (distributed by Biosoft, 22 Hills Road, Cambridge) and Systat version 3.2 (distributed by Systat Inc., 2902 Central Street, Evanston, IL60201, USA).

Preparation of materials

Carbonate solution - pH 10.5, 1.5 molar

This was prepared by adding 152g sodium carbonate (Na_2CO_3) and 20g sodium bicarbonate ($NaHCO_3$) to 1000ml distilled water, and mixing thoroughly. The solution was stored at room temperature.

Phosphate buffer solution (PBS) - pH 7.2

Disodium hydrogen orthophosphate (Na_2HPO_4)	8.74g
Sodium dihydrogen orthophosphate ($NaH_2PO_4.2H_2O$)	6.00g
Sodium chloride (NaCl)	9.00g
Sodium azide (NaN_3)	1.00g
Gelatine	1.00g

Distilled water to a volume of 1 litre.

The solution was warmed to 50°C to dissolve the gelatine, mixed well, and kept at room temperature.

Dextran-coated charcoal suspension

500mg charcoal and 50mg dextran were made up to 100ml with PBS. The suspension was stirred at 0°C in an ice/water bath for at least 30 minutes before use.

Chromatography

Sephadex LH20 (Sigma, St Louis, Missouri, USA) was used for chromatographic assays. This was soaked for at least 24 hours in either benzene/methanol 85:15 v/v prior to use in the chromatographic separation of the oestrogens, or in petroleum ether:toluene:methanol 85:14:1 v/v prior to progesterone chromatography.

Assay methods

Oestriol

Saliva oestriol (E3)

The saliva specimens, which had been stored at -40^{o}C, were thawed and then shaken thoroughly. Samples were then centrifuged for 10 minutes at 2000 relative centrifugal force (rcf). 250µl aliquots of the clear supernatant were pipetted into glass tubes ready for extraction. Samples were assayed either singly or in duplicate, as described in the appropriate chapters.

125µl of sodium carbonate buffer and 3.0 ml ether were added to each tube and the tubes were then vortexed for 5 minutes, after which they were frozen at -40^{o}C. The ether fraction was decanted into a set of glass tubes and evaporated at 45^{o}C using a stream of air. 500µl phosphate-buffered saline (PBS) were added to each tube to reconstitute, and the tubes were vortexed at 40^{o}C. 200µl aliquots were then taken for assay.

The standard solution was prepared by double dilution in ethanol. For each assay a standard curve was prepared using triplicates of each of 0, 10, 20, 40, 80, 160 and 320 pg E3 in an ethanol diluent. Two tubes were

also included for the determination of the non-specific binding (NSB). The contents of the standard tubes were then evaporated at 40°C. An extra set of tubes (24) were prepared by adding sodium carbonate buffer (125µl) and ether (3ml) to each tube, vortexing, freezing, decanting and evaporating in exactly the same way as for the unknown sample tubes above. 500µl PBS were added to each of the tubes, which were vortexed at 40°C for 5 minutes. 200µl from each tube were then used to reconstitute the standard curve tubes, which were vortexed at 40°C for 5 minutes.

Labelled E3 containing 10,000 counts per minute (cpm) (100µl) and antiserum at the appropriate dilution (100µl) were then added to the standards and the unknowns. Antisera was omitted from the NSB tubes. The volumes of all the tubes were then made up to a 600µl total with PBS. The tubes were gently vortexed and incubated overnight at 4°C. The tubes were then immersed in an ice/water mixture at 0°C for 1 hour. 200µl dextran-coated charcoal were added to each tube, the tubes were vortexed, left for 15 minutes and then centrifuged at 2000rcf for 15 minutes. 500µl aliquots of the supernatant were pipetted into counting vials to which were added 2.5ml scintillation fluid. The radioactive content of each vial was determined by counting for 10 minutes or to 10,000cpm in the liquid scintillation counter.

Calculations The values obtained for non-specific binding in cpm were subtracted from all the other counts prior to any calculations. The cpm in the unknown tubes were inversely proportional to the amount of hormone present. The data was processed on a computer using a logit/log transformation *(Rodbard et al, 1969)*, the final levels of E3 being expressed in nmol/L. The final volume used for calculation was equivalent to 100µl of saliva per reaction tube.

Recovery 90% (n=20, range 84-96%, *Darné,1987*). This value was used in the final calculation to correct for losses.

Blanks There was no significant difference between the cpm levels of the zero tubes of the dose response curve and that of the two blank tubes.

Sensitivity 12 fmol per tube *(Darné, 1987)*

Specificity Table 5.2 below

Table 5.2

Comparative cross reactions of the oestriol antiserum (%)

Oestriol	100.0
17ß-oestradiol	1.270
17α-oestradiol	0.005
Oestrone	0.017
Progesterone	<0.001
Androstenedione	<0.001
Cortisol	<0.001
Corticosterone	<0.0001

Precision

Interassay coefficients of variation were calculated using 3 pools of saliva (means 1.12, 2.42 and 4.61 nmol/L) in 20 assays, and were 8.0%, 12.3% and 7.5% respectively,

Intra-assay coefficients of variation were calculated using 4 samples (means 0.72, 1.13, 2.52 and 5.50 nmol/L) replicated 20 times, and were 6.0%, 4.2%, 6.1% and 5.5% respectively.

Plasma E3

The plasma specimens, stored at -40°C, were thawed and duplicate 100µl aliquots were mixed with 50µl sodium carbonate buffer and 3ml ether.

The extraction from then on was as previously described for the assay of saliva E3.

After reconstitution with 500µl PBS, 50µl of this dilution were used in the assay. The volumes were made up to 600µl with PBS after addition of antiserum and radioactive tracer. The dose response curve used was the same as that used in the saliva assay. The final volume used for calculation was equivalent to 10µl of plasma per reaction tube.

Recovery 90% *(Darné, 1987)*

Blanks There was no significant difference between the blank tubes and the zero tubes of the dose response curve.

Precision

Interassay coefficients of variation were calculated using 2 pools of plasma (means 13.1 and 28.9 nmol/L) in 10 assays, and were 10.0% and 8.2% respectively,

Intra-assay coefficients of variation were calculated using 3 samples (means 9.7, 29.0 and 49.3 nmol/L) replicated 20 times, and were 9.2%, 5.4% and 4.2% respectively.

Plasma oestradiol

The plasma specimens, stored at -40°C, were thawed and duplicate 100µl aliquots were mixed with 50µl sodium carbonate buffer and 3ml of ether. The extraction from then on was as previously described for the assay of saliva E3.

After reconstitution with 500µl PBS, 30µl of this dilution were used in the assay. The volumes were made up to 600µl with PBS after addition of

antiserum and radioactive tracer. Thereafter the assay was as described for saliva oestriol. The dose response curve used was prepared using triplicates of 0, 10, 20, 40, 80, 160 and 320 pg oestradiol, and duplicate NSB tubes. The final volume used for calculation was equivalent to 6.0µl of plasma per reaction tube.

Recovery 90% (Darné, 1987)

Blanks There was no significant difference between the blank tubes and the zero tubes of the dose response curve.

Sensitivity 10 fmol per tube

Specificity Table 5.10

Precision

All the samples for the study (described in Chapter 10) were assayed in a single batch. Darné et al, *(1987)* quoted an interassay coefficient of variation of 10.8% using the same assay procedure.

Intra-assay coefficients of variation were calculated using 3 samples (means 21.4, 56.9 and 107.4 nmol/L) replicated 20 times, and were 4.8%, 4.5% and 5.5% respectively.

Progesterone

Saliva progesterone

The procedure and volume of saliva used were identical to that already described for the estimation of E3 in saliva. The dose response curve was prepared using triplicates of each of 0, 10, 20, 40, 80, 160 and 320pg progesterone, and duplicate NSB tubes. Equilibration, separation of the bound from the 'free' component and final liquid scintillation counting were performed as previously described for oestriol.

Recovery 90% This value was used in correcting for losses.

Blanks There was no significant difference between the cpm levels of the blank tubes and those of the zero tubes of the dose response curve.

Sensitivity 16 pmol per tube *(Darné, 1987)*

Specificity - Table 5.3 below

Table 5.3

Comparative cross reactions of progesterone antisera (%)

Progesterone	100.0
5α-pregnanedione	22.0
Pregnenolone	5.6
11-deoxycortisol	1.8
Corticosterone	1.3
11-deoxycorticosterone	0.8
Cortisol	0.3
17α-hydroxyprogesterone	0.17
17ß-oestradiol	<0.1
Oestriol	<0.1

Precision

Interassay coefficients of variation were calculated using 3 pools of saliva (means 1.10, 2.07 and 3.34 nmol/L) in 20 assays, and were 14.0%, 12.6% and 9.4% respectively,

Intra-assay coefficients of variation were calculated using 4 samples (means 1.01, 1.74, 2.73 and 3.25 nmol/L) replicated 20 times, and were 11.2%, 7.6%, 5.0% and 6.1% respectively.

Plasma progesterone

In view of the high concentration of plasma progesterone in pregnancy, 100µl plasma were used in the initial extraction, and then a dilution was performed following extraction. Reconstitution following ether

extraction was performed with 500µl PBS, and 100µl aliquots were then transferred into a new set of tubes and the volume made up to 1000µl with PBS. 40µl of this dilution were then used in the assay and made up to a volume of 400µl before addition of the radioactivity and antiserum. (The standard curve was prepared exactly as for the saliva progesterone assay.) The final volume used after dilution was equivalent to 0.8µl plasma per reaction tube.

Recovery 90%

Blanks There was no significant difference between the blank tubes and the zero tubes of the dose response curve.

Specificity

The cross reactivity of the antiserum with 5α-dihydroprogesterone was 22%. The levels in plasma of 5α–dihydroprogesterone between 20 and 40 weeks of pregnancy have been reported to be between 15%-22% of the reported progesterone levels *(Parker et al, 1979)*. Further steps to purify the extract were not taken, and therefore slight overestimation of plasma progesterone remains a possibility.

Precision

Interassay coefficients of variation were calculated using 3 pools of plasma (means 132, 300 and 416 nmol/L) in 16 assays, and were 10.3%, 8.3% and 6.7% respectively,

Intra-assay coefficients of variation were calculated using 3 samples (means 130, 259 and 448nmol/L) replicated 20 times, and were 9.3%, 8.1% and 9.0%) respectively.

Cortisol

Saliva cortisol

The saliva samples were prepared as for the oestriol assay but no extraction procedure was necessary. 30µl aliquots were pipetted in duplicate into the reaction tubes and the volume made up to 400µl with PBS. The tubes were then heated at 70°C for 10 minutes to inactivate any possible traces of CBG in saliva. The rest of the assay procedure was exactly the same as for the saliva oestriol assay, using the same volumes of labelled cortisol and antibody. The standard solution for the dose response curve was prepared with tubes in triplicate containing 0, 25, 50, 100, 200 and 400pg cortisol, and a duplicate set of tubes for NSB. The volume used for calculation was 30µl per reaction tube.

Blanks There was no significant difference between the blank tubes and the zero tubes of the dose response curve.

Sensitivity - 32 fmol per tube *(Darné, 1987)*

Specificity - Table 5.4 below

Table 5.4

Comparative cross reactions of the cortisol antiserum (%)

Cortisol	100.0
21-deoxycortisol	50.8
11-deoxycortisol	15.3
Corticosterone	2.8
Cortisone	2.0
Deoxycortisone	<0.6
Aldosterone	<0.6
Progesterone	2.4
17ß-oestradiol	<0.6
Oestrone	<0.6
Oestriol	<0.6
Prednisone	2.0

The cross-reactivity of the antiserum with 21-deoxycortisol and 11-deoxycortisol is assumed to have no significant effect on saliva cortisol levels, as the levels in plasma are extremely low. Investigations on the levels of 21-deoxycortisol and 11-deoxycortisol in saliva have not been carried out. The cross-reactivity of the antiserum with P was thought to be of no significant consequence because of the markedly higher concentration of cortisol in saliva compared with P. Cortisone is present in saliva, but some plasma 'free' cortisol is converted to cortisone in the salivary glands *(Brooks and Brooks, 1984)* and the overall result is that saliva cortisol measurements probably slightly underestimate plasma 'free' cortisol, *(Vining et al, 1983)*. In saliva, corticosterone levels are less than 5% of cortisol levels, and are therefore unlikely to be of any significant consequence, *(McGarrigle, unpublished data)*.

Precision

Interassay coefficients of variation were calculated using 4 pools of saliva (means 1.53, 5.85, 13.83 and 23.16 nmol/L) in 25 assays, and were 14.2%, 11.0%, 8.1% and 7.5% respectively.

Intra-assay coefficients of variation were calculated using 4 samples (means 2.42, 5.73, 13.95 and 21.80 nmol/L) replicated 20 times, and were 11.2%, 8.0%, 5.8% and 6.5% respectively.

Plasma Cortisol

Duplicate 100µl plasma samples were taken initially. After ether extraction and reconstitution with 500µl of PBS, 100µl aliquots of this solution were transferred to a new set of tubes. The volume was then increased to 1000µl with PBS and the tubes vortexed for 5 minutes. 30µl of

this dilution were used in the assay. The assay was then carried out exactly as for the preceding assays. The final volume used after dilution was 0.6µl plasma per reaction tube.

Recovery 90%

Blanks There was no significant difference between the blank tubes and the zero tubes of the dose response curve.

Specificity High cross reactivity of the antiserum with 21-deoxycortisol and 11-deoxycortisol is not a significant problem in plasma of normal patients as both precursors are present in very small concentrations *(Cope, 1972; Dörr et al, 1989)*. Corticosterone is a minor constituent of normal human plasma, the concentration being about 10% that of cortisol *(Bondy and Upton, 1957)*. Hence the specificity of the cortisol assay is unlikely to be affected to any significant extent in the population studied.

Precision

Interassay coefficients of variation were calculated using 2 pools of plasma (means 340 and 770 nmol/L) in 6 assays, and were 7.6% and 5.4% respectively,

Intra-assay coefficients of variation were calculated using 4 samples (means 148, 243, 562 and 751 nmol/L) replicated 20 times, and were 6.9%, 6.4%, 5.3% and 4.2% respectively.

Dehydroepiandrosterone sulphate (DHEAS)

A 40µl aliquot of plasma was thoroughly mixed with 2ml PBS. 50µl of this solution were then transferred into a second set of tubes and vortexed for 5 minutes with 1 ml PBS. 100µl of the second dilution was used in the assay.

A dose response curve was prepared using triplicate points of each of 0, 25, 50, 100, 200 and 400pg DHEAS per tube. Triplicate NSB values were also estimated. After evaporation of the ethanol in the standard curve tubes the volume of all tubes was adjusted to 400µl with PBS. The tubes were again vortexed and 100µl DHEA antiserum and 100µl labelled DHEA (containing 10,000 cpm) were added to each tube. Equilibration, separation of bound and free fractions and counting of residual radioactivity were as described for the preceding assays. The final volume used for calculation was equivalent to 0.1µl per reaction tube.

Blanks There was no significant difference between the blank tubes and the zero tubes of the dose response curve.

Sensitivity 114 fmol per tube *(Darné, 1987)*

Specificity Table 5.5 below

Table 5.5 Comparative cross reaction of DHEA antiserum (%)

DHEA	100.0
DHEAS	100.0
Testosterone	<0.1
Androstenedione	<0.1
Progesterone	<0.1
Oestradiol	<0.1
Cortisol	<0.1

The DHEA antiserum used cross reacts 100% with DHEAS. However, the concentration of DHEA is much lower than that of DHEAS throughout pregnancy. At 26 weeks of gestation the reported mean concentrations of DHEA and DHEAS are 4 and 1400 ng/ml respectively *(Buster et al, 1979)*. It is well established that the use of a dilution method and DHEA antiserum

gives rise to a reliable assay for the measurement of plasma DHEAS *(Buster and Abrahams, 1972)*.

Precision

All the samples for the study (described in Chapter 9) were assayed in 2 batches. Darné et al, *(1987)* quoted an interassay coefficient of variation of 9.6% using the same assay procedure.

Corticosterone binding globulin

100µl of each serum sample were assayed in triplicate with one aliquot of each sample serving as the non-specific binding blank. 600µl dextran-coated charcoal were added to each tube. After vortexing, these tubes were incubated for 30 minutes at 45°C, and centrifuged for 10 minutes at 2000rcf. This step stripped the samples of the endogenous steroids.

500µl of each supernatant were transferred to a new tube. As a separate step each nonspecific binding serum blank was incubated for an additional 30 minutes at 60°C, to inactivate the binding by CBG to less than 3µg/100ml. Non-specific binding by albumin was shown to be less than 2µg/100ml, whether samples were preheated to 60°C or not, *(Moore et al, 1978)*. The serum blanks were otherwise treated identically as the other samples.

100µl tritiated cortisol were added to each tube, including the serum blanks, and the tubes were then vortexed and incubated for 30 minutes at 37°C. The tubes were then placed in an ice/water bath, and after 15 minutes 600µl aliquots cold dextran-coated charcoal were added to each tube. The tubes were vortexed, incubated for 30 minutes at 4°C, and then centrifuged

at 2000rcf for 10 minutes. 500µl aliquots of the supernatants were transferred into tubes for counting. The final volume used for calculation was equivalent to 71.4µl per reaction tube.

Calculation The cpm in the unknown sample tubes were directly proportional to the CBG levels in the sample. The number of counts in the nonspecific binding serum blanks were subtracted from the mean of the counts of the duplicate serum samples. The resulting value was then multiplied by 33600, which is the dilution/volume factor to correct the result to a volume of 1L.

Sensitivity 30 nmol/L

Precision

Interassay coefficients of variation were calculated using 2 pools of plasma (means 423 and 1050 nmol/L) in 4 assays, and were 6.6% and 5.1% respectively.

Sex Hormone Binding Globulin

This assay was performed by immunoradiometric assay using a kit, and following the procedure as per the kit instructions. All the reagents were brought to room temperature, and reconstituted using distilled water where necessary. Serum samples were diluted to 1:400 using the buffer provided. Standards and control sera were diluted 1:100 with assay buffer. 100µl aliquots of sample were added in duplicate to test tubes. A dose response curve, NSB tubes, total counts, and low and high controls were also set up. Anti-SHBG antiserum 100µl and [^{125}I]anti-SHBG antibody 100µl were added to all the tubes. The tubes were vortexed and incubated for one hour at room temperature. The total tubes were set aside for later counting of radioactivity.

500µl of a solid-phase donkey anti-rabbit IgG antiserum, which was being stirred using a magnetic stirrer to keep the solid phase homogenously in suspension, were added to all tubes except for the total count tubes. The tubes were vortexed and incubated for 15 minutes at room temperature. They were then re-vortexed and 2ml 0.9% saline were added immediately to all tubes except for the total count tubes, and centrifuged for 15 minutes at room temperature at 2000rcf. The supernatant was decanted, and the residual activity in each tube was counted for 2 minutes or until 10,000 counts had accumulated.

Calculation

The amount of radioactivity measured is directly proportional to the concentration of SHBG in the samples.

Quality Control The expected values for the SHBG controls were 9.0-12.2 nmol/L for the low, and 81.0-106.0 nmol/L for the high. The values obtained in this assay were 9.5 and 93.5 nmol/L for the low and high controls respectively.

Blanks There was no significant difference between the blank tubes and the zero tubes of the dose response curve.

Sensitivity - 0.5 nmol/L

Specificity - no human serum protein is known to cross-react with the combination of antibodies employed in this present assay, *(Hammond and Robinson, 1984; Cunningham and McKenna, 1988).*

Precision

All the samples for the study (described in Chapter 9) were assayed in a single batch. The quoted kit interassay coefficient of variation for pregnancy sera over 5 assays performed by 2 technicians was 1.99-9.98%.

Human chorionic gonadotrophin

The assay was performed by radioimmunoassay using a kit and following the kit method. The ß-hCG-antiserum and iodinated hCG were reconstituted with distilled water. hCG calibrators were mixed with hCG-free male human serum, to obtain the appropriate calibration points (A to F) for the dose response curve.

Tubes were prepared in duplicate for total counts, NSB, A (maximum binding) through to F calibration tubes, and also for serum samples and controls. 200µl of the zero calibrator A was pipetted into the NSB and A tubes, and 200µl of each of the remaining calibrators were pipetted into tubes B to F. 200µl of the unknown samples and controls were pipetted into their respective tubes. 100µl ß-hCG antiserum were added to all the tubes except the NSB and total tubes, and vortexed. The tubes were then incubated for 30 minutes at 37°C. 100µl [^{125}I] hCG were added to all the tubes, which were then vortexed and centrifuged for 15 minutes at 3000rcf. The supernatant was decanted and the precipitate was retained for counting. Each tube was counted for 2 minutes.

Calculations The hCG concentrations were calculated from a logit/log representation of the calibration curve.

Blanks There was no significant difference between the blank tubes and the zero tubes of the dose response curve.

Sensitivity 5 mIU/ml

Specificity The antiserum, being raised against the hCG beta subunit, is highly specific for the hCG molecule, with an extremely low crossreactivity to other naturally occurring protein hormones that may be present, (Table 5.6). Neither protein, lipaemia, bilirubin nor haemolysis have any effect on the assay.

Table 5.6 Comparative cross reactivity of ß-hCG antiserum (%)

ß-hCG	100.0
Follicle stimulating hormone	0.1
Luteinising hormone	0.2
Thyroid stimulating hormone	0.02

Precision

All the samples for the study (described in Chapter 9) were assayed in a single batch. The quoted kit interassay coefficient of variation for 5 samples in 20 assays was 3.6-6.6%.

Human placental lactogen

The assay was performed by radioimmunoassay using a kit and following the kit instructions. The buffered [^{125}I] hPL and the hPL calibrators were reconstituted using distilled water, as instructed. Plain tubes in duplicate were prepared for the total counts and the NSB's. hPL-antibody-coated tubes were labelled A through to G in duplicate, and further hPL-antibody-coated tubes were labelled in duplicate for the unknown samples and the controls.

25µl of the zero calibrator (maximum binding) A were pipetted into the NSB and A tubes. 25µl of each remaining calibrator (B to G) were pipetted into their appropriate tubes. 25µl of the unknown samples and controls were pipetted into their tubes. Care was taken to pipette directly to the bottom of the tubes. Buffered [^{125}I] hPL (1 ml) was added to every tube and vortexed. The tubes were incubated for 2 hours at 37°C in a waterbath. The tubes were then decanted, and allowed to drain for 2 or 3 minutes, taking care to

remove residual droplets before counting for 2 minutes. The results were calculated using a logit/log transformation.

Blanks There was no significant difference between the blank tubes and the zero tubes of the dose response curve.

Sensitivity 0.26µg/ml

Specificity Table 5.7

Precision

All the samples for the study (described in Chapter 9) were assayed in a single batch. The quoted kit interassay coefficient of variation for 6 samples in 20 assays was 4.9-15.9%.

Table 5.7 Comparative cross reactivity of hPL antiserum (%)

Human placental lactogen	100.0
Chorionic gonadotrophin	ND
Follicle stimulating hormone	0.04
Growth hormone	0.0003
Luteinising hormone	ND
Prolactin	0.006
Thyroid stimulating hormone	0.012

[ND = the apparent concentration was below the detection limit of the assay, even though the hormone was assayed at levels far above those which one would expect to encounter in a clinical situation.]

The assay is virtually free of protein and other matrix effects.

Prolactin (PRL)

This assay was performed by radioimmunoassay using a kit and following the kit instructions. Buffered [^{125}I] PRL and the prolactin calibrators

were reconstituted using distilled water. Tubes were prepared in duplicate, exactly as for the hPL assay, with plain tubes for totals and NSB's, and PRL antibody-coated tubes for the calibrators A-G, unknown samples and controls.

200µl of calibrator A (maximum binding) were pipetted into tubes A and the NSB tubes. 200µl of the calibrators B to G were pipetted into the appropriate tubes, and 200µl of unknown sample and controls were pipetted into their respective tubes. Care was taken to pipette directly to the bottom of the tubes. Buffered [^{125}I] PRL (1 ml) was then added to every tube, and vortexed gently. The tubes were incubated for 18 hours at room temperature, having been covered with parafilm to prevent evaporation. The tubes were then decanted, and allowed to drain for 2-3 minutes, taking care to remove residual droplets before counting for 2 minutes. Calculations were carried out using a logit/log representation of the calibration curve.

cpm levels of the those of

Blanks There was no significant difference between the blank tubes and the zero tubes of the dose response curve.

Sensitivity 3.7 ng/ml

Specificity Table 5.8

Table 5.8 Comparative cross-reaction of PRL antibody (%)

Prolactin	100.00
Thyroid stimulating hormone	0.5
Human growth hormone	ND
Luteinising hormone	0.4
Follicle stimulating hormone	0.6
Human chorionic gonadotrophin	0.3

(Abbreviations as for Table 5.7)

Precision

All the samples for the study (described in Chapter 9) were assayed in a single batch. The quoted kit interassay coefficient of variation for 3 samples in 18 assays was 5.0-9.1%.

Chromatographic method - saliva and plasma

Oestrone, Oestradiol and Oestriol Assays

Chromatography was performed on Sephadex LH20 columns. The Sephadex was prepared by soaking for 24 hours in benzene/methanol 85:15 v/v. It was then transferred to the glass columns using a Pasteur pipette, was allowed to settle, and washed twice with 2ml of the benzene/methanol mixture.

The saliva specimens were thawed, mixed and centrifuged for 15 minutes. 500µl aliquots of the clear supernatant were pipetted into glass extraction tubes, mixed with 40µl distilled water containing 2000 cpm of each of labelled E1, E2 and E3, and vortexed for 1 minute. They were then allowed to equilibrate in extraction tubes for 20 minutes. 5 ml of freshly distilled diethyl ether were added to each tube and the tubes were vortexed for 5 minutes. The tubes were frozen, and the ether layer was then decanted and evaporated. The residue was reconsitituted in 125µl of benzene:methanol mixture (85:15). This was then added to the Sephadex LH20 columns and the columns developed with further benzene:methanol mixture.

The appropriate eluting fractions for E1, E2 and E3, had been previously identified as being 1.0-1.8 ml, 2.0-3.0 ml, and 3.4-5.2 ml respectively. The appropriate fractions were collected and evaporated. Reconstitution of each fraction was then performed with 500µl PBS by vortexing the reaction tubes at 40°C for 5 minutes. 100µl aliquots were transferred to counting vials for recovery estimation. Individual recoveries, after volume correction, were calculated as a percentage of the initial labelled steroid added.

Dose response curves for E1, E2 and E3 were prepared in triplicate for each assay at the following concentrations:- 0, 10, 20, 40, 80, 160 and 320 pg/tube. A triplicate set of tubes were included in each assay for the estimation of the non specific binding. Also, to maintain similarity between the dose response curve and the unknowns, the mean equivalent of radioactivity remaining in the unknown reaction tubes (calculated from individual recoveries) was added to all the reaction tubes in the dose response curve.

From then on, until final computing, the assays for estimation of E1, E2, and E3 were as described for saliva E3. For the final computing, because of the additional chromatographic step, individual recoveries were used for each tube after volume adjustment. The final volumes for calculation were equivalent to 350, 350 and 100µl for saliva E1, E2, and E3 respectively. The final levels were expressed in pmol/L.

For the assay of E1, E2 and E3 in plasma, duplicate 100µl volumes of each sample were mixed with 40µl distilled water containing 2000cpm of each of tritiated E1, E2 and E3 (for recovery estimation). The tubes were vortexed, equilibrated for 20 minutes and extracted with 2.0ml of diethyl ether.

Otherwise the procedure was identical to that described for the estimation of the same steroids in saliva. The final volumes for calculation were equivalent to 10, 4 and 8µl for plasma E1, E2, and E3 respectively.

The volumes used for the assays following chromatography, after reconstitution of the residue in 500µl of PBS, are shown below (Table 5.9).

Table 5.9

Volume (µl) used in saliva and plasma assays following chromatography.

	E1	E2	E3	P
Saliva	350	350	100	100
Plasma	50	20	40	25

Assay characteristics for oestrone, oestradiol and oestriol in plasma and saliva

Recovery E1 - mean 80% (range 63%-92%)

E2 - mean 73% (range 58%-86%)

E3 - mean 71% (range 55%-84%)

Blanks There was no significant difference between the blank tubes and the zero tubes of the dose response curve for the E1, E2 or E3 assays.

Sensitivity

E1 - 10 fmol per tube

E2 - 10 fmol per tube

E3 - 12 fmol per tube

Specificity Tables 5.2 and 5.10

Table 5.10

Comparative cross reactions of oestrone and oestradiol antisera

	Anti-oestrone(%)	Anti-oestradiol (%)
Oestrone	100.0	0.17
17ß-oestradiol	1.4	100.0
17α-oestradiol	<0.3	0.17
Oestriol	-	0.81
DHEAS	<0.3	-
Androstenedione	<0.3	<0.003
Testosterone	<0.3	<0.003
Progesterone	<0.3	<0.003
17α-hydroxyprogesterone	<0.3	<0.003
Cortisol	<0.3	<0.003
Aldosterone	<0.3	<0.003

A high degree of specificity was further ensured by the additional step of chromatography.

Precision

Oestrone - saliva

Interassay coefficients of variation were calculated using 2 pools of saliva (means 192 and 456 pmol/L) in 10 assays, and were 10.7% and 9.5% respectively.

Oestrone - plasma

Interassay coefficients of variation were calculated using 2 pools of plasma (means 19.5 and 31.6 nmol/L) in 10 assays, and were 9.2% and 8.4% respectively.

Oestradiol - saliva

Interassay coefficients of variation were calculated using 2 pools of saliva (means 107 and 329 pmol/L) in 10 assays, and were 11.4% and 8.1% respectively.

Oestradiol - plasma

Interassay coefficients of variation were calculated using 2 pools of plasma (means 25.3 and 42.7 nmol/L) in 10 assays, and were 9.6% and 8.7% respectively.

Oestriol - saliva

Interassay coefficients of variation were calculated using 2 pools of saliva (means 1.91 and 4.72 nmol/L) in 10 assays, and were 11.7% and 8.6% respectively.

Oestriol - plasma

Interassay coefficients of variation were calculated using 2 pools of plasma (means 14.3 and 30.9 nmol/L) in 10 assays, and were 9.6% and 9.1% respectively.

Progesterone

Chromatography was performed on Sephadex LH20 columns. The Sephadex was prepared by soaking for 24 hours in petroleum ether (80-100°C BP): toluene:methanol (85:14:1) mixture. It was then transferred to the glass columns using a Pasteur pipette, was allowed to settle, and washed twice with 2ml of the petroleum ether:toluene:methanol mixture.

Duplicate 200µl volumes of saliva were mixed with 40µl distilled water containing 2000cpm of tritiated progesterone. After equilibration for 20 minutes, 100µl aliquots of carbonate solution were added and the tubes extracted with 3ml diethyl ether. After freezing, the ether was decanted, evaporated and the residue dissolved in 150µl of petroleum ether:toluene:methanol mixture and applied to the Sephadex LH20 columns. The columns were then developed with the same mixture. The fraction eluting between 1.6-3.2ml (containing progesterone) was collected,

evaporated and the residue reconstituted in 500µl PBS. 200µl aliquots from each tube were transferred to counting vials for recovery estimation, and 100µl aliquots from each tube were assayed for their P content, using the same assay procedure as described previously for saliva progesterone.

Plasma P was assayed in a similar way using duplicate 25µl aliquots of plasma. After mixing with tracer progesterone, extraction and chromatography, the residue was reconstituted in 500µl PBS. 200µl aliquots from each tube were transferred to counting vials for recovery estimation, and 25µl aliquots from each tube were assayed for their P content.

The final volumes used for calculation were equivalent to 1.25 and 100 µl per reaction tube for plasma and saliva respectively.

Recovery mean 79% (range 64-91%)

Blanks There was no significant difference between the blank tubes and the zero tubes of the dose response curve.

Sensitivity 16 pmol per tube

Specificity Table 5.3

A high degree of specificity was further ensured by the additional step of chromatography.

Precision

Progesterone - saliva

Interassay coefficients of variation were calculated using 3 pools of saliva (means 1.40, 1.97 and 5.86 nmol/L) in 10 assays, and were 10.4%, 10.6% and 7.2% respectively.

Progesterone - plasma

Interassay coefficients of variation were calculated using 2 pools of plasma (means 234 and 459 nmol/L) in 10 assays, and were 10.1% and 9.2% respectively.

6

Saliva Oestriol and Progesterone in the Second and Third Trimesters of Pregnancy - Oestriol:Progesterone Ratios in relation to Term and Preterm Labour

Introduction

The onset of labour in sheep and some other mammalian species is preceded by a rise in the maternal plasma oestrogen to progesterone ratio. As discussed in Chapter 2, no consistent change has been demonstrated in the plasma unconjugated oestradiol:progesterone ratio before the onset of labour in women. However, plasma unconjugated steroid concentrations consist of both the protein bound steroid and the biologically available unbound 'free' steroid, and the percentage binding of the oestrogens and of progesterone to plasma proteins differs considerably (Table 6.1, *McGarrigle and Lachelin, 1983; Darné, 1987*). Consequently, changes in the ratios of the unbound unconjugated steroids are not immediately apparent when measuring total plasma unconjugated steroid concentrations.

Table 6.1 Percentage 'free' oestradiol, oestriol and progesterone in pregnancy, expressed as the saliva:plasma ratio x 100.

Hormone	Saliva x100 / Plasma	(Range)	Number of paired samples
Oestradiol	0.68%	(0.34-1.40)%	15
Oestriol	16.4%	(11.1-21.7)%	15
Progesterone	2.00%	(1.63-2.40)%	19

Studies in pregnant women have shown a good correlation between saliva levels and unbound unconjugated levels in plasma both for P *(Poteczin et al, 1981; Darné, 1987)* and for E3 *(Vining et al,1983; Lachelin and McGarrigle, 1983)*. Thus, the levels of unconjugated hormones in saliva provide a direct estimate of the biologically active portion of plasma steroids.

This study was developed following the results of previous work at University College Hospital. It had been found that there was a rise in the saliva oestriol:progesterone (E3:P) ratio from <1 to >1 in all the 20 women studied in the weeks preceding the spontaneous onset of labour at term *(Darné et al,1987)*. Furthermore, an inappropriately high saliva E3:P ratio, which was above the 90th centile for the gestation in all of the 13 women studied, was reported in women who went into idiopathic preterm labour with intact membranes. In contrast, the women who had had prolonged rupture of membranes (PROM) prior to preterm labour had an E3:P ratio less than 1 and on or below the 50th centile for the gestation in the 1-4 days before delivery *(Darné et al,1987)*.

The aims of this study were threefold:

1) To obtain serial saliva samples from 20 weeks gestation onwards from a group of normal women who went into spontaneous labour at term, in order to obtain a normal range, which could be compared with previous findings and with the results from the other subjects in the study.
2) To obtain samples from women who were admitted in preterm labour or threatened preterm labour, in order to determine the saliva E3:P ratios.
3) To obtain serial saliva samples from 20 weeks gestation onwards from women who subsequently went into preterm labour, in order to determine whether the E3:P ratio could be useful as a predictive biochemical test.

Materials and Methods

Women were recruited to the study from the antenatal clinic, where all the women attending for their first or 'booking' appointment were asked if they were willing to participate in the study. Only those women who had no freezing facilities at home were excluded. Recruiting patients for the study was continued for one year. The aim was to recruit as large a number of women as possible, in order to have a serial collection from that small proportion of women who would subsequently go into preterm labour. Women were also recruited at the time of their admission to UCH in threatened or possible preterm labour.

The women were asked to collect a saliva sample three times each week from 20 weeks gestation until they delivered. They were provided with appropriate containers and labels, and were asked to store their samples in their domestic freezers until they were able to give them to us. The samples were stored in the laboratory at -40^{O}C. The women who agreed to participate in the study received standard UCH obstetric care, which in most cases meant shared care with their general practitioners. No special advice or treatment was offered.

1331 women were recruited to the study in total.
615 women collected 1 or more saliva samples.
134 women provided reasonably complete collections.
8 of the 134 women (5.97%) went into spontaneous preterm labour.

The samples of 28 normal women who went into spontaneous labour at term, and who had reasonably complete collections were chosen at random and assayed. The samples from all of the women who went into spontaneous preterm labour were assayed. All of the samples were assayed for saliva E3 and P. All of the preterm samples were assayed in duplicate.

Table 6.2 Normal subjects' (1-28) details including parity (P-primiparous, M-multiparous), gestation (in weeks and days), mode of delivery (NVD-normal vaginal delivery, LSCS-lower segment cesarean section), sex of infant (male..M, female..F), birthweight (in grams), and other pregnancy/delivery details. [* subjects results excluded in calculations for Fig. 6.5; ¶ subjects results excluded in calculations for Fig. 6.6]

Subject	Parity	Gestation	Mode of delivery	Sex	Birthweight	Pregnancy and delivery details
1	P	38 +2	NVD	M	3160	Small vaginal bleed at 33+4 weeks.
2*	P	41 +4	NVD	F	2740	Small vaginal bleed at 30 weeks.
3	P	41 +1	NVD	M	3460	
4*	P	41 +0	LSCS	F	3740	CS..maternal pyrexia, fetal tachycardia , slow progress.
5	M	38 +2	NVD	M	2880	Small pv bleed at 32+2 weeks. Grade 1 placenta praevia.
6	P	40 +1	NVD	M	3510	
7	P	40 +0	NVD	M	2890	
8	M	40 +4	NVD	M	3950	
9	P	40 +0	NVD	F	3120	
10	M	40 +2	NVD	F	3720	
11	P	39 +5	NVD	M	3400	
12	M	38 +2	Forceps	F	2740	Spotting X4 between19 and 23 weeks gestation.
13	M	39 +6	NVD	F	2980	
14	P	40 +2	NVD	F	3000	Urinary tract infection at 30-31 weeks gestation, treated with ampicillin.
15	P	41 +0	NVD	M	3060	
16	P	40 +5	Forceps	M	2940	
17	P	41 +2	Forceps	F	3500	
18	M	41 +3	NVD	F	3840	
19	M	42 +1	NVD	M	4340	
20	P	38 +4	LSCS	M	3140	CS.. brow presentation.
21	P	39 +3	NVD	F	3350	
22	P	41 +0	NVD	F	3600	
23	M	41 +0	NVD	M	3640	Painful tightenings 24-25 weeks gestation.. no cervical change.
24	P	39 +1	Forceps	F	3610	
25	P	38 +0	LSCS	F	3200	CS at onset of labour, as patient wished to avoid antenatal herpes screening.
26*¶	M	39 +0	NVD	M	3620	Diarrhoea and vomiting at 33 weeks.
27	P	41 +4	NVD	F	3780	
28	P	40 +2	NVD	M	3300	

Table 6.3 Preterm delivery subjects' details including parity, gestation (weeks+days), mode of delivery (NVD..normal vaginal delivery, (LS)CS.. (lower segment) cesarean section), sex and birthweight of infant (M..male, F..female, weight in grams), time between spontaneous rupture of the membranes (SROM) and delivery (hours), and other pregnancy or delivery details. (Rx- treatment, B/P-blood pressure, IUFD-intrauterine fetal death)
[* indicates those women who provided serial collections]

Subject	Parity	Gestation	Delivery	Sex	Weight	SROM	Pregnancy and delivery details
1*	0+1	36+0	LSCS	F	2300	104	Pyrexial, contracting, offensive liquor. CS..fibroid obstructing head descent.
2*	0+2	35+0	LSCS	M	2130		CS failure to progress in 2nd stage (maternal childhood rickets).
3*	0+0	36+2	LSCS	F	2040		CS..narrow pelvic outlet and fetal distress on CTG. Birthweight<10th centile.
4*	0+1	35+6	NVD	F	2240		
5*	1+0	31+2	LSCS	F	1780		CS first stage..compound presentation with cord prolapse.
6*	0+2	36+0	NVD	M	1940		
7*	2+2	35+1	LSCS	F	2220		Threatened preterm labour from 27/52 Rx tocolytics. CS..bicornuate uterus.
8*	0+0	33+0	NVD	M	2270	>72	
9	3+1	31+0	NVD	F	2050		Threatened 1st trimester abortion. Tightenings 22-24 weeks Rx oral ritodrine.
10	0+1	35+3	NVD	F	2270	48	
11	2+3	29+2	NVD	F	1110	79	Threatened 1st trimester abortion. Intermittent bleeding from 18/52 (low placenta).
12	0+1	34+1	NVD	F	2490	426	Raised B/P at 23/52 Rx methyl dopa. Baby congenital toxoplasmosis.
13	0+0	25+5	NVD	F	890	108	Cord prolapse and IUFD at 8 cms dilated first stage.
14	0+0	30+4	NVD	F	1840	696	In utero transfer with SROM. B/P intermittently raised..no Rx.
15	1+1	25+2	NVD	M	780		
16	1+0	29+2	LSCS	F	1560	576	SROM following failed amniocentesis Rx ritodrine, antibiotics and indomethacin.
17	3+4	35+5	NVD	F	2900		
18	8+0	26+1	NVD	M	960	188	Intermittent vaginal bleeds from 14/52 from low placenta.
19	6+1	35+4	NVD	M	2060		Amniocentesis. Chest pain Rx antibiotics at 33/52. Baby weight <10th centile.
20	0+0	33+0	NVD	F	2040	61	Threatened 1st trimester abortion. Urinary tract infection at 20/52 Rx antibiotics.
21	2+1	34+1	Forceps	M	2140	37	?Abnormal right fetal kidney on ultrasound. Forceps for fetal distress 2nd stage.
22	0+0	34+3	NVD	M	2120		Late booker. Preterm labour 33+6/52, initially settled with ritodrine.
23	0+1	34+0	NVD	M	2300	62	
24	1+1	26+2	CS	M	1210		Late booker. CS .. footling breech + preterm.
25	0+1	35+0	NVD	M	1970		Late booker. Pregnancy first noted by dentist at a routine dental appointment!
26	1+1	35+1	NVD	F	2410		Multiple admissions for abdo pain ?cause.. not thought to be preterm labour.
27	1+0	34+3	NVD	M	1810		
28	2+0	27+4	NVD	F	1260		
29	0+0	36+1	NVD	F	3040	504	
30	2+1	31+1	NVD	F	1390	134	Threatened abortion at 14/52.
31	3+0	35+6	Forceps	M	2450		Threatened preterm labour from 26+/52.. Rx tocolytics. Forceps.. fetal distress.

Table 6.4 Summary of the clinical details of the normal women who laboured at term, those who went into idiopathic preterm labour with intact membranes, and those who laboured preterm following prolonged rupture of the membranes (PROM).

Group	No. of subjects	Age at delivery (years)	Gestation at delivery (weeks+days)	Birthweight (g)
Term delivery				
Mean (SD)	28	31 (4)	40+1	3360 (400)
Range		22 - 39	38+0 to 42+1	2740 - 4340
Idiopathic preterm				
Mean (SD)	17	32 (8)	33+2	1960 (500)
Range		19 - 42	25+2 to 36+2	780 - 2900
PROM and preterm				
Mean (SD)	14	30 (5)	32+0	1900 (630)
Range		23 - 38	25+5 to 36+1	890 - 3040

The normal group comprised 19 primiparous and 9 multiparous women, who had 21 normal vaginal deliveries, 3 cesarean sections, and 4 forceps deliveries, and gave birth to live healthy infants (14 male and 14 female) of normal weight (Tables 6.2 and 6.4).

The preterm group consisted of 31 women, 17 of whom went into idiopathic preterm labour (mean gestation 33 weeks and 2 days) and 14 of whom went into spontaneous preterm labour (mean gestation 32 weeks) following prolonged spontaneous rupture of membranes. Cesarean sections were performed in 7 women, forceps deliveries in 2 women and the remaining 22 women had normal vaginal deliveries, (Table 6.2). Only 8 of these women (subjects 1-8) had collected serial saliva samples from around 20 weeks gestation onwards.

Results

Saliva oestriol and saliva progesterone in normal women.

Saliva oestriol levels for the 28 normal women during the last 22 weeks prior to the spontaneous onset of labour are shown in Fig. 6.1. The same results are also plotted by gestation in Fig. 6.2. The saliva progesterone levels for the same women are shown during the last 22 weeks prior to the onset of labour and plotted by gestation in Figs. 6.3 and 6.4 respectively. Both the saliva oestriol and progesterone measurements showed a trend of increasing levels with increasing gestation. The increase in the saliva oestriol levels was more pronounced than that of saliva progesterone during the last 4-6 weeks before the onset of labour.

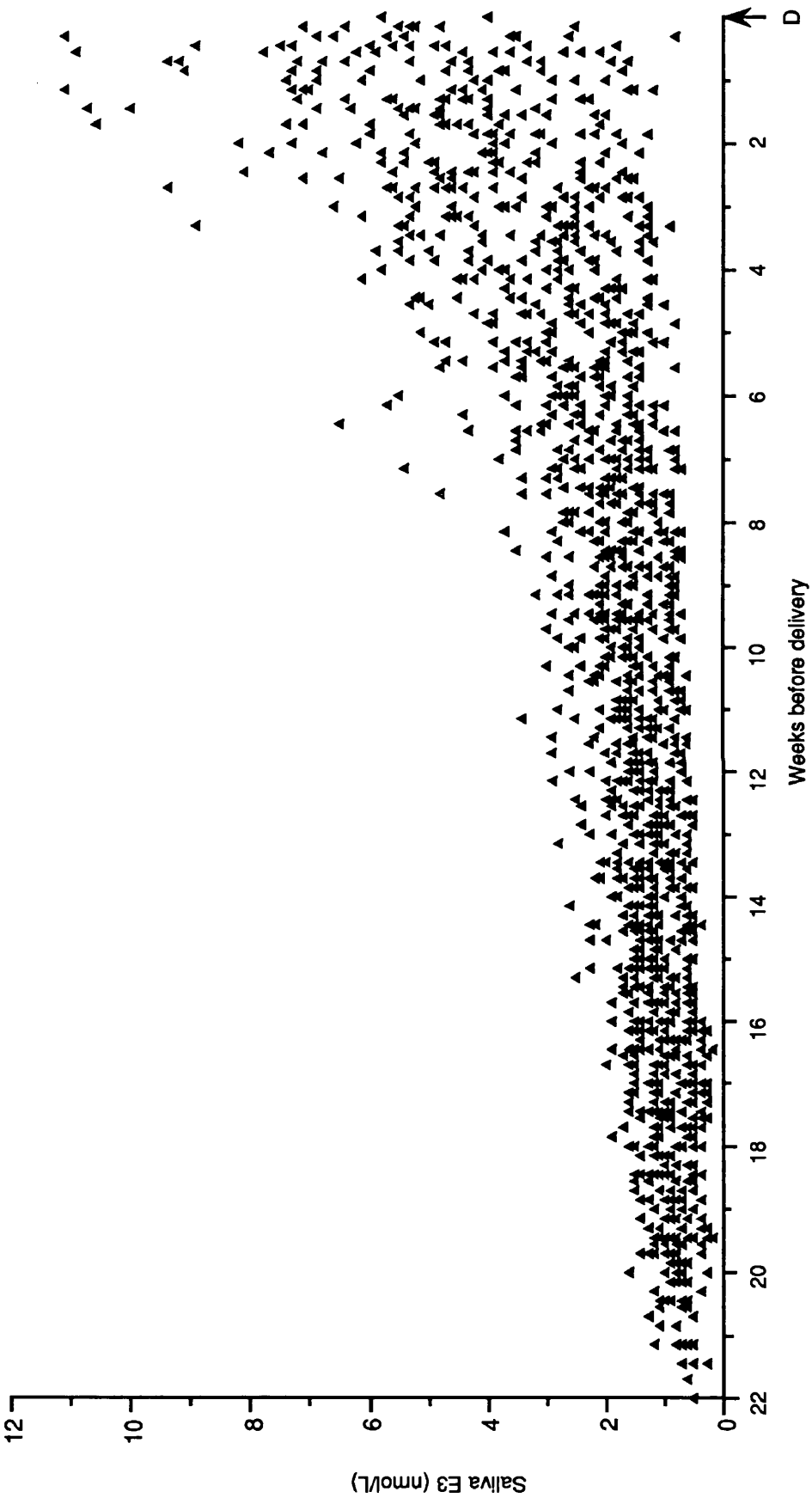


Fig. 6.1 Saliva E3 levels (nmol/L) in 28 normal women during the last 22 weeks of pregnancy.

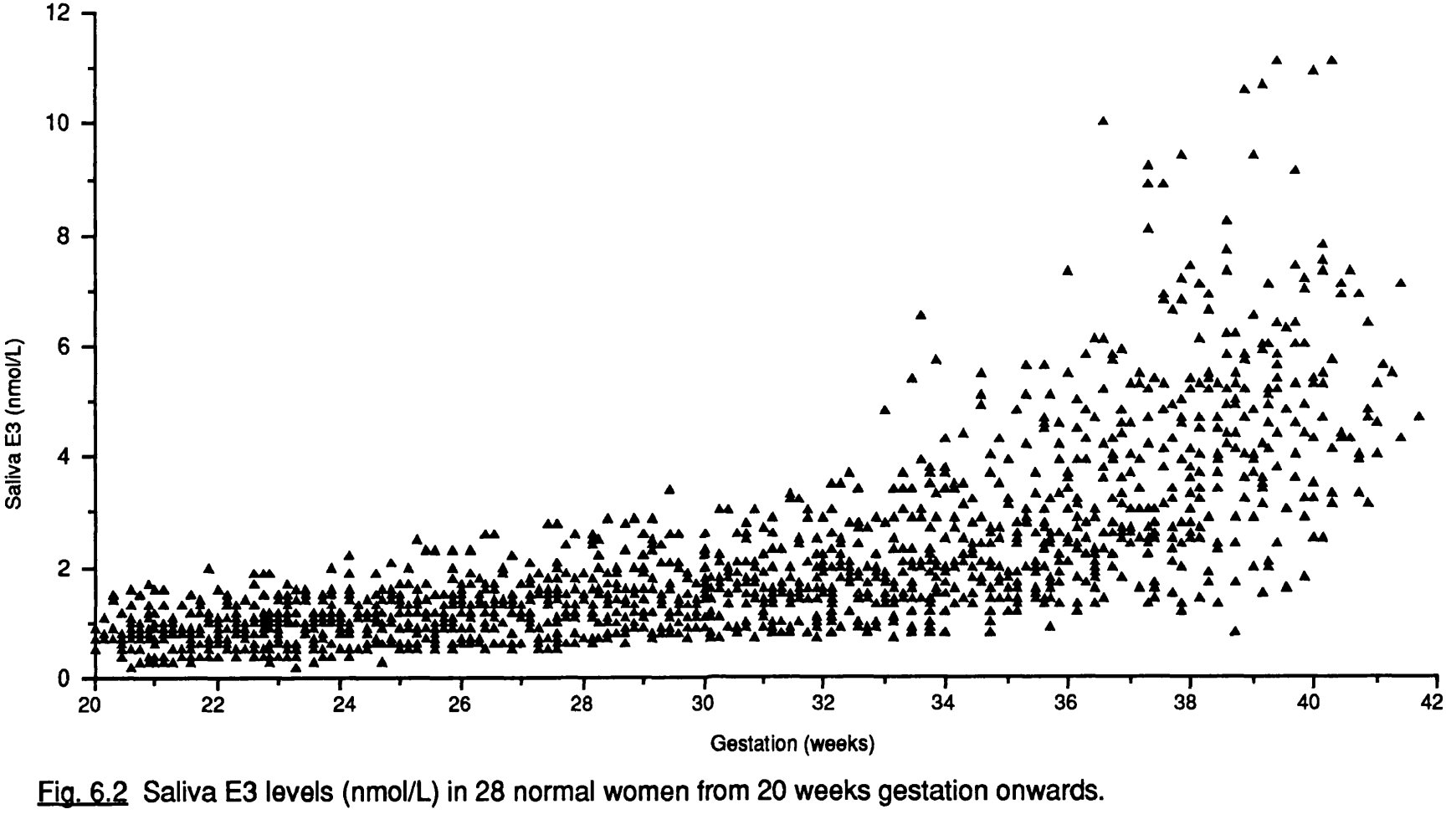


Fig. 6.2 Saliva E3 levels (nmol/L) in 28 normal women from 20 weeks gestation onwards.

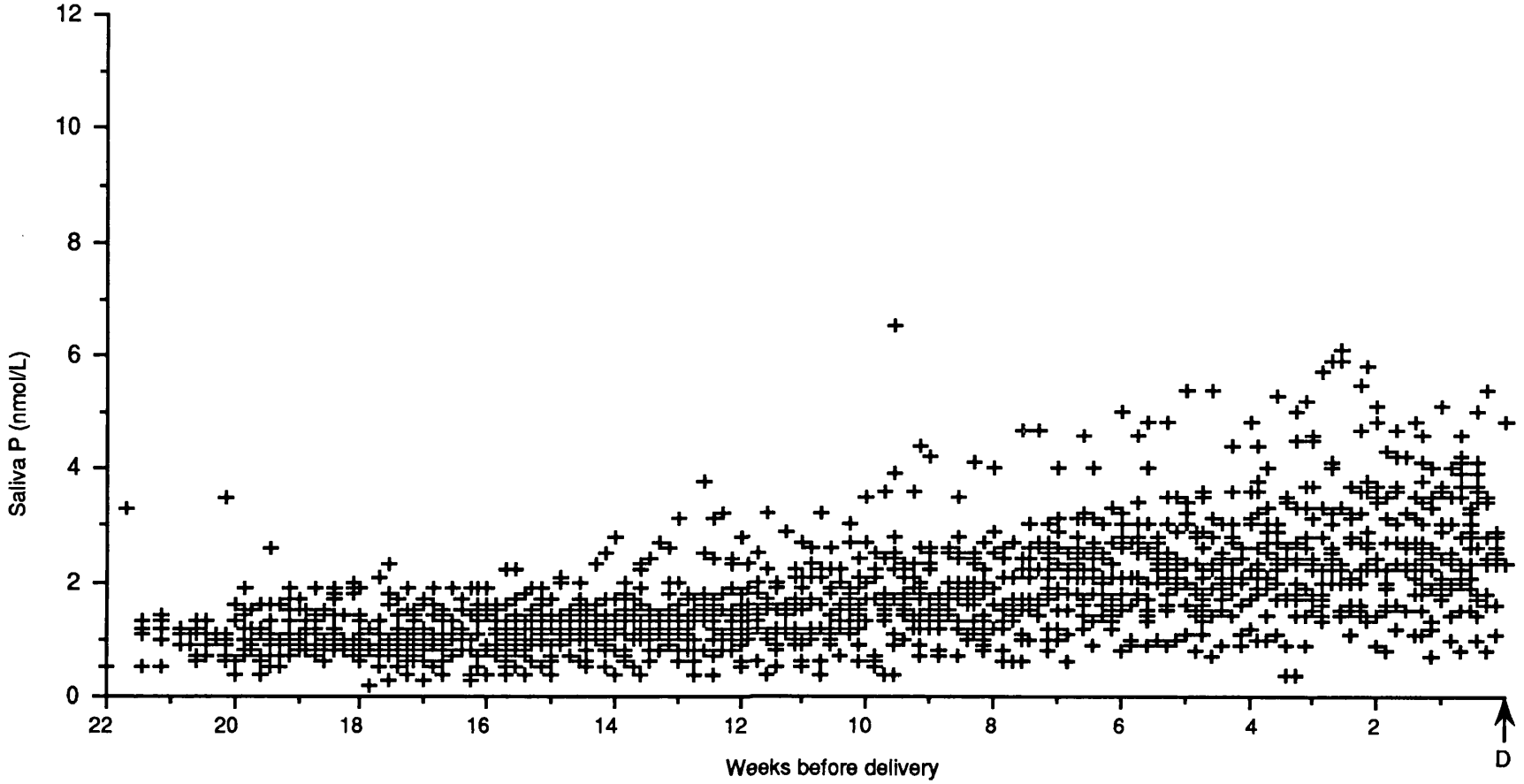


Fig 6.3 Saliva P levels (nmol/L) in 28 normal women during the last 22 weeks of pregnancy.

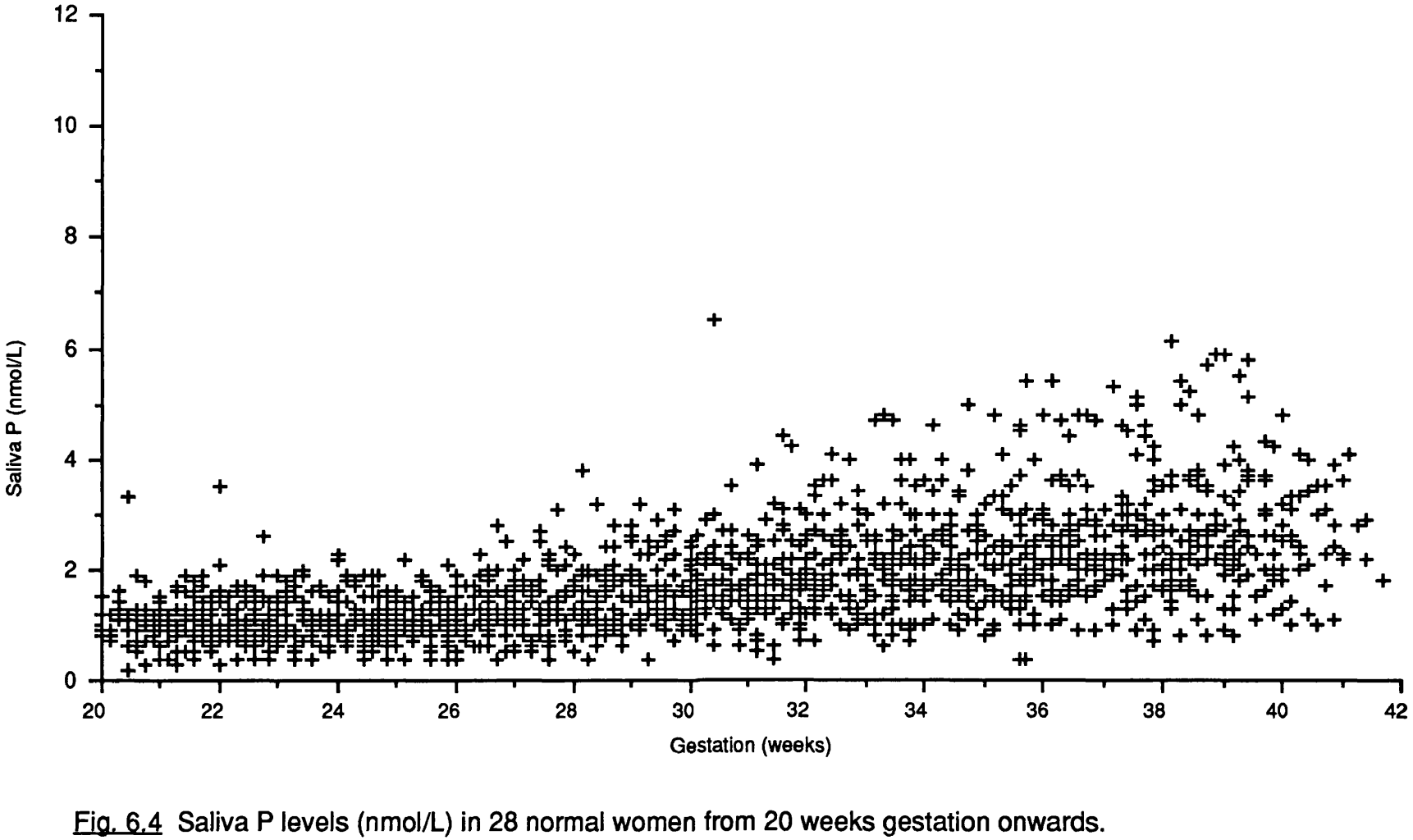


Fig. 6.4 Saliva P levels (nmol/L) in 28 normal women from 20 weeks gestation onwards.

The median saliva oestriol and progesterone levels in 25 normal women during the 18 weeks prior to the onset of labour are shown in Fig. 6.5. The median saliva oestriol and progesterone levels in 27 normal women plotted by gestation are shown in Fig. 6.6. The medians were calculated for 2 samples/patient/week. Three women were excluded from the calculations for Fig. 6.5. Two of the women did not have samples for the week prior to delivery and the other woman had omitted too many samples to be included. In Fig. 6.6 only the latter woman was omitted from the calculations.

The overall percentage increase in saliva oestriol during the last 18 weeks prior to labour was 533% with half of the rise occurring in the last 4-5 weeks before delivery. The overall percentage increase in saliva progesterone during the last 18 weeks prior to labour was 267%, but the increase was steady throughout those 18 weeks, with half the rise having occurred by 9-10 weeks before delivery.

It can be seen from the graphs that the median saliva E3 and P levels were similar, and were very closely related until about 5 weeks before delivery, after which the median E3 levels began to increase much more rapidly than the median P levels, which continued with a slow increase until about 3 weeks prior to delivery when it levelled off slightly.

Saliva E3:P ratios in normal women.

The E3:P ratio for each saliva sample was calculated and the results are shown in Figs. 6.7 and 6.8. The median E3:P ratio together with the 5th, 10th, 90th and 95th centiles were also calculated (Figs. 6.9 and 6.10) . There was a rise in the E3:P ratio with gestation and with the approach of labour from approximately unity to above unity, demonstrating the increasing dominance of saliva E3.

Fig 6.5 Median saliva E3 and P levels in 25 normal women during the last 18 weeks of pregnancy. (Medians calculated for 2 samples/patient/week)

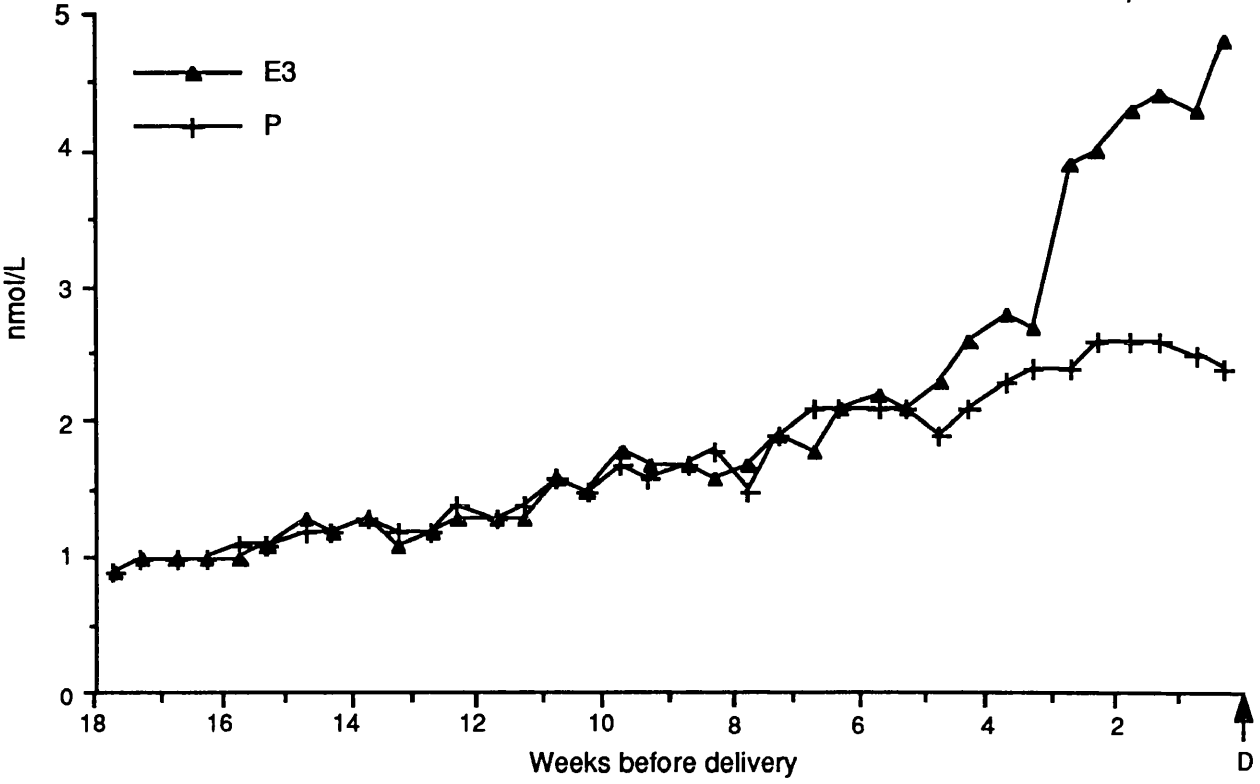
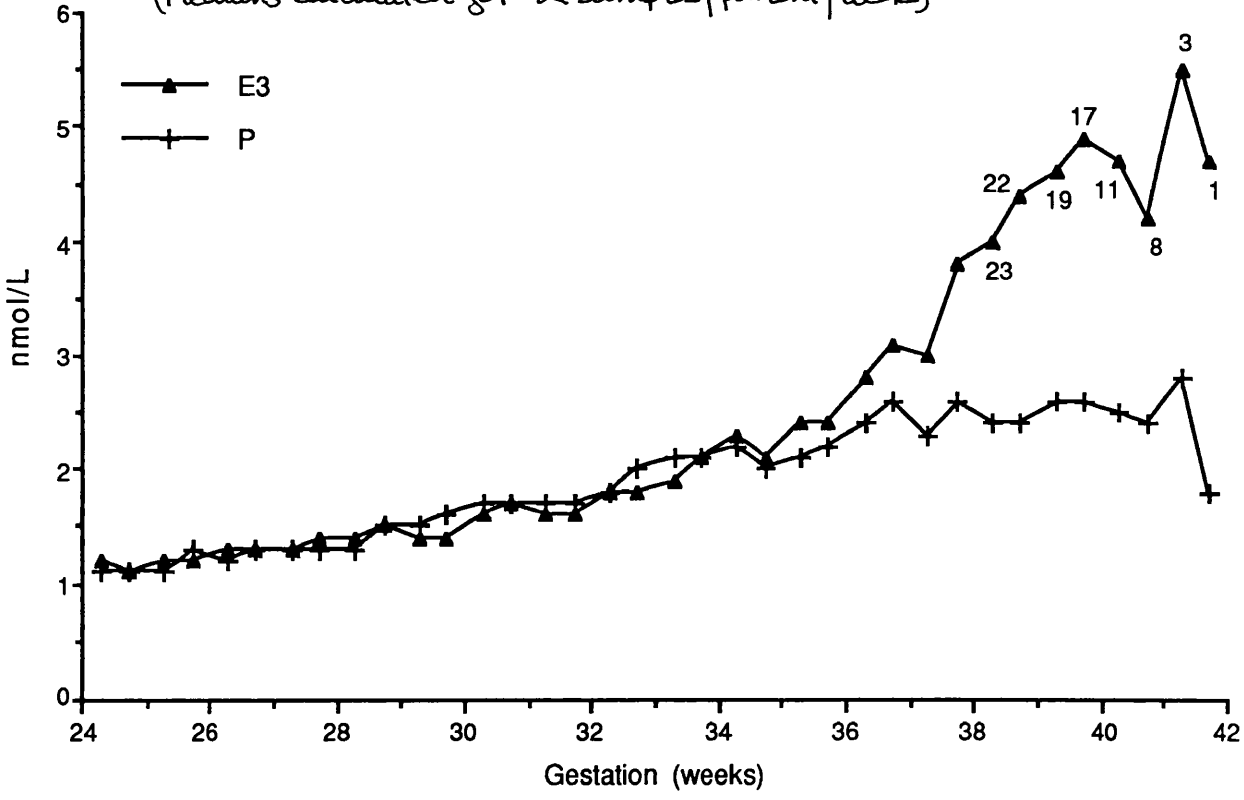


Fig. 6.6 Median saliva E3 and P levels in normal women from 24 weeks gestation onwards. [n=27 for both E3 and P, unless otherwise indicated on graph]
(Medians calculated for 2 samples/patient/week)



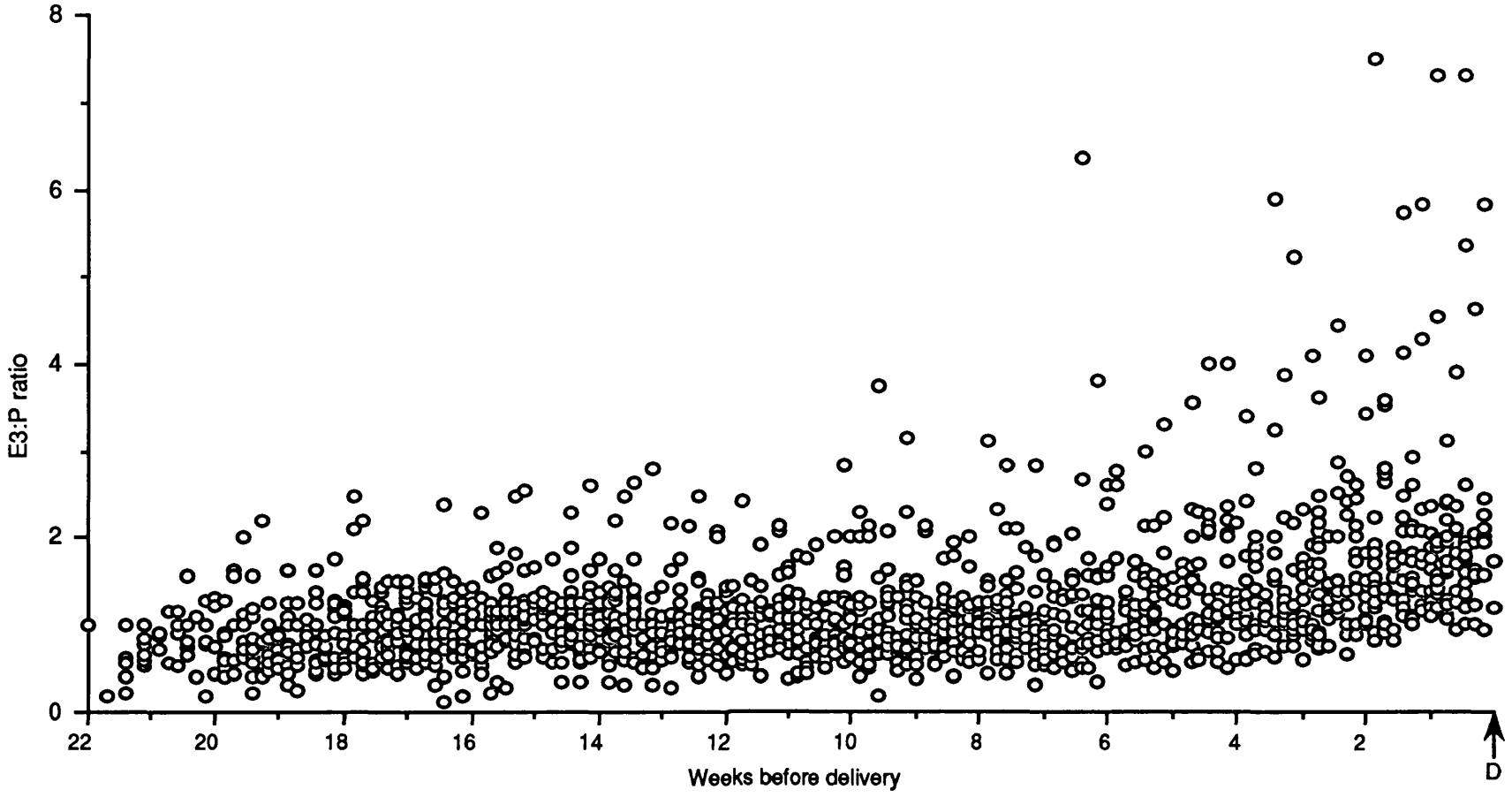


Fig. 6.7 Saliva E3:P ratios in 28 normal women during the last 22 weeks of pregnancy.

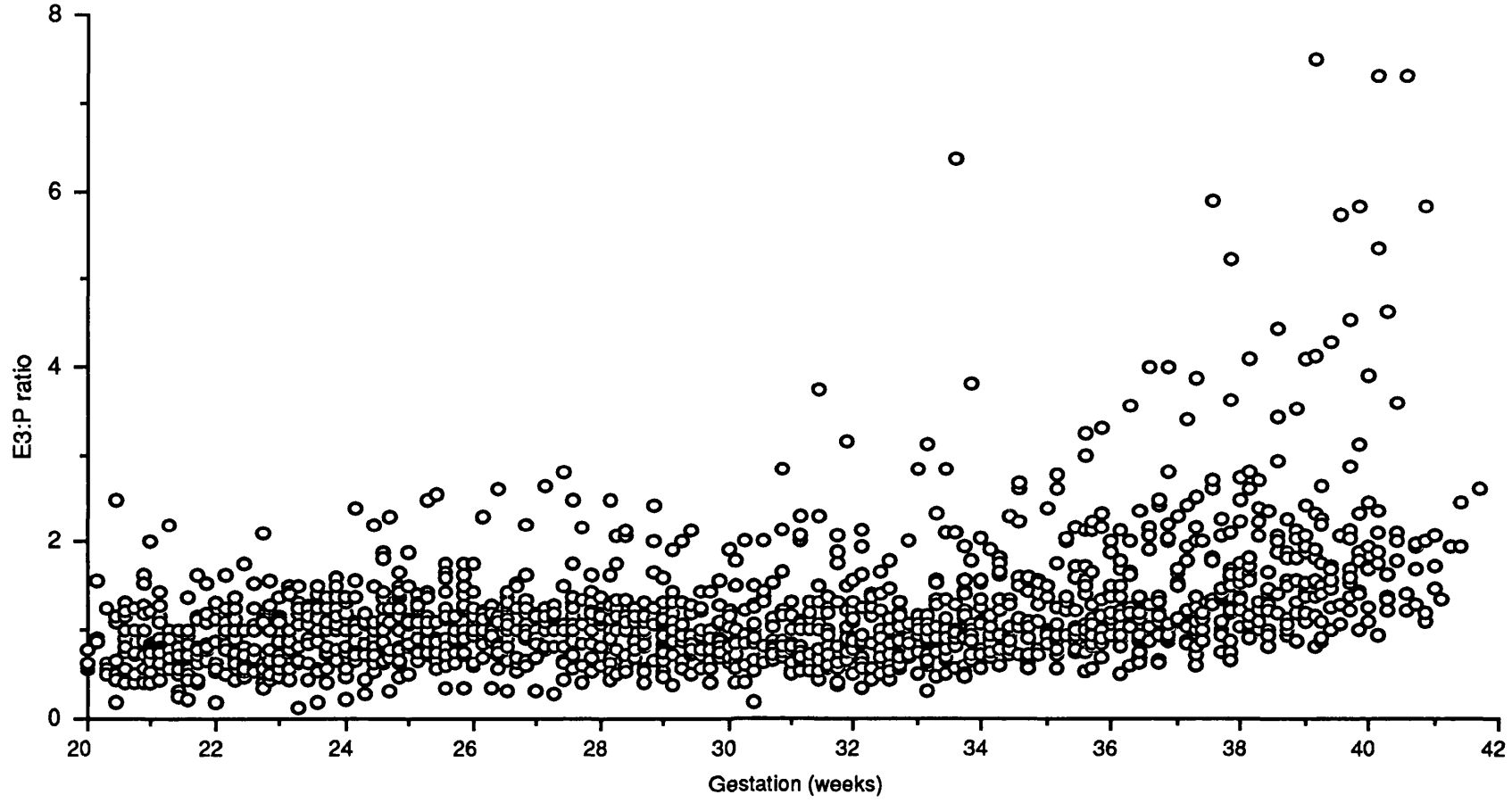


Fig. 6.8 Saliva E3:P ratios in 28 normal women from 20 weeks gestation onwards.

Fig. 6.9 Median saliva E3:P ratios with 5,10,90 and 95 centiles in 25 normal women during the last 18 weeks of pregnancy.

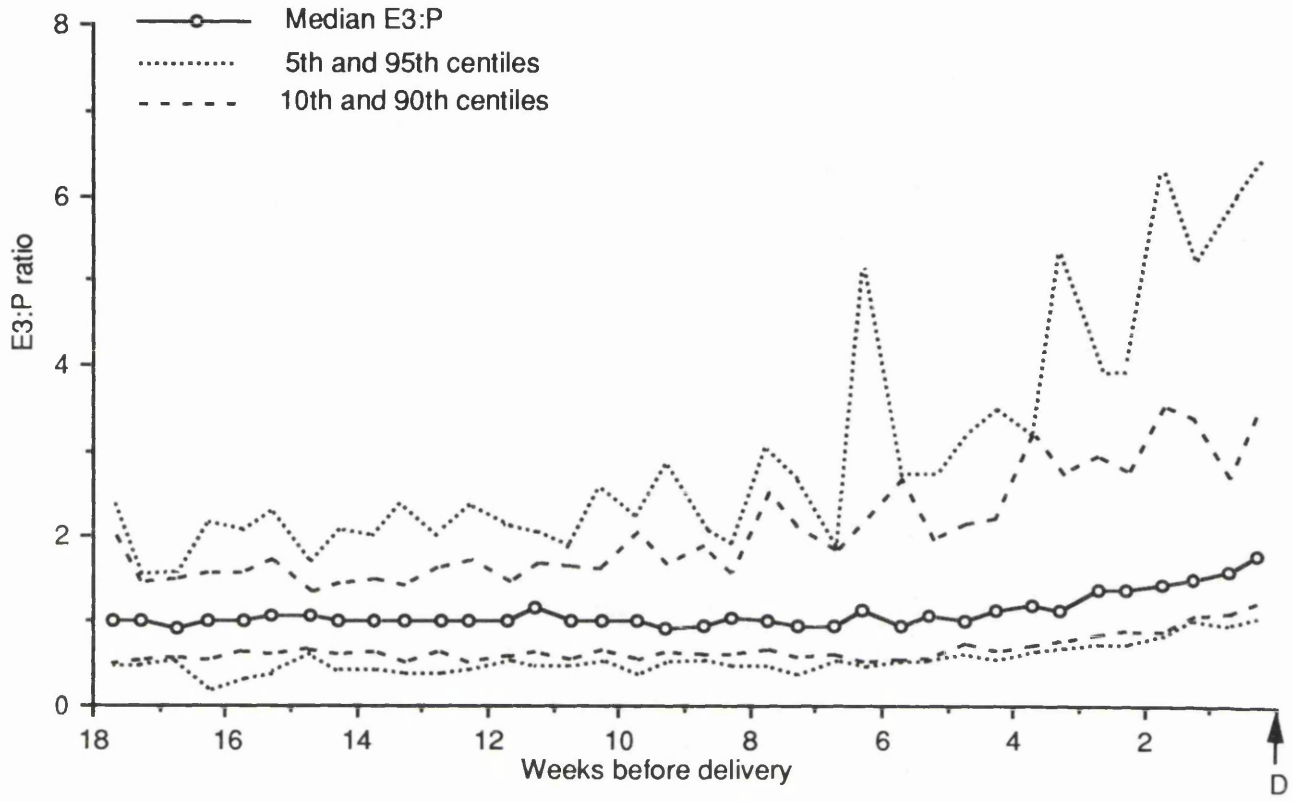
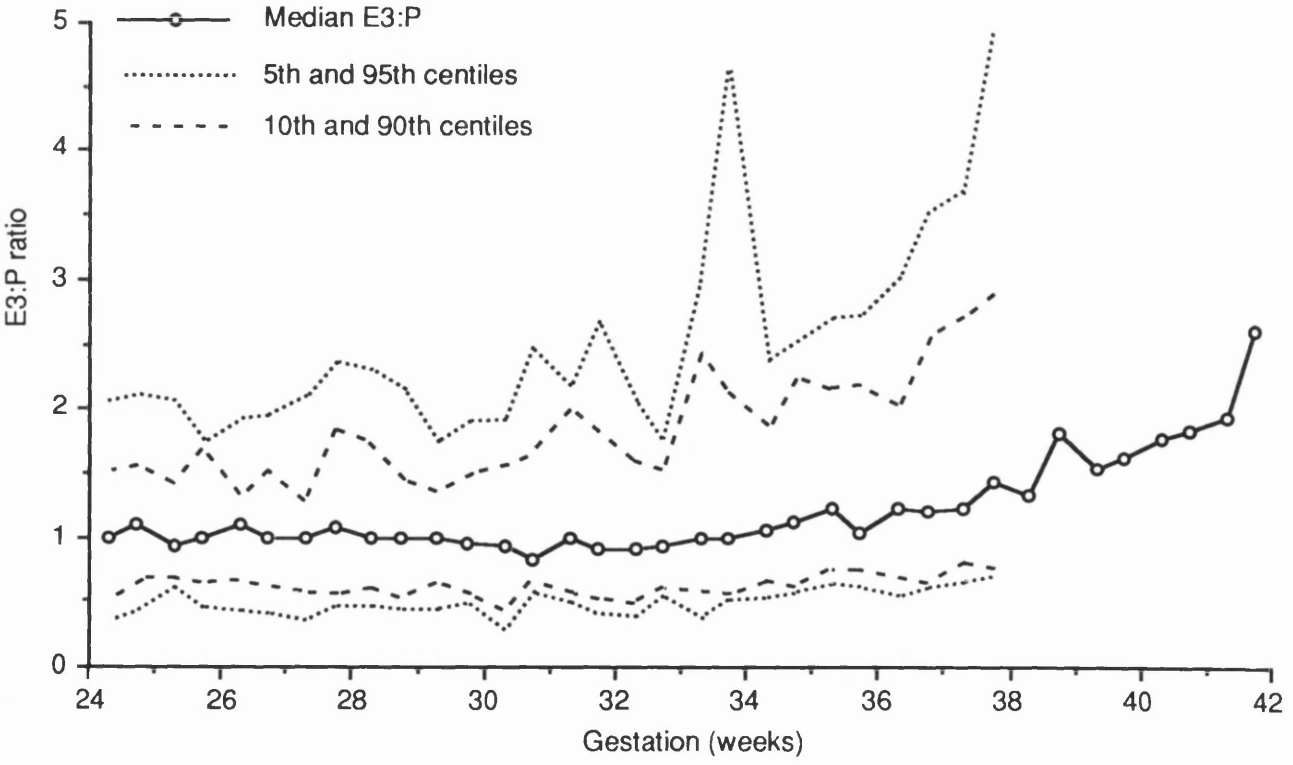


Fig. 6.10 Median saliva E3:P ratios with 5,10,90 and 95th centiles in normal women from 24 weeks gestation onwards (n=27 until 38 weeks gestation, and decreases as in Fig. 6.6 thereafter).



The pattern described was seen in 19 out of 28 (67.9%) of the women studied, with the increase in ratio occurring between 1 and 6 weeks before delivery. Three of the 9 remaining women (10.7% of the women studied) showed an increasing E3:P ratio but in 2 of these cases the E3 started at a lower level compared to the P and therefore only reached unity by the weeks immediately preceding delivery, and in the third woman the E3 was higher than the P throughout pregnancy. Six women (21.4% of the women studied) did not show an increasing E3:P ratio with increasing gestation.

Spontaneous Preterm Labour

Samples from 31 women in spontaneous preterm labour were assayed. The mean E3, P and E3:P ratio in the last 0-5 days prior to delivery were calculated for each woman (Table 6.5). The results were then plotted in relation to the median E3:P ratios for normal women (Figs. 6.11 and 6.12). These women were considered in two groups, depending on whether they had had an idiopathic preterm labour with intact membranes, or prolonged rupture of membranes prior to the onset of labour. The clinical details of the two groups were comparable (Table 6.4).

When plotted by gestation, 8 out of the 17 women (47%) who went into idiopathic preterm labour with intact membranes, had an E3:P ratio which was on or above the 90th centile for the gestation. 6 out of the 17 women (35.3%) had an E3:P ratio above the 95th centile. 16 out of 17 women (94.1%) had a ratio above the median for the gestation. When plotted by weeks prior to delivery, 7 out of 17 women (41.2%) had E3:P above the 95th centile, and all 17 women were on or above the median for the gestation.

Table 6.5 Preterm labour subjects (1-31) mean saliva E3 (nmol/L), mean saliva P (nmol/L), and mean saliva E3:P ratio in the last 0-5 days prior to delivery, together with the length of time that the membranes were ruptured and the gestation (weeks + days) at delivery. [* indicates the women who provided serial collections]

Subject	No. of samples	E3	P	E3:P	Hours of ROM	Gestation
1*	2	6.29	2.08	3.02	104	36+0
2*	1	2.25	1.43	1.57		35+0
3*	2	4.63	3.82	1.21		36+2
4*	3	4.70	1.10	4.27		35+6
5*	2	1.70	1.45	1.17		31+2
6*	1	3.68	1.73	2.13		36+0
7*	4	4.70	2.80	1.68		35+1
8*	2	0.80	2.48	0.32	>72	33+0
9	2	3.70	0.93	3.98		31+0
10	1	3.29	2.38	1.38	48	35+3
11	3	1.22	1.60	0.76	79	29+2
12	3	1.17	2.57	0.45	426	34+1
13	1	1.14	1.03	1.11	108	25+5
14	1	1.71	2.25	0.76	696	30+4
15	1	1.23	0.64	1.92		25+2
16	5	1.27	1.50	0.85	576	29+2
17	1	5.26	1.44	3.65		35+5
18	4	1.90	0.47	4.04	188	26+1
19	1	1.98	0.34	5.82		35+4
20	1	11.12	3.91	2.84	61	33+0
21	1	1.00	1.12	0.89	37	34+1
22	2	4.95	1.31	3.78		34+3
23	2	2.26	1.43	1.58	62	34+0
24	1	2.60	2.10	1.24		26+2
25	1	3.50	1.90	1.84		35+0
26	1	6.50	3.30	1.97		35+1
27	1	2.90	1.60	1.81		34+3
28	1	2.70	1.00	2.70		27+4
29	3	4.63	2.50	1.85	504	36+1
30	4	0.84	1.22	0.69	134	31+1
31	1	3.14	1.83	1.72		35+6

Fig. 6.11 Median saliva E3:P ratios with 5th, 10th, 90th and 95th centiles in normal women from 24 weeks gestation onwards, together with the mean E3:P ratios in the 0-5 days prior to preterm delivery in 17 women with idiopathic preterm labour (solid triangles), and 14 women with prolonged rupture of the membranes (PROM) prior to the spontaneous onset of labour (open triangles).

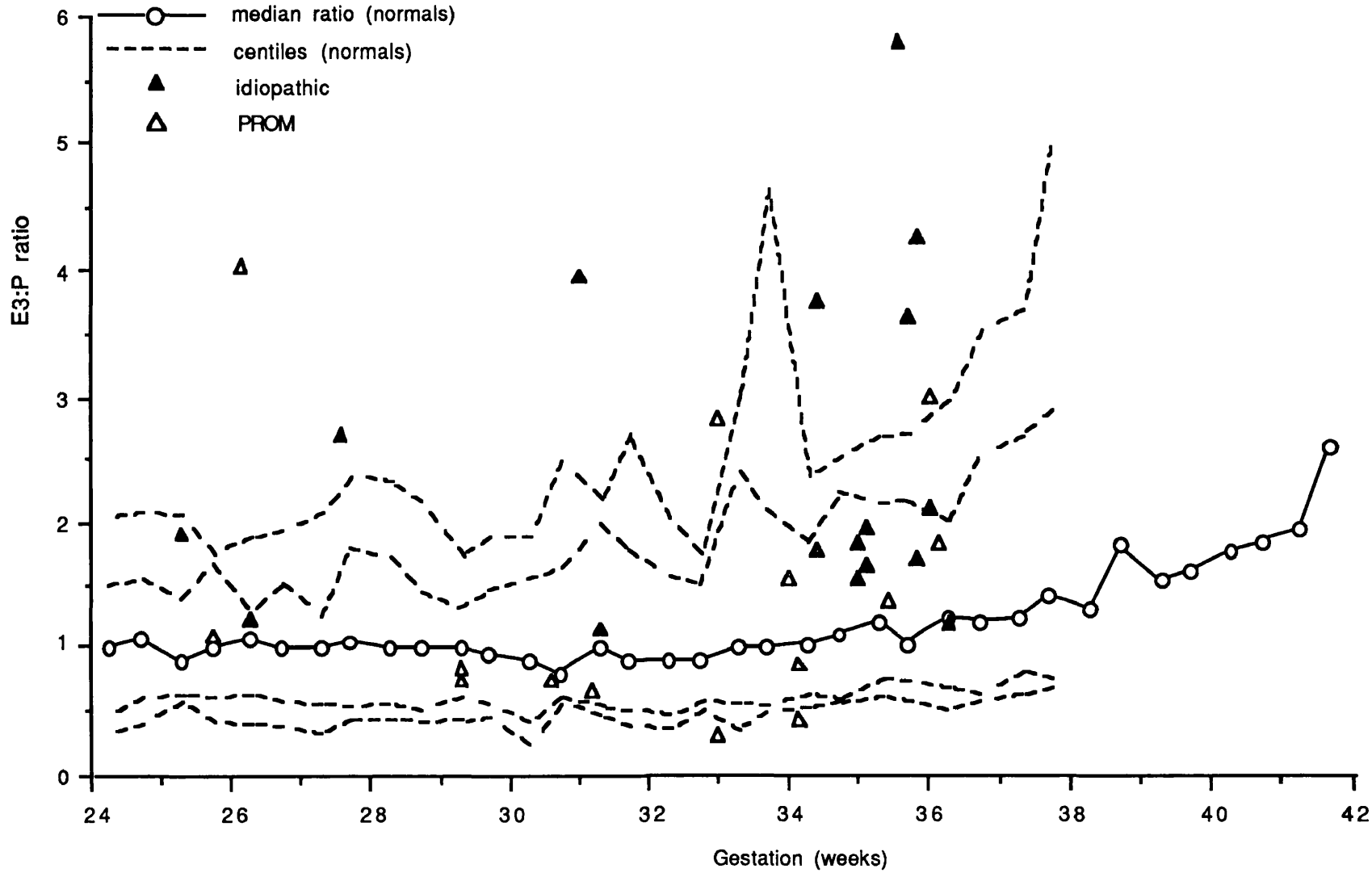
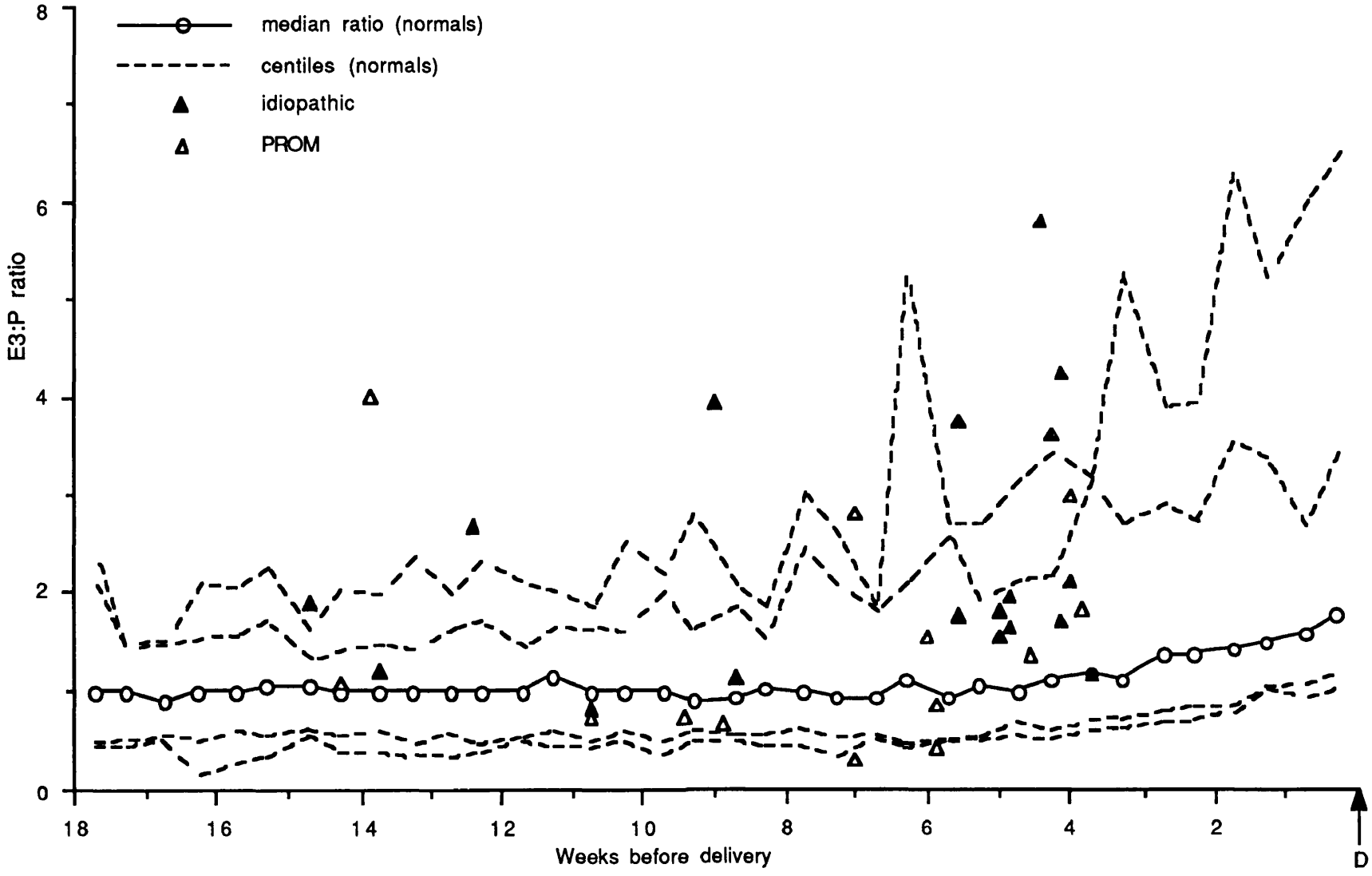


Fig. 6.12 Median saliva E3:P ratios with 5th, 10th, 90th and 95th centiles in normal women during the last 18 weeks of pregnancy, together with the mean E3:P ratios in the 0-5 days prior to preterm delivery in 17 women with idiopathic preterm labour (solid triangles), and 14 women with prolonged rupture of the membranes (PROM) prior to the spontaneous onset of labour (open triangles).



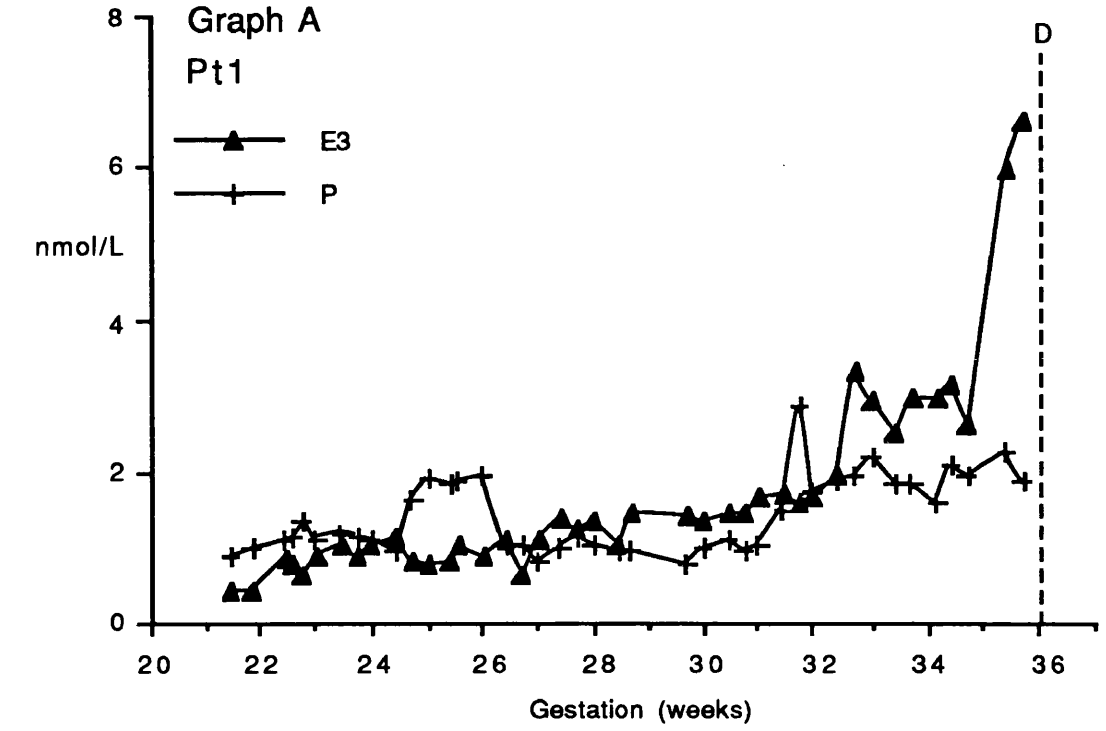
The 14 women with preterm labour following prolonged rupture of the membranes had ratios which were distributed evenly about the median whether plotted by gestation or weeks to delivery, 7 being above and 7 below the median. Three women had levels above the 95th centile for the gestation and 2 women had levels below the 5th centile for the gestation.

Serial collections from women who laboured preterm

Serial saliva collections were received from 8 women, 2 of whom had had prolonged rupture of the membranes prior to delivery (patients 1 and 8). The levels of saliva E3, P and their respective ratios are shown in Figs. 6.13 - 6.20.

Although all the women in idiopathic preterm labour with intact membranes had mean ratios (for the last 0-5 days prior to delivery) which were on or above the median for the gestation, only 2 of the 6 women (patients 4 and 6) had mean ratios on or above the 90th centile for the gestation. Patient 4 had an uneventful pregnancy and was asymptomatic until the onset of labour in spite of a consistently raised E3:P ratio throughout her pregnancy. A rise in E3:P ratio was present in 3 out of the 6 women (patients 2,6 and 7). No change in the ratio prior to delivery occurred in patients 3 and 4, although there was a rise in E3 terminally in both women. Patient 5 showed neither a surge in oestriol levels nor an acute change in the E3:P ratio, which simply showed a gradual, gentle rise with gestation. When there was a rise in the E3:P ratio it tended to occur about 1-2 weeks before the onset of labour.

Fig. 6.13 A) Saliva E3 and P levels in a woman during pregnancy prior to preterm labour and delivery (D) at 36 weeks gestation, following prolonged rupture of the membranes. B) Saliva E3:P ratio in the same woman during the weeks prior to delivery (D).



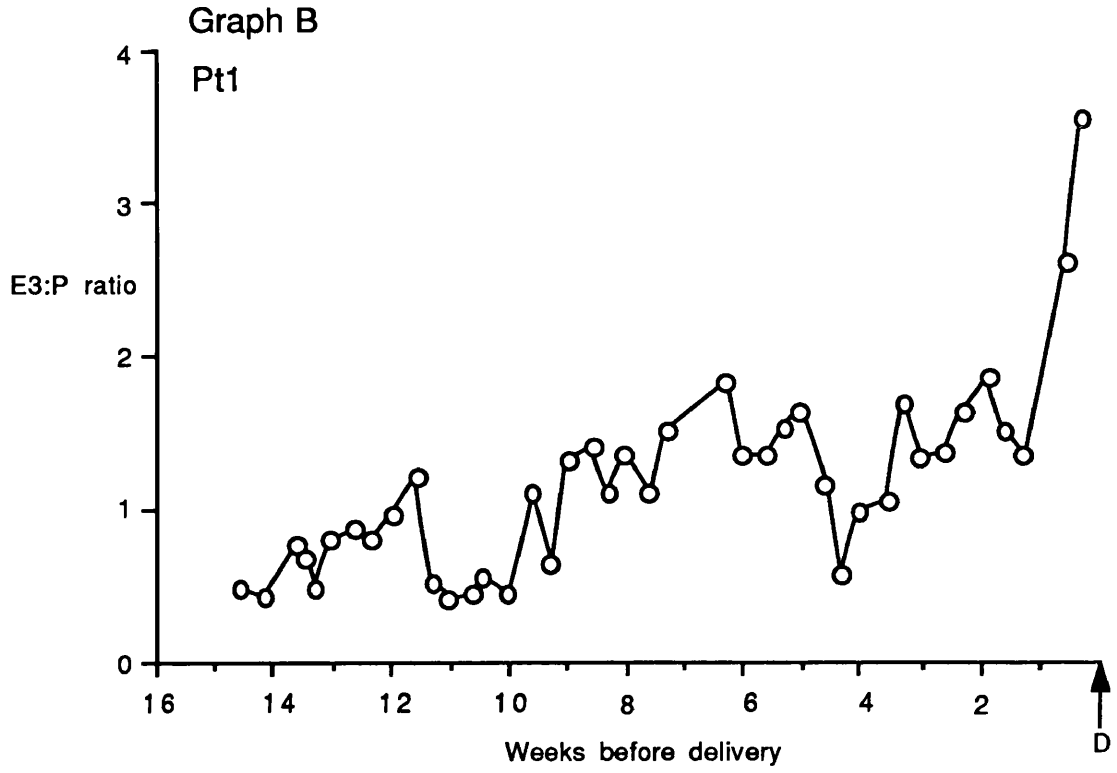
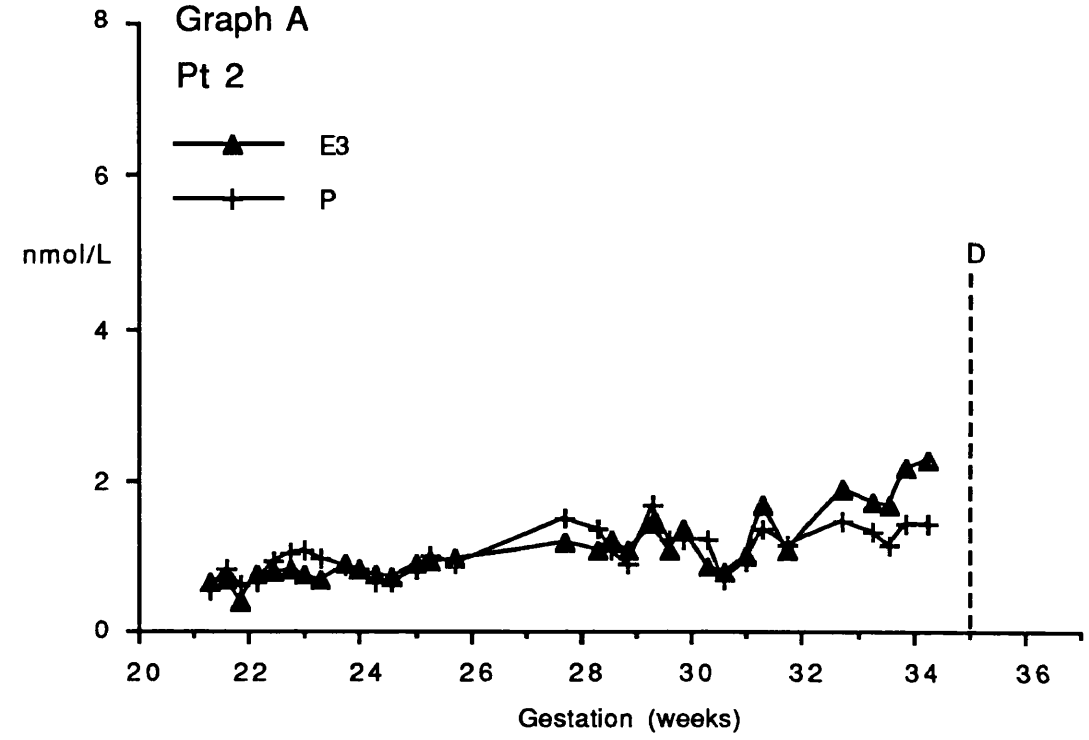


Fig. 6.14 A) Saliva E3 and P levels in a woman during pregnancy prior to idiopathic preterm labour and delivery (D) at 35 weeks gestation. B) Saliva E3:P ratio in the same woman during the weeks prior to delivery (D).



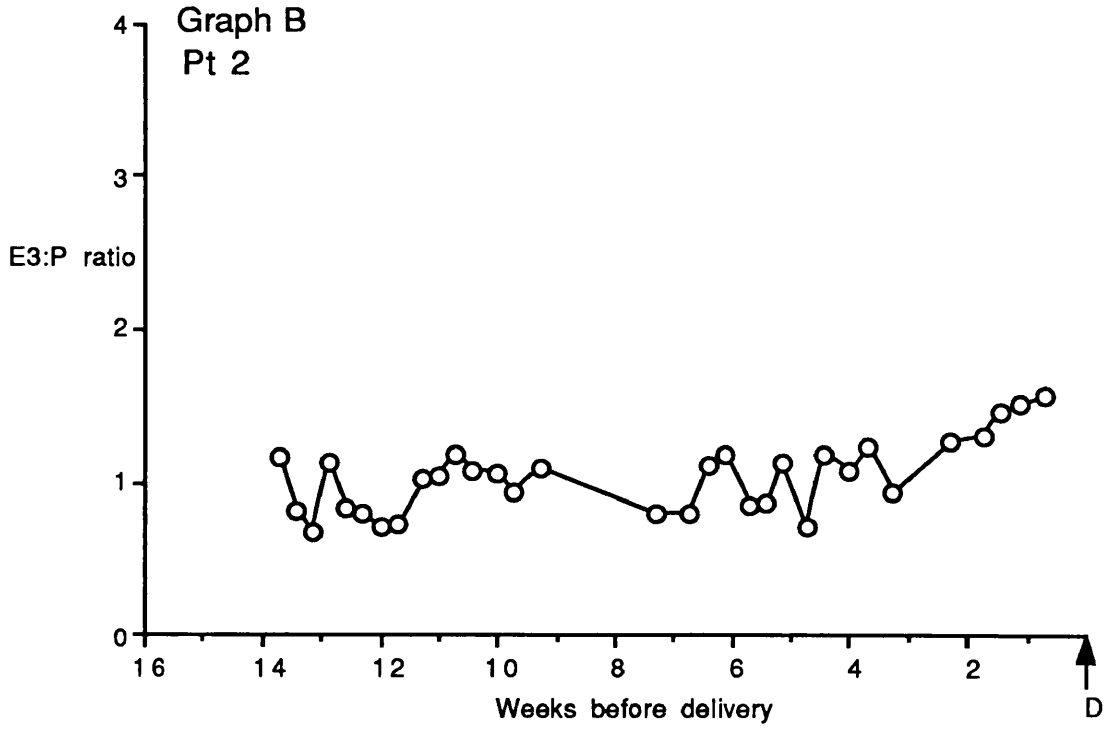
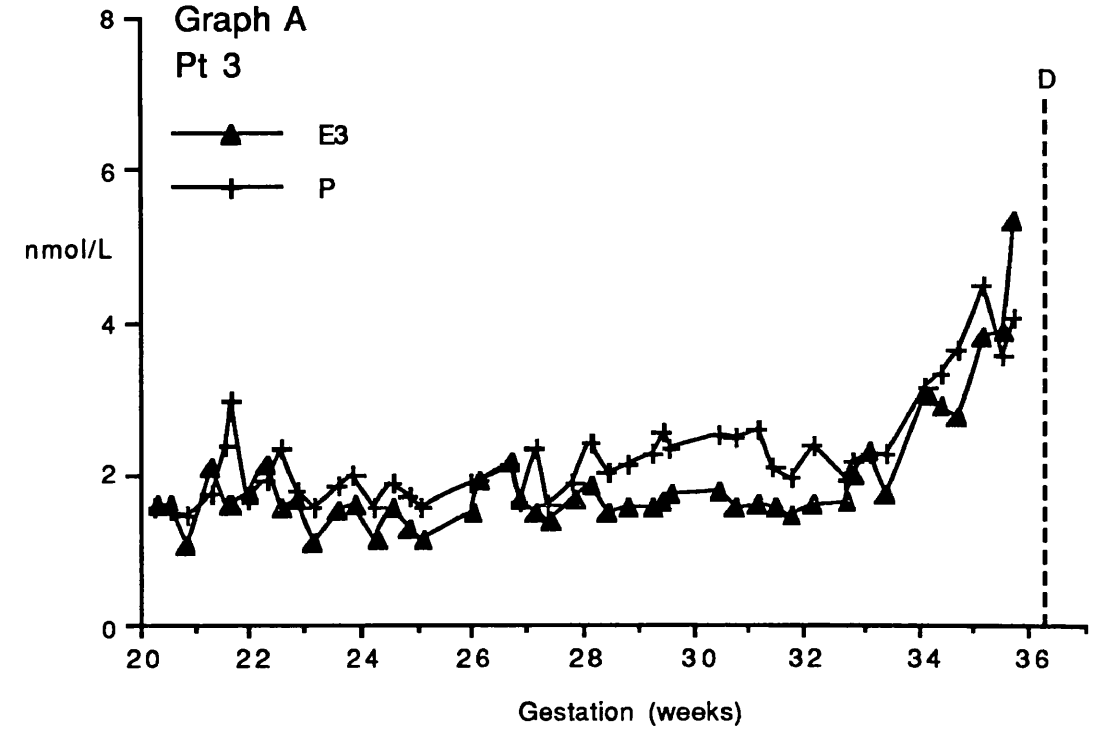


Fig. 6.15 A) Saliva E3 and P levels in a woman during pregnancy prior to idiopathic preterm labour and delivery (D) at 36 weeks and 2 days gestation. B) Saliva E3:P ratio in the same woman during the weeks prior to delivery (D).



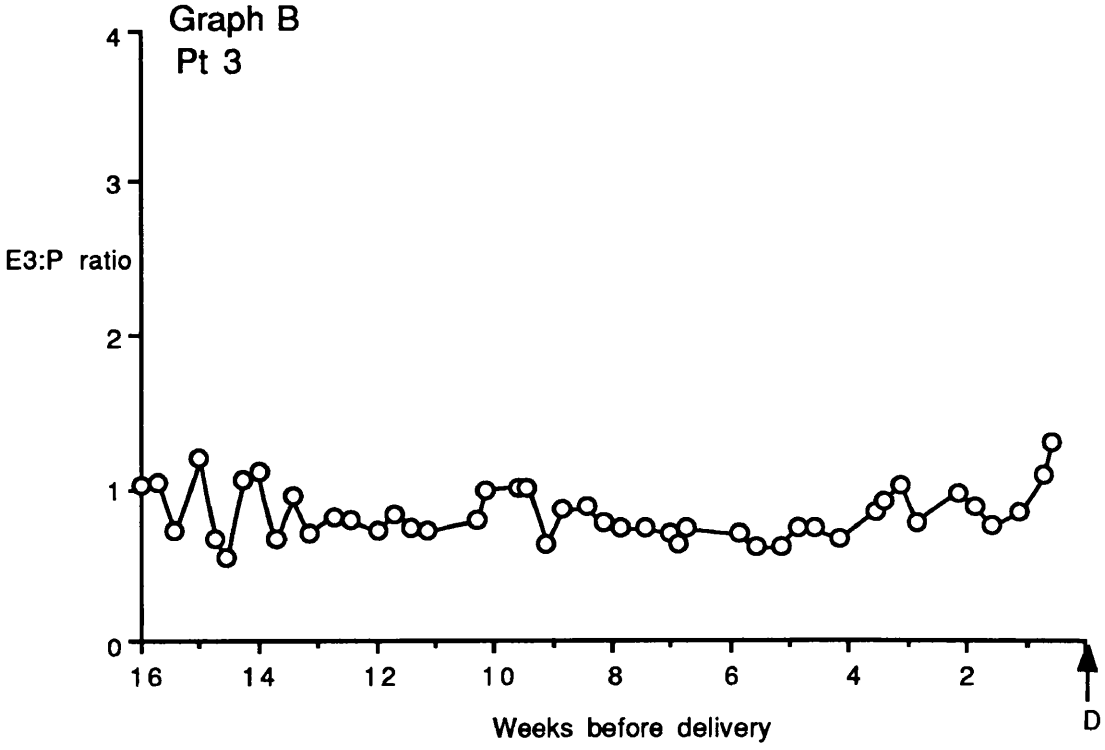
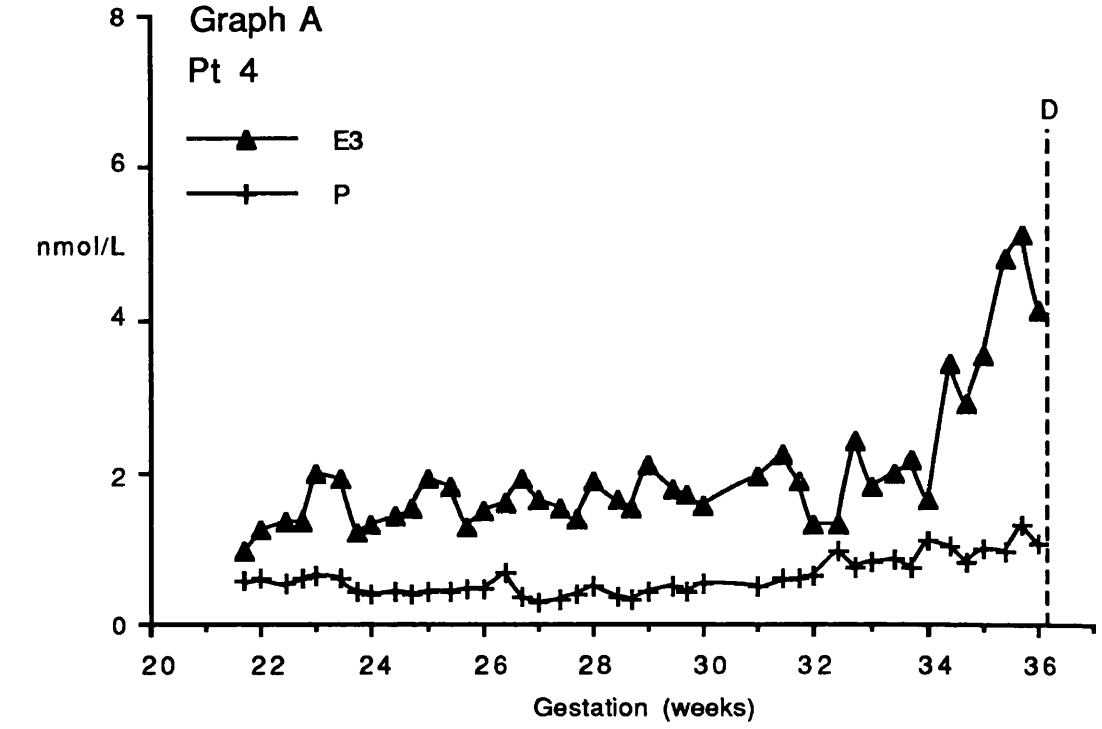


Fig. 6.16 A) Saliva E3 and P levels in a woman during pregnancy prior to idiopathic preterm labour and delivery (D) at 36 weeks and 1 day gestation. B) Saliva E3:P ratio in the same woman during the weeks prior to delivery (D).



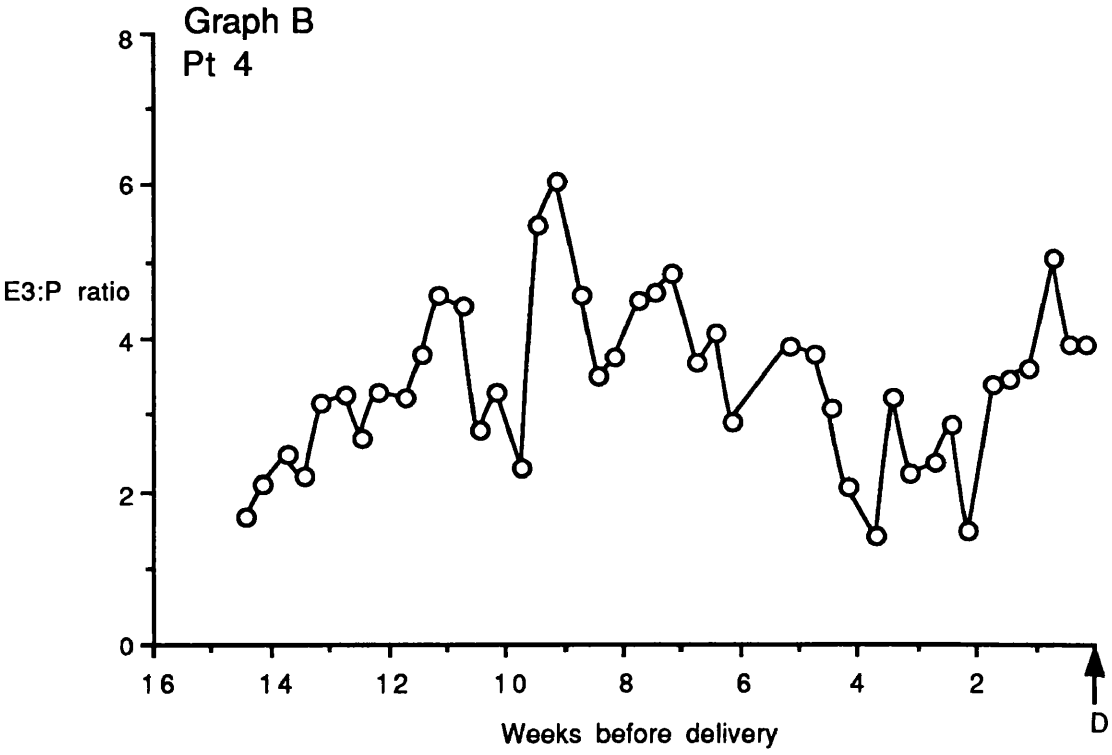


Fig. 6.17 A) Saliva E3 and P levels in a woman during pregnancy prior to idiopathic preterm labour and delivery (D) at 31 weeks and 2 days gestation. B) Saliva E3:P ratio in the same woman during the weeks prior to delivery (D).

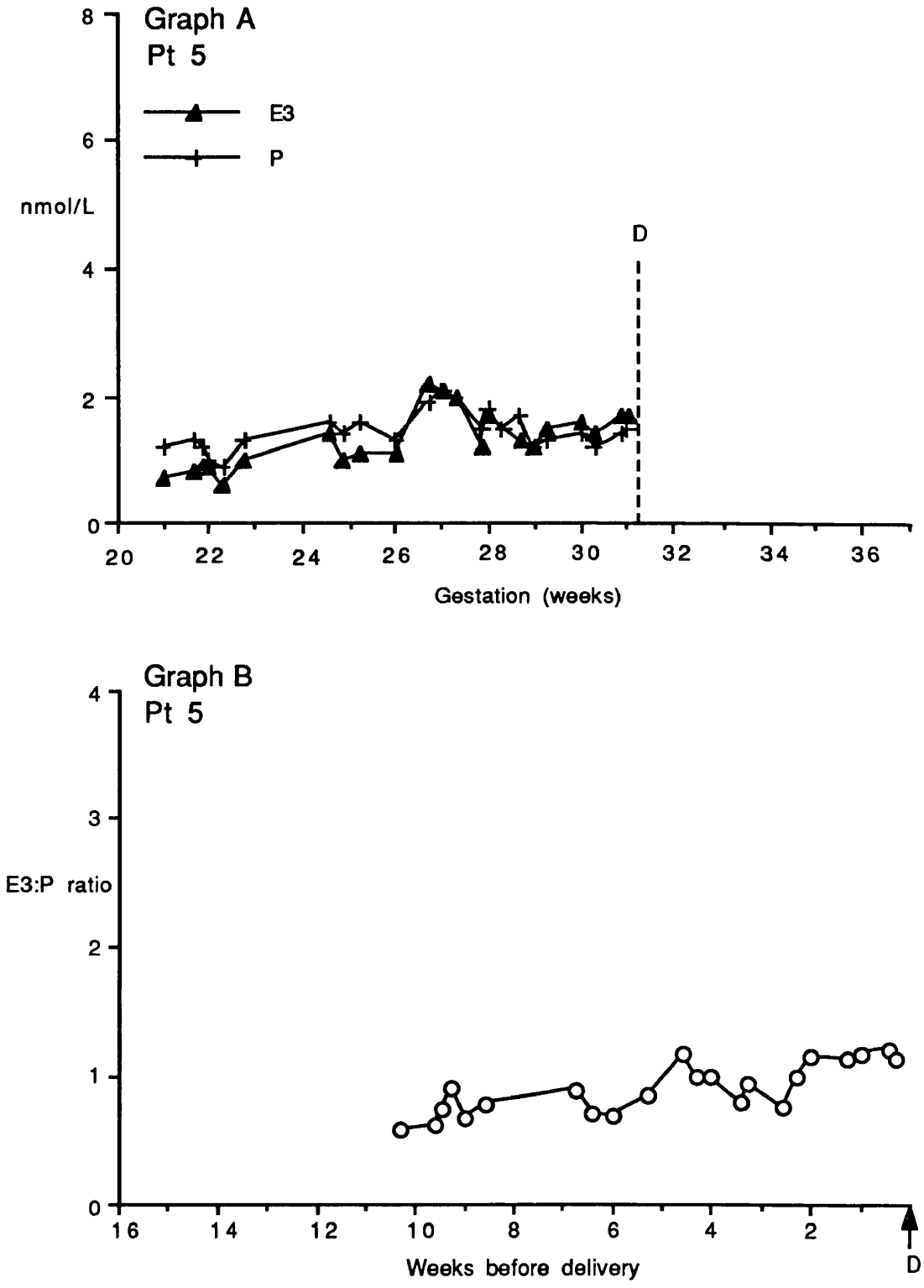


Fig. 6.18 A) Saliva E3 and P levels in a woman during pregnancy prior to idiopathic preterm labour and delivery (D) at 36 weeks gestation. B) Saliva E3:P ratio in the same woman during the weeks prior to delivery (D).

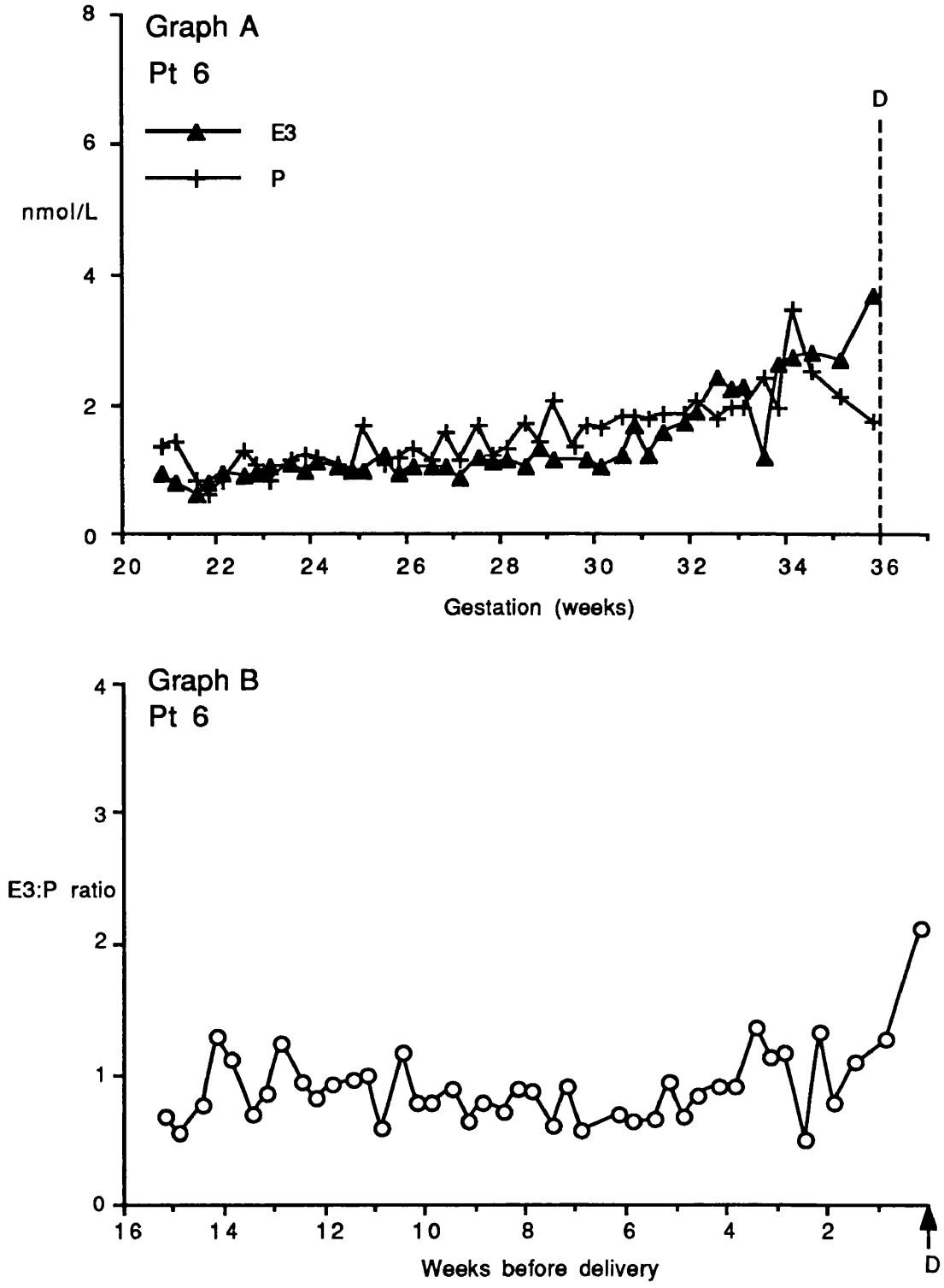


Fig. 6.19 A) Saliva E3 and P levels in a woman during pregnancy prior to idiopathic preterm labour and delivery (D) at 35 weeks and 1 day gestation. B) Saliva E3:P ratio in the same woman during the weeks prior to delivery (D).

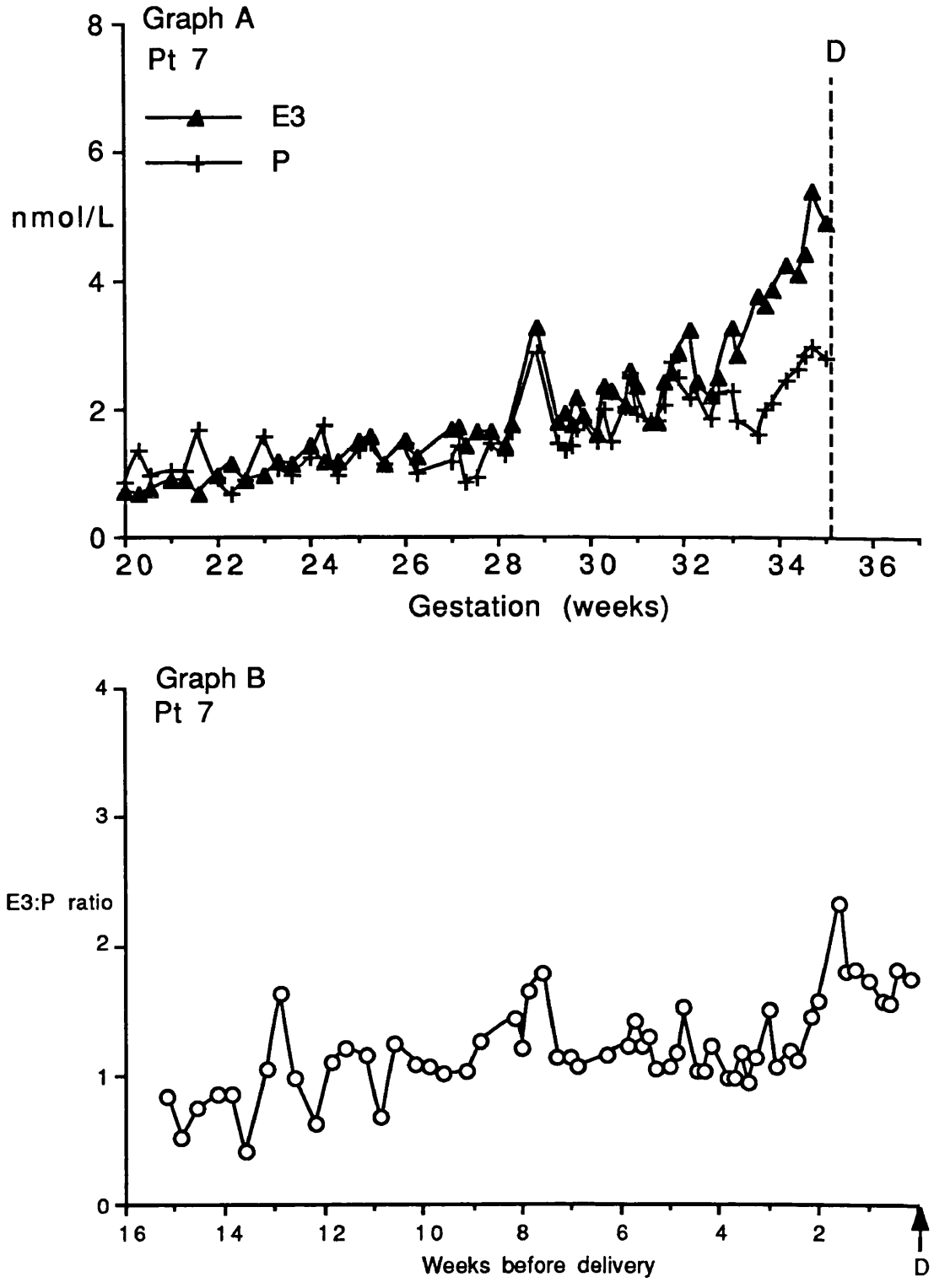
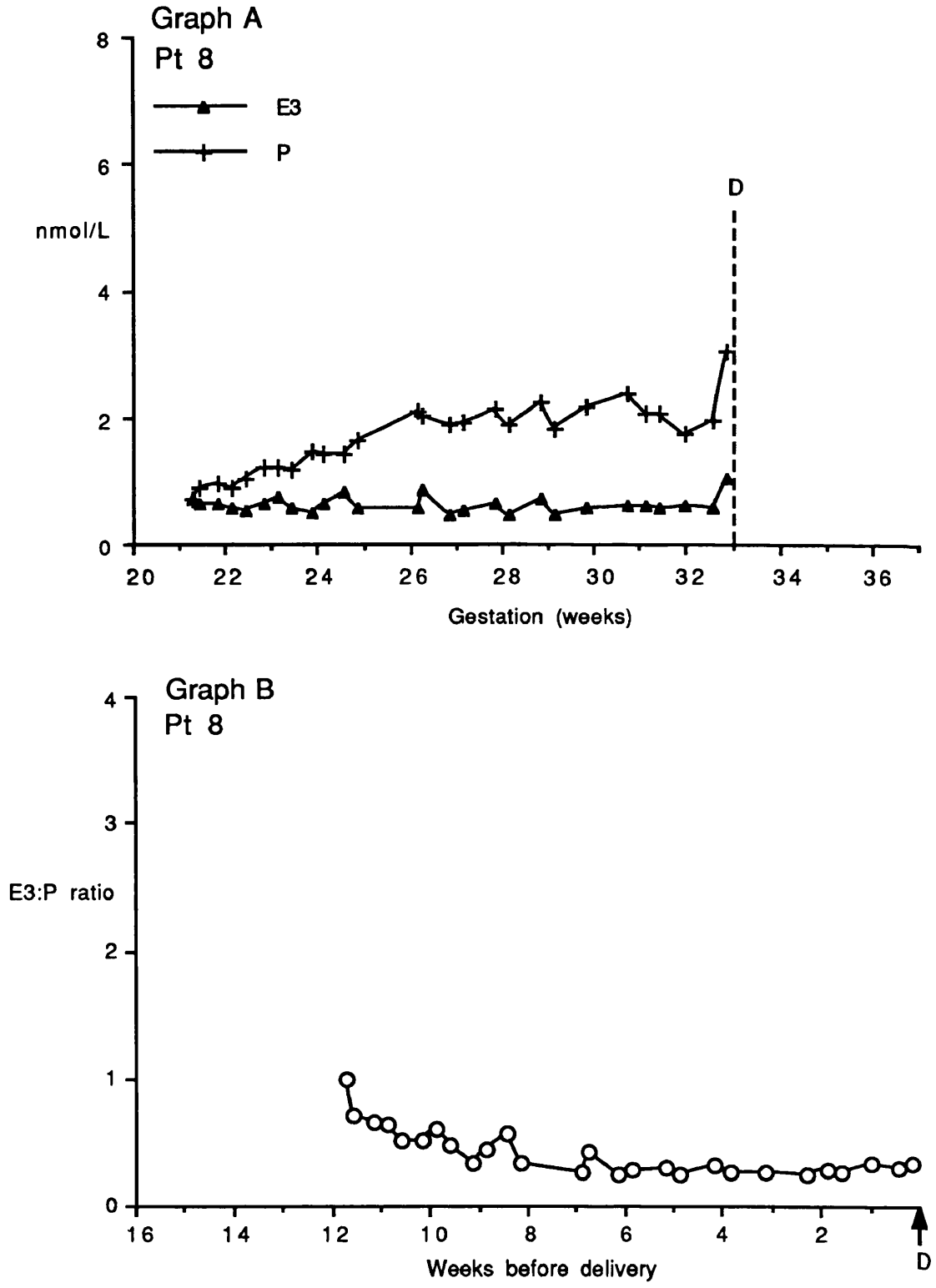


Fig. 6.20 A) Saliva E3 and P levels in a woman during pregnancy prior to preterm labour and delivery (D) at 33 weeks gestation, following prolonged rupture of the membranes. B) Saliva E3:P ratio in the same woman during the weeks prior to delivery (D).



Of the 2 women with preterm labour following prolonged rupture of membranes, patient 1 showed a rise in the E3:P ratio above the 90th centile occurring about one week prior to delivery, whereas patient 8 had a ratio which was below the 5th centile at the onset of labour.

Discussion

Spontaneous labour at term

The levels of saliva P in this study mimicked the known plasma P pattern in pregnancy *(Tulchinsky et al, 1972)*, and were in agreement with the saliva levels in the few previous studies performed in the second or third trimesters of pregnancy *(Connor et al,1982; McGarrigle and Lachelin, 1984; Lewis et al, 1987; Darné et al, 1987)*. Similarly, the E3 levels obtained were also in accordance with previous plasma *(Tulchinsky et al, 1972; Buster et al, 1976)* and saliva results *(Robinson et al, 1981; Vining et al, 1983; Evans et al, 1984; Truran et al, 1984; Lachelin and McGarrigle, 1984; Darné et al, 1987)*. A rise in the E3:P ratio before the spontaneous onset of labour at term occurred in the majority (≈68%), although not all, of the women studied.

That a rise in free oestrogen should precede the onset of labour accords with the known effects of oestrogen on myometrial activity. Rising P concentrations are thought to inhibit and rising oestrogen concentrations to enhance the synthesis of prostaglandins in uterine tissue *(Csapo,1977)*, the formation of gap junctions in myometrium *(Garfield et al, 1980)*, and the formation of oxytocin receptors and adrenoceptors in the myometrium *(Alexandrova and Soloff, 1980; Roberts et al, 1981)*. However, most previous studies have focused on measurements of E2 in relation to P in pregnancy

and labour, as E3 had been thought to be a weak oestrogen. It is now apparent that E3 may be as active as E2 in specific target tissues. For example, E3 is equipotent to E2 in its oestrogenic effects when administered to rats continuously rather than as a single injection *(Anderson et al, 1975)*, and binds to myometrial oestrogen receptors *(Bergink, 1980; Wiegerinck et al, 1983)*, and is as effective as E2 in stimulating production of prostaglandin $F_{2\alpha}$ by human endometrial cells in culture *(Schatz et al, 1984)*. Katzenellenbogen *(1984)* found that E3 is effective, although slightly less potent than E2, in stimulating production of P receptor synthesis by human endometrial cells in culture.

Darné et al *(1987)* found that all the patients they studied had a rising E3:P ratio prior to the advent of labour, whilst in our study although the majority of women showed this pattern, there were exceptions. The saliva P and E3 levels tended to be closely related until the E3 surge, and the relationship between the hormones seemed to be more important than the absolute levels in an individual woman. This close relationship suggested the possibility of a common controlling mechanism or the control of one hormone level by the other, and deserved further investigation (Chapter 9).

The surge in E3 levels prior to the onset of labour seems likely to be due to increased fetal adrenal activity, the trigger to which is not yet known. Evidence to support increased fetal adrenal activity prior to the spontaneous onset of labour is that increased adrenal weights were noted in babies delivered preterm following idiopathic preterm labour compared to babies delivered preterm following antepartum haemorrhage *(Anderson et al, 1971)*. Certainly, in the present study, all of the women who went into idiopathic preterm labour with intact membranes had saliva E3 levels which were above the median for the gestation, supporting the idea of increased

fetal adrenal activity. The relationship between fetal adrenal size as measured by ultrasound examination and saliva E3 levels is investigated in Chapter 6.

Amongst the 28 normal women, there were 9 women who did not have an E3:P ratio rising above unity prior to delivery, although 3 of these women did show an increasing E3:P ratio. One possible explanation for the women who did have rising E3 levels is that the sensitivity of these women to the hormonal environment was in some way different, possibly as a result of different receptor levels, or because of the involvement of other factors.

It has been shown that in women in preterm labour with intact membranes, 25% had microorganisms present in the amniotic fluid, and 75% of the infections were subclinical *(Bobitt et al, 1981)*. The incidence of preterm labour in women with intact membranes was increased from 5.4% to 11.9% if histopathologically confirmed chorioamnionitis, which was usually subclinical, was present *(Guzick and Winn, 1985)*. Women in whom Chlamydia trachomatis was detected by screening before 19 weeks gestation, had a significantly shorter gestation prior to delivery (35.9 weeks compared to 39.4 weeks in uninfected women) *(Martin et al, 1982)*. All of these studies suggest that in a proportion of women going into labour either at term or preterm, subclinical infection may play a role in precipitating the onset of labour.

Bejar et al *(1981)* showed that several organisms possess greater phospholipase activity than that of the membranes, and hypothesized that infection of the extraplacental membranes by these organisms might initiate the cascade causing release of free arachidonic acid and a marked increase in synthesis and release of PGE_2, which would thereby initiate labour.

Lamont et al *(1985)* provided evidence to support this theory by showing that amnion cells (*in vitro*) exposed to the products released by intact bacteria or from disintegrated bacteria resulted in up to a six-fold increase in PGE_2 output. Thus, bacterial infection may bypass or be synergistic with the increase in the oestrogen to progesterone ratio which normally occurs prior to the onset of labour.

Spontaneous preterm labour.

All of the women who went into spontaneous idiopathic preterm labour with intact membranes had E3:P ratios above the 50th centile for their gestation, and 47% had levels above the 90th centile for their gestation. Thus, the finding of Darné et al *(1987)* that the rise in saliva E3:P ratios, which occurs in normal women near term, occurs inappropriately early in many women who labour preterm, is confirmed. However, the proportion of women in which the ratio was above the 90th centile was considerably smaller in the present study (47% versus 100%). An innappropriately raised E3:P ratio for the gestation in some women who go into spontaneous preterm labour would accord with previous studies, which have shown that women who labour spontaneously preterm have significantly more gap junctions than women delivered by cesarean section either preterm or at term *(Garfield and Hayashi, 1981)*, and they also have increased numbers of oxytocin receptors *(Fuchs et al, 1984)*.

The women who went into spontaneous preterm labour following prolonged rupture of the membranes had ratios which were spread evenly about the median. This adds credence to the idea that the aetiology of preterm labour in women with intact membranes and those with PROM may be different, although it seems likely that there is some degree of overlap with the presence of occult infection.

Saliva E3:P ratios as a predictive test?

The number of women who delivered preterm having provided an adequate serial saliva collection was disappointing, although predictable in retrospect. Women who deliver preterm are more likely to live in poor social circumstances, are often single parents, or very young, with a limited education. Their problems may be considerable and mean that they are less likely to attend properly for their routine antenatal care. Understandably, they are not easily motivated to take part in a study such as this one, which offered no benefit either to themselves or to the health of their baby.

When there was a rise in the saliva E3:P ratios in the women who delivered preterm, having collected samples serially, it occurred between 1-2 weeks prior to delivery. In spite of the small numbers obtained, this study has established that the saliva E3:P ratio is unlikely to be a definitive predictive test for preterm labour with membranes intact, as 9 out of 17 women had a ratio between the 50th and 90th centile for their gestation. However, it might be a useful test in women who have recurrent idiopathic preterm labours in order to assess whether they might benefit from progesterone treatment.

Possible criticisms of the study

Many of the women who delivered preterm with intact membranes prior to 32 weeks gestation were treated with either oral or more commonly intravenous ritodrine. The effect of intravenous ritodrine on plasma steroid hormones has been investigated by several groups. Ylikorkala et al *(1978)* found a significant decrease in plasma E3 and P levels, as well as a significant decrease in the P:E2 ratio compared to a control group. Hanssens et al *(1983)* found no change in plasma unconjugated P levels, a significant fall in total plasma E3 levels, (although the fall was within the

limits of normal variation), and a significant fall in unconjugated E2 levels. Schreyer et al *(1989)* found that ritodrine given intravenously, intramuscularly or orally resulted in a significant decrease in plasma unconjugated E2, E3 and P levels. However, no studies have been done to investigate the effect of ritodrine on 'free' or saliva steroid levels; if such an effect existed, it could clearly bias the preterm labour results.

Another possible criticism is that the women in our study collected their samples at any time of the day convenient to them, and did not necessarily collect their samples at the same time each day (although they were encouraged to do so). Whilst no diurnal variation has been demonstrated in saliva oestriol, it has been suggested that the mean level of the first morning sample is higher and the variance greater than in samples collected during the rest of the day *(Besch et al, 1982: Vining et al, 1983)*. The activity of the patient during the day may also have an effect.. oestriol levels being higher in recumbent patients *(Cusick et al, 1986)* and lower following food and drink *(Kirkish et al, 1986)*. None of these factors were controlled for in our study, and it would probably be true to say that the preterm labour women were more likely to be recumbent than the normal women. However, if this was a significant cause of bias in the study, both the idiopathic preterm group and the prolonged rupture of membranes group might have been expected to have raised E3:P ratios. It therefore seems unlikely that these factors influenced the results significantly.

Final Comment

This study showed an increasing saliva E3:P ratio in the majority of women prior to the onset of labour, and an inappropriately raised E3:P ratio for the gestation in some women prior to the spontaneous onset of preterm labour. These findings are in accordance with established knowledge about the

onset of labour. Whilst the results would not support the use of saliva E3:P ratios as a predictive screening tool for preterm labour, saliva E3:P ratios might provide additional useful information in women who have recurrent preterm labours, and who may possibly benefit from treatment with progesterone.

Another possibility arising from the study is that it may be possible to induce labour in women in a more gradual and physiological way by the administration of oestriol over the course of several days, thus raising the 'free' E3:P ratio and hopefully causing those changes which are necessary for the onset of labour to occur.

7

Serial Adrenal Ultrasonography in the Fetus and the Neonate, and the Relationship of Fetal Adrenal Size to Maternal Plasma and Saliva Oestriol and Progesterone Levels

Introduction

The adrenal glands were first described in 1563 by Bartholomaeus Eustachius *(Jones, 1957)*. In the late 1800's, the adult adrenal cortex was categorized, by Arnold (1866) and Gottschau (1883), into three histological zones according to the arrangement of the connective tissue stroma . Later, it was realized that there were structural differences between the fetal/neonatal adrenal and that of the adult. Starklowa and Wegrzynowski (1910) were the first to document the difference when they described, in the fetal adrenal, the presence of a special region of the cortex... the fetal cortex *(Sucheston and Cannon, 1968)*.

The fetal cortex develops from columnar mesothelial cells in the adrenal groove (just medial to the urogenital ridge) during the third week following fertilisation. The 'definitive' zone is formed by a further proliferation of mesothelial cells from the same area, some three weeks later. These latter cells come into direct contact with the ventral portions of the fetal cortex and multiply and spread along the surface of the gland *(Uotila, 1940)*. Cells of the fetal cortex are relatively large, acidophilic and contain abundant cytoplasm, whereas cells forming the 'definitive' cortex are smaller, basophilic with prominent nuclei and little cytoplasm, *(Sucheston and Cannon, 1968)*. The morphological characteristics of cells forming the 'definitive' zone remain relatively undifferentiated during the first trimester. In contrast, the cells of the fetal zone show electron microscopic findings characteristic of steroidogenic activity by the seventh week. The definitive

zone does not show similar signs of activity until the late second or early third trimester *(Johannison, 1968)*. The adrenal medulla is derived embryologically from sympathetic elements which invade the interior of the primitive adrenal cortex at around 7 weeks gestation *(Uotila, 1940)*.

At term, the fetal zone occupies some 80% of the adrenal cortex. However, it has been shown by postmortem histopathological studies to undergo marked involutional changes during the early weeks and months of extrauterine life *(Bech et al, 1969)*. During this time, involution of the fetal zone is accompanied by proliferation and development of the definitive zone. Adrenal weight decreases during the first 3 months of extrauterine life and then increases once more with further growth of the definitive zone *(Tähkä, 1951)*. Since such a large part of the fetal adrenal cortex persists only during the fetal period, it has been assumed for some time to have important specific functions during pregnancy.

In the last 25 years, various studies have suggested that fetal adrenal activity may be an important factor in relation to the spontaneous onset of labour. Anderson et al *(1969)* demonstrated that in anencephalic pregnancies without hydramnios, the longer the pregnancies were prolonged beyond term, the smaller were the fetal adrenals in size. Also, the amount of adrenal cortical tissue (in particular the fetal zone) that was present was proportionately less. In a later study, it was noted that the mean adrenal weight in infants who were born preterm without any apparent reason was higher than the mean fetal adrenal weight of those delivered preterm in association with antepartum haemorrhage *(Anderson et al, 1971)*. Furthermore, it is known that the steroidogenic activity of the fetal zone of the fetal adrenal cortex provides the precursors for placental production of oestriol, and that the capacity of placental enzymes is not considered to be

the rate limiting factor in sustaining the level of oestriol biosynthesis *(Oakey, 1970)*. The increase in adrenal weights in babies born preterm for no apparent reason might therefore have been coincident with a surge in oestriol production, such as that found in the majority of women prior to the spontaneous onset of labour at term; and also in some women prior to the onset of preterm labour (Chapter 6).

Ultrasound has been shown to provide a useful method of evaluating the size and appearance of the adrenal glands in both fetal life and in the neonate *(Lewis et al, 1982; Oppenheimer et al, 1983)*. Hata et al *(1988)* suggested that measurement of the fetal adrenal gland *in utero* might be a diagnostic tool in the management of high risk pregnancies, particularly those with intra-uterine growth retardation (IUGR). It has been shown to be helpful in the diagnosis of neonatal pathology such as adrenal hemorrhage *(Nordshus and Monn, 1980)* and hyperplasia *(Ghiacy et al, 1985)*.

Most of the studies described so far have involved single ultrasound measurements of the adrenal glands at different stages of gestation or in babies of different ages. Only one group *(Hata et al, 1988)* has presented any sequential study results; and even then, only one adrenal parameter was measured in the neonate. Few studies have been reported which assess the relationship between hormonal measurements during pregnancy and adrenal ultrasound measurements *(Hata et al, 1987; Matsumura et al, 1987; Hauffa et al, 1988)*. No studies have been reported involving maternal salivary hormone measurements in relation to fetal adrenal size.

The study was divided into three parts:

A) Serial fetal adrenal ultrasonography in the second and third trimesters of pregnancy - relation to maternal oestriol and progesterone levels in plasma and saliva

Serial ultrasonographic assessments of the size and appearance of the adrenal glands were performed from 24 weeks gestation until term, and simultaneous maternal plasma and saliva samples were obtained at each ultrasound visit in order to measure oestriol and progesterone levels. The aim was to document more thoroughly the changes in fetal adrenal size and appearance throughout the second and third trimesters of pregnancy, and to assess whether there was any relationship between fetal adrenal size and maternal plasma and saliva hormone levels. A relationship between adrenal size and oestriol would be interesting, and possibly even useful as a predictive test assuming a surge in oestriol production occurs in some women prior to the onset of labour and that a corresponding increase in fetal adrenal size could be detected.

B) Comparison of fetal adrenal size in term pregnancies with adrenal size in the one day old neonate

This section of the study was originally part of the pilot study, which was being carried out in order to decide on which days following birth to perform the serial scans in the neonates. However, as the comparison between late pregnancy and day 1 of neonatal life was interesting, these results have been included in the chapter. The babies in this part of the study also had a variable number of scans at a variety of different neonatal ages, the results of which are not included here.

C) Serial adrenal ultrasonography in the neonate

The aim of this part of the study was to evaluate changes in the size and appearance of the adrenal glands during the first 6 weeks of neonatal life in normal babies studied serially.

Materials and Methods

A) Serial fetal adrenal ultrasonography in the second and third trimesters of pregnancy - relation to maternal oestriol and progesterone levels in plasma and saliva

Women were recruited to the study from the antenatal clinic or from the ultrasound department following their routine anomaly scan at 19 weeks gestation. Women who were already known to have complicated pregnancies, or those who had previously had complicated pregnancies were usually excluded. Two women (subjects 8 and 24) were included in spite of problematic previous pregnancies, because they were to be scanned serially during the current pregnancy as part of their planned antenatal care.

Scans were organized every 4 weeks from 24 weeks gestation onwards. During each visit, paired plasma and saliva samples were collected simultaneously. Oestriol and progesterone levels were measured in all the samples obtained. Gestation was calculated using the patients dates unless the gestation by early ultrasound scan differed by 14 or more days.

45 women were recruited in total, and their individual details are shown in Table 7.1. All the women completed the study, but some of them had their scans and/or plasma and saliva samples either missing or wrongly timed. To calculate means, only those women who had a full series of scans and a complete collection of samples at within a week of 24, 28, 32, 36 and when possible 40 weeks gestation were included. 32 women could be included using these criteria, although 17 of the 32 women had delivered prior to 39-41 weeks and therefore the number of women in the calculated means for 273-287 days gestation was only 15.

All 45 women gave birth to healthy babies (31 males and 14 females) with a mean (± SD) birthweight of 3.47 (± 0.49) kg. The 32 women with complete data had babies (22 males and 10 females) with a mean (± SD) birthweight of 3.51 (± 0.44) kg.

The plasma and saliva samples were stored at -40°C prior to assay. All the samples were assayed in duplicate for oestriol and progesterone.

Ultrasound scans were performed using a real-time linear array scanner (Hitachi EUB240) with a 3.5 MHz transducer. The uterus was scanned to assess the lie of the fetus, and thus enable a longitudinal section of the fetal spine to be visualized. The transducer was rotated through 90° to obtain a transverse section of the fetal abdomen (showing fetal spine, stomach and umbilical vein and where possible the bifurcation of the vein); the adrenal could then be visualized by moving the transducer slightly cephalad. The measurements of the kidney in transverse section were obtained by moving the transducer slightly caudally *(Jeanty et al, 1984)*.

Table 7.1 Individual antenatal subjects' (S) details including parity, gestation (gest) at delivery (days), onset of labour spontaneous (S), spontaneous but augmented with syntocinon during the first or second stage (Sa), or induced (I), mode of delivery (NVD - normal vaginal delivery, (LS)CS - (lower segment) cesarian section, el - elective), sex of babies (M - male, F - female), weight of babies (in grams), and other pregnancy or delivery details (U/S - ultrasound, PIH - pregnancy induced hypertension, OP - occipito-posterior, SROM - spontaneous rupture of the membranes).

S	Parity	Gest	S/Sa/I	Delivery	Sex	Weight	Pregnancy and delivery details
1*	0+0	278	Sa	Forceps	F	3370	Elective forceps for maternal back problems
2	1+1	283	Sa	NVD	F	2970	
3*	0+0	279	Sa	NVD	F	3160	? decreased liquor on U/S at 36 weeks gestation
4*	0+0	292	I	NVD	M	2800	Induced because post term
5	0+0	282	Sa	NVD	F	3070	
6*	1+0	267	S	NVD	M	2940	
7	1+1	278	S	NVD	M	3520	
8*	1+3	283	S	NVD	M	3020	Cervical suture inserted at 14 weeks and removed at 37 weeks gestation
9*	0+1	275	S	NVD	M	3600	
10*	0+0	271	I	NVD	M	3900	Induced because had had SROM for 24 hours
11*	0+0	292	S	NVD	M	3940	Epileptic on carbamazepine 600mg orally daily
12*	1+0	286	S	NVD	M	3900	
13	1+0	264	S	NVD	M	2220	Uneventful pregnancy. Birthweight below 5th centile - Chinese origin
14*	1+0	283	Sa	LSCS	F	3640	Prolonged 2nd stage and failed Kiellands forceps delivery
15	1+2	285	S	NVD	M	4000	Mild PIH at term
16*	1+0	292	I	NVD	F	3980	Two admissions with hyperemesis gravidarum. Induced because post dates
17*	1+0	275	S	NVD	M	3120	
18*	0+0	282	S	NVD	F	3200	
19	0+0	285	Sa	NVD	M	4100	Bleeding ?vaginally at 32 weeks. Settled with no treatment. No obvious cause
20*	2+1	258	I	NVD	F	2820	Induced following SROM at term
21*	0+0	280	S	NVD	M	3400	
22	0+0	276	Sa	NVD	M	2740	
23*	1+0	296	S	NVD	M	3700	
24	1+0	246		El LSCS	M	3100	CS for unexplained fetal tachycardia antenatally & previous stillbirth
25*	0+0	289	Sa	NVD	M	3760	Treated with amoxycillin at 31 weeks gestation for urinary infection

Table 7.1 continued...

S	Parity	Gest.	S/Sa/I	Delivery	Sex	Weight	Pregnancy and delivery details
26*	1+3	291	S	NVD	M	4360	
27*	1+1	279	I	NVD	F	3680	Treated with amoxycillin at 27+ weeks gestation for urinary infection
28	0+0	283	S	NVD	F	3140	
29*	0+0	284	S	LSCS	M	3220	CS for brow presentation in labour
30	0+0	288	S	NVD	M	3450	Mild hypertension at 38 weeks, no proteinuria
31*	0+1	290	I	NVD	M	3540	Induced for PIH with proteinuria
32*	1+0	282	S	NVD	M	3510	Threatened abortion at 12 weeks gestation
33	2+2	269		LSCS	M	3930	Elective LSCS for placenta praevia brought forward following SROM
34*	0+2	277	Sa	LSCS	M	3800	CS for fetal distress in first stage
35*	0+0	281	Sa	Forceps	M	3420	Forceps for failure to progress in 2nd stage
36*	0+0	272	S	NVD	M	3720	
37*	1+1	260	I	NVD	M	2980	Induced for PIH with proteinuria evident from 37 weeks gestation
38	1+0	290	I	NVD	M	4380	Induced because post term
39*	0+0	280	Sa	Forceps	F	3740	Kiellands forceps for OP position and delay in second stage
40*	0+1	280	Sa	NVD	M	2950	
41*	0+2	277	S	Forceps	M	3840	Forceps for delay in second stage and fetal distress on CTG
42	0+0	287	S	NVD	F	3480	
43*	0+1	286	I	LSCS	F	3260	Induced because post term and aged 40 years. CS for fetal distress in 1st stage
44*	2+1	266		El LSCS	M	3360	Elective CS due to rising titre of anti-E antibodies
45*	1+0	278	S	NVD	F	4660	

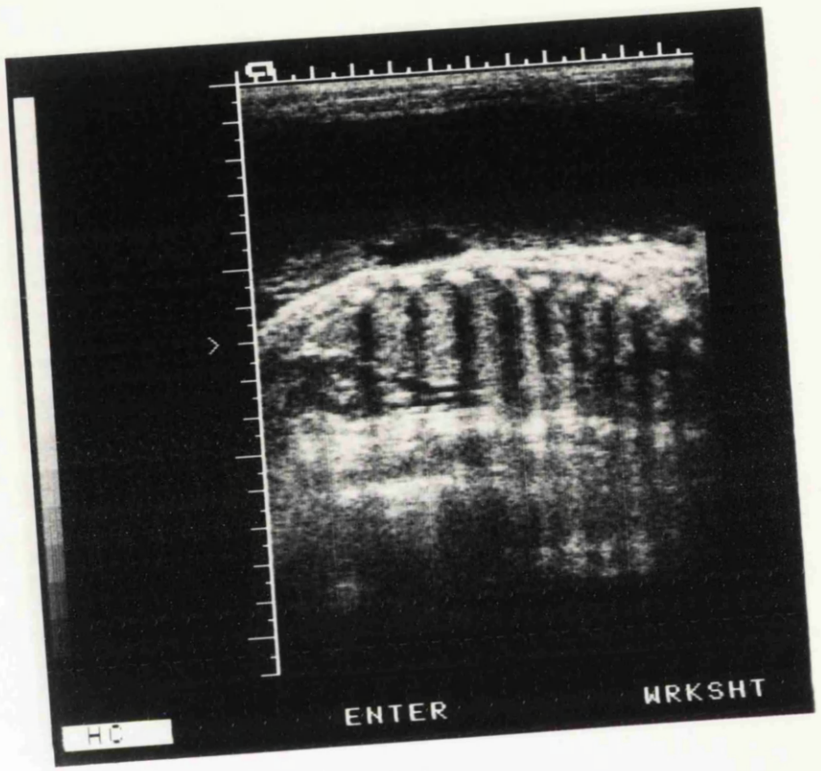
To obtain longitudinal measurements of the kidney, the fetal spine was viewed again in longitudinal section, and the transducer was moved slightly to each side of the spine in order to view the kidneys. The adrenals could then be seen by angling the transducer medially. It was found that an accurate longitudinal section of the adrenal was technically more difficult to obtain due to interference from the acoustic shadowing from the ribs (Fig. 7.1), and for this reason the rather sparse number of readings obtained in longitudinal section (Raw data, Table R.7.1) are not included in the composite results.

In the transverse plane, the maximum transverse and anteroposterior diameters, circumference and area of the adrenal were measured in the same section. Similar measurements were made of the kidney for comparative purposes. Both right and left sides were measured where possible, although the feasibility of this depended on the position of the baby in the uterus at the time of scanning. During the scan, routine antenatal screening measurements of head circumference, abdominal circumference and femur length were made to confirm normal growth, and an assessment of liquor volume was made.

B) Comparison of fetal adrenal size in term pregnancies with adrenal size in the one day old neonate

Nine women were recruited from the antenatal clinic. They were scanned once in late pregnancy between 37-40 weeks gestation and the babies were then rescanned on day 1 following birth. All the women had a spontaneous onset of labour and gave birth to healthy infants (4 male, 5

Fig. 7.1 Ultrasound picture, with corresponding line drawing, of a fetal adrenal (at 37 weeks gestation) in longitudinal section, demonstrating the acoustic shadowing from the ribs. [A - adrenal, R - rib, S - superior]



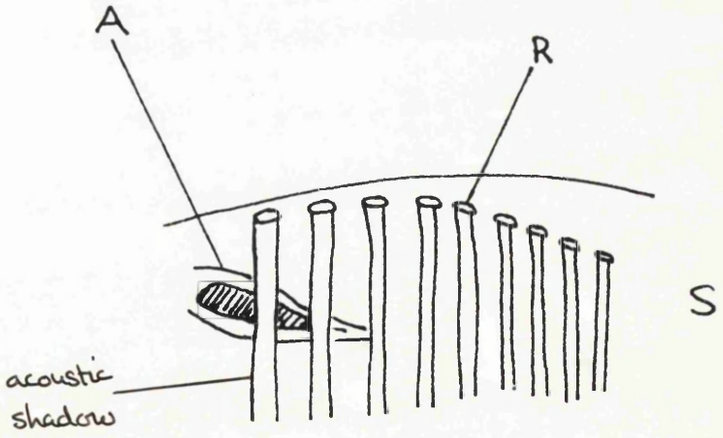


Table 7.2 Details of individual women having fetal adrenal scans 30 days or less prior to delivery and neonatal scans on day 1 following birth. [Abbreviations as for table 7.1 except gestation is in weeks + days]

Subject	Gest. at scan	Gest. at del.	S/Sa	Delivery	Sex	Weight
1	37+5	41+0	S	NVD	M	3780
2	37+1	39+6	S	NVD	F	2860
3	38+0	40+5	S	NVD	F	3520
4	37+5	39+4	S	NVD	F	3100
5	38+1	42+3	S	NVD	F	3180
6	38+2	40+2	S	NVD	F	3540
7	39+6	42+3	S	LSCS	M	4760
8	37+5	40+4	S	NVD	M	3240
9	39+1	40+2	S	NVD	M	3810

Table 7.3 Individual delivery details of neonates having serial ultrasound scans. [Abbreviations as for Table 7.1 except gestation is in weeks +days]

Subject	Gest.	S/Sa	Delivery	Sex	Weight
1	41+1	S	NVD	F	3880
2	41+3	Sa	Forceps	F	3790
3	39+0	S	NVD	F	2940
4	39+3	S	Forceps	M	2760
5	39+2	S	NVD	M	3080
6	39+4	S	NVD	F	2750
7	39+3	Sa	NVD	F	3060
8	39+0	S	NVD	F	3400
9	38+6	Sa	NVD	M	3340
10	39+6	Sa	NVD	M	3530
11	40+3	S	NVD	F	3440
12	37+5	Sa	Forceps	M	3160

female) with a mean (± SD) birthweight of 3.53 (± 0.56) kg (Table 7.2). The antenatal scan was performed using the Hitachi EUB240 and the neonatal scan was performed using an Acuson 128. The same parameters were measured as described in sections A and C of the study.

C) Serial adrenal ultrasonography in the neonate

12 women and their babies were recruited from the postnatal wards. Only women who had had a spontaneous onset of labour and a vaginal delivery following a normal pregnancy, and who gave birth to a healthy neonate (mean ± SD birthweight, 3.26 ± 0.37kg; 5 male infants, 7 female infants) were included (Table 7.3). No women withdrew their babies from the study once they had agreed to take part.

Serial adrenal scans were performed on days 1, 3, 5, 10-11, 21-22, and 41-45 of neonatal life. One woman was unable to bring her baby for a scan on day 10-11, but otherwise a complete series of scans was obtained. The scans were performed using a high-resolution, real-time, computerized, phased-array sector scanner (Acuson 128) with a 5 MHz transducer.

The right adrenal was visualized with the baby in the supine position using the liver as an acoustic window; the left adrenal was visualized with the baby in the right lateral decubitus position using the spleen as an acoustic window *(Kangarloo et al, 1986).* The longitudinal views of the adrenal were obtained by visualizing the kidney longitudinally and then angling the transducer about 30° medially. Having obtained a longitudinal view of the kidney, the transverse view of the kidney was obtained by rotating the transducer through 90°. The adrenal was seen in transverse section by identifying the upper pole of the kidney and angling the transducer slightly. The same measurements were made as in the

antenatal part of the study. In the transverse plane, the maximum transverse and anteroposterior diameters, circumference and area were measured in the same section; in the longitudinal plane, the length of the adrenal was measured from the apex to the midpoint of the base of the gland (Fig 7.2). Both right and left adrenals were measured wherever possible. Similar measurements were made of the kidneys for comparative purposes.

Variability

The variability of the adrenal and kidney ultrasound measurements were assessed in the following way. Five consecutive measurements of each parameter were made during the course of a single examination of an adrenal and kidney. Twelve adrenals and kidneys were measured in this way at 24-26 weeks gestation, and a further 12 adrenals and kidneys were assessed at 36-40 weeks gestation. The results could not be hidden from the ultrasonographer as they appeared on the screen after setting the cursors, but it was ensured that cursors were not moved after the figures had appeared. The medians (and ranges) of coefficients of variation obtained for each measurement are shown in Table 7.4.

The variability was assessed in the same way for 12 adrenals and kidneys in 1-3 day old neonates and a further 12 adrenals in 41-45 day old neonates. The ultrasonographer was unable to see the results of these measurements which were covered and recorded by the author. The medians and ranges of coefficients of variation obtained for each measurement are shown in Table 7.5.

All of the ultrasound scans in this chapter were performed by the same experienced ultrasonographer (Alison Thomas, DCR DMU).

Fig. 7.2 Diagrammatic representation of ultrasound appearance of neonatal adrenal gland in transverse section (on the left) and longitudinal section (on the right). [ap - anteroposterior diameter, t - transverse diameter, l - length]

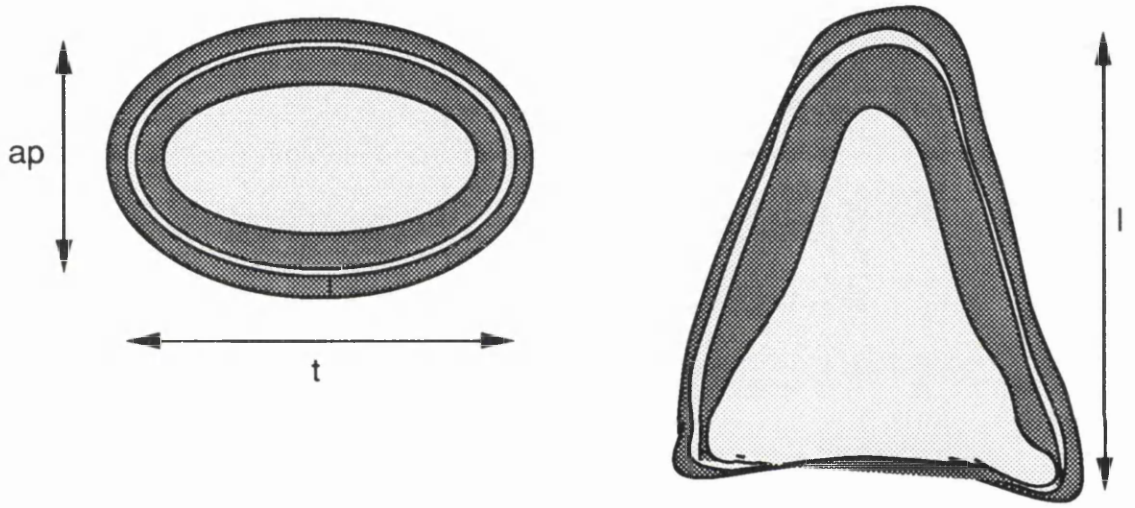


Table 7.4 Medians (and ranges) of coefficients of variation (%) of adrenal and renal parameters in fetuses at 24-26 weeks and 36-40 weeks gestation.

	Transverse	Anteroposterior	Circumference	Area
Adrenal				
24-26 weeks	6.3 (4.4-8.8)	8.6 (6.5-17.8)	3.8 (2.3-8.5)	10.7 (0.0-19.2)
36-40 weeks	6.2 (3.8-11.9)	8.4 (4.4-19.9)	5.3 (3.2-17.4)	13.3 (2.1-39.8)
Kidney				
24-26 weeks	5.7 (1.8-7.0)	6.0 (2.6-15.2)	4.7 (2.5-10.1)	9.8 (3.7-22.2)
36-40 weeks	4.5 (3.8-7.6)	6.8 (3.5-9.8)	3.6 (2.5-6.3)	7.4 (4.8-13.4)

Table 7.5 Medians (and ranges) of coefficients of variation (%) of adrenal and renal parameters in 1-3 day old and 41-45 day old neonates.

	Transverse	Anteroposterior	Circumference	Area	Length
Adrenal					
Day 1-3	6.8 (5.0-11.1)	11.1 (4.9-18.2)	6.1 (3.1-9.8)	12.6 (7.3-22.5)	6.6 (2.4-10.6)
Day 41-45	8.5 (4.1-18.4)	10.6 (3.6-19.1)	7.5 (4.8-15.4)	15.1 (5.4-29.2)	8.0 (1.9-16.0)
Kidney					
Day 1-3	5.7 (2.1-11.5)	5.7 (1.9-11.8)	4.8 (2.4-6.6)	9.1 (3.0-15.6)	3.0 (1.7-6.6)

None of the parameters measured were of a normal distribution as confirmed by the Kolmogorov-Smirnoff one sample test using a standard normal distribution. Statistics were therefore carried out using Wilcoxon signed ranks test, Kruskal-Wallis one-way analysis of variance and Spearman rank correlation coefficients.

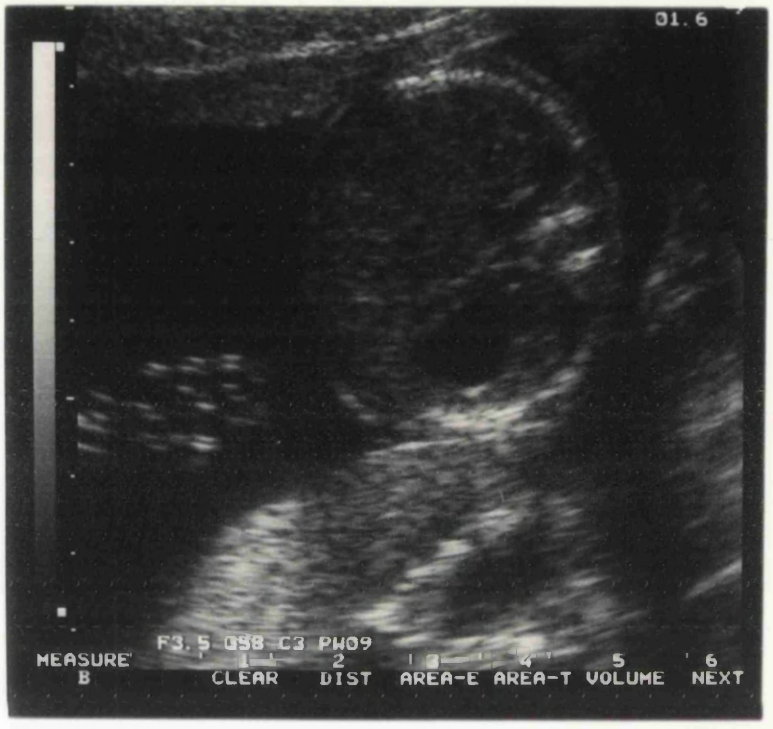
Results

A) Serial fetal adrenal ultrasonography in the second and third trimesters of pregnancy - relation to maternal oestriol and progesterone levels in plasma and saliva

Most commonly, the adrenals have a bean-shaped shaped appearance in the transverse plane, although occasionally they are oval or spindle-shaped. In the longitudinal plane, the most common shape is that of an indented triangle, although an inverted Y shape was also observed. The main features were of an echogenic centre surrounded by a more hypoechogenic periphery (Figs. 7.3 and 7.4). The differences in echogenicity tended to become more marked as gestation progressed, and the proportion of gland occupied by the echogenic centre also tended to increase with gestation. Towards term, it was sometimes possible to see all the layers described for the one day old neonate.

Right-sided measurements were obtained in 53% of the scans, left-sided measurements in 32%, and both sides could be measured in 15% of the scans. The ability to obtain a measurement depended purely on the position of the fetus in the uterus at the time of the scan. There was no significant difference between the right and left adrenals or kidneys for any

Fig. 7.3 Ultrasound picture, with corresponding line drawing, of a right fetal adrenal (at 20 weeks gestation) in transverse section. [R - rib, A - adrenal, Sp - spine, S - stomach]



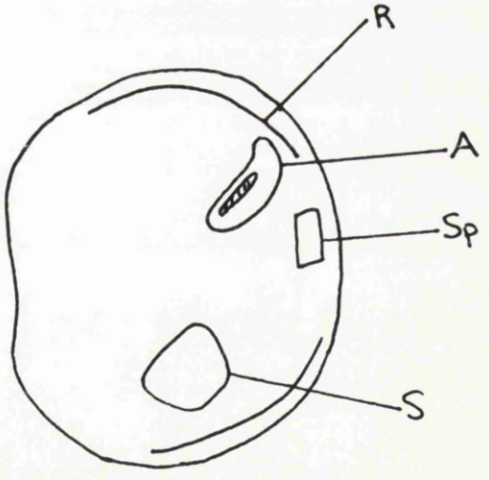
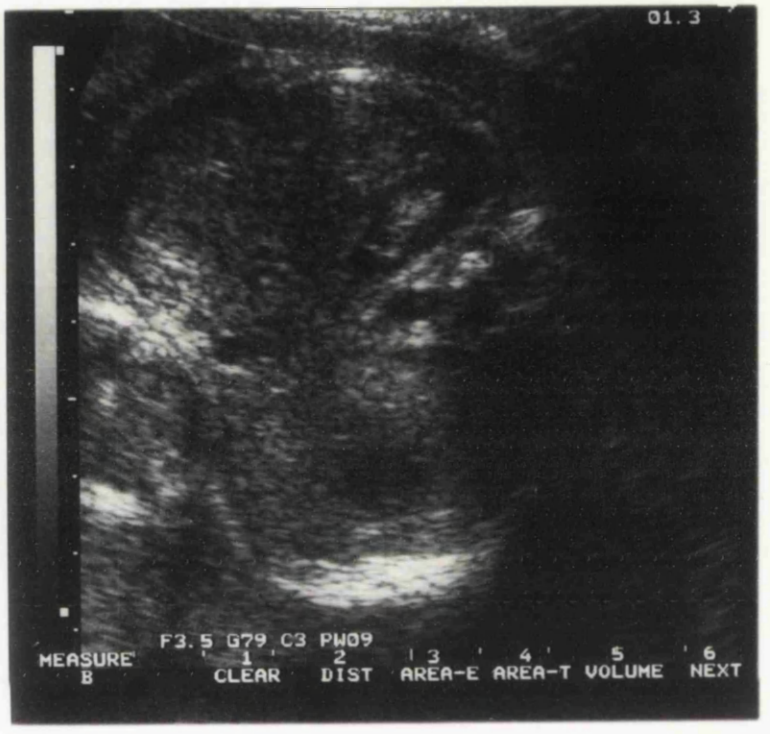
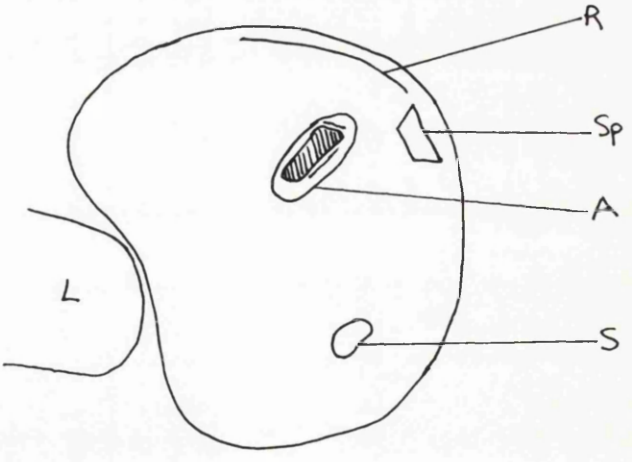


Fig. 7.4 Ultrasound picture, with corresponding line drawing, of a right fetal adrenal (at 38 weeks gestation) in transverse section. [R - rib, Sp - spine, A - adrenal, S - stomach, L - limb]





parameter measured. Neither was there any significant difference between the adrenals or kidneys of male and female fetuses.

The measurements for each adrenal parameter and the corresponding renal parameters are shown in Figs. 7.5 - 7.8. The median adrenal and renal measurements for each gestation are shown in Table 7.6. (These values were calculated using the right adrenal measurements where possible, and the left adrenal where no right adrenal measurement was obtained.) There was an approximately linear increase in adrenal parameters with gestation. The kidneys also increased in size in an approximately linear fashion. There were no significant changes in the mean adrenal:kidney ratios throughout gestation as tested by Kruskal-Wallis analysis of variance.

The results of saliva and plasma oestriol (E3) and progesterone (P) measurements are shown in Figs. 7.9, 7.10 and Table 7.7. Progesterone levels in plasma and saliva increased gradually throughout gestation, whereas there was a surge in both plasma and saliva oestriol levels from 32 weeks gestation onwards. The median percentage 'free' progesterone, expressed as the saliva/plasma ratio x 100, was 0.83% (range 0.3-2.52%, n=200 paired samples). The median percentage 'free' oestriol was 9.0% (range 4.0-18.0%, n=200 paired samples). The interindividual variation in percentage 'free' of both hormones was quite high, but within individual subjects the variability was considerably less.

There was a significant correlation between adrenal and renal parameters throughout gestation (Spearman correlation coefficients (r_s) were 0.85, 0.74, 0.87, and 0.84 for transverse diameter, anteroposterior diameter, circumference and area respectively, $p<0.0001$). Similarly, there

Fig. 7.5 Adrenal (graph A) and kidney (graph B) transverse diameters (in transverse section) measured in 45 normal women at 4 weekly intervals throughout pregnancy from approximately 24 weeks gestation onwards.

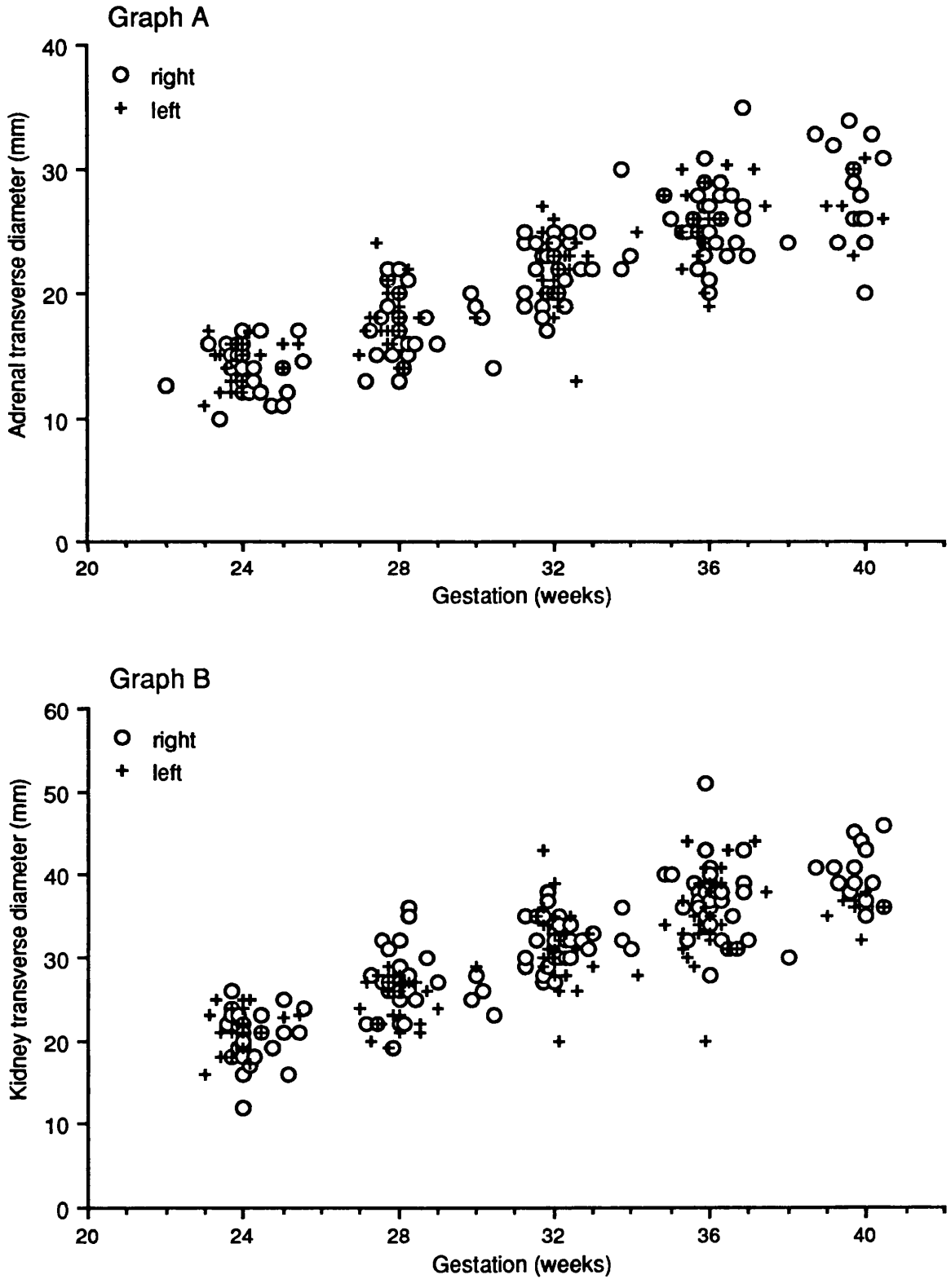


Fig. 7.6 Adrenal (graph A) and kidney (graph B) anteroposterior diameters (in transverse section) measured in 45 normal women at 4 weekly intervals throughout pregnancy from approximately 24 weeks gestation onwards.

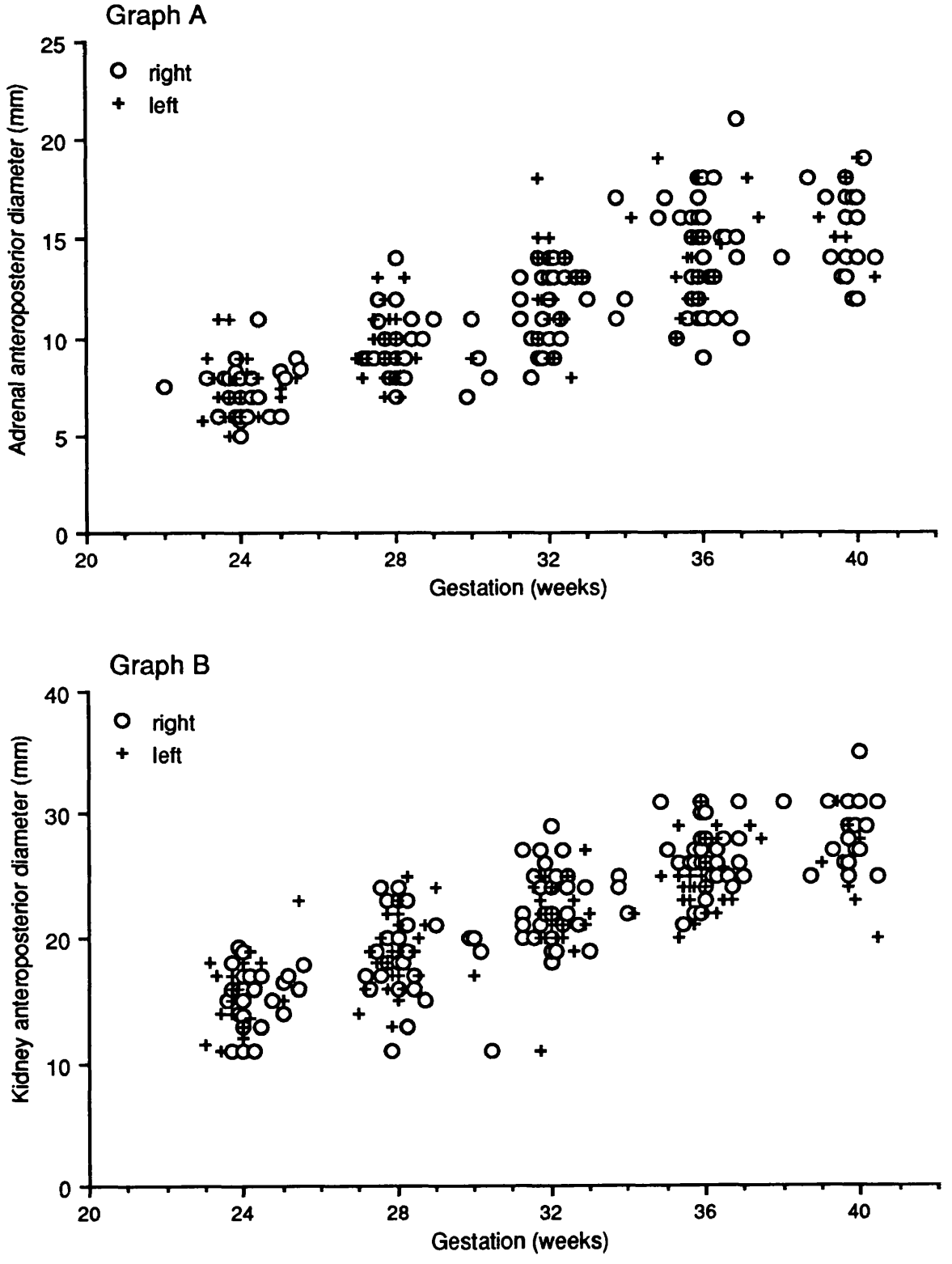
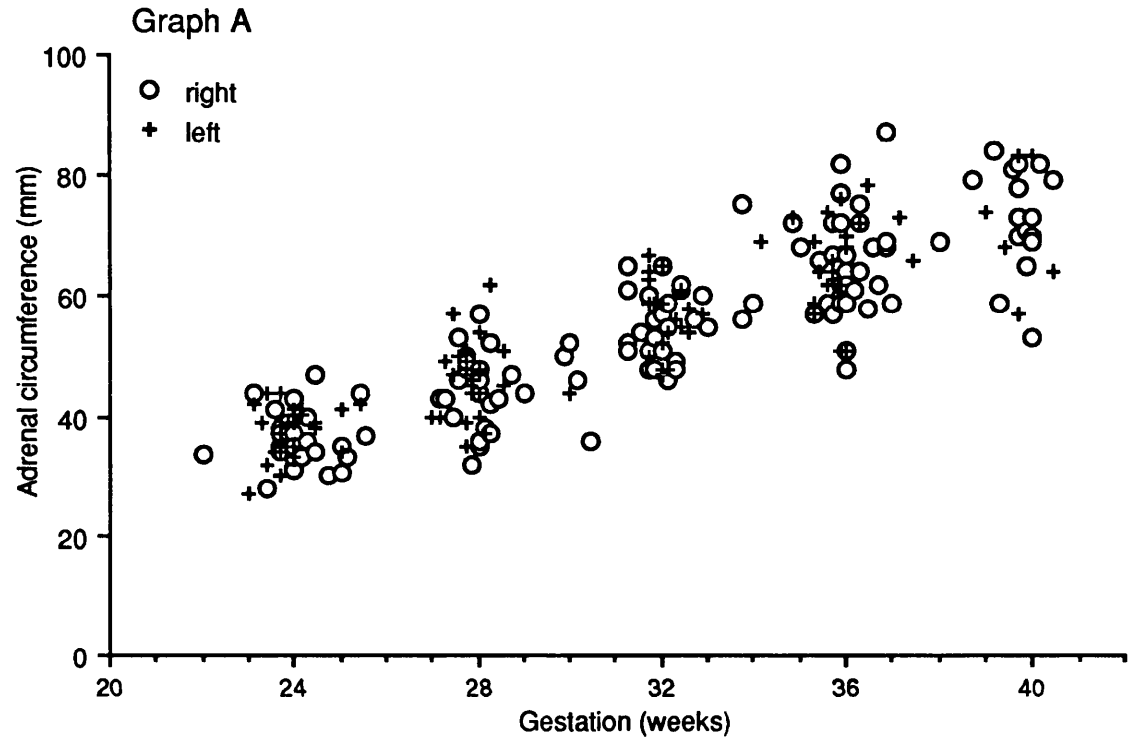


Fig. 7.7 Adrenal (graph A) and kidney (graph B) circumferences (in transverse section) measured in 45 normal women at 4 weekly intervals throughout pregnancy from approximately 24 weeks gestation onwards.



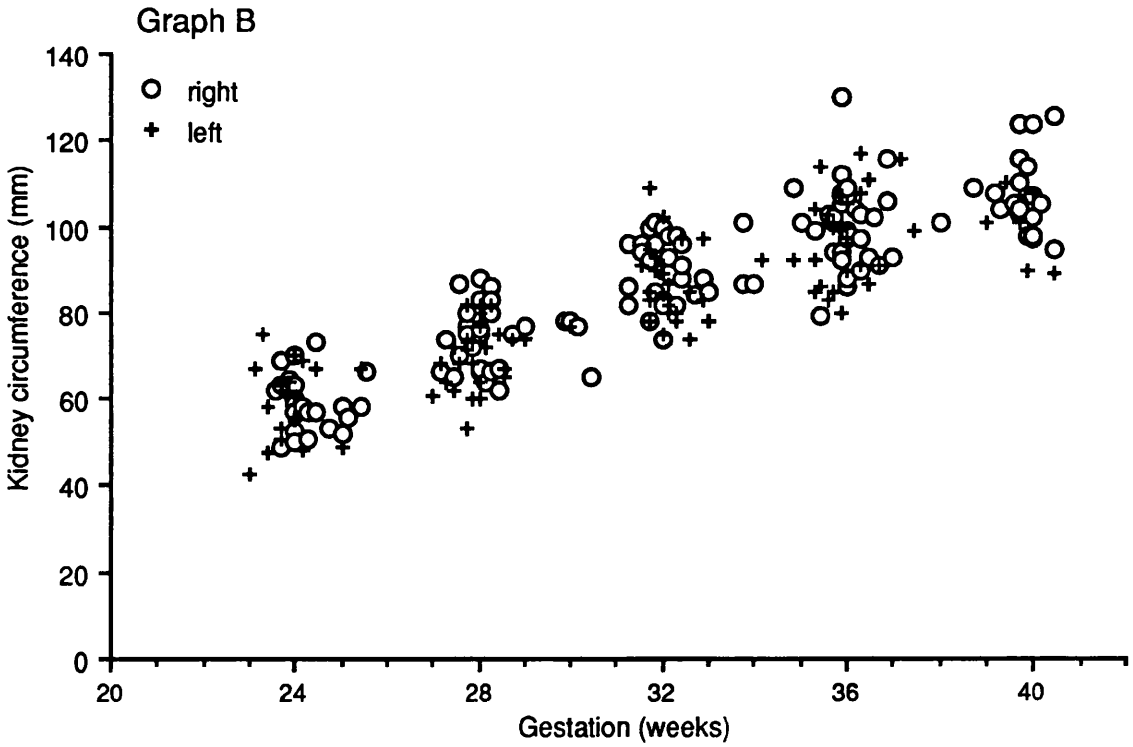
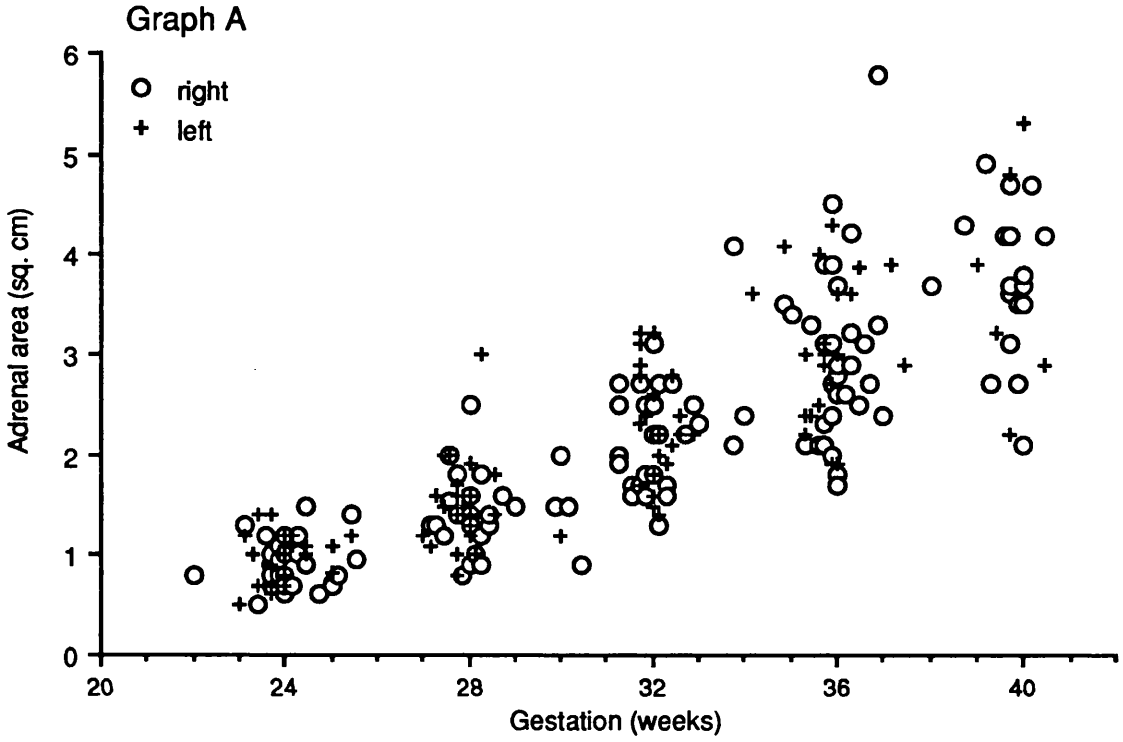


Fig. 7.8 Adrenal (graph A) and kidney (graph B) areas (in transverse section) measured in 45 normal women at 4 weekly intervals throughout pregnancy from approximately 24 weeks gestation onwards.



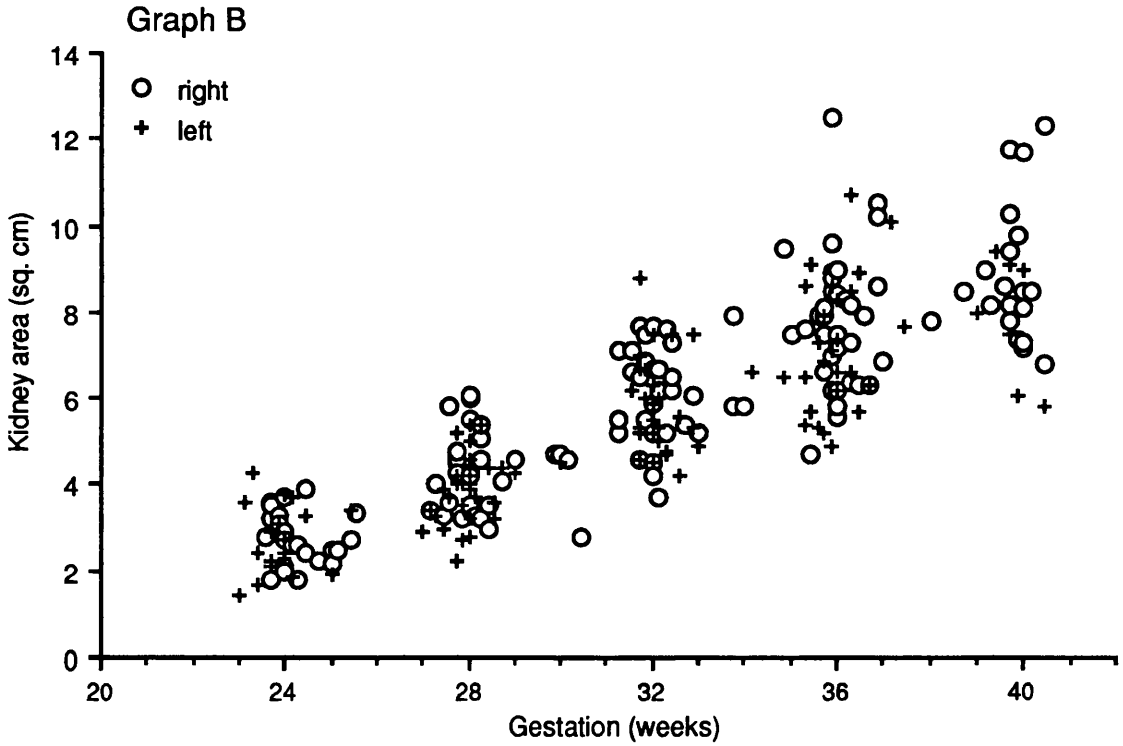
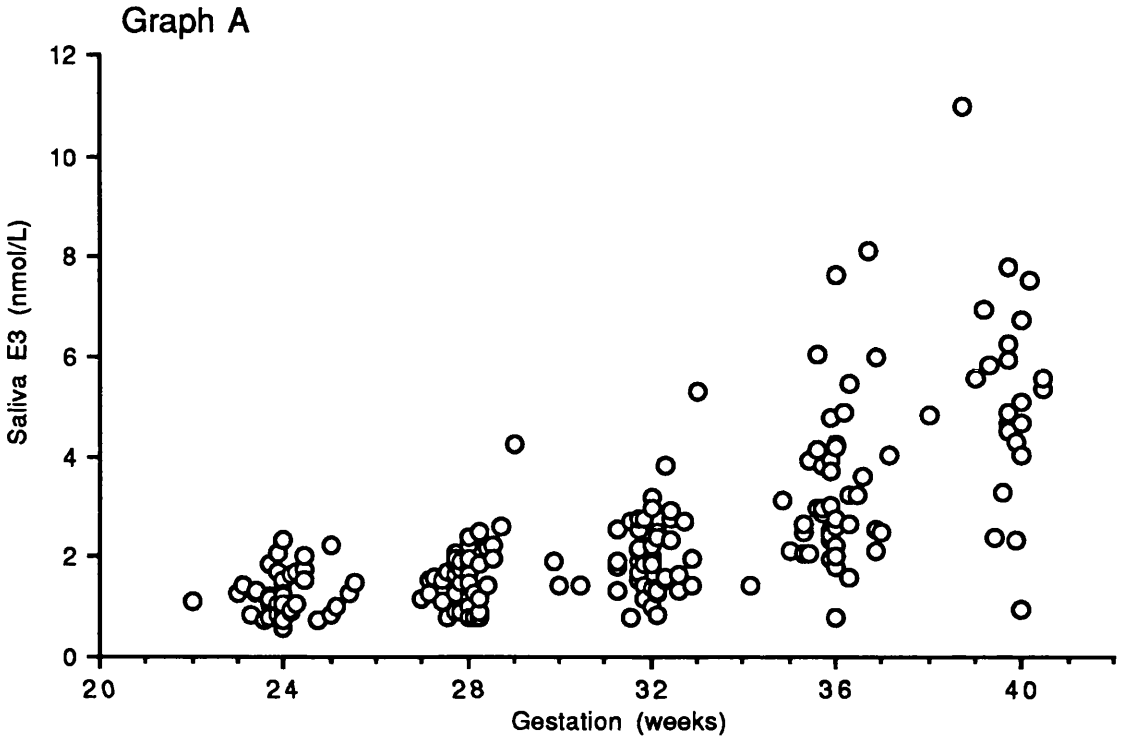


Table 7.6 Median [range] adrenal and renal measurements in 32 normal women, taken at 4 weekly intervals from 168 ± 7 days gestation onwards. (All measurements are in mm except those for area, which are in sq. cm.) (** n=15 women)

	Transverse	Anteroposterior	Circumference	Area
Adrenal (Adr)				
161-175	14.5 [10 - 17]	7.0 [5 - 11]	36.5 [27 - 44]	0.90 [0.5 - 1.4]
189-203	17.0 [13 - 24]	9.0 [7 - 14]	44.0 [35 - 57]	1.45 [0.9 - 2.5]
217-231	22.5 [17 - 27]	12.0 [8 - 15]	55.5 [46 - 65]	2.20 [1.3 - 3.2]
245-259	26.0 [20 - 35]	13.0 [10 - 21]	64.0 [51 - 87]	2.85 [1.8 - 5.8]
273-287 **	27.0 [20 - 34]	16.0 [12 - 19]	73.0 [53 - 82]	3.70 [2.1 - 4.7]
Kidney (Kid)				
161-175	21.0 [12 - 26]	15.0 [11 - 19]	59.5 [43 - 75]	2.70 [1.4 - 4.3]
189-203	27.0 [21 - 32]	18.0 [13 - 24]	73.0 [60 - 88]	3.95 [2.7 - 6.1]
217-231	32.0 [20 - 43]	22.0 [18 - 29]	88.5 [74 -109]	6.00 [3.7 - 8.8]
245-259	37.0 [31 - 51]	26.0 [21 - 31]	102.5 [79 -130]	7.90 [4.7 -12.5]
273-287 **	39.0 [35 - 46]	28.0 [26 - 35]	105.0 [97 -126]	8.50 [7.2 -12.3]
Adr:Kid ratio				
161-175	0.68 [0.47-1.08]	0.50 [0.46-0.79]	0.60 [0.47-0.83]	0.30 [0.19-0.58]
189-203	0.64 [0.50-0.86]	0.53 [0.38-0.85]	0.63 [0.50-0.79]	0.37 [0.23-0.56]
217-231	0.69 [0.53-1.00]	0.52 [0.36-0.68]	0.61 [0.52-0.74]	0.36 [0.21-0.73]
245-259	0.69 [0.56-0.90]	0.51 [0.37-0.76]	0.63 [0.52-0.84]	0.37 [0.21-0.73]
273-287 **	0.72 [0.57-0.89]	0.52 [0.41-0.66]	0.68 [0.54-0.78]	0.44 [0.28-0.55]

Fig. 7.9 Saliva oestriol (E3) levels (graph A) and saliva progesterone (P) levels (graph B) measured in 45 normal women at 4 weekly intervals throughout pregnancy from approximately 24 weeks gestation onwards.



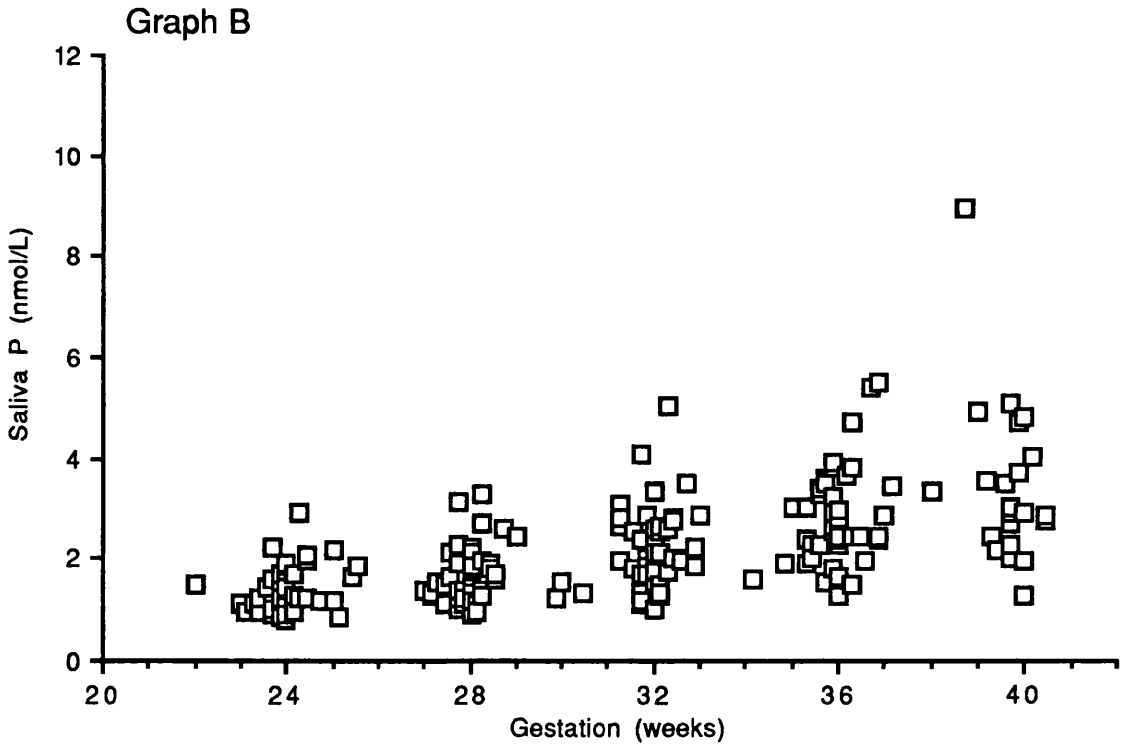
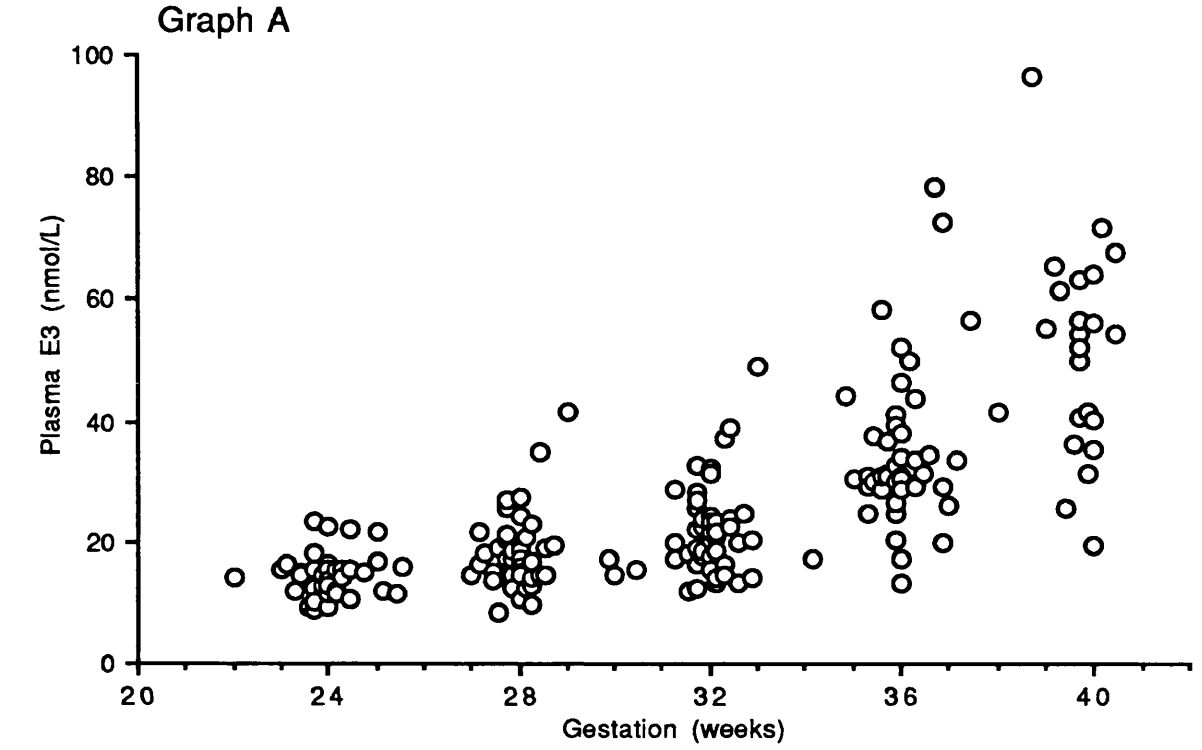


Fig. 7.10 Plasma oestriol (E3) levels (graph A) and plasma progesterone (P) levels (graph B) measured in 45 normal women at 4 weekly intervals throughout pregnancy from approximately 24 weeks gestation onwards.



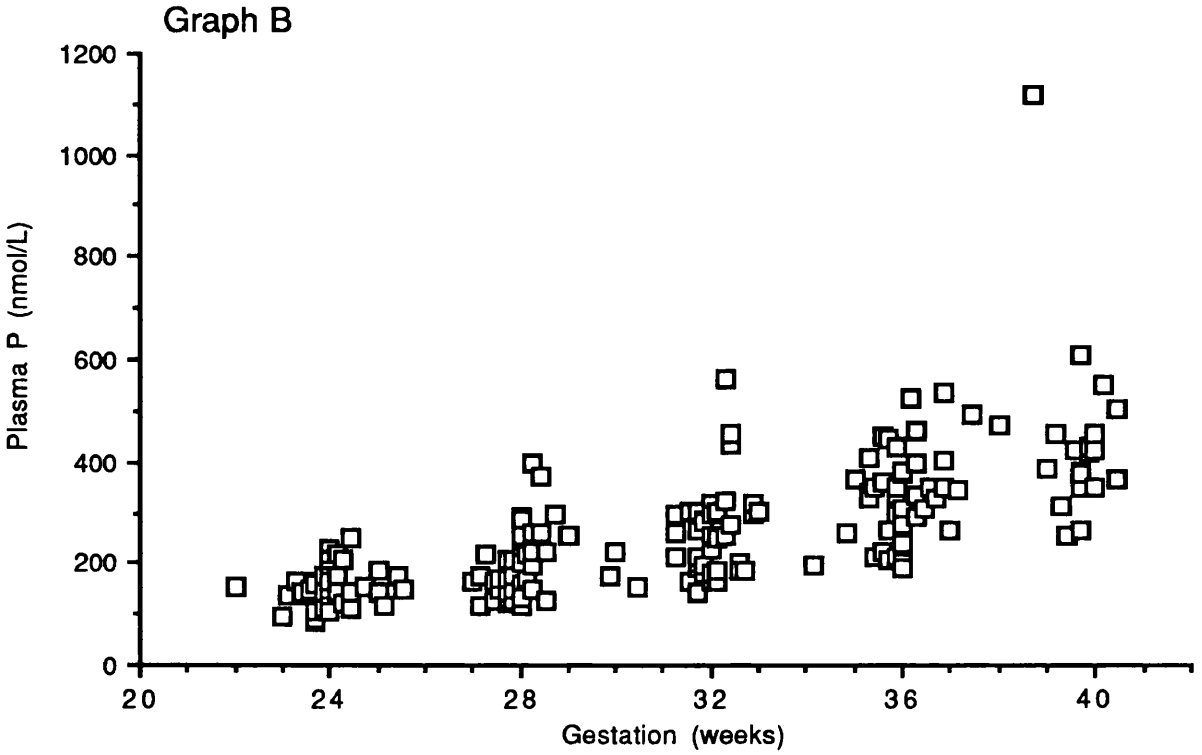


Table 7.7 Median [range] saliva and plasma oestriol (E3) and progesterone (P) levels in 32 normal women, measured at 4 weekly intervals from 168 ± 7 days gestation onwards. (All measurements are in nmol/L)
(* n=15 women)

	Saliva E3	Saliva P	Plasma E3	Plasma P
161-175	1.17 [0.59 - 2.34]	1.20 [0.82 - 2.25]	14.3 [9.2 - 23.3]	149 [84 - 227]
189-203	1.51 [0.77 - 4.26]	1.43 [0.91 - 3.12]	17.2 [8.2 - 41.8]	173 [115 - 299]
217-231	1.90 [0.78 - 5.33]	2.02 [1.01 - 4.09]	21.1 [12.1 - 49.3]	253 [143 - 456]
245-259	2.96 [0.80 - 8.10]	2.60 [1.30 - 5.50]	30.9 [13.3 - 78.5]	330 [201 - 534]
273-287*	4.87 [0.96 - 7.78]	2.96 [1.29 - 5.09]	52.1 [19.4 - 71.8]	381 [253 - 613]

Table 7.8 Spearman correlation coefficients between adrenal and renal parameters and oestriol and progesterone levels in plasma and saliva. (n=32 women, $p < 0.0001$ in all cases)

	Saliva E3	Plasma E3	Saliva P	Plasma P
Adrenal				
Transverse	0.68	0.69	0.70	0.75
Anteroposterior	0.62	0.66	0.62	0.68
Circumference	0.69	0.72	0.69	0.76
Area	0.67	0.72	0.67	0.74
Kidney				
Transverse	0.66	0.72	0.68	0.74
Anteroposterior	0.62	0.66	0.67	0.70
Circumference	0.68	0.72	0.71	0.74
Area	0.68	0.71	0.71	0.74

was a significant correlation between the adrenal parameters and the saliva and plasma levels throughout gestation (r_s ranged from 0.62-0.76, $p<0.0001$), as well as between the kidney parameters and the saliva and plasma E3 and P levels throughout gestation (r_s range 0.62-0.74, $p<0.0001$), Table 7.8.

However, when the results within each gestation period were considered separately, there was no correlation between any adrenal and renal parameters except circumference. There was a significant but weak correlation between adrenal and renal circumference at 24, 28 and 32 weeks gestation (r_s = 0.36, 0.48, and 0.39 respectively, $p<0.05$). Furthermore, there was no correlation within each gestation period between any of the adrenal or renal parameters and the hormone levels in either plasma or saliva. In particular, there was no significant correlation between saliva or plasma E3 and any adrenal parameter.

B) Comparison of fetal adrenal size in term pregnancies with adrenal size in the one day old neonate

Adrenal and renal measurements for the antenatal and day 1 neonatal scans are shown in Table 7.9. (All the measurements shown are for the right adrenal and kidney.) There was a significant decrease in size for every adrenal parameter between the scans. Whilst the median kidney measurements tended to be decreased in the neonatal scans, the decrease was not significant for any parameter.

Table 7.9 Median [range] adrenal and renal measurements for 9 neonates studied antenatally (mean ± SD..... 18 ± 6 days before delivery) and on day 1 of neonatal life. [* denotes significant difference from corresponding antenatal measurement]

	Transverse		Anteroposterior		Circumference		Area	
Adrenal								
Antenatal	28.0	(20.0 - 33.0)	17.0	(10.0 - 21.0)	75.0	(52.0 - 83.0)	4.00	(1.80 - 4.60)
Postnatal	21.7	(17.6 - 28.9)*	12.7	(11.1 - 16.2)*	52.4	(49.4 - 70.0)*	2.08	(1.75 - 3.20)*
p value		<0.01		<0.03		<0.02		<0.02
Kidney								
Antenatal	40.0	(35.0 - 47.0)	25.0	(23.0 - 31.0)	105.0	(98.0 - 116.0)	8.00	(7.00 - 11.00)
Postnatal	37.0	(29.0 - 42.5)	26.0	(18.1 - 28.9)	95.7	(81.0 - 111.8)	6.60	(4.95 - 9.35)

C) Serial adrenal ultrasonography in the neonate

The shape of the adrenal was as described in part A of the study. The ultrasound appearance is of a central hyperechogenic area surrounded by a hypoechogenic zone. The outer edge of the gland is delineated by a thin hyperechogenic line surrounded by a hypoechogenic area (Fig. 7.11). After a few days these layers are less easy to distinguish as the gland becomes smaller and the differences in echogenicity less marked. The main features are then an echogenic central area surrounded by a less echogenic periphery.

The right adrenal gland could be seen and measured in every assessment, but full left adrenal measurements were possible in only 86% of neonates, although the gland could be visualized in 91% of cases. There was no significant difference between the right and left adrenals or kidneys for any of the parameters measured.

The measurements for each adrenal parameter and the corresponding renal measurements are shown in Figs. 7.12 - 7.16 and Table 7.10. There was a marked decrease in adrenal size in the first 5 days of life, and in contrast there was little change in kidney size over the same time period. The mean percentage changes in adrenal and renal measurements during the first 6 weeks of life are shown in Fig. 7.17. There was a significant decrease in every adrenal parameter between days 1 & 3 ($p<0.02$), 3 & 10-11 ($p<0.03$), 5 & 21 ($p<0.03$) and 5 & 41-45 ($p<0.001$). In contrast, the kidneys had significantly increased in size between days 1 & 41-45 ($p<0.01$) for all parameters except the anteroposterior diameter.

Fig. 7.11 Ultrasound pictures of the adrenal gland of a one day old neonate in transverse (upper) and longitudinal (lower) section.

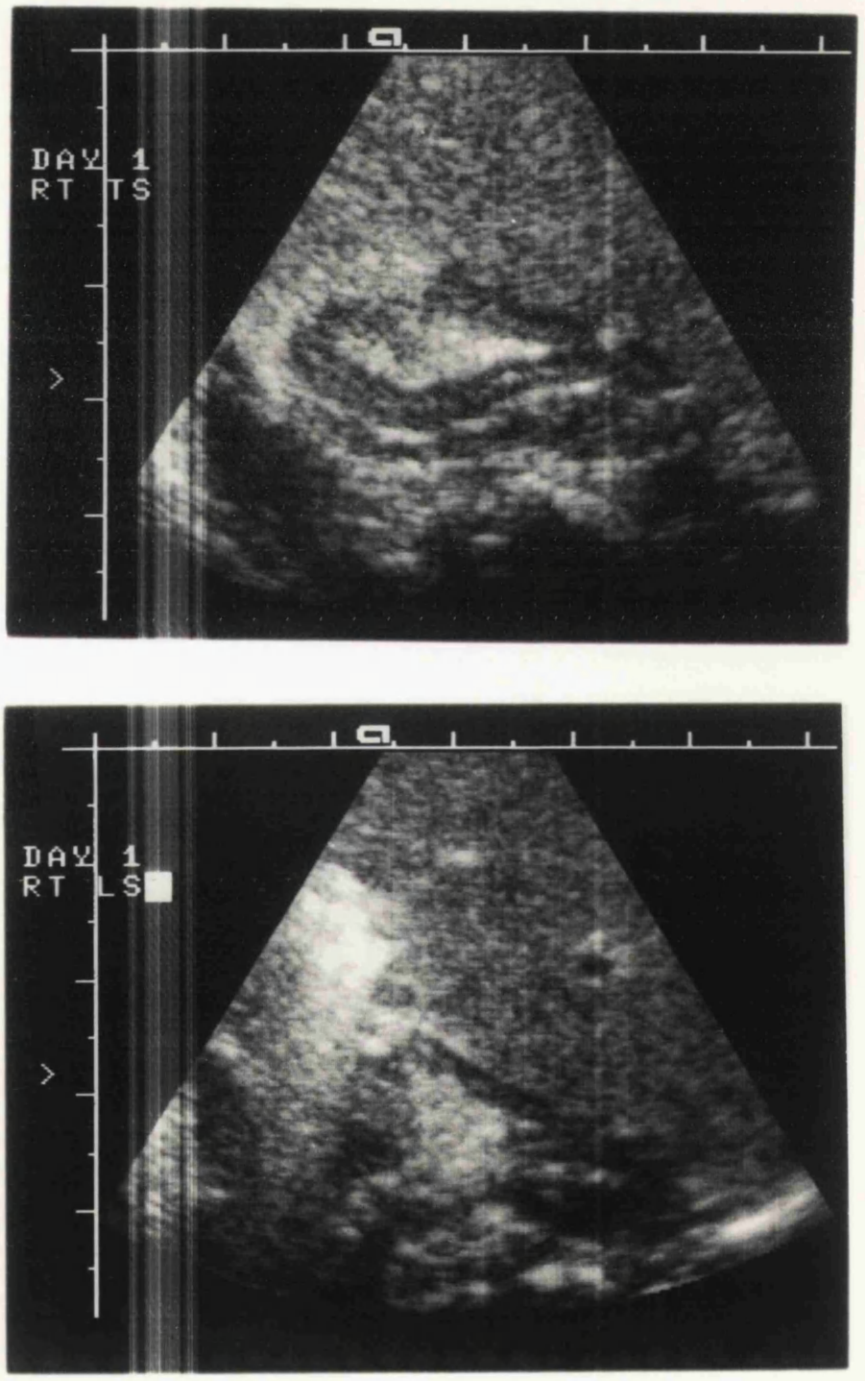
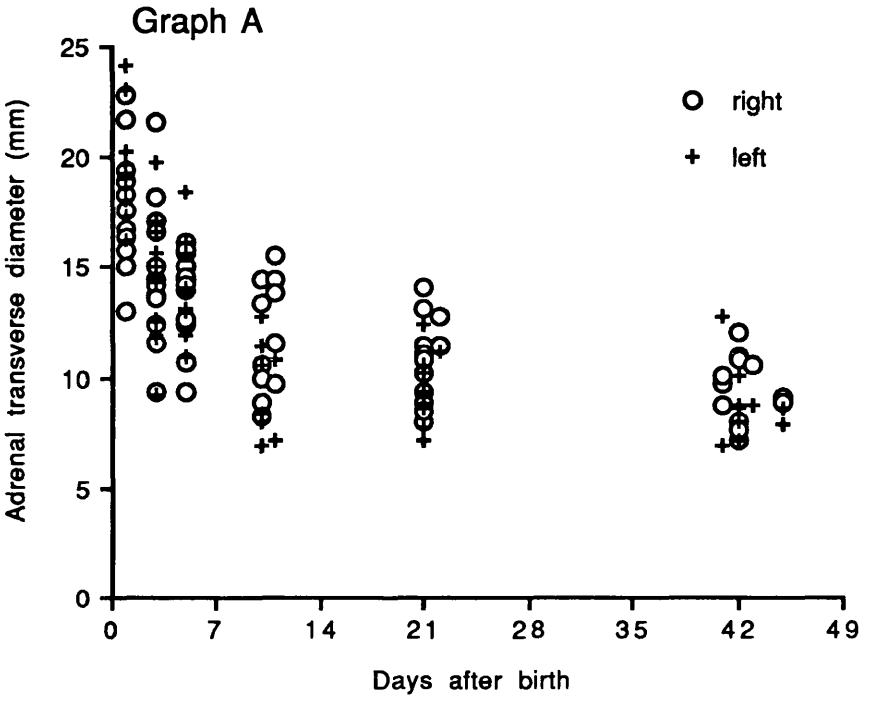


Fig. 7.12 Adrenal (graph A) and kidney (graph B) transverse diameters (mm) (in transverse section) in 12 normal neonates during the first 6 weeks of neonatal life.



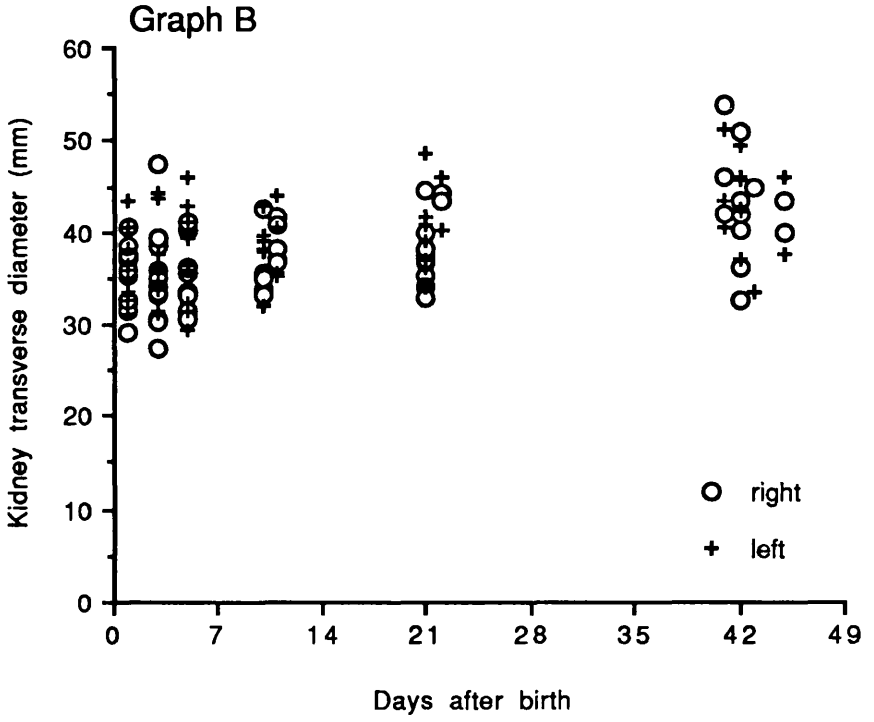


Fig. 7.13 Adrenal (graph A) and kidney (graph B) anteroposterior diameters (mm) (in transverse section) in 12 normal neonates during the first 6 weeks of neonatal life.

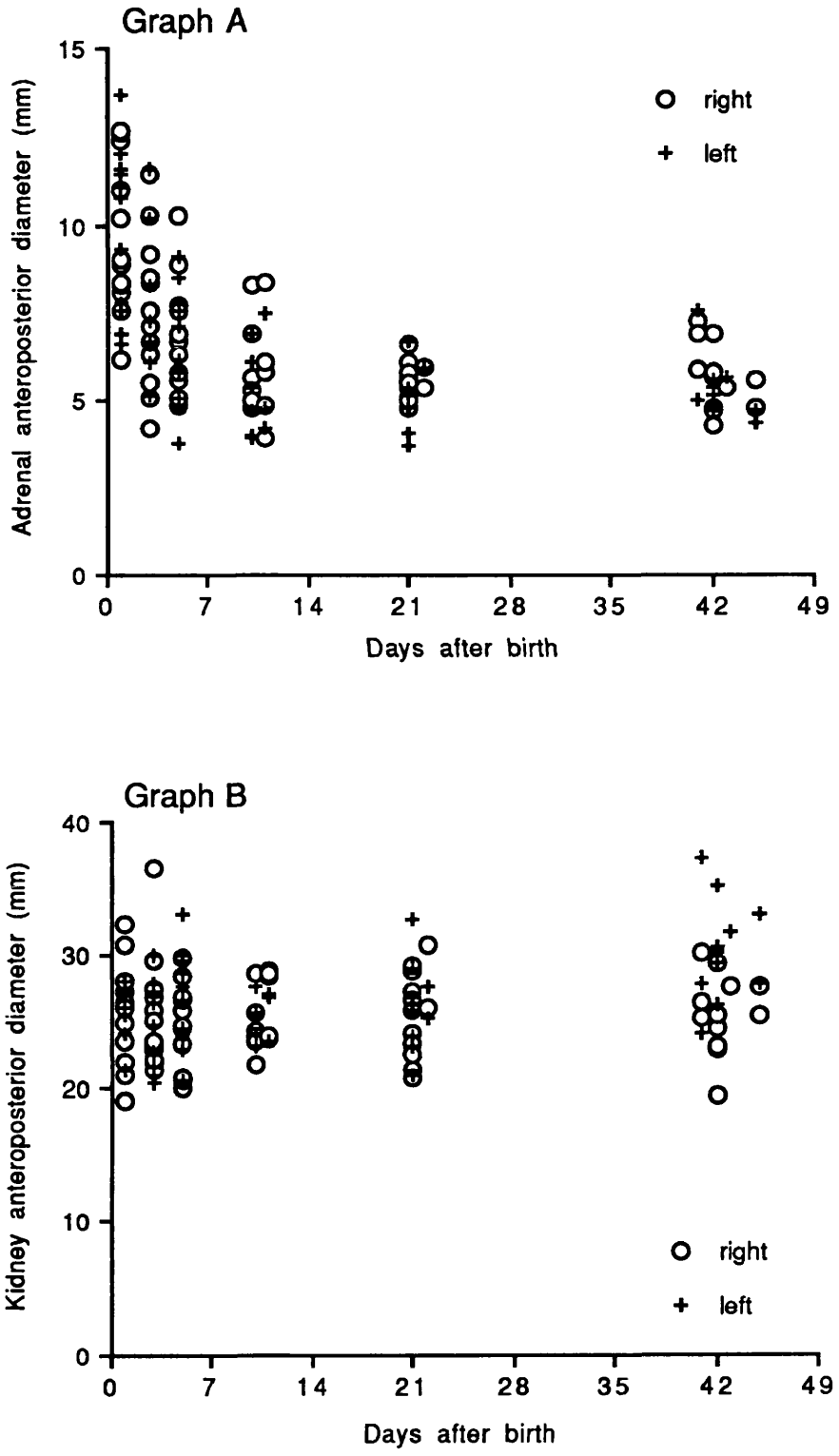


Fig. 7.14 Adrenal (graph A) and kidney (graph B) circumferences (mm) (in transverse section) in 12 normal neonates during the first 6 weeks of neonatal life.

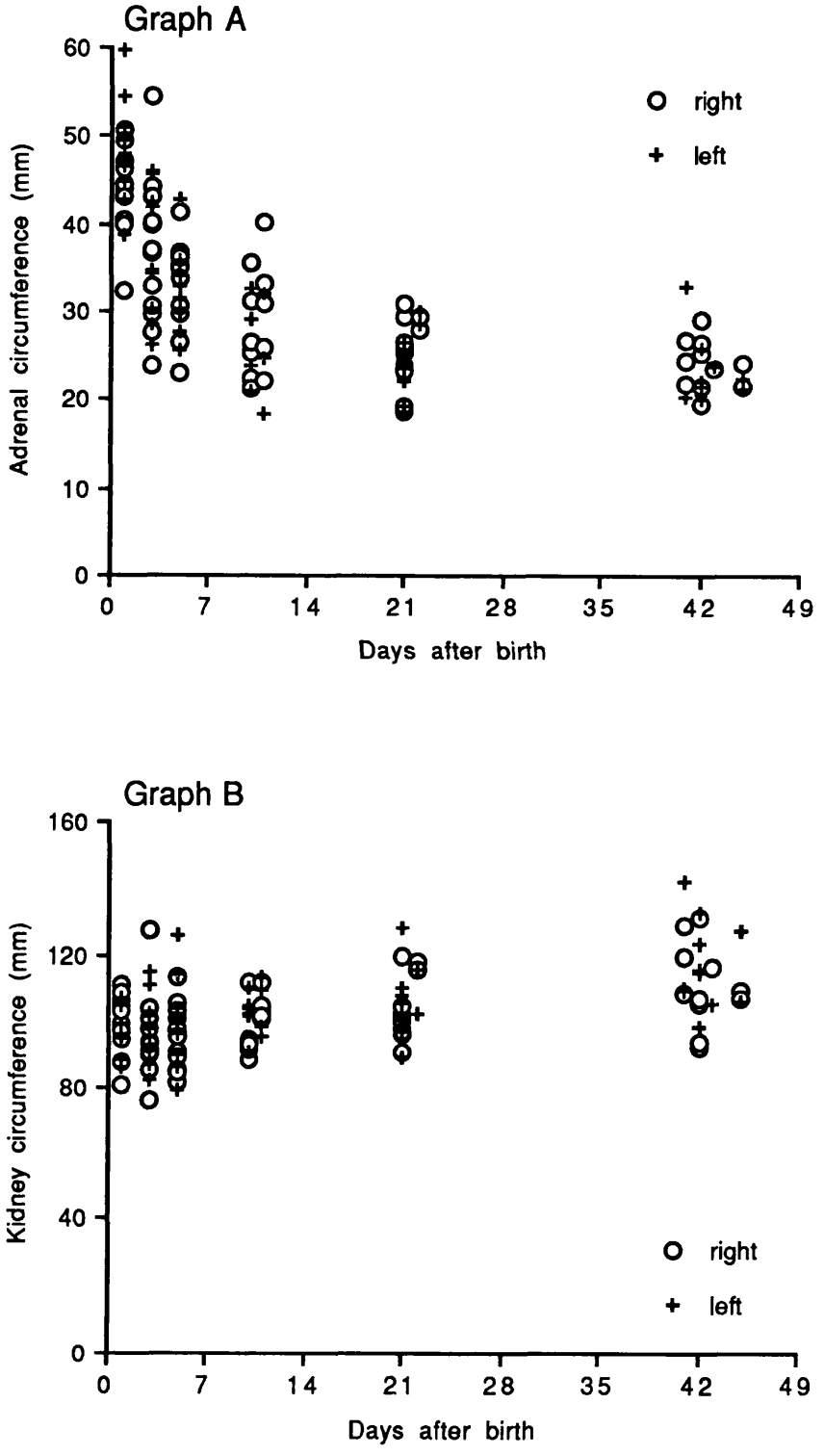


Fig. 7.15 Adrenal (graph A) and kidney (graph B) area (cm^2) (in transverse section) in 12 normal neonates during the first 6 weeks of neonatal life.

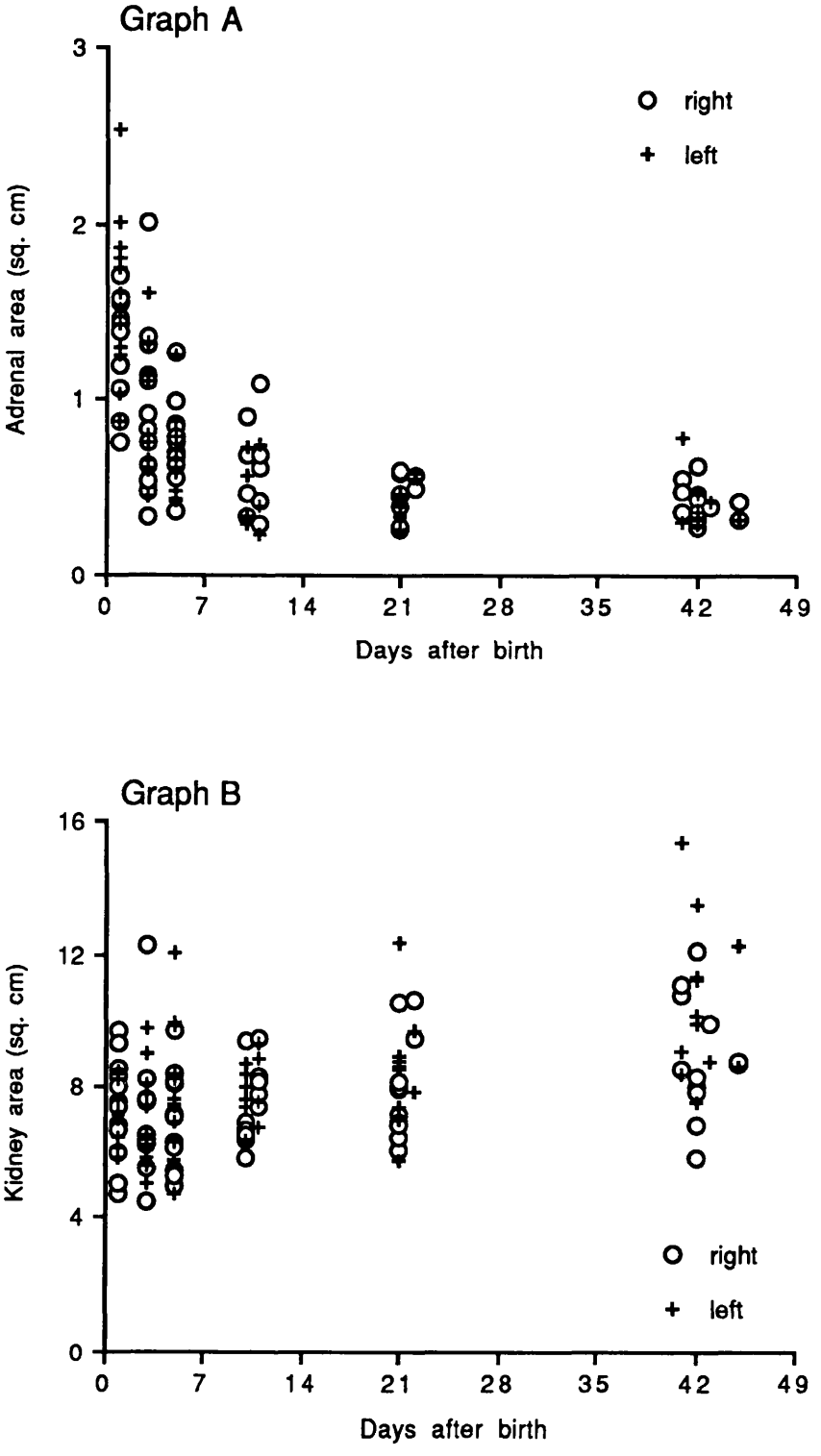


Fig. 7.16 Adrenal (graph A) and kidney (graph B) length (mm) (in longitudinal section) in 12 normal neonates during the first 6 weeks of neonatal life.

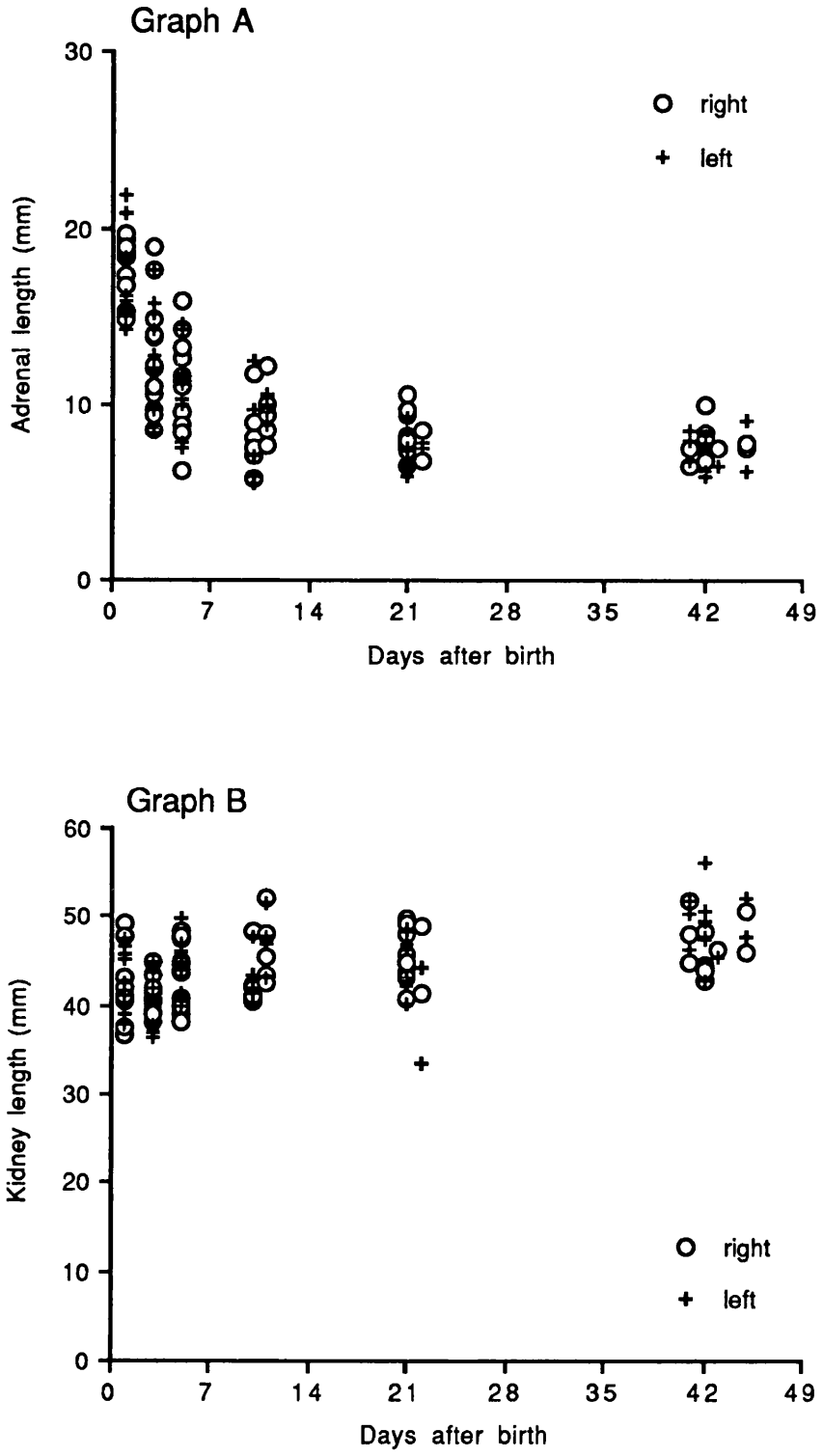
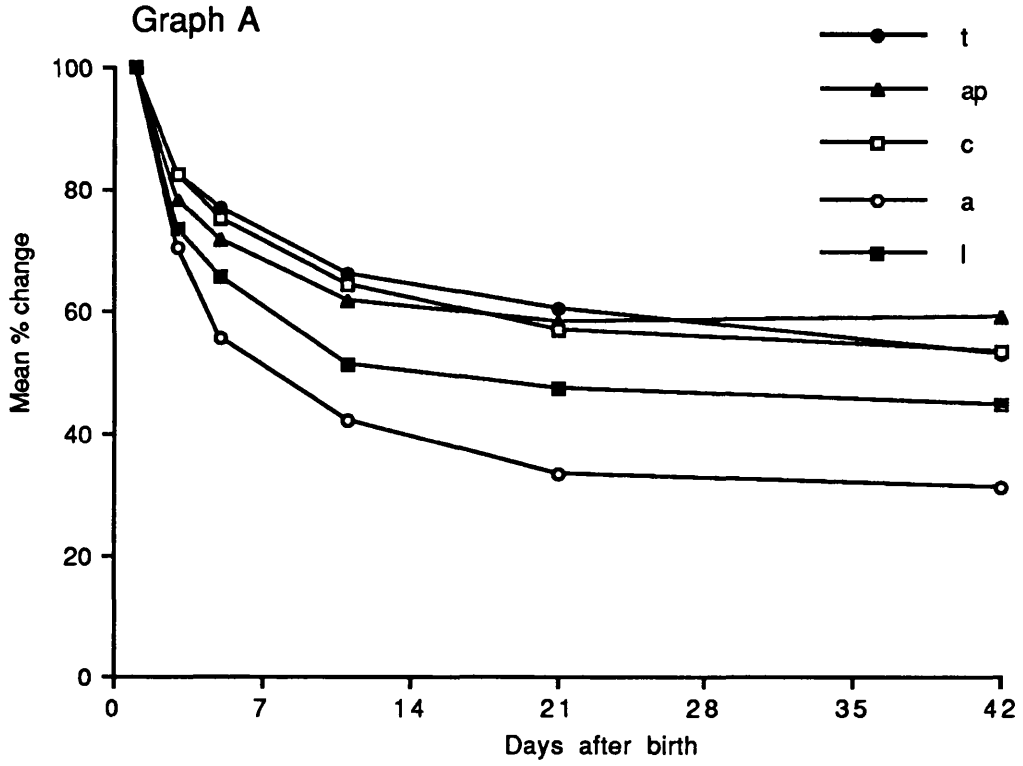
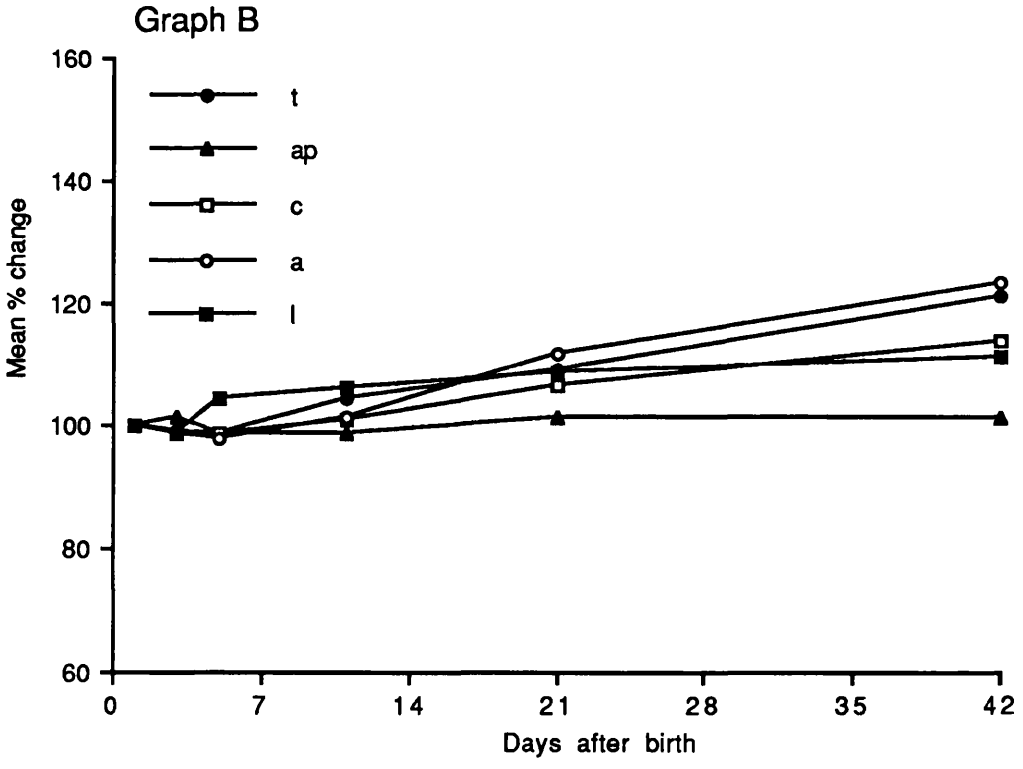


Table 7.10 Median [range] right adrenal and renal measurements in 12 neonates studied serially during the first 6 weeks of life. (All measurements are in millimetres unless otherwise indicated) [n=11 for the mean values on day 11]

Day	ADRENAL PARAMETERS									
	Transverse		Anteroposterior		Circumference		Area (sq. cm)		Length	
1	18.0	[13.0 - 22.8]	9.0	[6.2 - 12.7]	44.6	[32.2 - 50.7]	1.44	[0.75 - 1.71]	17.9	[14.9 - 19.6]
3	14.4	[9.3 - 21.6]	7.4	[4.2 - 11.4]	36.9	[24.0 - 54.4]	0.88	[0.34 - 2.01]	12.2	[8.6 - 19.0]
5	14.4	[9.3 - 16.2]	6.8	[4.9 - 10.3]	35.1	[23.0 - 41.3]	0.73	[0.36 - 1.27]	11.6	[6.2 - 15.9]
11	11.5	[8.2-15.5]	5.7	[3.9 - 8.4]	26.4	[21.4 - 40.1]	0.46	[0.29 - 1.09]	8.6	[5.8 - 12.3]
21	10.9	[8.0 - 14.1]	5.6	[4.8 - 6.6]	25.7	[18.6 - 30.8]	0.47	[0.26 - 0.60]	8.1	[6.5 - 10.6]
42	9.4	[7.2 - 12.0]	5.7	[4.3 - 7.3]	23.9	[19.6 - 29.1]	0.41	[0.27 - 0.62]	7.7	[6.6 - 10.0]
	KIDNEY PARAMETERS									
	Transverse		Anteroposterior		Circumference		Area (sq. cm)		Length	
1	36.4	[29.0 - 40.4]	25.6	[19.1 - 32.3]	98.6	[80.4 - 111.1]	7.45	[4.71 - 9.74]	41.6	[36.8 - 49.2]
3	34.5	[27.4 - 47.5]	24.3	[21.5 - 36.6]	93.6	[75.9 - 127.3]	6.46	[4.51 - 12.30]	41.1	[38.2 - 44.9]
5	33.5	[30.5 - 41.0]	25.3	[20.1 - 29.7]	96.4	[81.9 - 113.7]	7.12	[4.97 - 9.67]	43.9	[38.1 - 48.3]
11	36.7	[33.2 - 42.4]	24.4	[21.8 - 28.7]	100.8	[88.3 - 111.8]	7.41	[5.79 - 9.44]	42.6	[40.6 - 52.2]
21	37.9	[32.9 - 44.5]	26.0	[20.9 - 30.8]	100.8	[91.2 - 119.6]	7.91	[6.02 - 10.64]	44.4	[40.7 - 49.8]
42	42.6	[32.6 - 53.8]	25.6	[19.6 - 30.2]	108.1	[92.2 - 130.9]	8.60	[5.80 - 12.10]	45.5	[42.8 - 51.8]

Fig. 7.17 Mean percentage changes in adrenal (Graph A) and renal (Graph B) measurements during the first 6 weeks of neonatal life. [t-transverse diameter, ap- anteroposterior diameter, c-circumference, a-area, l-length in longitudinal section]





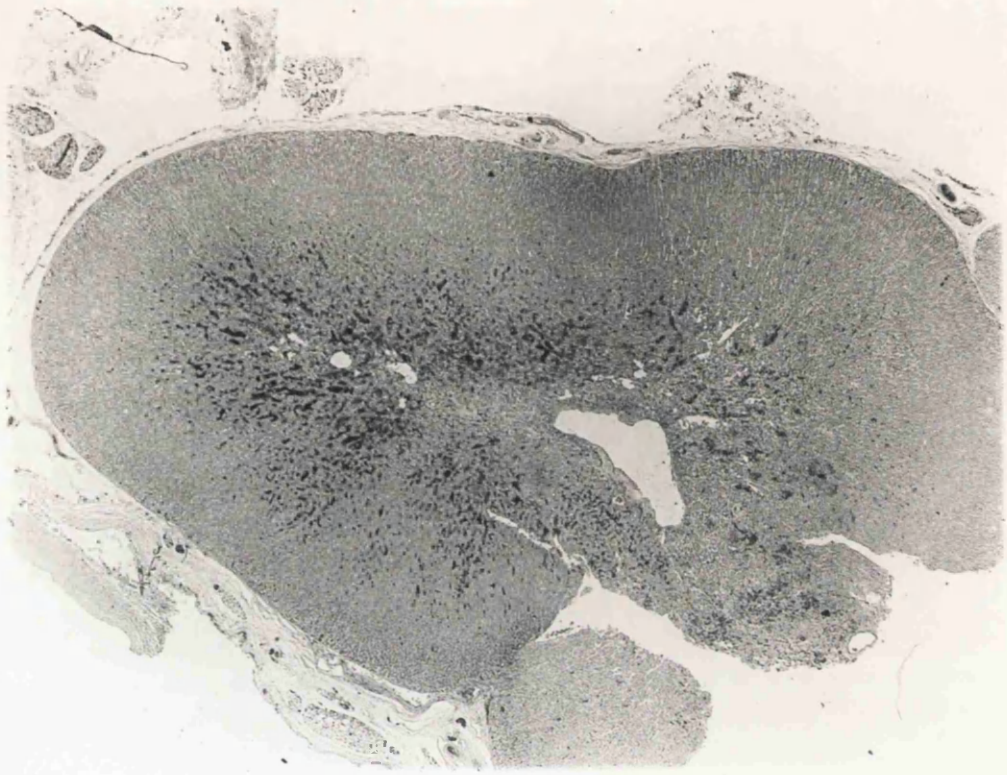
There was a significant but weak correlation between adrenal transverse diameter, anteroposterior diameter, circumference and area, and birthweight (r_s = 0.42, 0.44, 0.58,and 0.62 respectively, $p<0.04$). Similarly, there was a weak correlation between kidney circumference and area, and birthweight (r_s = 0.44 and 0.43 respectively, $p<0.04$).

Discussion

Ultrasound measurement of the fetal and neonatal adrenal, although not easy, is less difficult than that of the adult adrenal gland because it is a relatively larger structure in the fetus and neonate (0.2-0.3% of total body weight in newborns compared with 0.01% of total body weight in adults), *(Morison, 1963; Bech et al, 1969)*. Also, there is less surrounding fat and the gland is nearer to the surface in the neonate.

The ultrasound appearance of the adrenal in the fetus and the neonate was found to be similar to that described by others, although only Hauffa et al *(1988)* commented on the thin echogenic outer rim, that was noted in the present study in late pregnancy and in the newborn. Most authors have suggested that the hyperechogenic centre of the adrenal represents the medulla and that the surrounding hypoechogenic zone is the fetal cortex, but in this study, it was found that the hyperechogenic area was too large to be the medulla alone. By correlating the appearance with postmortem macroscopic and histological adrenal sections (Fig. 7.18), it was concluded that the hyperechogenic central area represents not only the central vein and the medulla (which is very small in neonates, *(Tähkä et al, 1951)* but also the

Fig. 7.18 Histological section of a neonatal adrenal gland in longitudinal section (magnification x25).



congested sinusoids of the inner part of the fetal cortex. The surrounding hypoechogenic zone appears to represent the less congested part of the fetal cortex, which is enclosed by the thin hyperechogenic definitive cortex and capsule. The hypoechogenic area outside the adrenal capsule probably represents the loose connective tissue and fat surrounding the gland.

Male and female adrenals were of equivalent size, and there was no significant difference between right and left adrenal measurements, in agreement with previous histopathological studies *(Tähkä et al, 1951; Schulz et al, 1961; Keene and Hewer, 1926).*

A) Serial fetal adrenal ultrasonography in the second and third trimesters of pregnancy - relation to maternal oestriol and progesterone levels in plasma and saliva

One or both adrenals could be visualized in all the fetuses that were studied from 24 weeks gestation onwards. It was difficult to obtain satisfactory views of the full craniocaudal extent of the gland whilst the fetus was *in utero*, and so the few results that were obtained are not presented. As previously described by Jeanty et al, *(1984)*, the main problem was interference from acoustic shadowing by the ribs. Fortunately, adult CT scanning of the adrenal has demonstrated that knowledge of the craniocaudal length of the gland is not necessary in clinical practice; all adult adrenal pathology is diagnosed in the transverse section *(Jeanty et al, 1984)*. However, some authors have chosen to measure parameters in longitudinal section *(Lewis et al, 1982; Hata et al, 1988)*, and whilst Lewis et al *(1982)* found rib interference to be a frequent problem, Hata et al *(1988)* were able to obtain satisfactory measurements in 100% of the patients studied using a similar technique.

There was a gradual approximately linear increase in adrenal size throughout gestation consistent with previous ultrasound findings *(Hata et al, 1985)* and with the growth and development of the gland noted histopathologically *(Keene and Hewer, 1926; Scammon, 1925).* The adrenal measurements are of the same order but slightly larger than those recorded by Jeanty et al *(1984).* However, their study had a cross-sectional design, with fewer subjects and the means were calculated over 4 week periods. No other study gives details of transverse section adrenal parameters. The adrenal:kidney ratio remained fairly constant from 24 weeks until term, indicating that the two organs were increasing in size at approximately the same rate.

Saliva oestriol and progesterone measurements showed a similar pattern to that found in Chapter 6; plasma and saliva levels are in agreement with those in Chapter 9. Matsumura et al *(1987)* looked at the correlation between various adrenal parameters and certain maternal plasma steroids including oestriol in a cross-sectional study of 100 women between 28 and 40 weeks gestation, and found a weak but significant correlation with plasma oestriol (range of correlation coefficients 0.40-0.47, $p<0.001$). Hata et al *(1987)* looked at the correlation between total urinary oestrogens and adrenal area (longitudinal section) in a study of 17 normal fetuses between 30 and 40 weeks gestation, and found a correlation coefficient of 0.65 ($p<0.001$). This was in very good agreement with the correlation coefficients obtained in this study between adrenal parameters and saliva or plasma oestriol levels. However, exactly comparable correlation coefficients were obtained between kidney parameters and hormone levels; they are really only indicative of the rising hormonal parameters and increasing organ size with increasing gestational age. The lack of correlation within each gestation

period indicated that the rate of fetal steroid biosynthesis and release is not reflected by adrenal size, as measured using ultrasonography, in the normal fetus, .

B) Comparison of fetal adrenal size in term pregnancies with adrenal size in the one day old neonate

It was interesting to find a significant fall in adrenal size of between 20% and 38% (depending on the parameter) between the fetal adrenal in late gestation and the one day old neonatal adrenal. A similar pattern is seen when comparing the 36-40 week gestation adrenal measurements from part A of the study, with the day 1 measurements of part C, although these were two different groups of women and babies.

A possible criticism of these findings is that different scan machines were used for the antenatal and neonatal scans. However, the fall in adrenal size was not matched by a similar fall in kidney size. Hata et al *(1988)* also noted a fall in adrenal area of 13% in measurements taken between 7 (or less) days prenatally and delivery. Whilst the adrenal weight has been found to increase approximately linearly in pregnancy, *(Scammon, 1925; Schulz et al, 1961)*; it has been shown histologically that involution of the fetal zone has started to occur during the last weeks of pregnancy *(Keene and Hewer, 1926; Benner, 1940)*.

These findings lend further support to the lack of a correlation between the hormone levels and adrenal size for a given gestation, particularly as an oestriol surge is occurring in most women at this time.

C) Serial adrenal ultrasonography in the neonate

In this part of the study, 6 serial measurements of the adrenals were made during the first 6 weeks of normal neonatal life in each of 12 babies. As in previous studies, it was found that measurement of the right adrenal was easier than that of the left, *(Kangarloo et al, 1986; Oppenheimer et al, 1983)*. Full measurement of the left adrenal was not possible in 14% of cases due to the presence of a full stomach or overlying bowel.

There was a rapid decrease in adrenal size in the first 5-10 days, followed by a slower decrease over the next few weeks, consistent with the involution of the fetal zone described in histopathologic studies. The finding of a median adrenal length of 17.9 mm on day 1 was similar to the mean length of 17 mm described by Oppenheimer et al *(1983)* for babies born at 36-40 weeks gestation. The percentage falls in size agreed approximately with those of Hata et al *(1988)*, who performed the only other reported serial study which looked at ultrasound changes in adrenal size; however, they measured only one parameter (in longitudinal section) for just the first 7 days of neonatal life.

Summary

In conclusion, this study confirmed that it is possible to visualize and to measure normal fetal and neonatal adrenal glands. There was an approximately linear increase in adrenal size during pregnancy, which was paralleled by the increase in kidney size. It would seem that there may be a decrease in adrenal size in the weeks immediately prior to the spontaneous onset of labour, although this finding needs more detailed investigation. The

adrenal glands decreased markedly in size during the first 6 weeks of neonatal life, whereas the kidneys continued to gradually increase in size.

There was no correlation between saliva or plasma oestriol or progesterone levels and any adrenal parameter for a given gestation. Indeed, it would seem that adrenal size may be decreasing at a time when the oestriol surge is occurring in most women. Therefore, even if it were possible to use hormonal parameters to predict the onset of labour, ultrasound measurement of the fetal adrenal would be unlikely to be of any value. Fetal adrenal size in women in preterm labour was not measured in this study, and still requires investigation. Contrary to our initial hypothesis, the fetal adrenal in these circumstances might possibly be smaller than normal for the gestation. However, even if fetal adrenals were significantly larger or smaller for the gestation in women going into preterm labour, the difference between fetal adrenal size compared to the fetal adrenals of normal women would be unlikely to be sufficient to be of use as a predictive test, because of the large range of fetal adrenal size, for a given gestation, as measured by ultrasound in normal women delivering at term.

Nevertheless, ultrasound can be useful in investigating adrenal pathology in the fetus and the neonate, although, as with other ultrasound investigations, it is necessary to have information on normal parameters as described in this chapter, in order to make a valid assessment.

8 The Absorption of Progesterone in Pregnancy

Introduction

Amongst the normal women studied in Chapter 6, 68% had a rise in the salivary oestriol:progesterone (E3:P) ratio prior to the spontaneous onset of labour at term. Furthermore, approximately 50% of the women who went into spontaneous idiopathic preterm labours, had a saliva E3:P ratio above the 90th centile, and all of the women who went into spontaneous idiopathic preterm labours, had saliva E3:P ratios above the median for their gestation. Whilst the main problem is knowing which subgroups of women are at risk of delivering preterm, it seems likely that treatment with progesterone may be of benefit in some cases.

Remarkably few previous studies have been carried out to look at the effect of treatment of preterm labour with progesterone, and the results obtained have been conflicting. The earliest studies were by Eichner et al, *(1951 and 1954)*, who looked at the treatment of preterm labour in women with spontaneous preterm rupture of the membranes using intramuscular progesterone and concluded that there might be some beneficial effect. However, Fuchs and Stakemann *(1960)* did not find any beneficial effect of intramuscular progesterone in the treatment of preterm labour, even when the patients were analysed in groups according to the predominant presenting symptoms- haemorrhage, ruptured membranes or rhythmic/constant pains. More recently, Erny et al *(1986)* carried out a trial using micronised progesterone (Utrogestan) and found a significant benefit of progesterone compared to placebo (decreased uterine activity in 80% cases and 42% cases respectively). This was the only study which monitored the change in progesterone levels achieved following treatment,

by taking a single plasma sample one hour after administration of progesterone.

If progesterone is to be an effective tocolytic agent for preterm labour, it would seem important to be able to give a dose which will reverse the raised E3:P ratio. Although many studies have been performed to look at plasma levels achieved following administration of progesterone in nonpregnant women, none have been reported in pregnant women. In pregnancy, there are much higher endogenous levels of corticosteroid binding globulin and progesterone, as well as many other physiological changes, which may influence the absorption and metabolism of any exogenous progesterone administered.

The aim of this study was to look at the absorption of progesterone in pregnancy and to determine the optimum route of administration by monitoring both plasma and saliva levels following oral and vaginal administration.

Materials and Methods

Women in early pregnancy were recruited to the study from gynaecological outpatients. They were all seeking termination of pregnancy. If they agreed to participate in the study, they were admitted to hospital, and the study was commenced, on the day prior to surgery. The women in late pregnancy were recruited from the antenatal clinic. If they decided to take part in the study, they attended the hospital during the day, went home overnight and returned the following morning to provide the final samples.

Eighteen pregnant women were recruited in total. All were healthy and had uncomplicated pregnancies.

Six women at 8-12 weeks gestation (PEP1-6) and six at 26-33 weeks gestation (PLP1-6) were studied following insertion of a 400mg progesterone pessary (Cyclogest) vaginally. Six women at 7-12 weeks gestation (MEP1-6) were given 400mg micronised progesterone orally (Utrogestan). Some of the women undergoing termination of pregnancy were given premedications on the morning of their operation, and some were prescribed dinoprostone (Prostin E2) 3 mg vaginally, which was administered after the first 12 hours of samples had been collected. The individual subjects details are shown in Table 8.1.

In addition, one woman, with an appalling obstetric history of 4 previous spontaneous second trimester abortions (between 20 and 28 weeks gestation), was admitted in preterm labour at 21+ weeks gestation, and was treated with 100mg intramuscular progesterone (Gestone) in addition to ritodrine. Although she is not strictly comparable with the other women studied, her results are included out of interest.

Paired plasma and saliva samples were obtained half-hourly for one hour prior to the administration of progesterone. Further samples were collected half-hourly for 3-4 hours and then hourly for a further 7-9 hours. If the women were awake during the night they collected further saliva samples. Between 1 and 4 paired final samples were taken hourly the following morning, the last sample being taken 24 hours post progesterone administration.

Table 8.1 Individual subjects' details including age (years), parity, gestation (weeks), height (m), weight (kg), and any drug treatment, other than progesterone, administered whilst the study was in progress. ['Cyclogest' pessary in early pregnancy = PEP, oral micronised 'Utrogestan' in early pregnancy = MEP, 'Cyclogest' pessary in late pregnancy = PLP]

Subject number	Age	Parity	Gestation	Height	Weight	Prostin	Premedication
PEP1	29	0+1	8+1	1.59	57.0	-	Ranitidine,temazepam, maxolon (oral) 08.00
PEP2	32	2+0	≈10	1.65	72.5	+	Omnopon, scopolamine intramuscularly (IM) 09.40
PEP3	21	0+0	≈12	1.65	80.0	+	Pethidine, phenergan (IM), +salbutamol nebuliser
PEP4	26	3+2	≈8	1.78	69.3	-	Temazepam (oral) 07.00
PEP5	21	0+0	9+3	1.57	51.0	+	Temazepam (oral) 08.45
PEP6	33	0+0	10+5	1.52	51.0	+	Temazepam (oral) 11.10
Mean (n=6)	27		9+5	1.63	63.5		
MEP1	29	3+2	7+2	1.63	50.0	-	-
MEP2	22	0+1	8+1	1.66	40.0	-	Omnopon, scopolamine (IM) 09.45
MEP3	24	0+0	≈10	1.73	67.9	+	Valium (oral) 08.45
MEP4	18	0+0	8+3	1.68	53.6	+	-
MEP5	18	0+0	12+1	1.59	55.0	-	-
MEP6	25	0+0	12+0	1.75	60.5	-	-
Mean (n=6)	23		9+5	1.68	54.5		
PLP1	37	0+1	26+2	1.57	62.5	-	-
PLP2	34	0+0	30+3	1.57	64.0	-	-
PLP3	22	1+2	32+2	1.77	75.0	-	-
PLP4	26	1+2	33+5	1.60	70.0	-	-
PLP5	35	0+0	26+1	1.82	79.2	-	-
PLP6	29	0+0	26+3	1.75	90.0	-	-
Mean (n=6)	31		29+2	1.68	73.5		

All samples were assayed in duplicate for progesterone. The saliva samples from 5 of the 6 women given 'Cyclogest' in early pregnancy were assayed for cortisol. High saliva progesterone levels were confirmed by assay following column chromatography. Statistical analyses were performed using a one-tailed Wilcoxon rank sum test for matched pairs. Correlation coefficients were calculated using linear regresssion.

Results

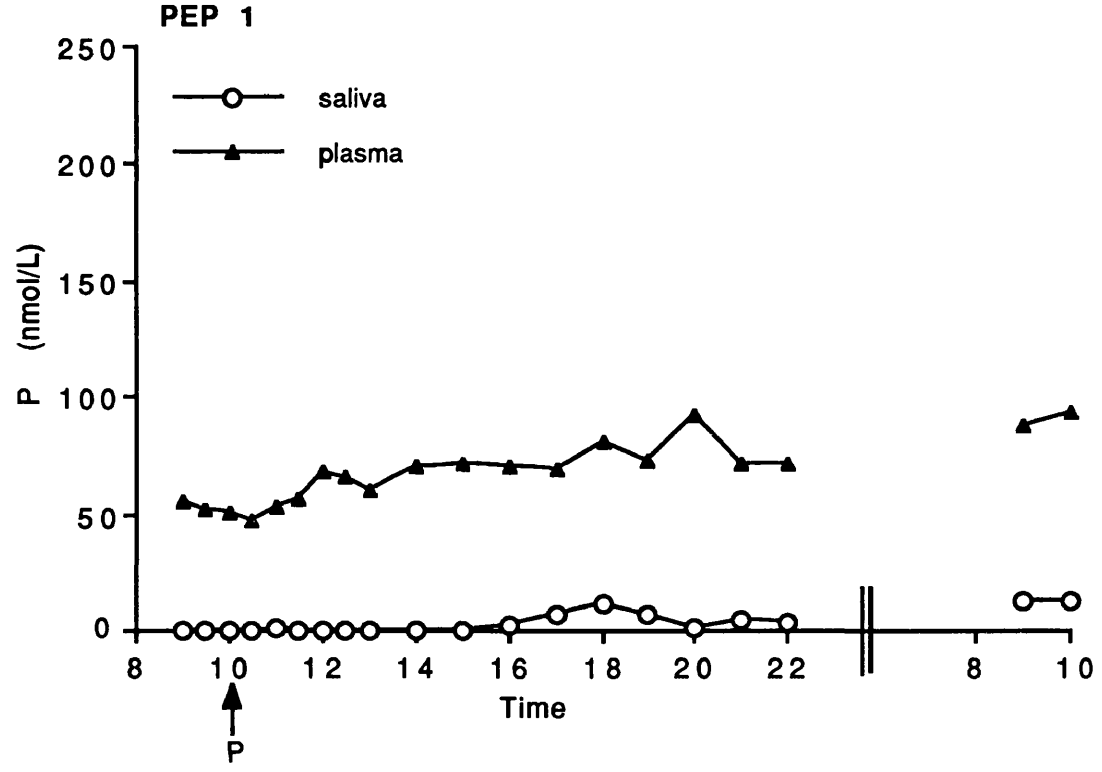
The saliva and plasma levels achieved following progesterone administration are shown in Figs. 8.1-8.4, and the results are also summarized in Table 8.2. The means and times of peaks are calculated using only the paired samples collected between 0 and 12 hours post dose and then at 24 hours post dose, which were available for all patients.

Plasma levels following progesterone administration rose significantly in all three groups ($p<0.025$). 'Cyclogest', 400mg vaginally, in early pregnancy gave a plasma mean individual peak of 110 nmol/L, which was a rise of 55 nmol/L above the baseline values. The same dose in later pregnancy, gave an increment above baseline of a similar order, with a peak rise of 87 nmol/L (baseline 204 nmol/L and mean individual peak 291 nmol/L). The levels remained significantly above baseline for 24 hours post dose in early pregnancy ($p<0.025$), and were above baseline levels for 10-12 hours post dose in later pregnancy, (this rise was not significant due to subject PLP2 having a high mean baseline, and to the large individual variation of time of peak).

Table 8.2 Baseline levels (nmol/L) , peak levels (nmol/L), and time peak attained (hours) following the administration of 400mg progesterone. ['Cyclogest' pessary in early pregnancy = PEP, oral micronised 'Utrogestan' in early pregnancy = MEP, 'Cyclogest' pessary in late pregnancy = PLP, * = higher saliva progesterone levels were attained between 12 and 22 hours post dose]

		SALIVA			PLASMA		
		Baseline	Peak	Time	Baseline	Peak	Time
PEP	1	0.48	13.10	24	53	93	24
	2	0.36	177.00	10	51	154	10
	3	0.55	53.60	2	48	102	9
	4	0.47	226.00	10	43	124	7
	5	0.55	2.60	10	52	73	22
	6	0.88	3.51	0.5	101	136	5
Geometric mean		0.53	25.20		55	110	
Range		0.36-0.88	2.60-226.00	0.5-24	43-101	73-154	5 - 24
MEP	1	0.62	5.26	3	66	733	3
	2	0.43	10.32	10	49	649	10
	3	0.77	20.20	2.5	98	1263	2.5
	4	0.38	2.54	1.5	37	237	1.5
	5	0.40	3.90	3	49	405	3
	6	1.21	14.68	1	113	1537	1
Geometric mean		0.58	7.36		63	668	
Range		0.38-1.21	2.54-20.20	1 - 10	37-113	237-1537	1 - 10
PLP	1	1.94	13.82	5	173	242	10
	2	2.62	35.22*	11*	342	442	2
	3	0.81	3.61	5	139	203	11
	4	1.87	3.64*	12*	288	358	1
	5	1.41	138.97	6	189	259	11
	6	1.37	7.15*	9*	161	303	12
Geometric mean		1.57	13.60		204	291	
Range		0.81-2.62	3.61-138.97	5 - 12	139-342	203-442	1 - 12

Fig. 8.1 Saliva and plasma progesterone (P) levels obtained in 6 subjects (PEP1-6) who were 8-12 weeks pregnant, following administration of a 400mg progesterone pessary vaginally.



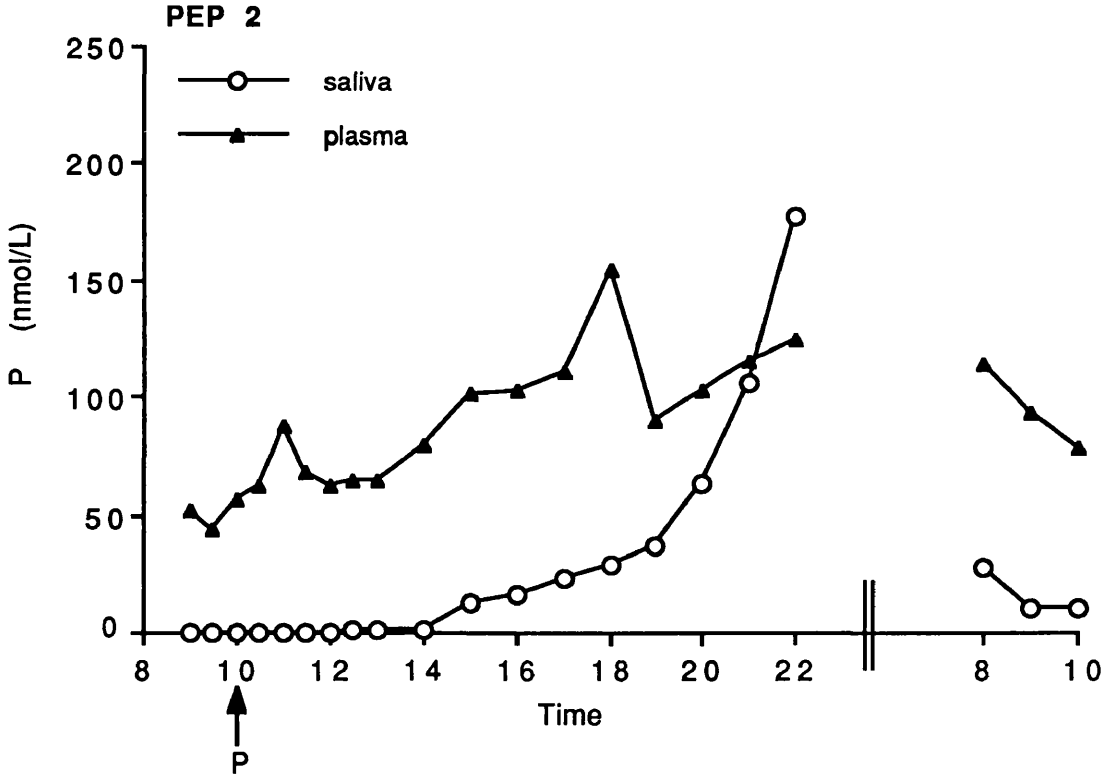
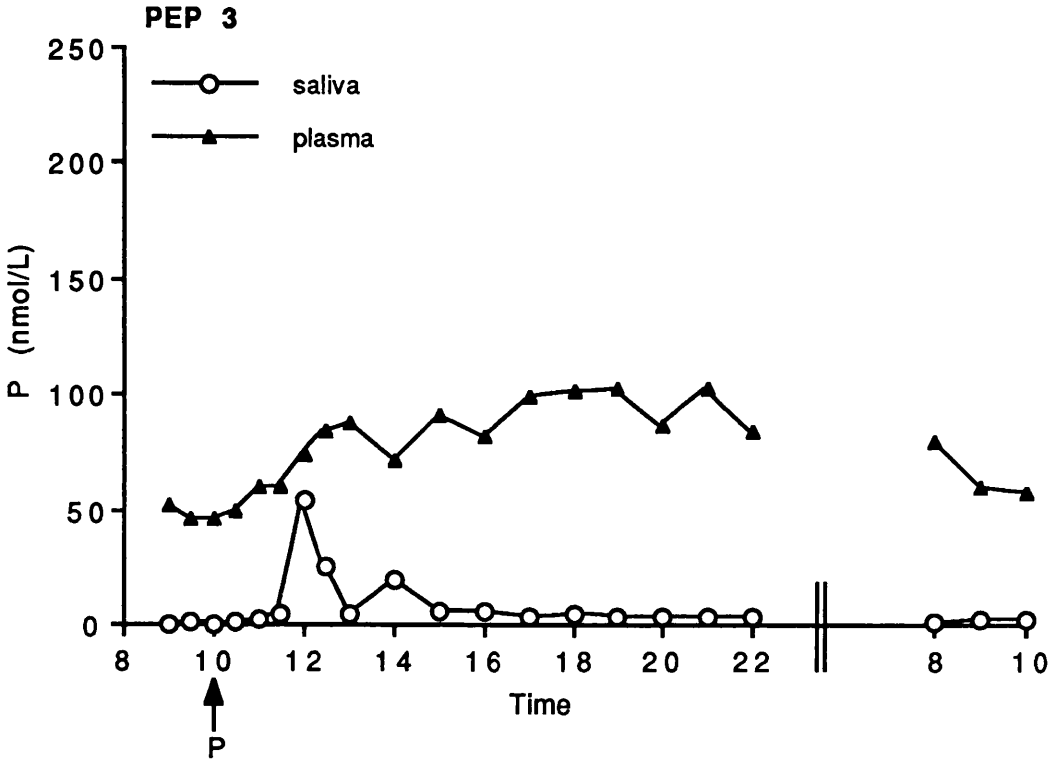
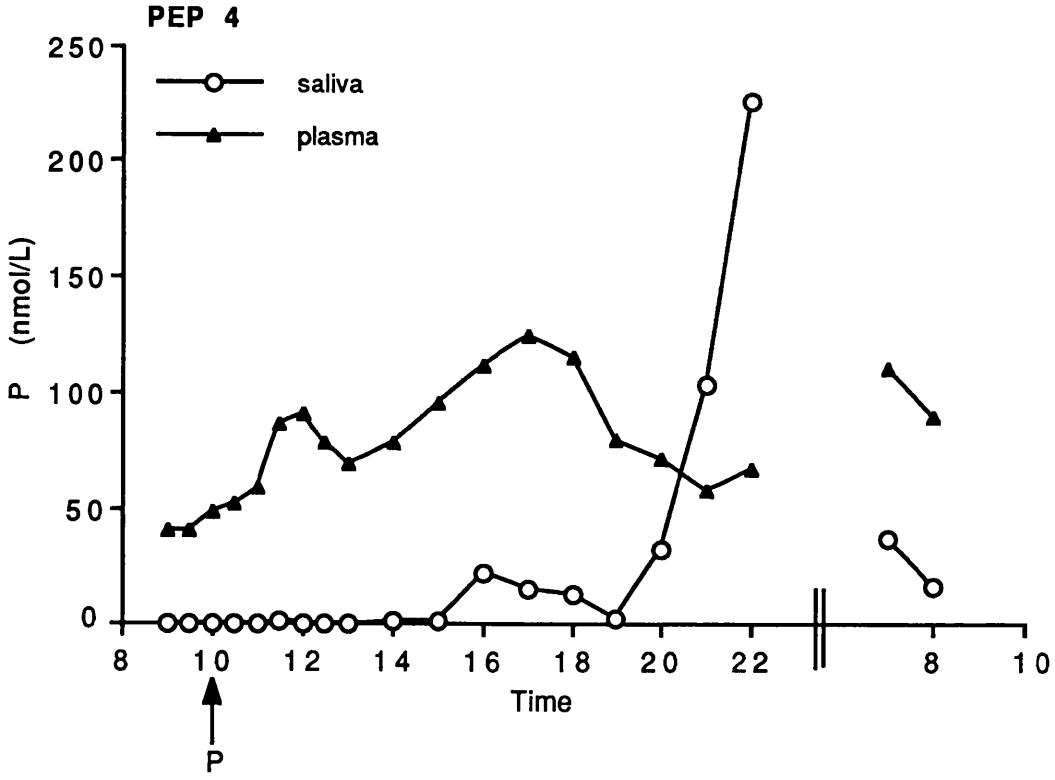


Fig. 8.1 continued





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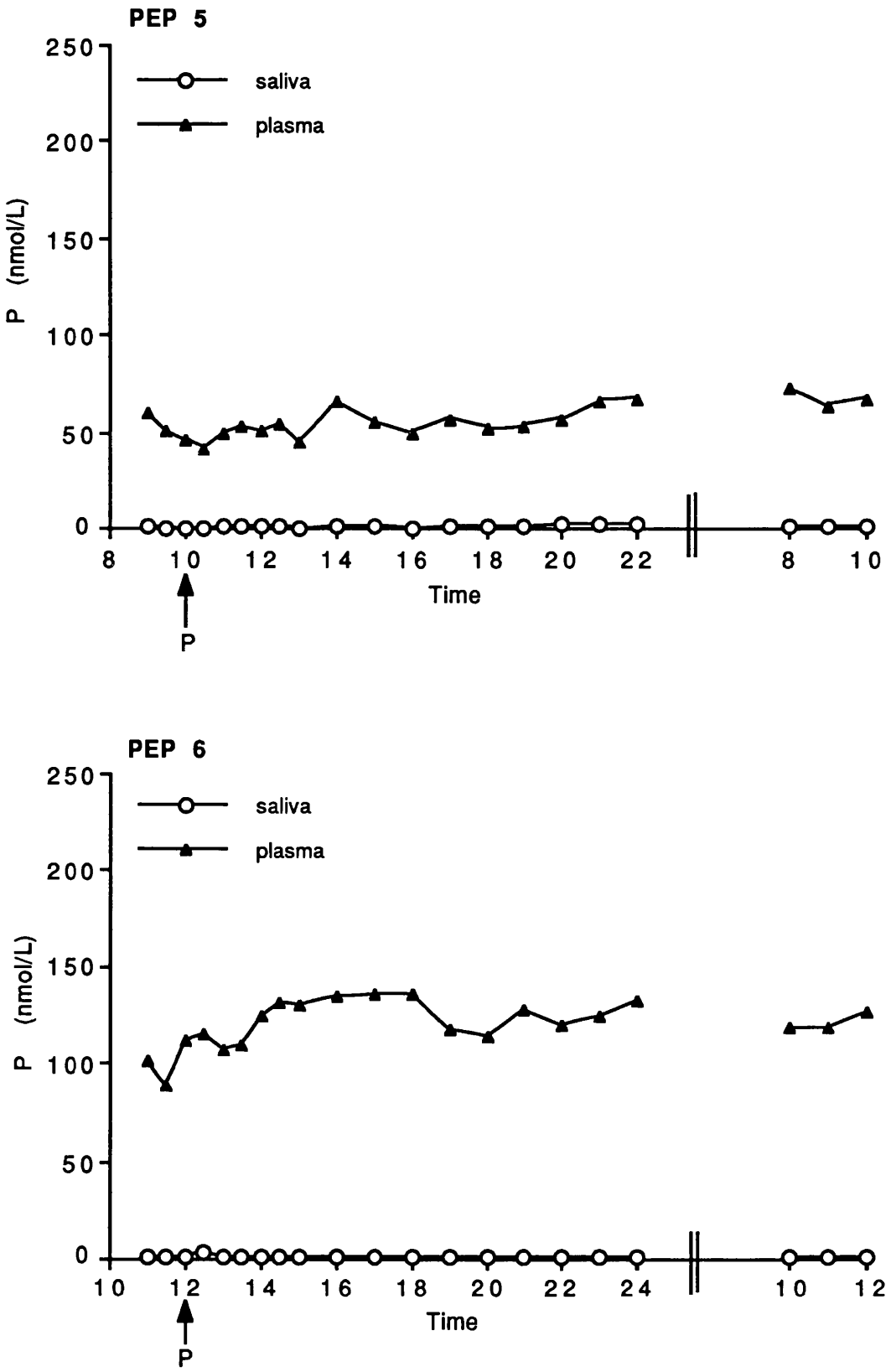
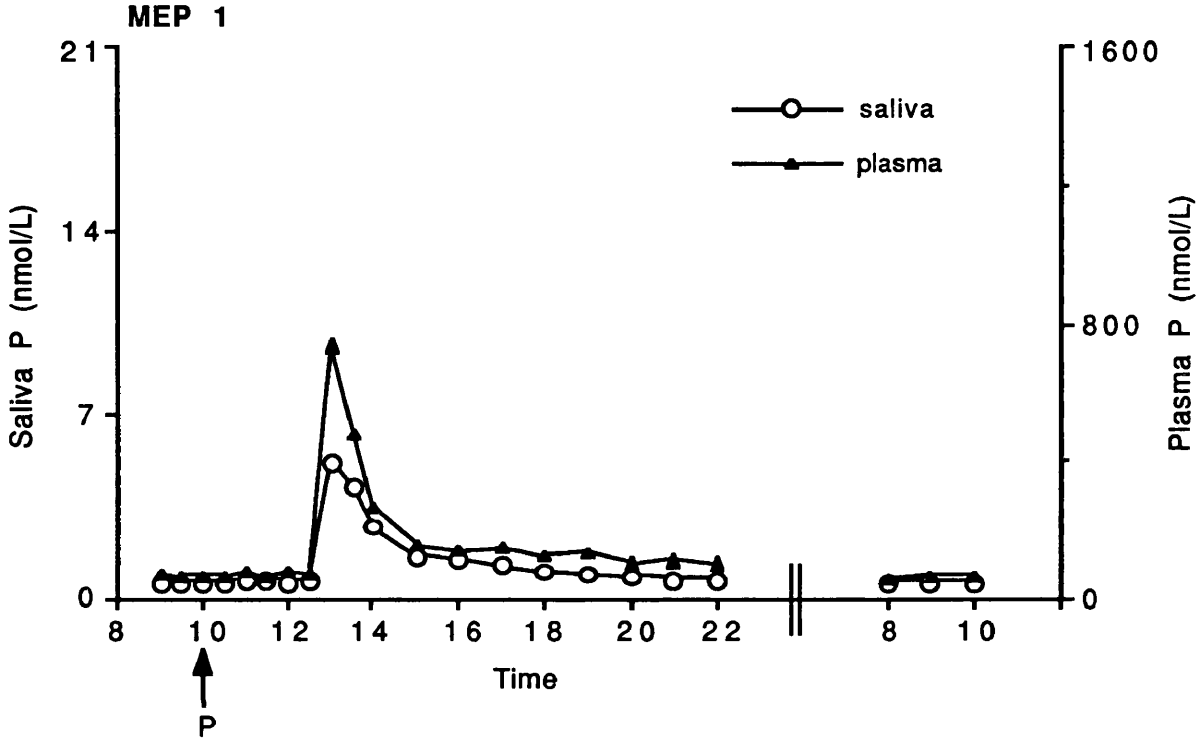


Fig. 8.2 Saliva and plasma progesterone (P) levels obtained in 6 subjects (MEP1-6), who were 7-12 weeks pregnant, following oral administration of 400mg micronised progesterone.



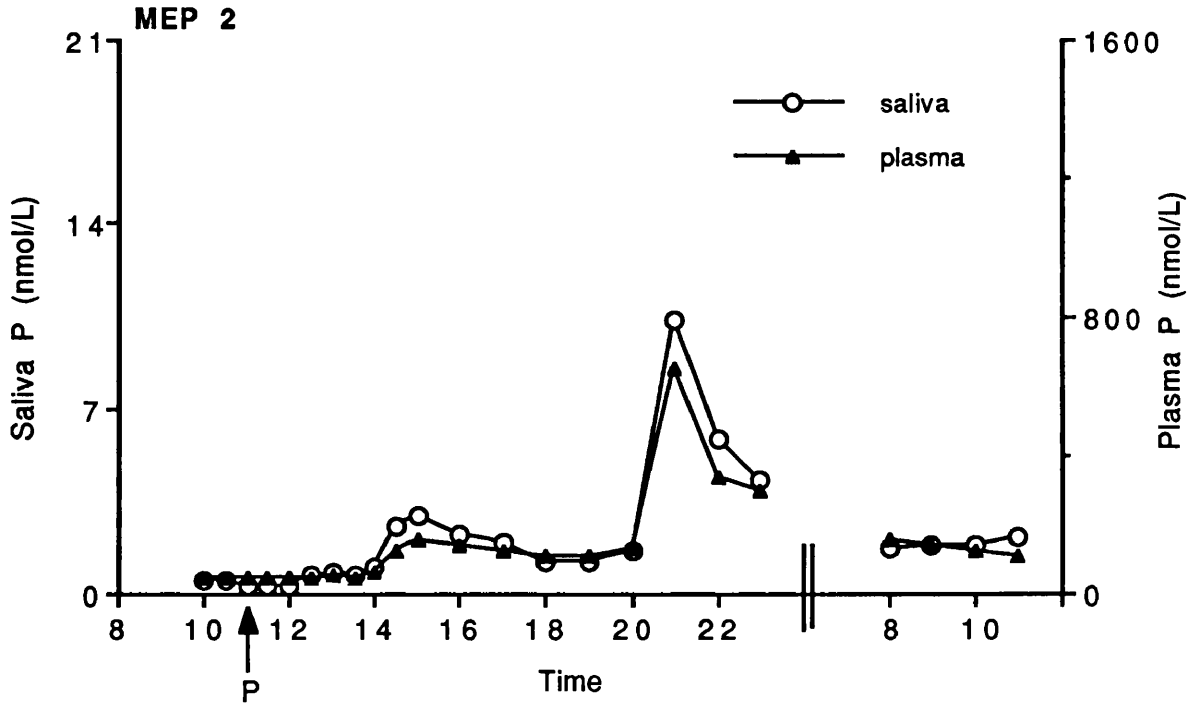
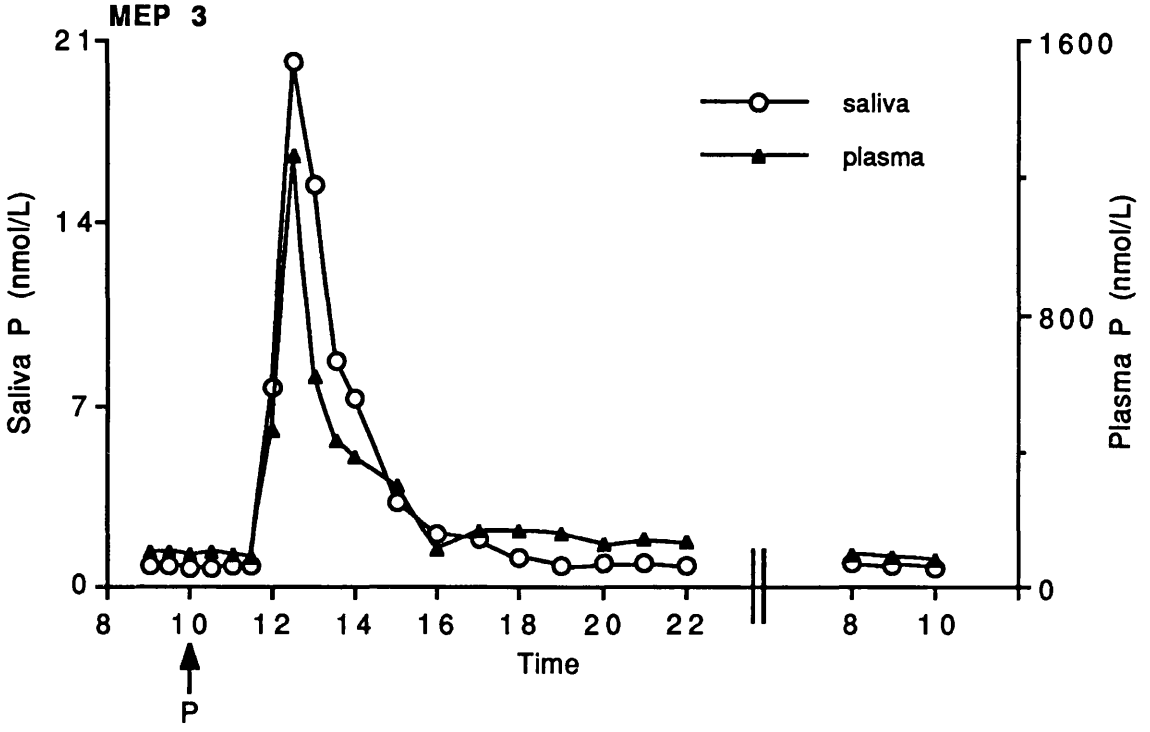
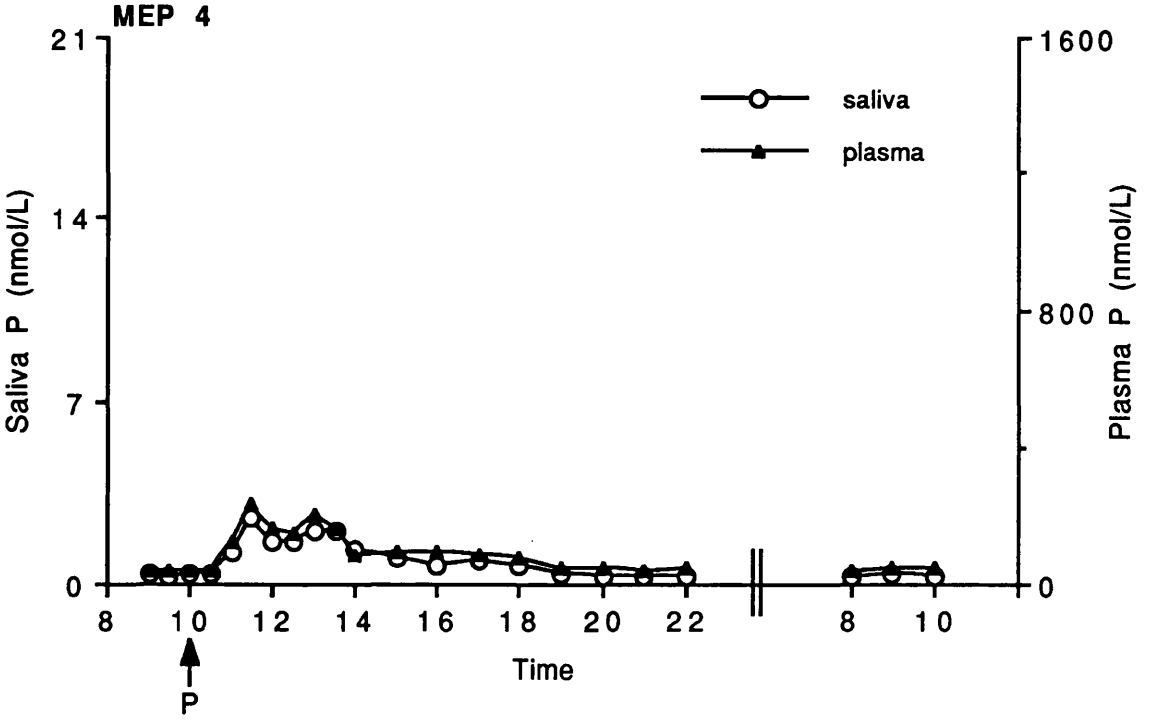


Fig. 8.2 continued





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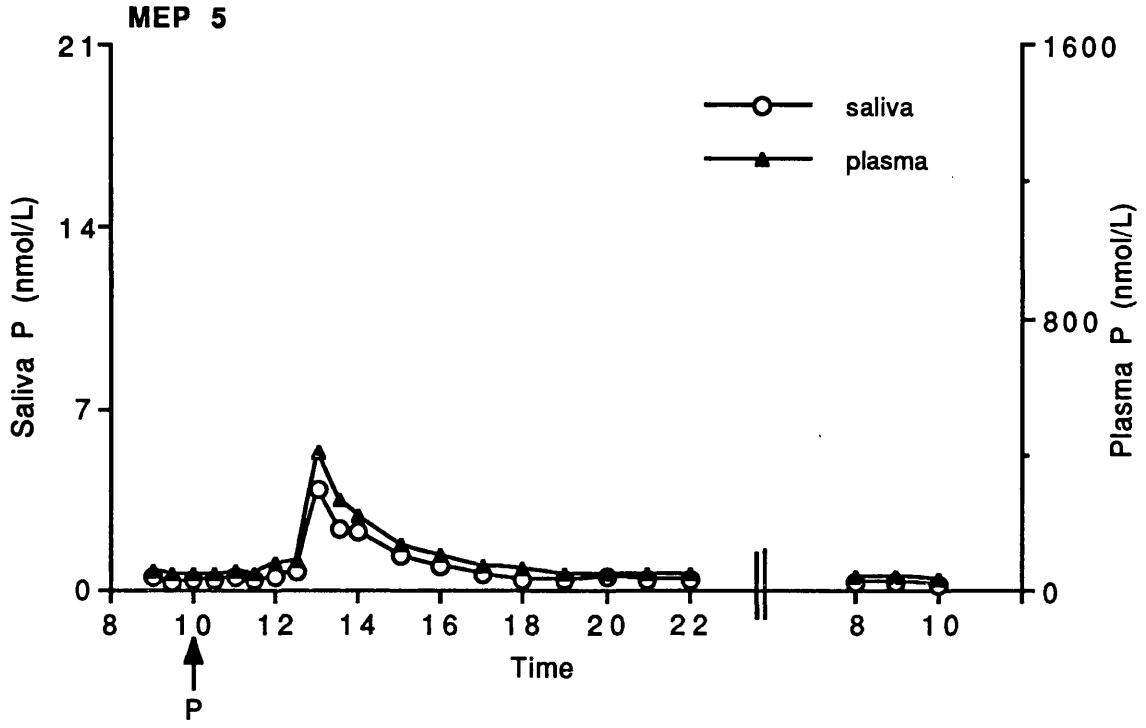


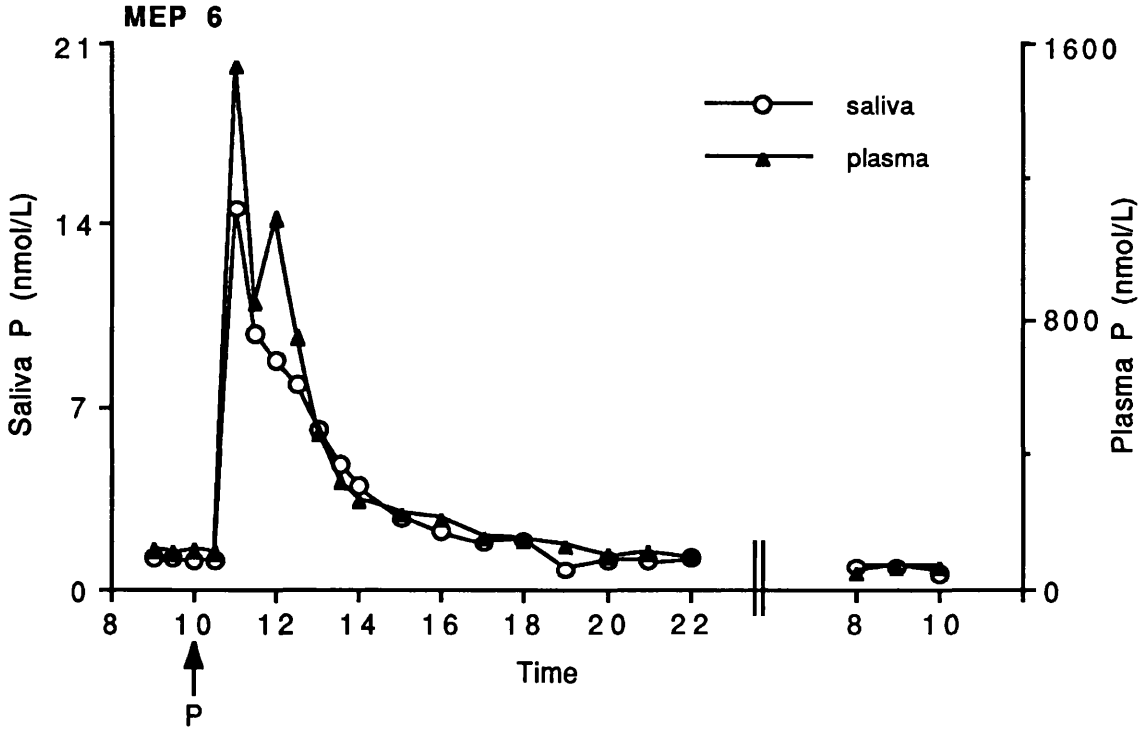
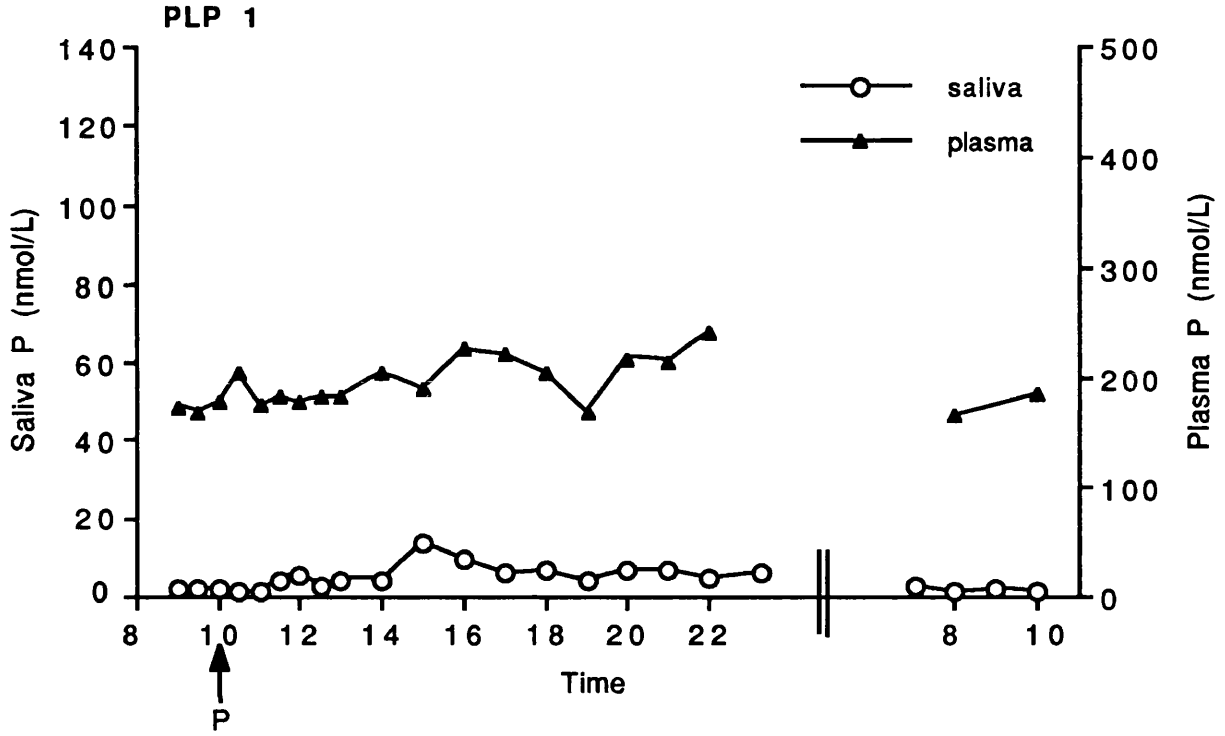


Fig. 8.3 Saliva and plasma progesterone (P) levels obtained in 6 subjects (PLP1-6) who were 26-33 weeks pregnant, following administration of a 400mg progesterone pessary vaginally.



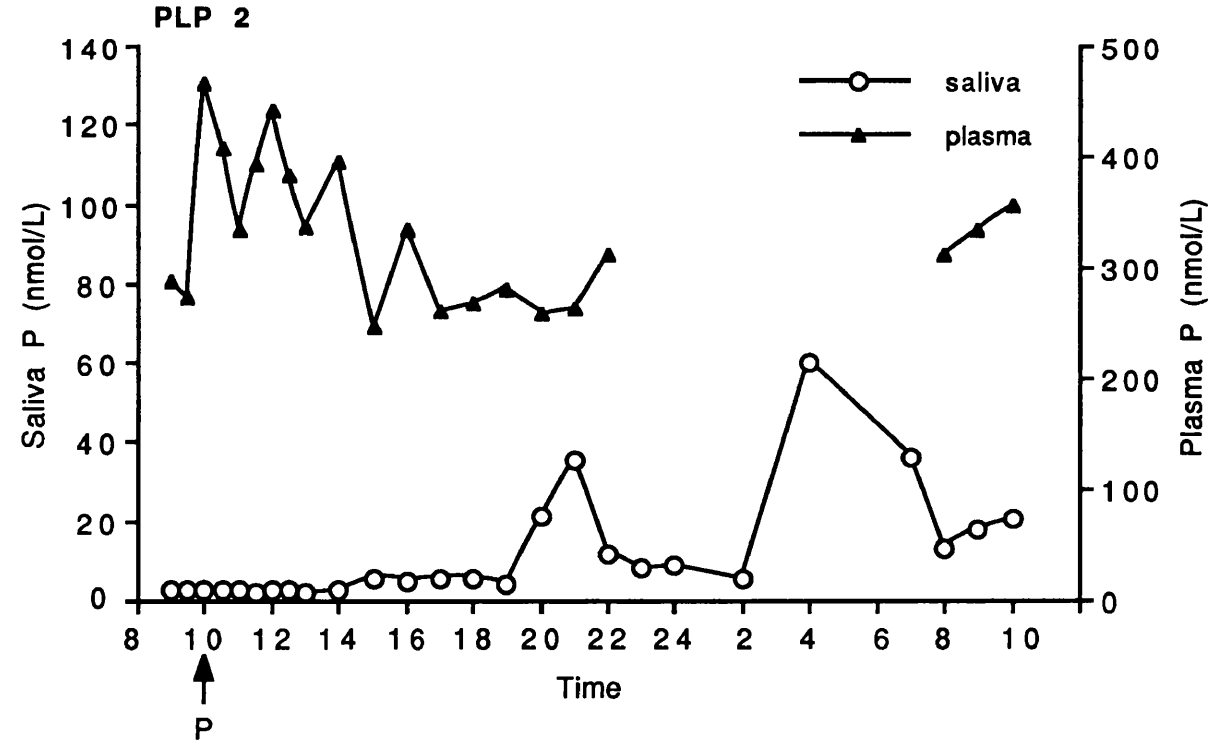
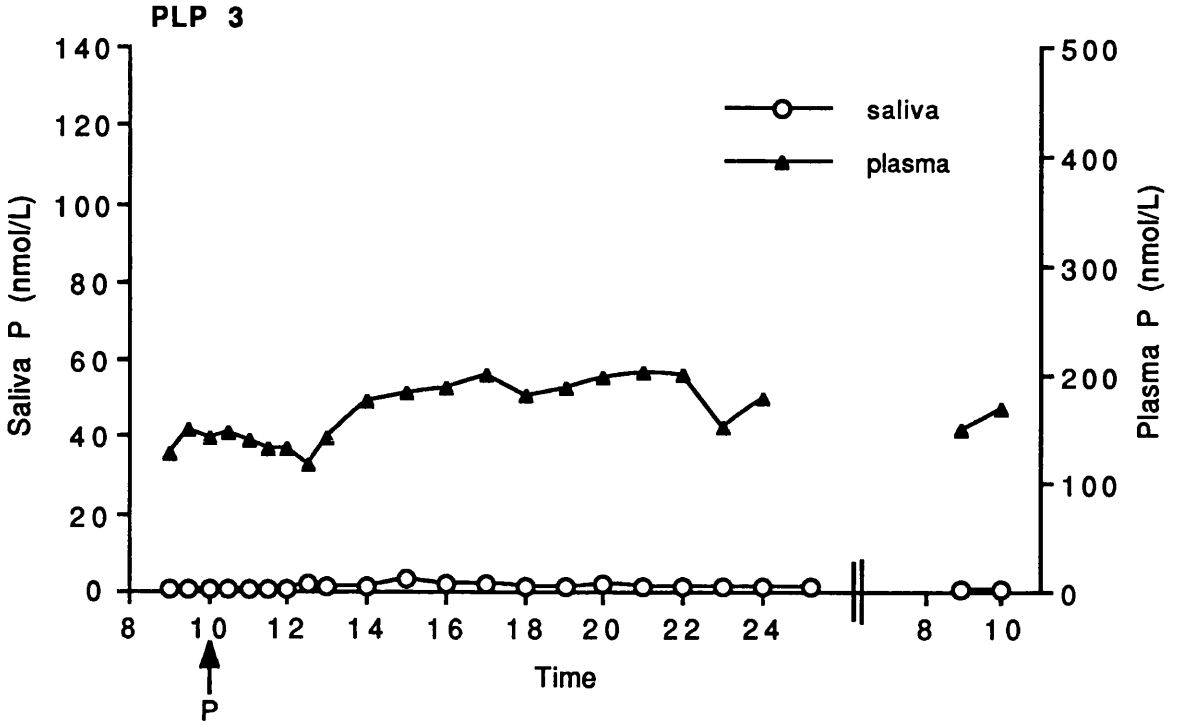


Fig. 8.3 continued



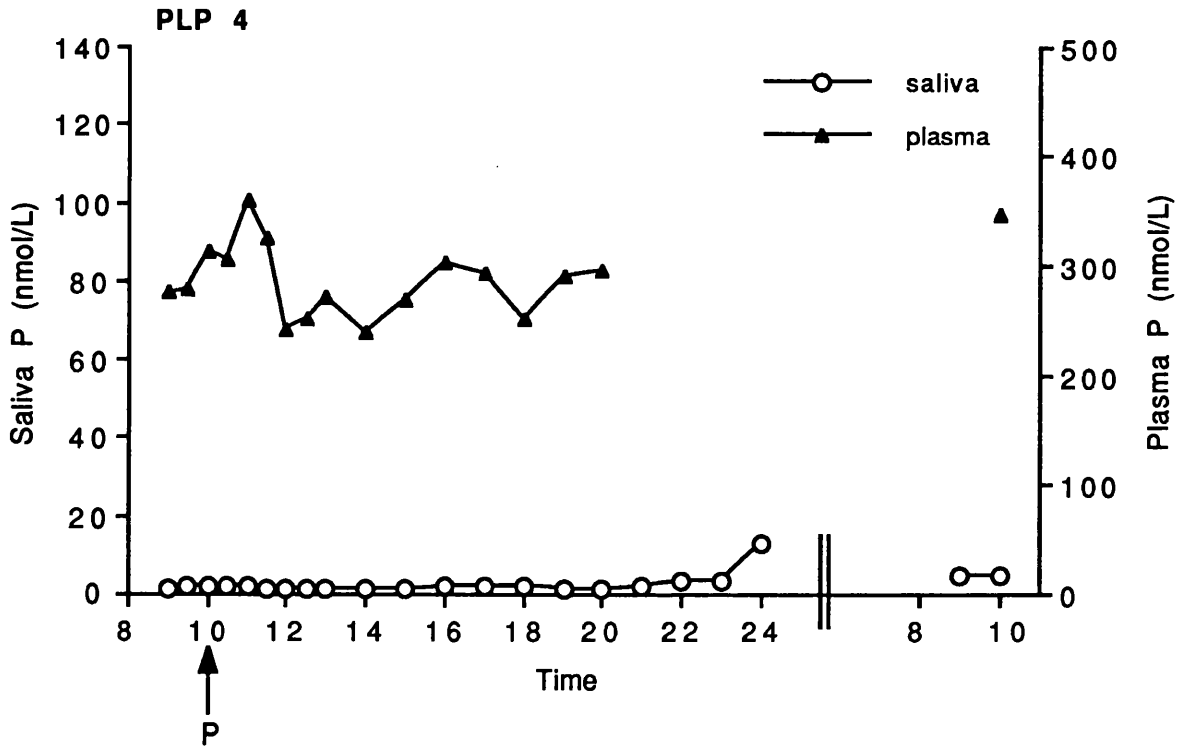
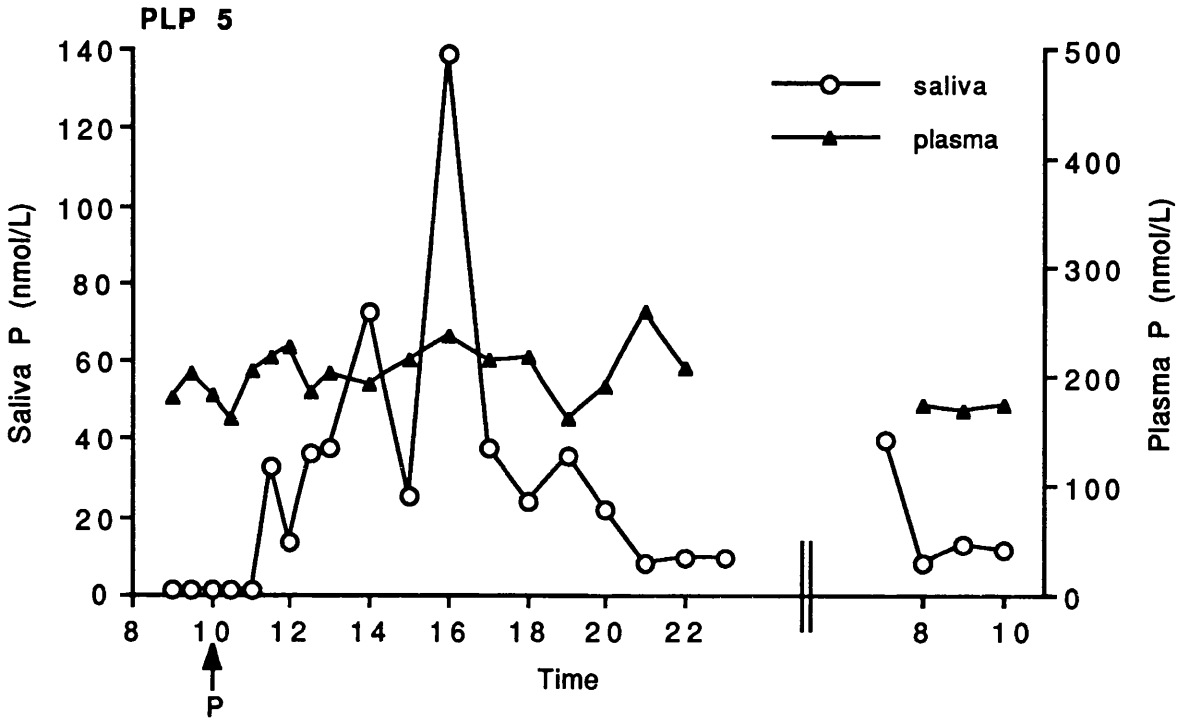
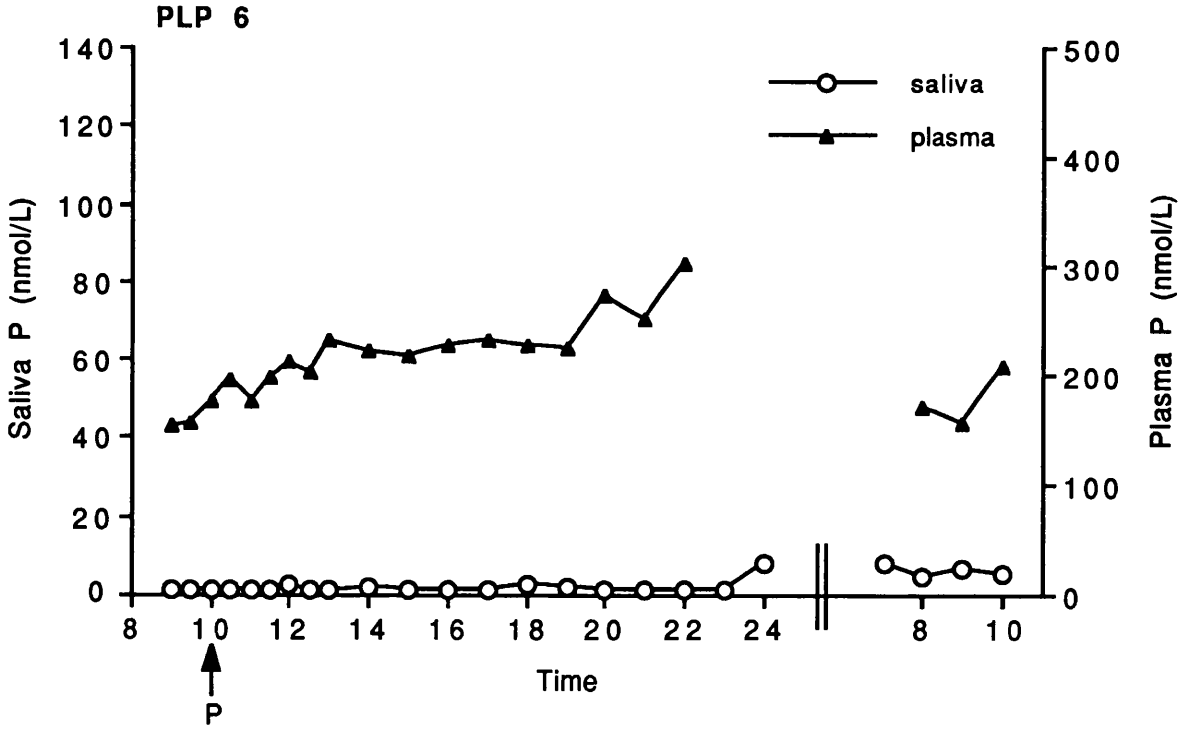


Fig. 8.3 continued





However, in early pregnancy 'Utrogestan' achieved much higher plasma mean individual peaks (668 nmol/L), than the vaginal pessaries, representing a peak rise of 605 nmol/L above baseline. The plasma progesterone levels remained significantly elevated for 8 hours post dose ($p<0.025$).

The saliva levels in all three groups were also significantly raised above baseline values ($p< 0.025$). With 'Utrogestan' the saliva levels were between 0.5 and 2.5% of the plasma levels, and thus reflected the levels of 'free' progesterone in plasma. The saliva mean individual peak was 7.36 nmol/L, representing a rise of 6.78 nmol/L above baseline, and the levels remained significantly above baseline for 7 hours post dose ($p<0.025$).

Unexpectedly high saliva levels were achieved with the 'Cyclogest' pessaries. The mean individual peaks in early and late pregnancy were 25.2 and 13.6 nmol/L respectively, representing rises of 24.67 and 12.03 nmol/L respectively. However, the individual variation of rise in saliva levels was so large as to make the calculated means of questionable value. The saliva levels remained significantly above baseline for 24 hours in early pregnancy and for 12 hours in later pregnancy ($p<0.025$).

The most notable feature of the saliva levels following administration of 'Cyclogest' was that, in contrast to the 'Utrogestan' subjects, saliva progesterone levels bore no relation to the changes in plasma levels, either in the individual peak levels achieved or in the time at which the peak level occurred. In fact, three of the six women given 'Cyclogest' had saliva peaks which were higher than the plasma levels taken at the same time. The correlation coefficient for plasma and saliva progesterone levels following 'Utrogestan' was 0.92, $p<0.0001$; whereas there was no significant

Fig. 8.4 Saliva and plasma progesterone (P) levels obtained in one subject, who was 21+ weeks pregnant, following the administration of 100mg progesterone intramuscularly.

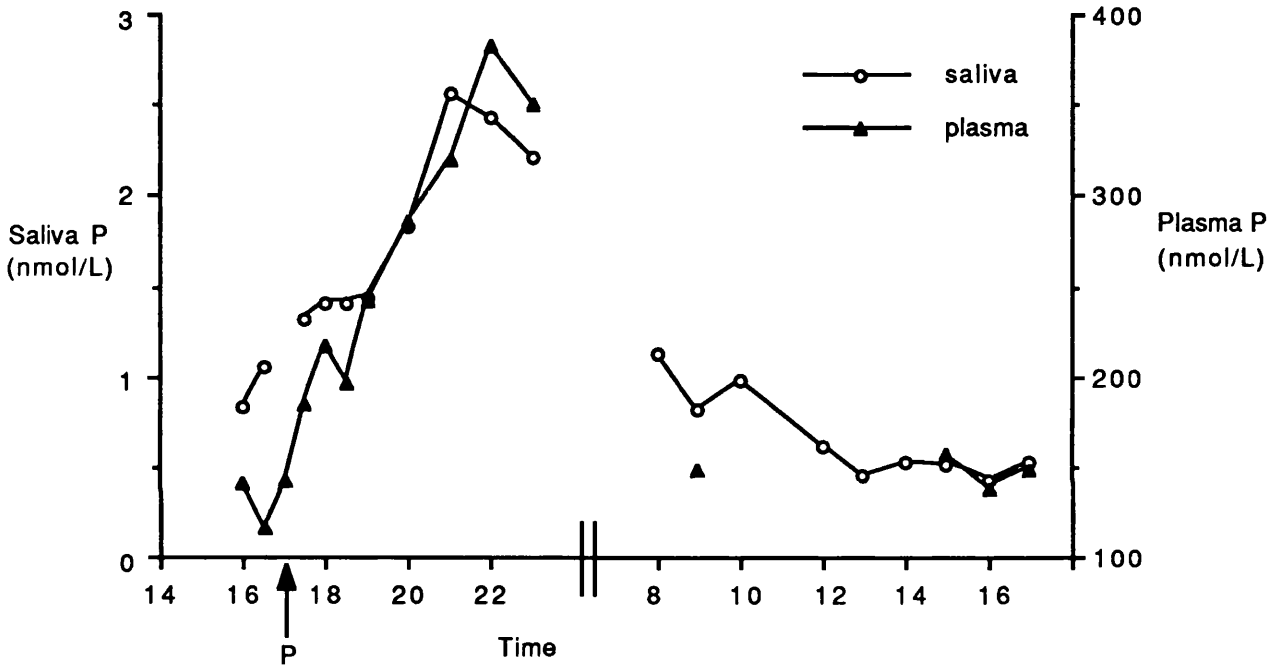
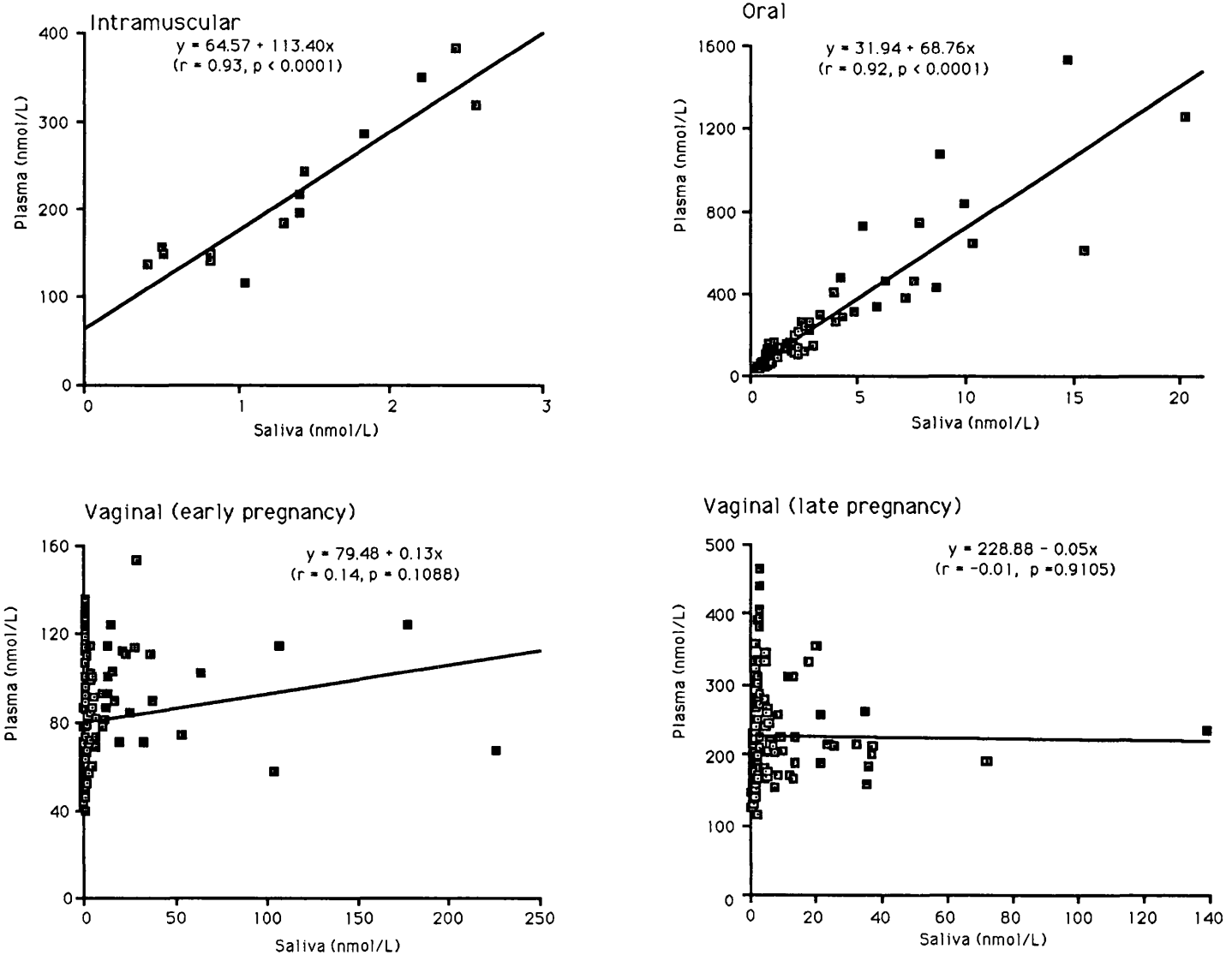


Fig. 8.5 The relationship between saliva and plasma values obtained following the administration of progesterone given via intramuscular, oral (micronised), and vaginal routes. The regression lines correlation coefficients (r), and probabilities (p) for each group are shown on the appropriate graphs.



correlation when progesterone was given vaginally as 'Cyclogest' in either early or late pregnancy (Fig. 8.5).

Saliva cortisol levels were measured in all the samples of the first 5 women given 'Cyclogest' in early pregnancy, to see if there was any relationship between the high salivary progesterone levels and cortisol, as both of these hormones bind primarily to the same binding site on corticosteroid binding globulin. No relationship was found, providing evidence against the hypothesis that the high saliva P levels were a result of displacement of P from CBG binding sites by high levels of cortisol.

In the single patient given 100mg intramuscular progesterone (Gestone), the saliva and plasma levels rose from baselines of 0.94 nmol/L and 133 nmol/L respectively to peaks of 2.56 nmol/L and 383 nmol/L respectively at 4 and 5 hours post administration (Fig. 8.4). There was excellent correlation between saliva and plasma levels ($r=0.93$, $p<0.0001$) with the saliva levels being 0.3-0.9% of plasma levels (Fig. 8.5).

None of the patients involved in the study suffered from any apparent side-effects.

Discussion

It is clear from this study that progesterone levels in pregnancy can be raised by the administration of progesterone via the oral, vaginal and intramuscular routes.

Intramuscular absorption was not studied in detail because it is a painful injection, requiring expertise for administration, and would not be an ideal or popular route with patients if long-term administration was required. However, intramuscular progesterone has been extensively used in the past, and does have the advantage that it avoids first-pass metabolism in the gut and liver, thus increasing bioavailability.

There was considerable interindividual variation in this study, similar to that found by many previous authors (Tables 8.3 and 8.4). Oral 'Utrogestan' produced higher peak levels which returned to baseline more quickly than those following vaginal 'Cyclogest'. The rise in plasma levels following 'Utrogestan' was probably comparable with the only other study in pregnancy, bearing in mind that in that study only one plasma sample was taken one hour post dose *(Erny et al, 1986)*. Although most other studies using 'Utrogestan' involved a lower dose, the rise in plasma levels obtained in this study was rather higher than that which would have been expected from looking at previous data (Table 8.3). However, previous studies usually involved postmenopausal women, and it has been suggested that there is a positive correlation between peak progesterone levels attained and baseline oestradiol levels *(Maxson et al, 1985)*.

Orally administered progesterone is extensively metabolized in the intestine to compounds with less progestational activity, and progesterone which is absorbed unchanged into the portal circulation is then exposed to rapid hepatic reductive metabolism *(Adlercreutz and Martin, 1980)*. For this reason, attention was concentrated for many years on the administration of synthetic progestogens, which did not have this problem. However, it would seem preferable to administer the natural hormone (particularly in

Table 8.3 Results of previous studies on the absorption of progesterone administered by the oral, sublingual and nasal routes.

Route		Dose (mg)	Form/Make	Baseline P (SEM/ range) (nmol/L)	Peak P (SEM/range/ *SD) (nmol/L)	Time of peak (hours)
Oral	1	400 stat	Utrogestan	323 (140-842)	484 (146-1812)	1
	2	100 stat	Utrogestan	(1.1-6.0)	55.6	1 - 4
	3	200 stat	Utrogestan	-	14.9 (3.7)	1 - 4
	4	100 mané} for 5	Utrogestan	1.4 (0.27)	22.7 (4.9) am	2.4 (1-4)
		200 nocté} days			47.7 (5.9) pm	3.6 (2-8)
	5	200 stat	Micronised in plain gelatin capsules	<3.2	54.0 (15.6)	2.8 (0.35)
	6	100 mané for 5 days	Pure P in capsule (Carnrick Labs.)	1.3 (0.14)	29.8 (4.7)*	1 - 3
	7a	100 stat	P on lactose carrier	-	27.3 (12.1-42.6)	2
	b	100 stat	P/cholesterol pivalate mixture on lactose	-	15.6 (12.1-19.0)	4
	8a	100 stat	Micronised P in a wax matrix	1.5 (0.5)	12.4 (1.4)	4 - 5
	b	200 stat	"	1.4 (0.5)	32.5 (10.1)	4 - 5
	c	100 stat	Micronised P in a gelatin capsule	3.1 (0.4)	31.9 (8.9)	2
	9a	200 stat	Plain milled P	<1.5	30.5 (7.9)	4
	b	200 stat	Micronised P	<1.5	42.0 (7.6)	3.2
	c	200 stat	Plain milled P in oil	<1.5	35.9 (9.5)	4.1
	d	200 stat	Micronised P in oil	<1.5	96.3 (22.3)	2
	e	200 stat	Micronised P in gelatin capsule	<1.5	35.6 (9.5)	4.1
Sublingual	1	50 stat	P in 1 ml suspension	2	12.6 (4.8)	0.5
	2a	50 stat	Micronised P in gelatin capsule	1.3 (0.4)	33.5 (5.6)	1
	b	100 stat	"	2.1 (0.4)	56.0 (12.0)	1 - 2
Nasal	1a	20 stat	Pronasone (P part in suspension, part	<1.5	7.9	0.5
	b	30 stat	in solution; contains 200mg/ml)	<1.5	13.0	4
	2a	20 stat	Ointment, 0.2ml of 100mg/ml,1 nostril	2.3 (0.9)	27.0 (4.0)	0.5
	b	30 stat	0.3ml of 100mg/ml, 1 nostril	3.2 (1.3)	18.8 (2.4)	0.5
	c	40 stat	0.4ml of 100mg/ml, 2 nostrils	5.5 (1.0)	26.9 (3.4)	0.5
	d	40 stat	0.4ml of 200mg/ml, 1 nostril	4.8 (1.2)	22.0 (6.7)	1

Table 8.3 cont.

Route		Dose (mg)	Time above baseline (hrs)	Time back at baseline (hrs)	Length of study (hrs)	Number and type of subjects	Author and Year
Oral	1	400 stat	-	-	1	29 women in ?preterm labour	Erny et al, 1986
	2	100 stat	12	24	48	4 women (day 7-8 of cycle)	Ottoson et al, 1984
	3	200 stat	8	24	24	5 women (follicular phase) and 1 man	Nahoul et al, 1987
	4	100 mané} for 5	3	>84	84	5 postmenopausal women	Padwick et al, 1986
		200 nocté} days	7			"	"
	5	200 stat	6	24	24	9 postmenopausal women and 1 man	Maxson et al, 1985
	6	100 mané for 5 days	96	-	96	5 postmenopausal women	Whitehead et al, 1980
	7a	100 stat	4 - 6	6 - 24	24	6 men	Kincl et al, 1978
	b	100 stat	10	>24		"	"
	8a	100 stat	>10	24	24	9 women (follicular phase)	}Chakmakjian and
	b	200 stat	>10	24	24	"	}Zachariah, 1987
	c	100 stat	>8	24	24	13 women (follicular phase)	}
	9a	200 stat	>6	-	6	6 postmenopausal women and 1 man	Hargrove et al, 1989
	b	200 stat	>6	-	6	"	"
	c	200 stat	>6	-	6	"	"
	d	200 stat	>6	-	6	"	"
	e	200 stat	>6	-	6	"	"
Sublingual	1	50 stat	7	24	24	5 postmenopausal women	Villanueva et al, 1981
	2a	50 stat	>8	24	24	8 women (follicular phase)	} Chakmakjian and
	b	100 stat	>8	24	24	"	} Zachariah, 1987
Nasal	1a	20 stat	4 - 6	8	8	8 women (follicular phase)	Steege et al, 1986
	b	30 stat	>8	-	8	"	"
	2a	20 stat	>2	6	6	6 women (follicular phase)	Dalton et al, 1987
	b	30 stat	>2	6	6	"	"
	c	40 stat	2 - 6	>6	6	4 women (follicular phase)	"
	d	40 stat	6	>6	6	"	"

Table 8.4 Results of previous studies on the absorption of progesterone administered by the subdermal, intramuscular, vaginal and rectal routes.

Route		Dose (mg)	Form/Make	Baseline P (SEM/ range) (nmol/L)	Peak P (SEM/range/ *SD) (nmol/L)	Time of peak (hours)
Subdermal	1a	200 stat	Compressed P in pellets, 11.8 x 3.2mm	<3	5.9	? (day 10)
	b	400 stat	"	<3	9.9	"
	c	600 stat	"	<3	13.5	"
Intramuscular	1	100 stat	P in arachis oil (25mg/ml)	1.1 - 6.0	192	8
	2	50 stat	P in suspension (50mg/ml)	0.9	28.1 (6.1)	24
	3a	50 stat	P in oil (Proluton)	<0.64	22.9 (10.2)	2-3days
	b	50 stat	"	<0.64	27.3 (12.1)	2 days
	4a	10 stat	P in arachis oil (5mg/ml)	1.6	22.3	4 - 8
	b	25 stat	P in arachis oil (25mg/ml)	<4.1	89	2 - 12
	c	50 stat	"	1.6	159	2 - 8
	d	100 stat	"	1.9	216.2	2 - 8
Vaginal	1	10mg/day	Ring (polysiloxane core with708mg P)	<3.0	14.0 (7.6-20.7)	5
	2a	50 stat	P in suspension (50mg/ml)	1.1 (0.2)	38.9 (9.9)	1 - 2
	b	"	"	1.1 (0.2)	20.8 (4.1)	1 - 2
	3a	25 stat	Pessary, glycerinated gelatin base	1.0	8.0 (3.8-14.0)	2-2.5
	b	25 stat	Pessary, cocoa butter base	0.8	12.3 (8.3-17.1)	1 - 3
	c	25 stat	Pessary, polyethylene glycol base	1.2	30.9 (23.2-39.7)	2 - 6
	4	100 stat	Micronised P in cocoa butter base pessary	3.0 (0.7)	26.1 (3.1)	2 - 5
	5	100 stat	Pessary, cocoa butter base	<1.6	42.9 (30.2-60.4)	2 - 4
	6	400 stat	Pessary, cocoa butter base	40	77.2	2 - 4
	7a	200 bd., 5 days	Pessary, inert wax base (Cyclogest)	<5.0	46.4 (17.2)*	-
	b	400 bd., 5 days	"	<5.0	53.8 (16.6)*	-
	c	400 bd., 8 days	"	38.2 (10.0)	64.5 (23.2)*	-
Rectal	1a	25 stat	Suppository, cocoa butter base	<2.3	20.3 (4.5-43.9)	2 - 6
	b	100 stat	"	<1.5	71.5 (47.7-165.0)	2 - 8
	2a	200 stat	Suppository, inert wax base (Cyclogest)	<2.3	62.4	2 - 6
	b	200 stat	Suppository, Witespol H15 base	<1.8	64.7	1 - 6

Table 8.4 cont.

Route		Dose (mg)	Time above baseline (hrs)	Time back at baseline (hrs)	Length of study (hrs)	Number and type of subjects	Author and Year
Subdermal	1a	200 stat	-	70 days	150 days	21 women (day 30-35 post partum)	Croxatto et al, 1982
	b	400 stat	-	100 days	150 days	22 women (day 30-35 post partum)	"
	c	600 stat	-	150 days	150 days	32 women (day 30-35 post partum)	"
Intramuscular	1	100 stat	>48	-	48	4 women (follicular phase)	Ottoson et al, 1984
	2	50 stat	>24	-	24	8 postmenopausal (PMP) women	Villanueva et al, 1981
	3a	50 stat	-	5-6 days	10 days	5 women (amenorrhoeic PCO)	Belisle, 1979
	b	50 stat	-	4 days	"	3 women (castrated)	"
	4a	10 stat	24	36	24-48	5 women (2 follicular phase + 3 PMP)	}Nillius and Johansson,
	b	25 stat	36	48	48	5 women (follicular phase)	} 1971
	c	50 stat	48	72	48-78	3 amenorrhoeic women	}
	d	100 stat	48	72	8 - 72	12 (8 follicular phase + 4 PMP)	}
Vaginal	1	10mg/day	-	-	18 days	8 women (follicular phase)	Bäckström et al, 1979
	2a	50 stat	24	-	24	4 postmenopausal women on premarin	Villanueva et al, 1981
	b	"	7	24	24	5 postmenopausal women (no drugs)	"
	3a	25 stat	8	24	24	5 women (follicular phase)	Price et al, 1983
	b	25 stat	8 - 12	24	24	"	"
	c	25 stat	8 - 12	24	24	"	"
	4	100 stat	-	24	24	13 women (follicular phase)	Chakmakjian, 1987
	5	100 stat	12 - 24	36	36	6 women (follicular phase)	Nillius et al, 1971
	6	400 stat	>8	-	8	8 women (luteal phase)	Myers et al, 1987
	7a	200 bd., 5 days	-	-	5 days	5 women (follicular phase)	Glazener et al, 1985
	b	400 bd., 5 days	-	-	"	"	"
	c	400 bd., 8 days	-	-	8 days	10 women (mid-luteal phase)	"
Rectal	1a	25 stat	12 - 24	-	24	6 women (follicular phase)	Nillius et al, 1971
	b	100 stat	12 - 36	-	12 - 48	"	"
	2a	200 stat	-	-	6	3 postmenopausal women and 3 men	Van der Meer et al,1982
	b	200 stat	-	-	6	"	"

pregnancy) rather than progestogens, which have some unwanted attributes as a result of their different chemical configuration.

Since then, it has been found that decreasing particle size by micronisation will increase aqueous dissolution in the intestine and so enhance absorption of progesterone *(Kincl et al, 1978)*. Also, the properties of the vehicle in which a drug is administered affect the degree of lymphatic absorption. With oils, the greater the degree of unsaturation of the fatty acid, the more rapid the onset of chylomicron synthesis. Most lipophilic molecules, that are transported through the lymphatics, reside in the triglyceride core of the chylomicron. Linoleic acid and arachis oil produce the highest concentration of chylomicron in the lymph in rats *(Cheema et al, 1987)*. Avoidance of the extensive prehepatic clearance of progesterone through absorption into the lymphatic rather than the portal circulation should enhance bioavailability, and a synergistic effect of micronisation and an oil base was found by Hargrove et al, *(1989)*. 'Utrogestan' consists of micronised progesterone in an arachis oil base, and does seem to have successfully overcome some of the problems associated with the large first pass effect.

Vaginal administration of progesterone should increase bioavailability by absorption directly into the systemic rather than the portal circulation. 'Cyclogest' consists of progesterone in a base, which is a mixture of mono-, di- and triglycerides of vegetable oils and polyoxyethylene glycerides. The peak rises in plasma levels were not statistically significantly different in early and late pregnancy following 'Cyclogest' administration, and were of a comparable level to previous studies using the same dose (Table 8.4).

However, the saliva progesterone levels attained bore no relationship to the plasma levels and were unphysiologically high. This finding was surprising, and in some paired samples the saliva levels even exceeded the plasma levels. Overall, this phenomenon seems more likely to be due to the pessary formulation than the route of administration, but further studies are necessary, and will be undertaken in the future.

No side effects were noted in any patient in this study. The main side effect of progesterone is said to be drowsiness, and indeed there is a case report of a subject who became unrousable for 2 hours following administration of 400mg micronised progesterone orally *(Arafat et al, 1988)*. Intramuscular progesterone has been documented as causing a severe thigh myositis *(Phipps et al, 1988)*.

No substance should be administered in pregnancy without very careful consideration of any possible teratogenic effects. Progesterone is a natural hormone, already present at high concentrations in pregnancy, and it therefore seems less likely to be teratogenic than synthetic derivatives of progesterone. The studies which have been performed to look for any possible teratogenic effect of exogenous administration of progesterone have failed to find an increased incidence of congenital malformations *(Michaelis et al, 1983; Resseguie et al, 1985; Check et al, 1986; Cunha et al, 1988; Scialli, 1988)*. Rock et al *(1985)* also found no increased risk of congenital malformations, but suggested that there was an increased risk (28.6%) of spontaneous abortion although the authors stressed that it was an uncontrolled historical analysis with relatively small sample numbers.

In summary, progesterone may be administered conveniently via the oral or vaginal route. However, further investigation of the optimum

formulation of pessary and dose regime in pregnancy for each route is necessary, before a controlled trial of the use of progesterone for the prevention or treatment of preterm labour could be performed.

9

A Serial study of the Interrelationships between Oestrogens, Progesterone, Dehydroepiandrosterone Sulphate, ß-Human Chorionic Gonadotrophin, Human Placental Lactogen and Prolactin in Maternal Plasma and Saliva in the 2nd and 3rd Trimesters of Pregnancy

Introduction

The fetus, the placenta and the 'fetoplacental unit' secrete a wide variety of steroid, protein and glycoprotein hormones. The production of these hormones varies with gestational age, allowing a relatively quiescent uterus throughout pregnancy until term and the onset of parturition.

Many studies, both *in vivo* and *in vitro*, have looked for relationships between the various hormones secreted, and tried to find possible controlling factors for the changing concentrations of the hormones with gestation. From these studies a variety of possible interrelationships or controlling factors have been suggested, and these are summarized below.

Human chorionic gonadotrophin (hCG)

The evidence for stimulation of fetal adrenal steroidogenesis by hCG is mixed. Lauritzen and Lehmann *(1967)* suggested that hCG is an adrenocorticotrophic hormone in the fetus, concerned with regulating the supply of fetal adrenal dehydroepiandrosterone sulphate (DHEAS) as a precursor for the production of oestrogens in the placenta. hCG has been reported to stimulate the conversion of cholesterol to pregnenolone and progesterone *(Villee et al, 1966)*, promote hydroxylation of placental steroids *(Troen, 1961; Varangot et al, 1965)*, and also promote placental aromatization and stimulate ß-hydroxysteroid dehydrogenase *(Tojo et al,*

1982). Significant increases in DHEAS synthesis occurred after the addition of hCG to isolated cells from the fetal adrenal *(Serón-Ferré et al, 1978),* and there was a significant increase in urinary DHEAS secretion in 4 out of 7 newborn infants after administration of exogenous hCG *(Lauritzen et al, 1969).*

However, in a study of chronically catheterized Rhesus monkeys, infusion of hCG had no effect on fetal or maternal plasma steroid levels *(Walsh et al, 1979).* In a more recent study by Fujieda et al *(1981)* hCG had no stimulating effect upon DHEA or DHEAS production, either in cultures of whole adrenals or in cultures of separated fetal zone and definitive zone cells. Johannison *(1968)* found morphological changes suggesting stimulation in the fetal zone of the human fetal adrenal after injecting hCG into the amniotic cavity, and atrophic changes after neutralizing circulating hCG with an antibody, when she studied women in pregnancies between 17 and 22 weeks gestation. Conversely, *in utero* injection of hCG in a woman in the third trimester with an anencephalic pregnancy failed to show stimulation of DHEAS secretion, and subsequent histochemical observations of the fetal adrenals failed to show signs of adrenal activation *(Honnebier et al, 1974).*

Human Placental Lactogen (hPL)

The role of hPL in pregnancy is still uncertain. However, hPL levels correlate with the functioning trophoblast mass *(Spellacy, 1973),* and third trimester hPL measurements have been used for some time as an indirect assessment of fetal well-being, which is largely dependent upon placental function.

hPL is known to have a variety of effects including lactogenic activity, weak somatotrophic effects, and effects on fat and carbohydrate metabolism *(Buster and Simon, 1989).* It has been suggested that the considerable inter-conversion of progesterone (P) and 20α-dihydroprogesterone (a weaker progestagen) which occurs in human gestation probably both in the placenta and in the fetus, might be partially under the control of hPL *(Josimovich, 1983).* However, in spite of these diverse actions, another suggestion is that hPL may be simply one of the waste products of the placenta *(Gordon and Chard, 1979).* Certainly there have been at least 7 documented cases of completely normal pregnancies in women with low or absent hPL levels *(Bradford and Hargreaves, 1978; Gaede et al, 1978; Nielson et al, 1979; Moshirpur et al, 1981; Borody and Carlton, 1981; Di Renzo et al, 1982; Barbieri et al, 1986).* Gaede et al *(1978)* found prolactin (PRL) and hCG levels to be raised in samples taken between 38 and 40 weeks gestation in a woman with low hPL; but this finding was not confirmed in subsequent reported cases where hCG and PRL levels were normal *(Nielson et al, 1979; Borody and Carlton, 1981).* Where they were measured, there was no abnormality in oestriol or progesterone levels.

Prolactin (PRL)

Like hPL, prolactin is a hormone to which multiple roles have been attributed, although none are as firmly established as its role in lactation and in the control of osmotic processes *(Josimovich, 1983).* Like hCG, prolactin has been considered to be a major fetal adrenocorticotrophic modulator in pregnancy, with the hypothesis being strengthened by the finding of abundant adrenal prolactin receptor activity in experimental animals *(Buster and Simon, 1989).* Cord PRL levels increase with advancing gestational age, and neonatal levels decrease during the first neonatal week; these changes correlate well with the increase in fetal adrenal weight during

pregnancy, and adrenal gland involution following birth *(Winters et al, 1975)*. Such findings were consistent with the view that PRL has a role to play in the control of fetal adrenal growth. In intact eels, ovine prolactin stimulates adrenal growth and partially retards involution of the adrenal induced by hypophysectomy *(Olivereau and Olivereau, 1970)*. Supporting evidence for an adrenal effect is that adrenal involution occurs after mid-gestation in the anencephalic fetus in association with low ACTH, prolactin and oestrogen levels *(Winters et al, 1975)*.

Although very high levels of PRL are present in the circulation and amniotic fluid, few investigations of the effect of PRL on placental function have been reported. Yuen et al *(1980)* found that pregnant women with prolactinomas, in whom long-term suppression of PRL was achieved using bromocriptine, had augmented hCG, oestriol (E3) and possibly oestradiol (E2) but not P levels compared to control pregnant women and suggested that prolactin had a role to play in the control of hCG and oestrogen secretion in the fetoplacental unit. Conversely, acute changes in PRL concentration had no effect on the secretion of P, E2 or hCG during early pregnancy *(Yuen et al, 1980; Ranta et al, 1980)*. At term, PRL incubated with placental explants suppresses hCG secretion *(Yuen et al, 1986)*. In another study using placental explants from term pregnancies, PRL at physiological concentrations increased P secretion and seemed to have a possible inhibitory effect on E2 secretion, providing further evidence for the modulation of placental steroid secretion by PRL *(Barnea et al, 1989)*. However, in the study on chronically catheterized Rhesus monkeys, infusion of PRL had no effect on fetal or maternal plasma steroid levels *(Walsh et al, 1979)*, and in a study on pregnant women at term, there was no correlation between fetal or maternal PRL levels and any of the steroids (which included

E2, P, DHEA, DHEAS and oestriol sulphate) measured *(Laatikainen et al, 1980).*

In non-pregnant women and men, PRL was thought to modulate the secretion of DHEAS, as an increase in PRL levels correlated with elevated concentrations of DHEAS *(Lobo et al, 1980).* Conversely, studies in pregnant women have suggested that the control of fetal production of DHEAS by the adrenals is not specifically PRL dependent *(Del Pozo et al, 1980; Lehmann et al, 1979).* In the study by Fujieda et al *(1981)* PRL did not have a stimulating effect upon DHEA or DHEAS production, either in cultures of whole adrenals or in cultures of separated fetal zone and definitive zone cells.

Another postulated role for PRL is the inhibition of fetal membrane prostaglandin production *(Tyson et al, 1985).* The increase in uterine prostaglandin gradients in labour was not associated with changes in the local concentrations of prolactin *(Davidson et al, 1987)*, although Rigg and Yen *(1977)* found a significant decrease in maternal peripheral prolactin levels during active labour. Bigazzi and Nardi *(1981)* suggested that PRL has a stimulating effect on uterine contractility, as they demonstrated an increase in the frequency and amplitude of rat uterus spontaneous contractions in response to PRL *in vitro.*

Steroids

A] Effect on hCG

At term, it has been found that hCG concentrations are slightly higher in women bearing female infants than in those bearing male infants *(Boroditsky et al, 1975; Spellacy et al, 1975; Danzer et al, 1980)*, and it has been suggested that this phenomenon is due to suppression of hCG by one

or more unknown factors of fetal origin *(Braunstein et al, 1980; Boroditsky et al, 1975; Danzer et al, 1980; Wilson et al, 1980).* More specifically, it has been suggested that hCG production is inhibited by a steroid originating in the fetal adrenal *(Haning et al, 1983). In vitro* studies using first trimester placentas showed a decrease in hCG release following the addition of progesterone to the medium *(Wilson et al, 1980; Maruo et al, 1986).* However, Belleville et al *(1978)* were unable to demonstrate this effect using P or E3 as a stimulator. The idea that P inhibited hCG production was favoured by Boroditsky et al *(1975)* although they found no correlation between maternal levels of P and hCG in pregnancy. However, a study on women in early pregnancy given 3 doses of 100mg progesterone intramuscularly showed in initial abrupt and significant rise in hCG following the first dose, with no further rise after the second and third doses *(Yosef et al, 1984).*

Lauritzen et al *(1969)* hypothesised that hCG stimulates the fetal adrenal, thus regulating DHEAS production, and that the oestrogen produced in the placenta as a consequence may increase the production of hCG according to the placental requirement for oestrogen precursors (ie DHEAS), and finally that DHEAS may in turn depress the production of hCG. However, the addition of DHEA or DHEAS to human midterm placenta *in vitro* did not result in a change in hCG secretion, although the oestrogen secretion was increased *(Voutilainen et al, 1981).*

B] Effect on Prolactin

It is known that administration of artificial oestrogens increases the serum concentration of prolactin in ovariectomized and postmenopausal women as well as in men *(Frantz et al, 1972; Yen et al, 1974).* Whether the increase in PRL levels in pregnancy is similarly due to increasing oestrogen

levels is not certain, but a positive correlation between oestradiol and PRL during pregnancy has been described *(Yuen et al, 1980)*. In mid-pregnancy DHEAS administered intravenously or intra-amniotically increases E2 levels in maternal serum and amniotic fluid, and also causes an increase in prolactin *(Ylikorkala et al, 1979)*.

However, a case report by Gipps et al *(1979)* described a pregnant woman with placental sulphatase deficiency who had very low or absent oestrogen concentrations and a prolactin in the middle of the normal range, indicating either that only small concentrations of oestrogens are necessary to sustain prolactin levels or suggesting the involvement of other factors *(Andersen,1982)*. Conversely, in the study by Biswas and Rodeck *(1976)* only those women with low oestrogen levels had prolactin levels below the normal range. Treatment with dexamethasone leading to lowered oestrogen concentrations did not affect prolactin levels *(Kauppila et al, 1979)*, and increasing oestrogen levels following the administration of DHEAS did not lead to a corresponding rise in prolactin in the study by Kauppila and Ylikorkala *(1980)*. Serial studies of oestrogen and prolactin have not shown a direct effect of oestrogens on prolactin *(Hertz et al, 1978; Egyed et al, 1978; Aspillaga et al, 1983)*.

Progesterone administration after oestrogen priming in non-pregnant women can cause an acute elevation of PRL levels *(Rakoff and Yen, 1978)*, but there is currently no conclusive evidence that P is an important physiologic regulator of PRL secretion *(Plosker et al, 1990)*.

C] Effect on other steroids

Buster et al *(1974)* looked at the effect of a large intravenous dose of DHEAS on plasma steroid levels in the second trimester of pregnancy and

found a rise in oestrone (E1) and E2 but not E3 following the infusion. When present in excess, DHEAS did not seem to exert any rate-limiting effect on placental steroidogenesis. Voutilainen et al *(1981)* also found a rise in oestrogen secretion following the administration of DHEA or DHEAS to midterm placenta in culture, and suggested that fetal adrenal 3ß-hydroxysteroid dehydrogenase might be inhibited by a placental factor. Wiener and Allen *(1968)* found that 20α-reductase could be inhibited by a number of steroids including oestriol and oestriol isomers, 16α-hydroxy-DHEA and oestrone, but that 3ß-hydroxysteroid dehydrogenase had fairly specific inhibitor requirements *in vitro* and appreciable inhibition could only be demonstrated by P itself and P metabolites. However, it is now considered that placental oestrogens may be present in sufficiently high concentrations *in vivo* to inhibit fetal adrenal 3ß-hydroxysteroid dehydrogenase and that it may be ACTH and oestrogens acting together which produce the characteristic fetal pattern of steroidogenesis *(Fujeida et al, 1982).*

The above summary gives an idea of the immense amount of conflicting data on the control of steroidogenesis and protein hormone synthesis by the placenta and fetoplacental unit. The aim of this study was to look again for possible interrelationships between oestrone (E1), oestradiol (E2), oestriol (E3), progesterone (P), dehydroepiandrosterone sulphate (DHEAS), sex hormone binding globulin (SHBG), human placental lactogen (hPL), ß-human chorionic gonadotrophin (ß-hCG) and prolactin (PRL) by studying the peripheral plasma levels serially in a group of women from 20 weeks gestation until delivery. As our previous work has suggested that it is the rise in the 'free' oestriol:progesterone ratio which may be of

significance prior to the onset of parturition, both saliva and plasma levels of E1, E2, E3 and P were measured.

The hope was that by measuring *all* of these hormones in the same samples, additional insight might be obtained into the various relationships and controlling factors. This is the first study which includes measurements of all of these hormones together, and which measures the 'free' as well as the total unconjugated oestrogens and progesterone. It was also hoped that serial measurements from a group of women throughout gestation would make apparent any subtle interrelationships which might be missed by a cross-sectional study design.

Method

Women were recruited to the study from the antenatal clinic when they attended for their booking appointment. Women with problematic pregnancies or previous problematic pregnancies were excluded, except for one subject who had had several previous preterm deliveries associated with a bicornuate uterus.

The women were asked to attend every 2 weeks from 20 weeks gestation until delivery. Gestation was calculated from the subjects last menstrual period unless their gestation by early ultrasound differed by 14 days or more. At each visit a 10ml blood sample was taken and the patient simultaneously provided a 3ml saliva sample. The plasma and saliva samples were stored at -40°C until assayed. The saliva and plasma samples were assayed for unconjugated oestrone, oestradiol, oestriol and

progesterone after Sephadex LH20 chromatography. The plasma samples were also assayed for dehydroepiandrosterone sulphate, sex hormone binding globulin, ß-human chorionic gonadotrophin, human placental lactogen and prolactin. The latter four hormones were assayed using commercial 'kit' methods.

20 women were recruited in total, and their individual details are shown in Table 9.1. All the women completed the study but some of them had missing samples and 1 woman delivered preterm. 12 women had a complete collection of plasma and saliva samples and normal pregnancies (apart from 1 woman having mild pre-eclampsia at term). These subjects are marked with an asterisk in Table 9.1 and were used to calculate the medians and centiles for each substance measured throughout gestation.

All 20 women gave birth to healthy babies (9 female, 11 male) with a mean ($\pm$ SD) birthweight of 3.32 ($\pm$0.46) kg. None of the 20 women smoked. The group of 12 women included 11 primiparous patients and 1 multiparous patient, and 10 of the 12 women had a spontaneous onset of labour. One woman was induced because of the onset of mild pre-eclampsia at term, and the other woman was induced following SROM for more than 24 hours without the onset of labour at term. The 12 women gave birth to 7 female and 5 male babies with a mean ($\pm$ SD) birthweight of 3.263 ($\pm$ 0.309) kg.

None of the parameters measured were of a normal distribution as confirmed by the Kolmogorov-Smirnoff one sample test using a standard normal distribution. Therefore statistics were carried out using Wilcoxon signed ranks test, Mann-Whitney U test, and Spearman rank correlation coefficients.

Table 9.1 Individual subjects' details including parity (P - primiparous, M - multiparous), gestation at delivery (weeks + days), spontaneous (S) or induced (I) onset of labour, sex of infant (M - male, F - female), birthweight (g) and any other pregnancy or delivery details. (LSCS - lower segment cesarean section, PIH - pregnancy induced hypertension, OP - occipitoposterior, SROM - spontaneous rupture of the membranes) [* indicates the 12 patients with complete collections of plasma and saliva]

Subject	Parity	Gestation	S/I	Sex	Birthweight	Pregnancy and delivery details
*1	P	38 +4	S	F	2710	Emergency LSCS in labour for failure to progress
*2	P	38 +6	S	M	3160	
3	P	39 +6	S	M	3140	Emergency LSCS in labour for brow presentation
*4	P	39 +3	S	F	3350	
*5	P	39 +2	S	F	3070	
6	P	41 +2	S	F	3600	
*7	M	40 +6	S	M	3640	
*8	P	39 +1	I	M	3100	LSCS following failed induction for mild PIH
*9	P	39 +1	S	F	3610	Forceps delivery
*10	P	38 +4	S	F	2910	
11	P	40 +8	S	M	3450	Mildly raised B/P at term- settled on resting
*12	P	38 +0	S	F	3200	Elective LSCS at patient request
13	M	40 +0	S	M	3800	
14	M	39 +0	S	M	3620	
15	M	35 +1	S	F	2220	Premature labour - bicornuate uterus
*16	P	37 +1	I	M	3320	Elective LSCS following failed induction following SROM
17	M	41 +3	I	M	4380	Induced for prolonged ROM
*18	P	41 +4	S	F	3780	
*19	P	40 +2	S	M	3300	
20	P	39 +4	S	M	3060	Keillands for OP position and fetal distress

Results

The results for all 20 women for each substance measured are presented in scattergram form in Figs. 9.1-9.7. The trend for all the unconjugated steroids was to rise gradually from 20 weeks gestation towards term, with both saliva and plasma oestriol rising more rapidly from about 34 weeks gestation onwards. In contrast, DHEAS, which is present in large quantities, fell over the same time period. SHBG levels appeared to rise initially and then remain approximately unchanged between 26-40 weeks gestation. Both PRL and hPL showed a rising trend, but the ß-hCG levels were very variable from patient to patient so that any trend is not immediately apparent.

In order to evaluate the trends more accurately, the medians, 10th and 90th centiles of each hormone were calculated for the 12 women who had complete collections from 22 weeks until term (Table 9.2). [It was not possible to calculate 5th and 95th centiles because of the small numbers.] Fig. 9.8 shows the median levels of saliva oestrone, oestradiol, oestriol and progesterone. The percentage increases in the saliva steroid levels were 273%, 289%, 399% and 230% respectively between 22 and 38 weeks pregnancy. The increase occurred gradually over the time period for oestrone and progesterone, whereas saliva oestradiol levels rose at a very slightly faster rate between 30 and 38 compared to 22 and 30 weeks gestation, and saliva oestriol showed a surge in levels from 34 weeks gestation until term.

The corresponding plasma medians are shown in Fig. 9.9. It can be seen that the changes in the unconjugated steroid levels in plasma reflect the changes seen in the saliva levels, with percentage increases in E1, E2,

Fig. 9.1 Saliva oestrone and oestradiol levels in 20 women between 19+ and 41+ weeks gestation.

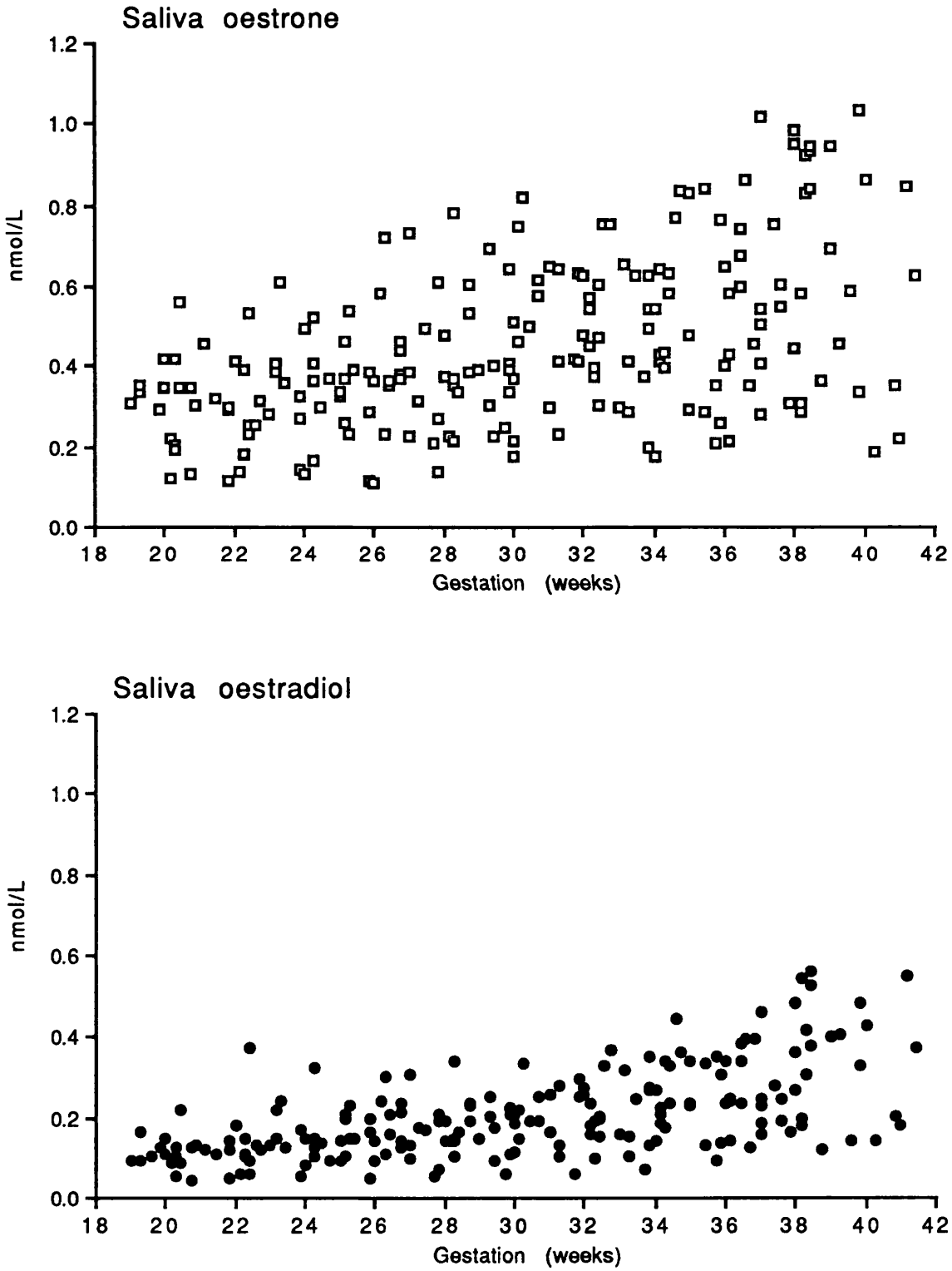


Fig. 9.2 Saliva oestriol and progesterone levels in 20 women between 19+ and 41+ weeks gestation.

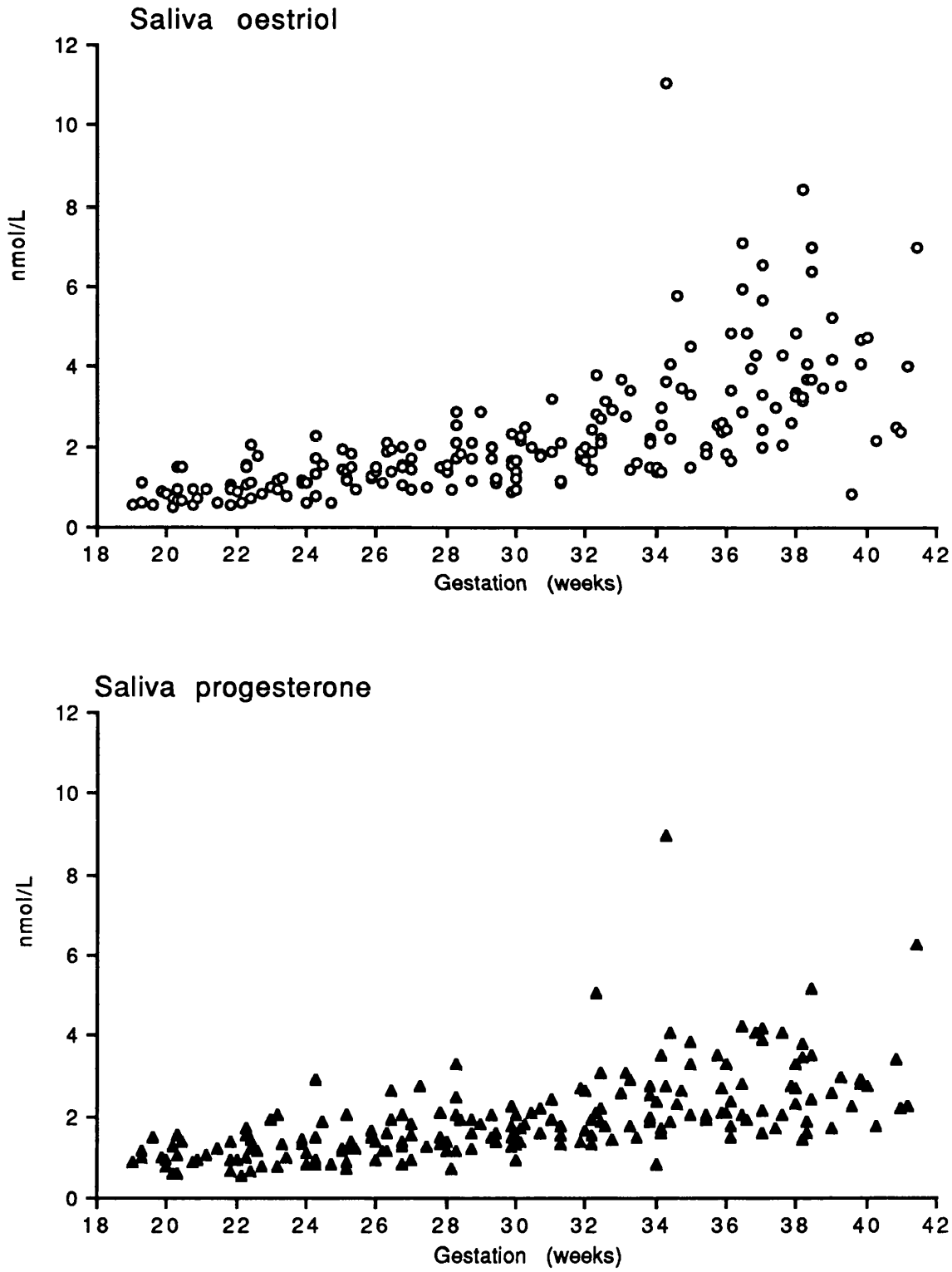


Fig. 9.3 Plasma oestrone and oestradiol levels in 20 women between 19+ and 41+ weeks gestation.

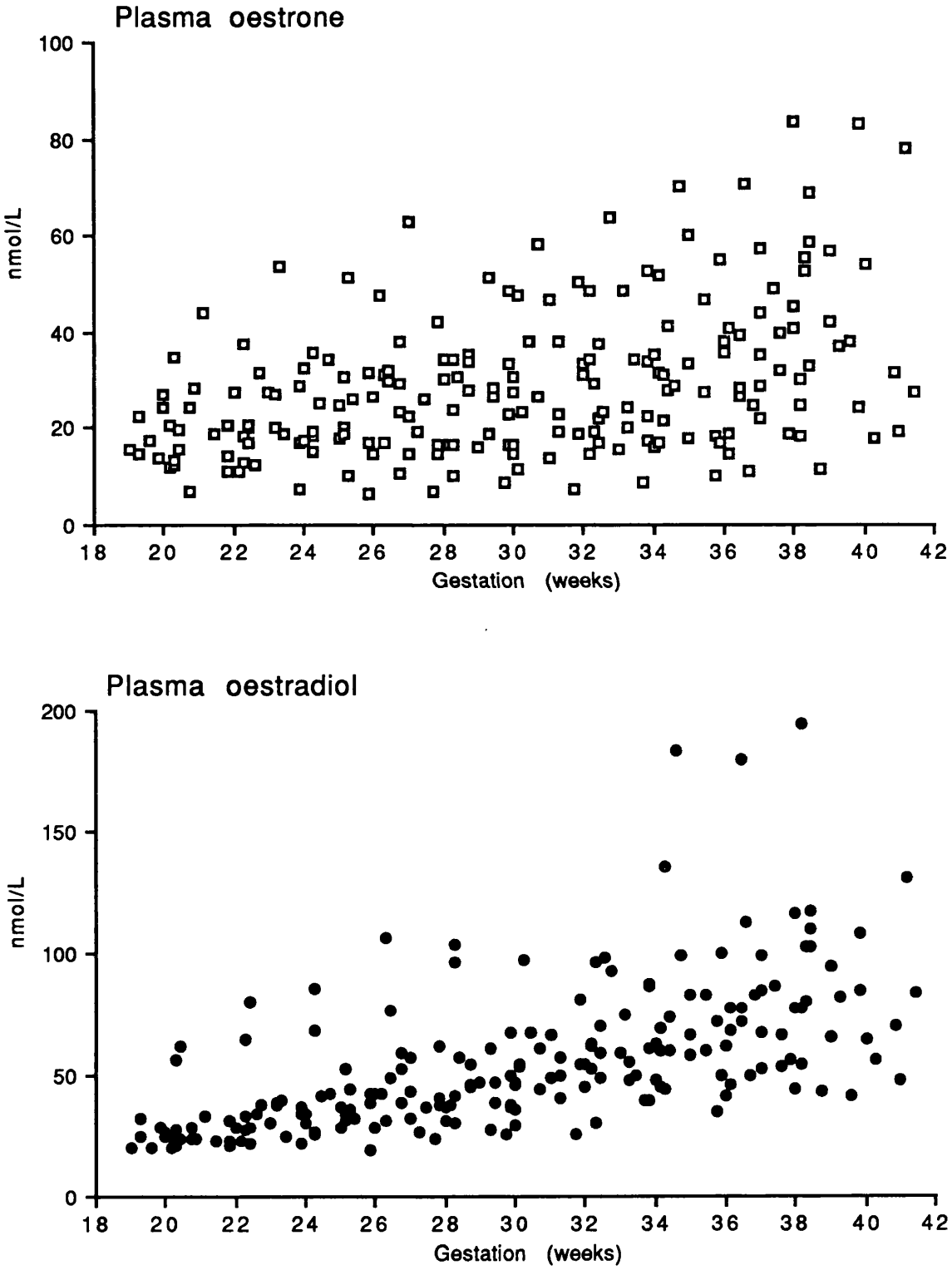


Fig. 9.4 Plasma oestriol and progesterone levels in 20 women between 19+ and 41+ weeks gestation.

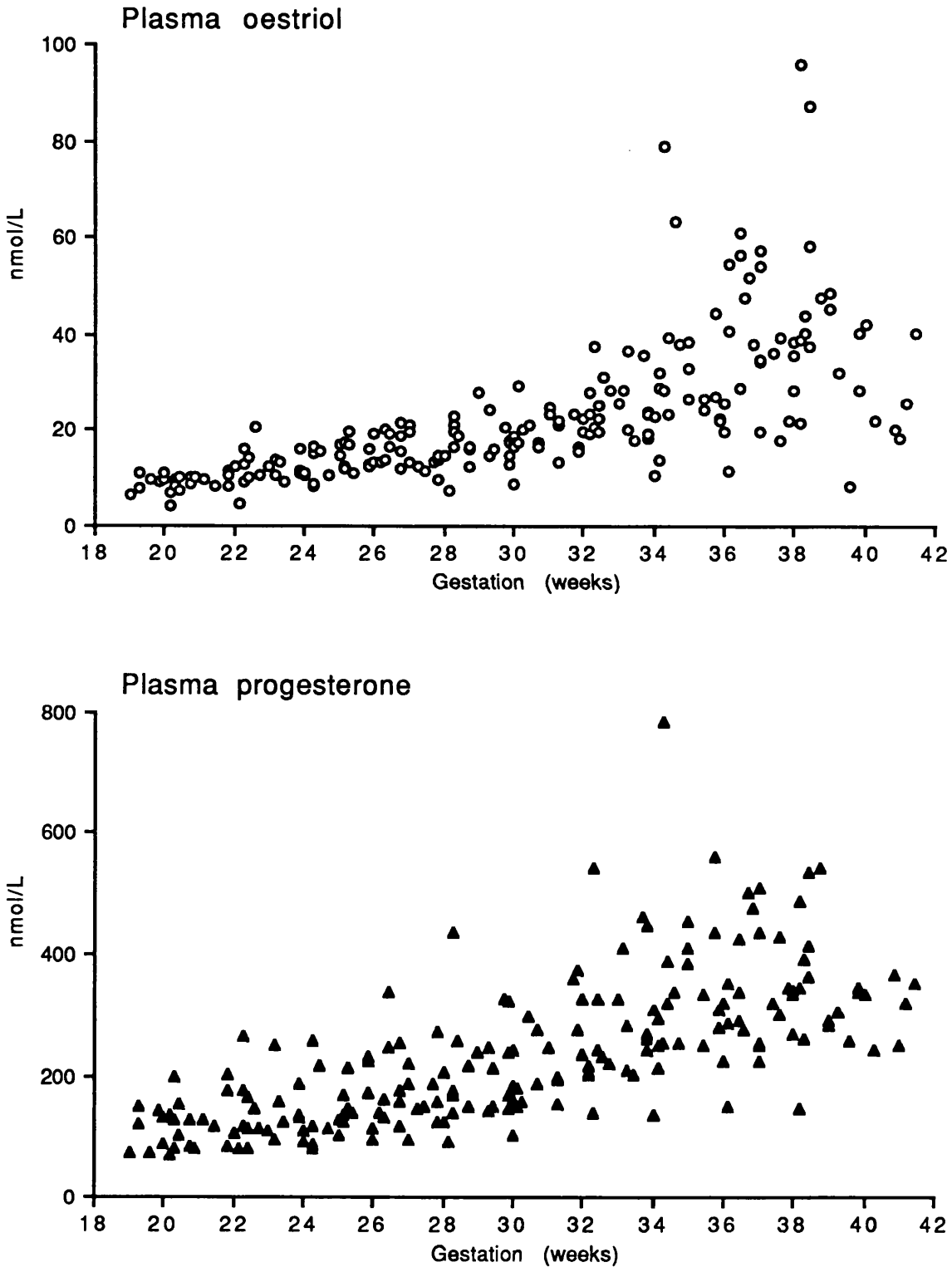


Fig. 9.5 Plasma levels of dehydroepiandrosterone sulphate (DHEAS) and sex hormone binding globulin (SHBG) in 20 women between 19+ and 41+ weeks gestation.

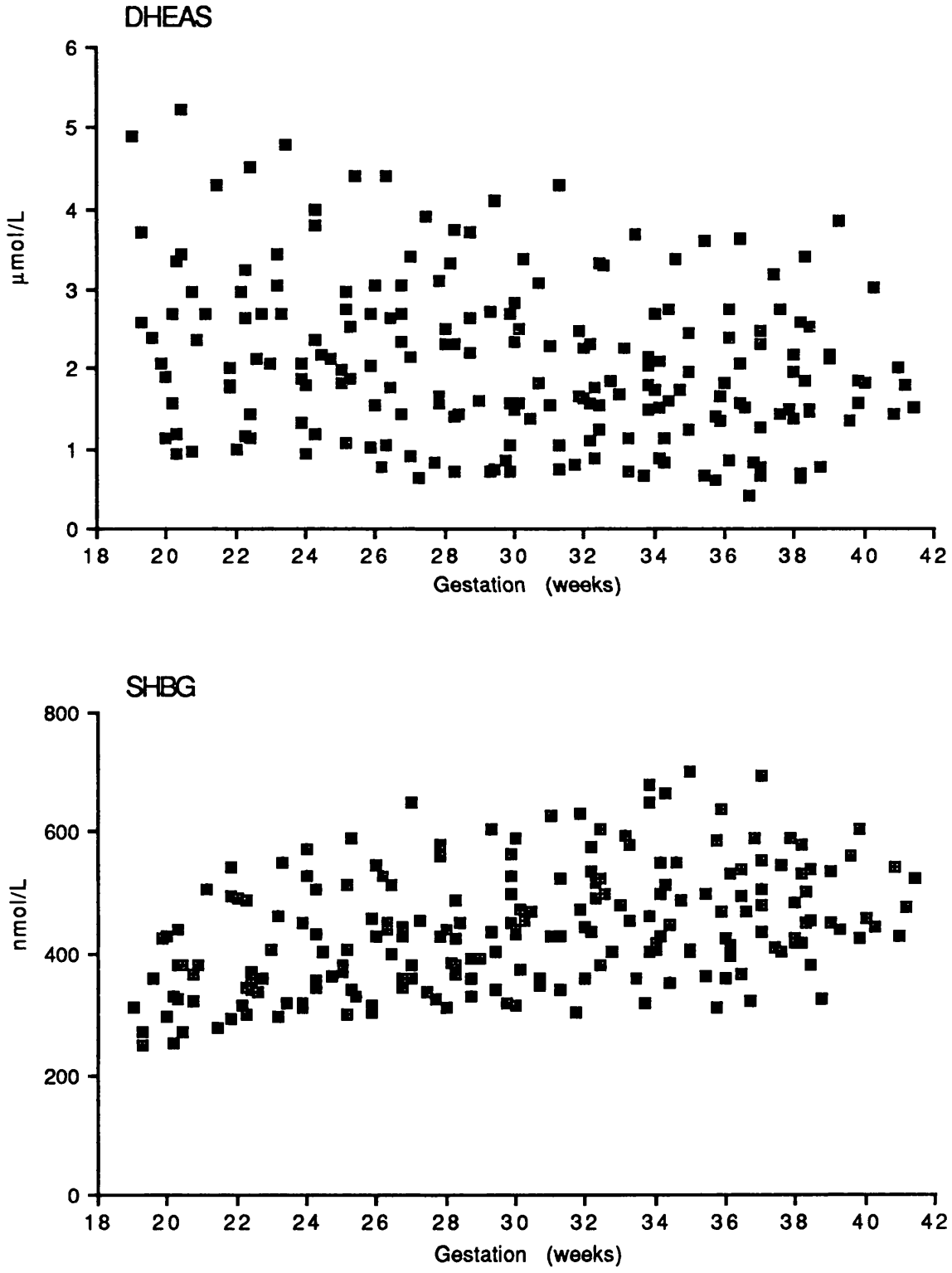


Fig. 9.6 Plasma levels of ß-subunit human chorionic gonadotrophin (ß-hCG) and human placental lactogen (hPL) in 20 women between 19+ and 41+ weeks gestation.

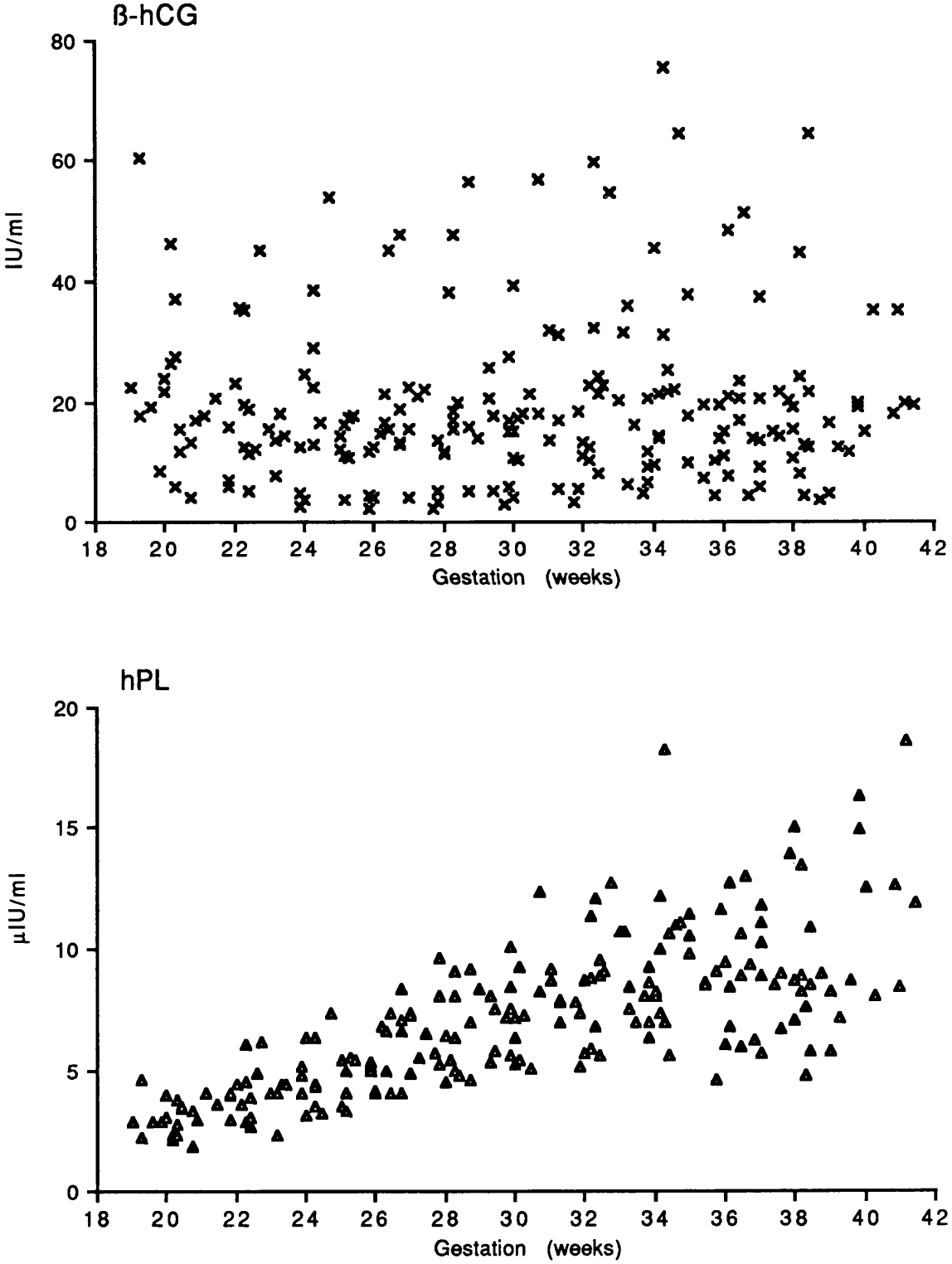


Fig. 9.7 Plasma levels of prolactin (PRL) in 20 women between 19+ and 41+ weeks gestation.

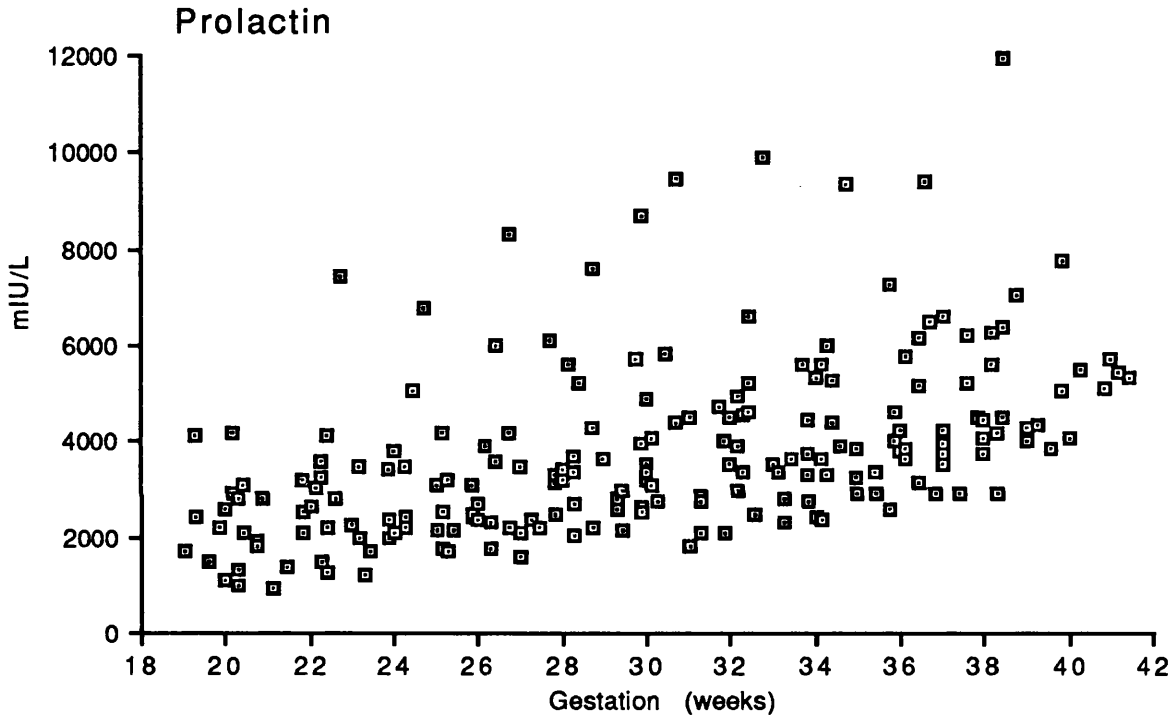
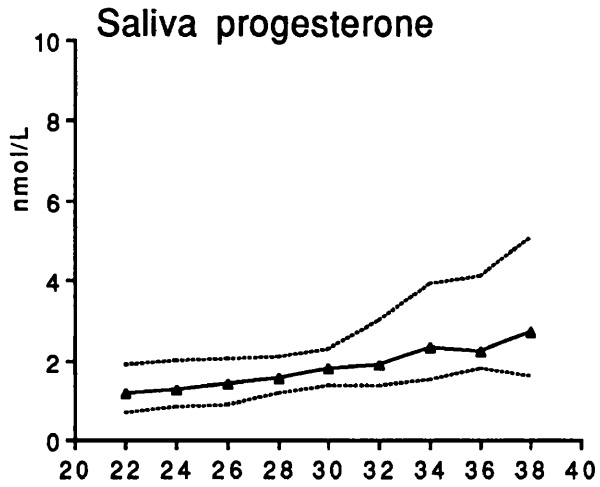
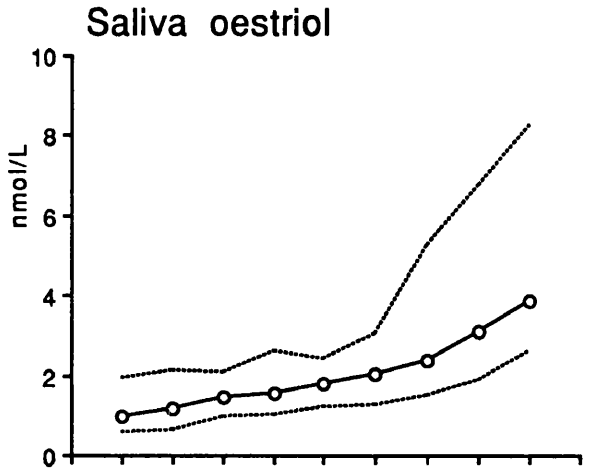


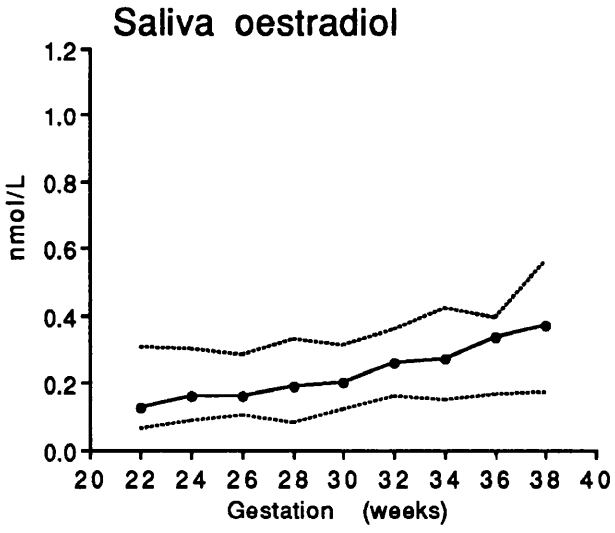
Table 9.2 Medians, 10th and 90th centiles for saliva and plasma E1, E2, E3, P and plasma SHBG, DHEAS, ß-hCG, hPL and PRL levels in normal women throughout gestation. (n=12 except for weeks 38 and 40 where n=10 and 2 respectively)
[SE1, SE2, SE3, SP - saliva oestrone, oestradiol, oestriol and progesterone; PE1, PE2, PE3, PP - plasma oestrone, oestradiol, oestriol and progesterone; SHBG-sex hormone binding globulin; DHEAS-dehydroepiandrosterone sulphate; ß-HCG - ß-human chorionic gonadotrophin; HPL - human placental lactogen; PRL-prolactin]

	SE1	SE2	SE3	SP	PE1	PE2	PE3	PP	SHBG	DHEAS	ß-hCG	hPL	PRL
Median 22	0.306	0.128	0.97	1.18	19.54	32.04	10.52	123.5	366.0	2.41	13.95	3.96	2369
Median 24	0.346	0.160	1.18	1.26	22.43	37.44	14.22	133.5	372.0	2.15	12.74	4.34	2473
Median 26	0.383	0.162	1.44	1.39	27.99	42.19	16.43	169.5	428.5	2.62	14.37	5.39	2565
Median 28	0.433	0.192	1.54	1.54	29.01	43.33	15.31	172.5	408.0	2.42	15.60	6.49	3232
Median 30	0.507	0.201	1.77	1.78	27.97	51.25	17.49	199.0	448.0	2.08	17.40	7.39	3266
Median 32	0.587	0.258	2.05	1.89	32.32	60.10	22.94	238.5	472.0	2.05	19.02	8.82	4014
Median 34	0.563	0.270	2.36	2.34	32.84	65.66	25.96	301.5	468.0	1.92	18.59	8.93	3784
Median 36	0.625	0.338	3.11	2.24	36.95	74.27	33.61	327.0	483.0	1.82	18.30	9.17	3830
Median 38	0.836	0.370	3.87	2.71	47.22	93.06	39.04	354.5	479.0	1.67	17.88	8.63	4347
Median 40	0.602	0.380	4.41	2.78	39.22	74.18	35.43	340.0	532.0	1.83	17.11	13.76	5911
10th cent. 22	0.158	0.062	0.59	0.67	11.36	21.74	8.12	87.2	283.6	1.53	6.11	2.40	1065
10th cent. 24	0.161	0.086	0.62	0.83	12.02	25.62	9.73	94.2	300.8	1.81	4.02	3.15	1364
10th cent. 26	0.164	0.103	0.97	0.86	11.69	29.65	11.22	101.7	320.8	1.46	4.08	4.02	1849
10th cent. 28	0.175	0.083	1.03	1.15	11.33	33.01	11.56	133.2	318.0	1.42	3.81	4.54	1766
10th cent. 30	0.259	0.124	1.21	1.33	12.89	38.58	15.14	151.4	323.2	1.42	4.76	5.09	2272
10th cent. 32	0.335	0.164	1.28	1.33	15.36	45.63	14.21	198.7	346.0	1.33	6.16	5.62	1923
10th cent. 34	0.264	0.148	1.49	1.51	17.26	42.65	18.08	213.0	354.4	1.49	7.36	6.01	2390
10th cent. 36	0.288	0.167	1.87	1.79	15.32	43.67	20.38	240.6	360.6	1.00	8.39	4.98	2680
10th cent. 38	0.322	0.175	2.60	1.59	20.13	45.25	22.65	260.0	383.2	0.70	5.10	4.91	2999
90th cent 22	0.511	0.307	1.94	1.87	40.42	66.98	18.58	193.9	533.2	4.45	37.67	5.79	6442
90th cent 24	0.585	0.302	2.12	1.99	47.71	75.43	19.05	240.5	566.0	4.55	43.21	6.77	6261
90th cent 26	0.668	0.282	2.06	2.02	47.47	92.24	21.05	251.6	578.8	4.41	39.06	7.98	7622
90th cent 28	0.765	0.331	2.62	2.06	54.50	89.55	20.69	266.8	629.0	3.85	46.39	9.53	6895
90th cent 30	0.799	0.313	2.42	2.25	56.28	88.62	28.01	316.2	600.4	3.88	46.21	11.42	8370
90th cent 32	0.755	0.358	3.06	2.98	58.60	96.26	30.41	361.6	630.8	4.01	47.88	12.36	8926
90th cent 34	0.818	0.421	5.28	3.92	63.78	158.32	56.15	437.3	670.4	3.60	54.72	11.85	8243
90th cent 36	0.856	0.392	6.75	4.13	67.33	159.47	59.71	449.3	682.7	3.63	47.18	12.96	8427
90th cent 38	1.014	0.561	8.28	5.06	67.95	186.62	94.86	531.6	681.7	3.13	61.89	13.88	11383

Fig. 9.8 Median saliva levels of oestrone, oestradiol, oestriol and progesterone in women between 22 and 38 weeks gestation. (n=12 except at 38 weeks gestation when n=10) Dotted lines signify the 10th and 90th centiles.







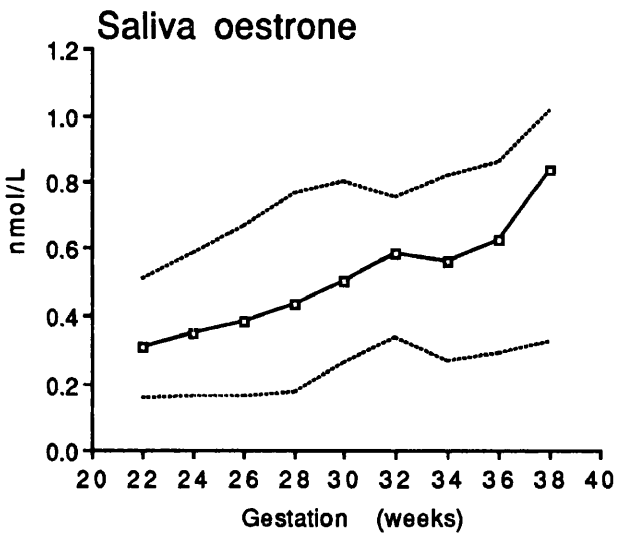


Fig. 9.9 Median maternal plasma levels of oestrone, oestradiol, oestriol and progesterone in women between 22 and 38 weeks gestation. (n=12 except at 38 weeks gestation when n=10) Dotted lines signify the 10th and 90th centiles.

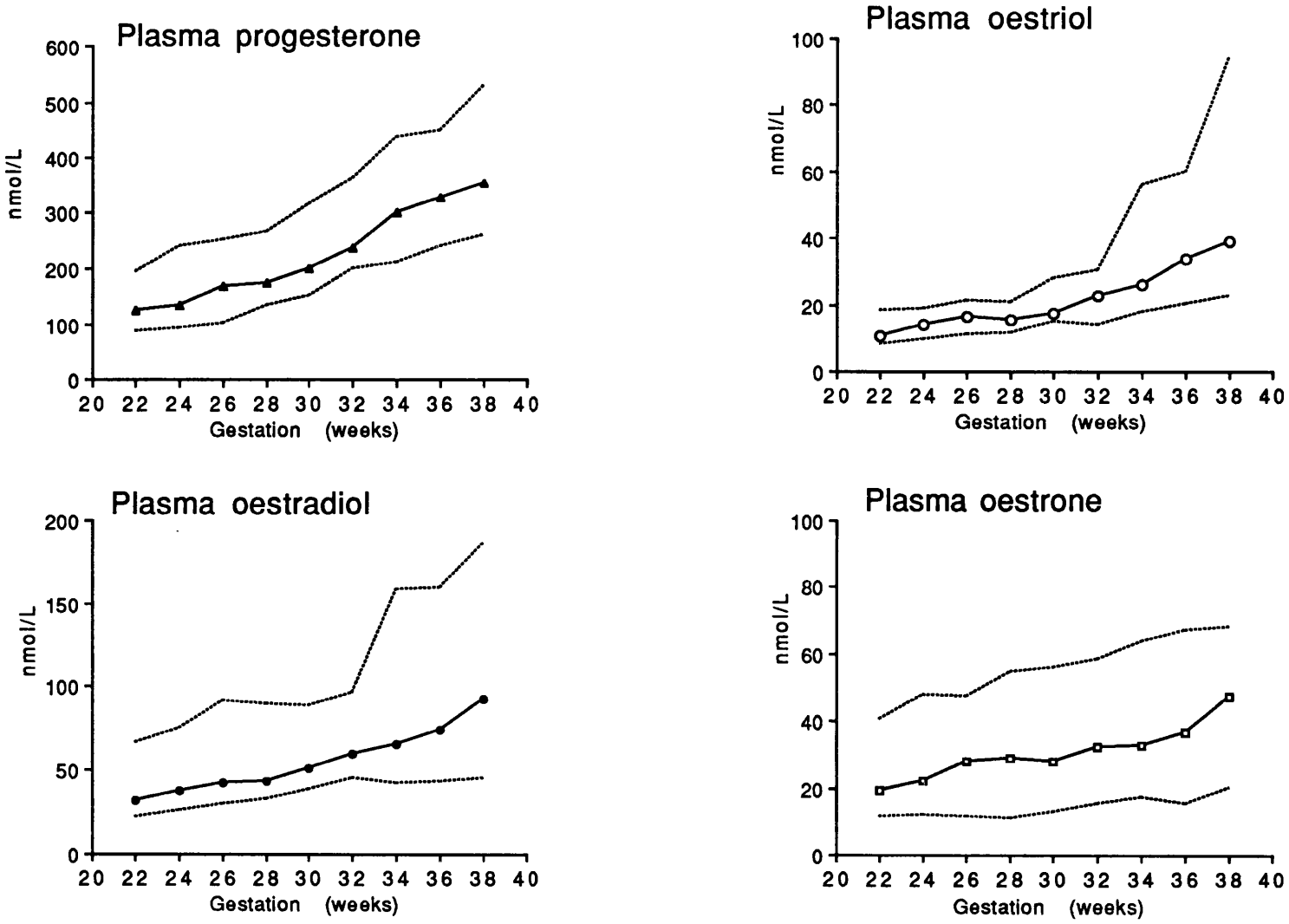
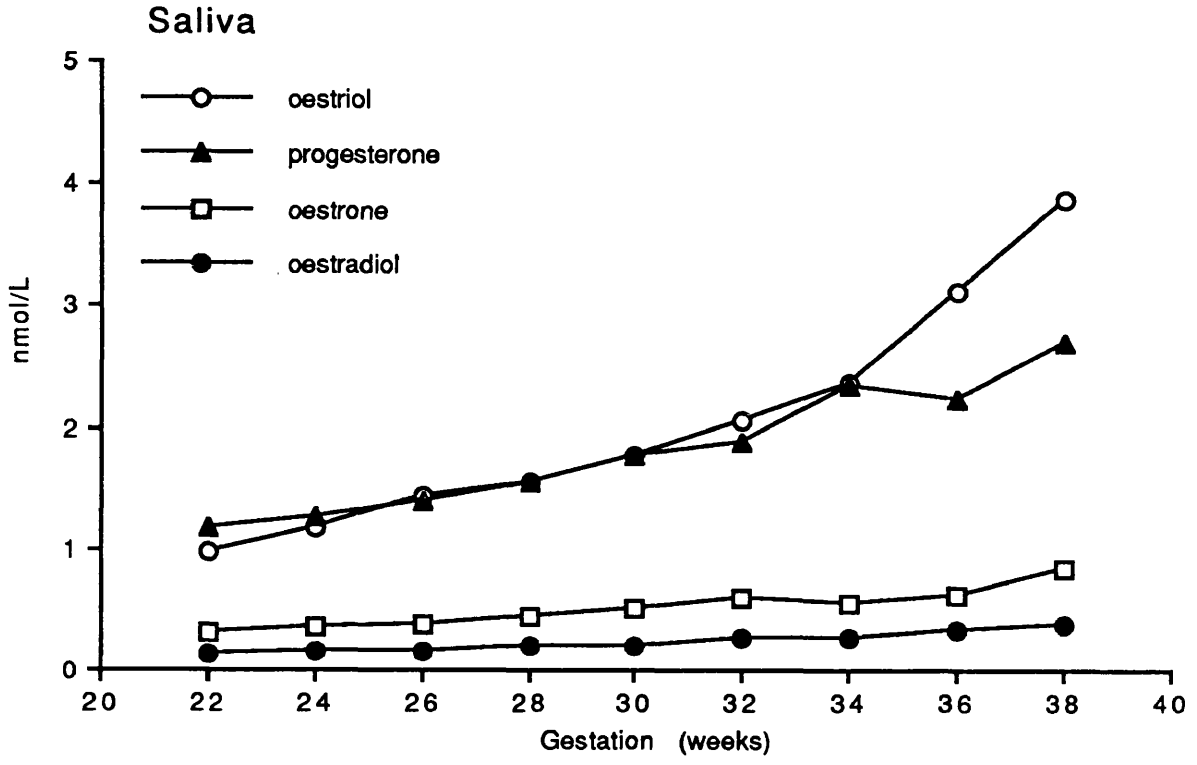
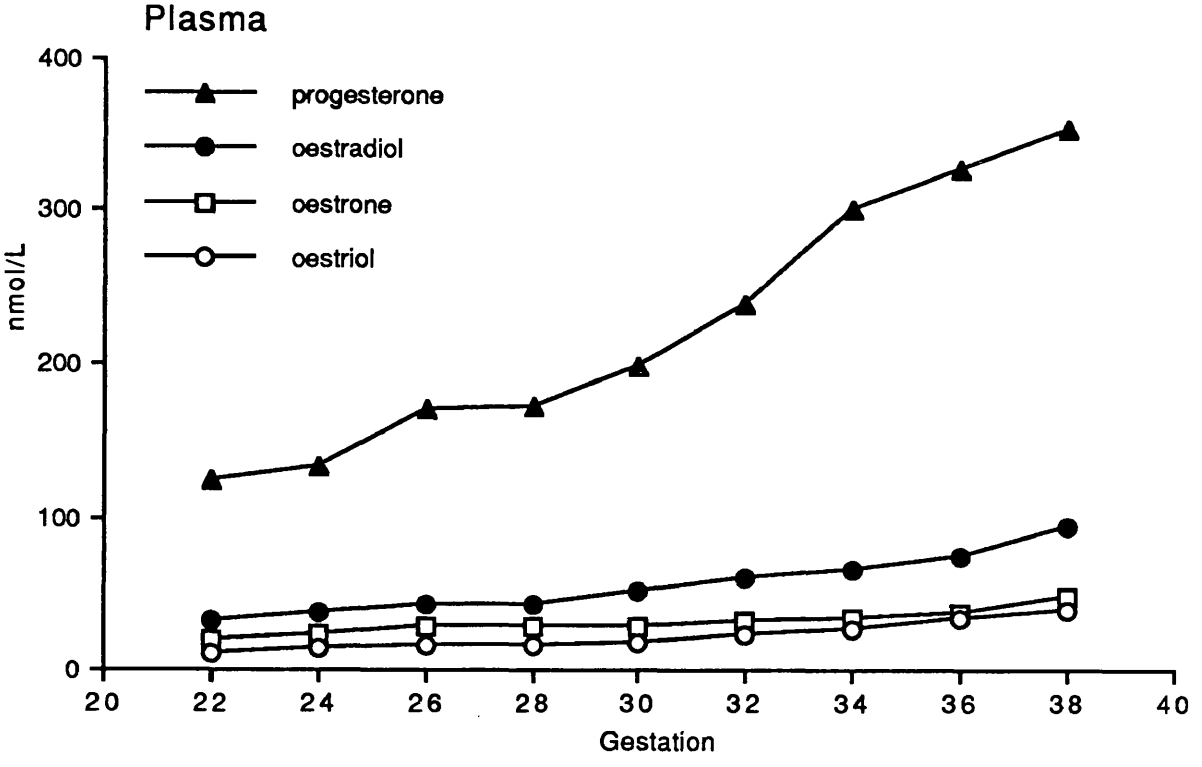


Fig. 9.10 Median saliva and plasma levels of oestrone, oestradiol, oestriol and progesterone in women between 22 and 38 weeks gestation. (n=12 except at 38 weeks gestation when n=10)





E3, and P of 242%, 290%, 371% and 287% respectively between 22 and 38 weeks pregnancy. Fig. 9.10 compares the relative levels of these unconjugated steroids in the saliva and the plasma. In plasma, unconjugated progesterone levels were considerably higher than the levels of any of the unconjugated oestrogens. Oestradiol had the highest levels of the unconjugated oestrogens in the plasma . However in saliva, oestriol and progesterone levels were at very similar levels and closely related until the oestriol surge, whereas oestradiol and oestrone levels were lower in comparison. The differences between plasma and saliva occur because of the differing percentages of the steroids which are bound to proteins in the plasma (Table 9.3).

Table 9.3 The median percentage 'free' and range of the oestrogens and progesterone for 20 women as expressed by the saliva/plasma ratio x100 . [n = number of paired samples]

Hormone	n	Median % 'free'	Range
Oestrone	201	1.50	0.75-4.17
Oestradiol	200	0.38	0.16-0.66
Oestriol	200	9.62	3.57-17.39
Progesterone	197	0.82	0.48-2.00

SHBG levels also showed a slight but significant increase between 22 and 38 weeks gestation to 131% of their level at 22 weeks. Most of the increase has occurred by 32 weeks gestation, after which the levels remained fairly constant. SHBG levels did not have any correlation with the percentage unbound unconjugated oestrogen. (Although the percentage bound of a hormone varied from individual to individual, when each

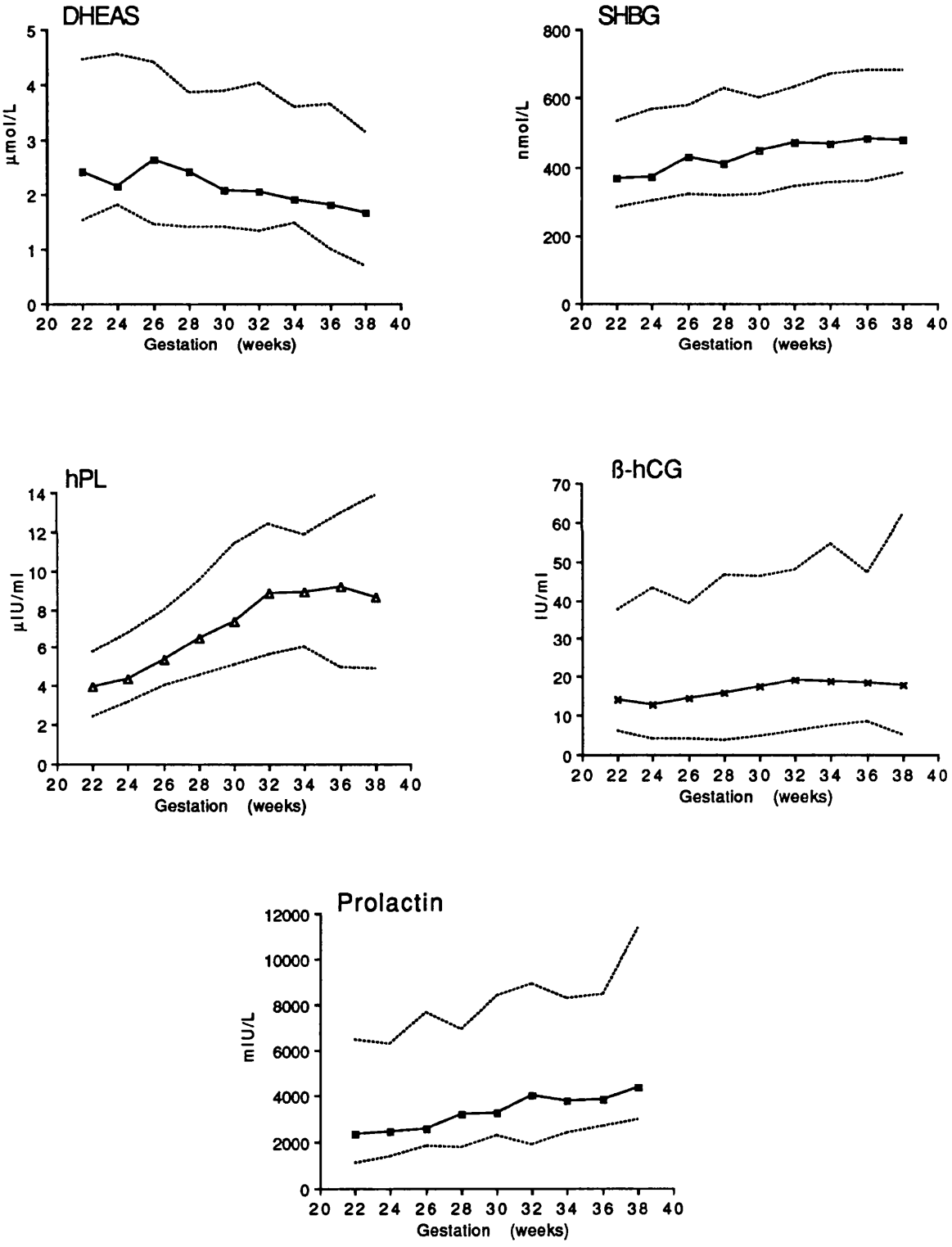
individual was considered separately the % bound tended to remain steady throughout the gestation period under study.) There was no correlation between the % unbound unconjugated E1, E2, E3 or P.

In contrast to the unconjugated steroids, DHEAS levels fell between 22 and 38 weeks to 69% of their original levels, with the decrease occurring gradually from 26 weeks gestation onwards, (Fig. 9.11). ß-hCG rose significantly by 136% between 22 and 32 weeks gestation. However, the levels then gradually decreased again and by 38 weeks gestation, although raised, the level was not significantly higher than at 22 weeks gestation. hPL levels rose from 22 weeks onwards to 218% of the 22 week levels, the majority of the rise occurring between 22 and 32 weeks gestation.

Finally, prolactin levels, which increased to 183% of the level at 22 weeks gestation, showed a steady rise throughout the time period studied.

There was no difference in the levels of most of the substances measured, between pregnancies with female and male fetuses. However, pregnancies with a female fetus did have significantly higher levels of saliva and plasma E3 (U=1846, p=0.008; U=1792, p=0.02); plasma E2 (U=1884, p=0.004); SHBG (U=1829, p=0.01); ß-hCG (U=1785, p=0.022); and hPL (U=1871, p=0.005), when the results throughout gestation were considered. In contrast, most of these differences were not apparent when analysed by each gestation period; although at 34 and 36 weeks gestation saliva and plasma E3 as well as hPL levels were still significantly higher in pregnancies with a female fetus (p values for 34 and 36 weeks... saliva E3 - 0.007 & 0.034, plasma E3 - 0.028 & 0.028, hPL - 0.019 & 0.012 respectively).

Fig. 9.11 Median maternal plasma levels of dehydroepiandrosterone sulphate (DHEAS), sex hormone binding globulin (SHBG), human placental lactogen (HPL), ß-human chorionic gonadotrophin (ß-HCG) and prolactin (PRL) in women between 22 and 38 weeks gestation. (n=12 except at 38 weeks gestation when n=10) Dotted lines signify the 10th and 90th centiles.



In order to analyse the possible interrelationships between the substances measured, Spearman correlations were performed. Firstly, all the results throughout pregnancy were analysed, and then an analysis for each two-week gestation period was carried out. In order for the correlation coefficients to be comparable between the 2 week gestation periods, only the same 12 women who were used in the calculation of the medians will be discussed. However, the correlation coefficients were also calculated using all the subjects results and broadly similar results were obtained.

The overall Spearman rank correlation coefficients (r_s) are shown in Table 9.4. Because of the large sample number (n=108), the coefficients which are on or above 0.195 and 0.254 are significant at the 5% and 1% levels respectively, even though the correlation might be considered poor.

The results in Table 9.4 can be summarised as follows:

1) Saliva E1, E2, E3 and P levels correlated with their respective plasma levels, (r_s = 0.702, 0.815, 0.913 and 0.898 respectively).
2) Saliva and plasma E1, E2 and E3 all correlated with each other (range r_s = 0.634-0.832), the only exception being the rather low correlations of plasma E1 with saliva and plasma E2 and E3 (range r_s =0.287-0.497).
3) P correlated with E2 and E3 in both plasma and saliva (range r_s =0.622-0.715).
4) hPL correlated with E3, P and E2 (range r_s = 0.548-0.665).
5) SHBG, DHEAS, ß-hCG, and PRL did not have correlation coefficients of 0.5 or more with any other substance.
6) There was some negative correlation between birthweight and saliva E1 (r_s = -0.509), DHEAS (r_s = -0.514) and ß-hCG (r_s = -0.522).

Table 9.4 Matrix of Spearman rank correlation coefficients between saliva and plasma E1, E2, E3 and P, and plasma SHBG, DHEAS, ß-hCG, hPL, PRL and birthweight for 12 women who gave samples every 2 weeks throughout gestation. [SE1, SE2, SE3, SP - saliva oestrone, oestradiol, oestriol and progesterone; PE1, PE2, PE3, PP - plasma oestrone, oestradiol, oestriol and progesterone; SHBG-sex hormone binding globulin; DHEAS-dehydroepiandrosterone sulphate; ß-HCG - ß-human chorionic gonadotrophin; HPL-human placental lactogen; PRL-prolactin; weight-birthweight of infant.]

	SE1	SE2	SE3	SP	PE1	PE2	PE3	PP	SHBG	DHEAS	ß-hCG	hPL	PRL	WEIGHT
SE1	1.000													
SE2	0.832	1.000												
SE3	0.634	0.751	1.000											
SP	0.463	0.677	0.705	1.000										
PE1	0.702	0.497	0.287	0.214	1.000									
PE2	0.740	0.815	0.818	0.622	0.420	1.000								
PE3	0.631	0.723	0.931	0.700	0.343	0.799	1.000							
PP	0.479	0.657	0.715	0.898	0.285	0.652	0.751	1.000						
SHBG	0.084	0.183	0.318	0.275	0.127	0.389	0.326	0.336	1.000					
DHEAS	0.280	0.104	-0.216	-0.275	0.124	-0.071	-0.273	-0.326	-0.316	1.000				
ß-hCG	0.414	0.316	0.290	0.265	0.437	0.355	0.296	0.222	-0.051	0.097	1.000			
hPL	0.464	0.548	0.635	0.634	0.226	0.619	0.665	0.641	0.233	-0.207	0.457	1.000		
PRL	0.159	0.210	0.440	0.426	0.232	0.365	0.432	0.439	-0.064	-0.457	0.451	0.489	1.000	
Weight	-0.509	-0.380	-0.031	-0.090	-0.355	-0.297	0.001	-0.056	0.409	-0.514	-0.522	-0.186	-0.145	1.000

The correlation coefficients for each two week gestation period were also calculated. Between weeks 22-36 inclusive, the sample number was 12 and therefore to be significant at the 5% and 1% level the correlation coefficient should be on or above 0.576 and 0.708 respectively. At 38 weeks gestation, n=10 and correlation coefficients of 0.632 and 0.765 are required for significance at the 5% and 1% level respectively.

The results for the 2 week gestation periods can be summarised as follows:

1) Saliva E3 correlated with plasma E3 at all gestations; saliva P correlated with plasma P at all gestations except 22 weeks, when the correlation was rather poor, (r_s = 0.389). However, for E1 and E2 the correlation between saliva and plasma was less consistent, although a good correlation was maintained in 5 out of 9 gestations periods for each. [The correlations for E1 were not significant at 22, 26, 28 and 30 weeks, (r_s = 0.399, 0.531, 0.441, and 0.497 respectively). The correlations for E2 were not significant at 24, 26, 34 and 36 weeks, (r_s = 0.490, 0.329, 0.182 and 0.545 respectively).]

2a) Saliva E1 correlated with saliva E2 in 7 out of 9 gestation periods, (correlation not significant at 30 and 38 weeks gestation) with the correlation coefficients ranging from 0.594 - 0.860. However, saliva E1 only correlated with plasma E2 on 2 occasions at 30 and 36 weeks gestation (r_s = 0.657 and 0.804 respectively). Plasma E1 did not correlate significantly with saliva or plasma E2 on any occasion.

2b) At 38 weeks gestation only, E2 correlated with E3 in both plasma and saliva, (range of r_s: 0.770-0.927). However, the same correlation was not found at other gestations with only sporadic significant correlations between saliva or plasma E2 and E3. [Significant correlations: plasma E2 with plasma E3 at 28 and 36 weeks gestation (r_s = 0.601

and 0.594); saliva E2 with plasma E3 at 22 weeks gestation (r_s = 0.692); saliva E3 with plasma E2 at 30 weeks gestation (r_s = 0.629).]

2c) There was no correlation between E1 and E3 in saliva or plasma. [Sole significant correlation: 30 weeks, saliva E1 with saliva E3, r_s = 0.643.]

3) There was also no significant correlation in saliva or plasma between P and E3, (sole exception: 38 weeks, plasma P and plasma E3, r_s = 0.636). At 38 weeks gestation only, there was a good correlation in saliva and plasma between P and E2. Prior to 38 weeks, there were sporadic correlations between saliva or plasma P and saliva or plasma E2. [Significant correlations: 24 weeks, saliva E2 with saliva P, r_s =0.594; 26 weeks, saliva E2 with saliva and plasma P, r_s =0.622 and 0.685; 34 weeks, plasma E2 with plasma P, r_s =0.629.] There was no correlation in saliva or plasma between P and E1 at any time.

4) hPL did not show any consistent significant correlations with E3, P, E2, or any other substance measured. [Significant correlations: 26 weeks, ß-hCG, r_s = 0.634; 34 weeks, saliva and plasma E3, saliva P, plasma E2, r_s = 0.630, 0.671, 0.734 and 0.587 respectively.]

5a) SHBG did not show any significant correlations with any other substance. [Sole significant correlation: 34 weeks, plasma E2, r_s = 0.594.]

5b) DHEAS had a significant correlation with saliva E1 in 7 of the 9 gestation periods, (correlation not significant at 30 and 38 weeks gestation) range of r_s 0.580-0.825). Otherwise there were no consistent correlations. [Significant correlations: 22 weeks, plasma E2, r_s = 0.615; 24 weeks, ß-hCG, r_s = 0.615.]

5c) ß-hCG also correlated with saliva E1 in 4 or the 9 gestation periods, (weeks 24, 32, 34, and 36, range of r_s 0.601-0.769). Otherwise there were no consistent correlations. [Significant correlations: weeks 22 and 24, plasma E1, r_s = 0.650 and 0.664; 32 and 34 weeks, saliva

and plasma E3, range of r_s 0.615-0.781; 24 weeks, DHEAS, $r_s = 0.615$; 26 weeks, hPL, $r_s = 0.634$: 36 weeks, plasma E2, $r_s = 0.594$.]

5d) Prolactin had no significant correlations with any other substance measured.

6) Birthweight showed significant negative correlations with saliva E1 in 8 out of 9 gestation periods, (22-36 weeks, range of r_s -0.580 to -0.923); and with ß-hCG in 6 out of 9 gestation periods, (24-34 weeks, range of r_s -0.657 to -0.776). Birthweight also correlated negatively with saliva E2 in 4 out of 9 gestation periods, (28, 32, 34 and 36 weeks , range of r_s -0.587 to -0.783). There were no other consistent correlations. [Significant correlations: 24 weeks, DHEAS, $r_s = -0.671$; 26 weeks, hPL, $r_s = -0.620$; 36 weeks, plasma E2, $r_s = -0.762$.]

Discussion

Comparison with previously established normal ranges

The values obtained for all the hormones measured were within the previously established normal ranges (references given below), and showed the same trends with advancing gestation unless otherwise stated.

E1 Loriaux et al, 1972; Lindberg et al, 1974;
De Hertogh et al, 1975; Turnbull et al, 1977

Tulchinsky et al *(1972)* like all the above authors found the same trend as in our study, but with rather lower levels throughout pregnancy.

E2 Loriaux et al, 1972; Tulchinsky et al, 1972;
De Hertogh et al, 1975; Turnbull et al,1977;
Buster et al, 1979; Allen and Lachelin, 1978;
Aspillaga et al, 1983

E3 Loriaux et al,1972; Tulchinsky et al,1972;
Lindberg et al, 1974; De Hertogh et al, 1975;
Buster et al, 1979; Allen and Lachelin, 1978;
Haning et al, 1983; Evans et al, 1984

P Tulchinsky et al, 1972; Allen and Lachelin, 1978;
Aspillaga et al, 1983; Haning et al, 1983

Most of these studies found rather higher levels of P than the levels in this study, but the trend throughout gestation was the same. Turnbull et al *(1977)* found similar levels to those in the present study, but was alone in demonstrating a rise to peak values (of ≈477nmol/L) at 36 weeks gestation followed by a fall in P levels (to ≈320nmol/L) towards term.

DHEAS Turnbull et al, 1977; Buster et al, 1979

[Buster et al found rather higher levels of DHEAS than in our study, with their mean levels at 26 and 40 weeks being approximately 4.1 and 2.2μmol/L respectively. He suggested that the fall in levels was due to the increasing metabolic clearance rate in pregnancy. Both groups demonstrated the same falling trend with gestation.]

SHBG Cannell et al, 1985

[Uriel et al *(1981)* in a serial study on 6 pregnant women described the trend of SHBG as rising to attain a plateau by 25-30 weeks, followed by a slight decline towards term. This was very similar to our findings except that we found no evidence of a decline towards term.]

ß-hCG Faiman et al, 1968; Braunstein et al, 1980; Danzer et al, 1980;
Kosasa, 1981; Aspillaga et al, 1983; Kletzky et al, 1985

[The finding of a secondary peak in ß-hCG levels around 34 weeks gestation *(Kosasa, 1981)* was confirmed by our findings of a statistically significant rise in ß-hCG between 22 and 32-34 weeks gestation, although it is debatable whether the relatively small rise noted in this study is of physiological importance. The levels then fell again slightly and by 38 weeks gestation were not significantly different from those at 20 weeks gestation.]

hPL Josimovich et al, 1970; Braunstein et al, 1980;
Ylikorkala, 1973

[The increase in levels with gestation found by Ylikorkala was slightly more rapid than that found in this study. The levels reported by Letchworth et al *(1978)* and Morrison et al *(1980)* were rather lower, with mean levels of 5.9 and ≈4.9 µg/ml respectively at 32 weeks gestation rising to 6.8 and ≈5.5 µg/ml respectively at term. (1 µg/ml=1 µIU/ml)]

PRL Hwang et al, 1971; Tyson et al, 1972; Rigg et al, 1977;
Kletzky et al, 1985

The levels reported by Biswas and Rodeck *(1976)* were rather lower throughout pregnancy than those found in both this study and the studies above; but in all the studies the same rising trend with gestation was noted.

Correlations with birthweight

In this study, when the results throughout gestation were considered together, there was a fairly weak negative correlation between birthweight and ß-hCG, DHEAS and saliva E1. The results analysed by 2 week gestation periods also showed consistent negative correlations between birthweight and ß-hCG and saliva E1 but not DHEAS. There was also

possibly a weak correlation between birthweight and saliva E2 mainly during the third trimester.

Previous work has suggested that there is a positive correlation between ß-hCG and birthweight when analysed for a given sex *(Obiekwe and Chard, 1982)* or that there is no correlation *(Said et al, 1984; Aspillaga et al, 1983)*. There has been very little sequential work on saliva oestrone in pregnancy performed to date, and neither confirmatory nor contradictory data on the relationship of either saliva E1 or DHEAS to birthweight could be found. Darné *(1987)* did not find a correlation between saliva E2 and birthweight in the mean of samples taken in the last 3 days prior to delivery.

Certainly none of the correlations with birthweight were very striking, although different results might be obtained with a larger sample number and by standardizing for gestation, parity and infant sex before analysis.

Correlations with sex of fetus

When the results throughout gestation were considered the oestriol, plasma oestradiol, sex hormone binding globulin, ß-hCG and hPL levels were significantly higher in women carrying female fetuses. When analysed within the 2 week gestation periods, only oestriol and hPL were significantly higher in pregnancies carrying female fetuses at 34 and 36 weeks gestation.

Many studies have shown higher levels of ß-hCG in pregnancies with female fetuses *(Boroditsky et al, 1975; Danzer et al, 1980; Obiekwe and Chard, 1982; Bremme et al, 1990)*. Bremme et al *(1990)* found no sex difference for oestriol or hPL. Hercz et al *(1989)* found no sex differences for oestradiol or hPL. However, the findings of Aspillaga et al *(1983)* were in

agreement with our data as they found that hPL levels were lower in male multiparous than in female primiparous pregnancies. No data on the sex differences in pregnancy for SHBG could be found, and androgen levels were not measured in this study. It could be argued that if oestrogen levels were higher in pregnancies with female fetuses, it was perhaps not altogether surprising to find higher SHBG levels as well. However, evidence against this hypothesis is the fact that there was no correlation between SHBG levels and oestrogen levels.

Saliva and plasma oestrogens and progesterone, and SHBG

That changes in saliva steroids should reflect changes in unconjugated plasma steroids is now well established, although the correlations were more consistent for E3 and P than for E1 or E2. The percentage of unbound unconjugated hormone as reflected in the saliva/plasma ratios were of the expected order, and the ratios for E3 and P were consistent with those in Chapter 7. Although of a similar order, the percentages of unbound unconjugated steroids were slightly lower than the ranges reported in some studies *(Tulchinsky, 1973; Freymann et al,1977; Poteczin et al, 1981; McGarrigle and Lachelin, 1983; Darné,1987)*, but were exactly similar to results from other studies *(Kundu et al, 1983; Vining et al, 1983; Evans et al, 1984; Butt,1984)*.

Anderson *(1974)* carried out an *in vitro* study which demonstrated that an increase in SHBG concentration led to a fall in the percentage of unbound E2. Wu et al *(1976)* also described a weak negative correlation between percentage 'free' E2 and SHBG. However, in this study, there was no correlation between SHBG levels and the percentage 'free' E2 levels, probably because any potential change in percentage of 'free' E2 caused by

a change in SHBG levels (of the magnitude in this study) is effectively buffered by the high capacity, low affinity binding of E2 to albumin. Also, it has previously been demonstrated that the percentage of 'free' E2 depends not only on SHBG concentrations but also on the levels of other steroids which may bind to SHBG with higher affinity *(Siiteri et al, 1982)*.

Interrelationships

When the results throughout gestation were considered the oestrogens correlated with each other (except for plasma oestrone), oestradiol and oestriol correlated with progesterone and hPL, and progesterone and hPL were correlated. However, there were no strong correlations involving DHEAS, ß-hCG, or prolactin. The results did not confirm the suggested interrelationships discussed in the introduction. Previous studies, which have been conducted by obtaining samples throughout gestation and measuring either steroid levels, or protein levels, or a combination of both, are summarized in Table 9.5. Correlations between ß-hCG and PRL, ß-hCG and hPL, ß-hCG and E3 (both positive and negative!), ß-hCG and P, PRL and E2 were found by various authors, but we were unable to confirm any of these findings. If all significant correlations had been considered (ie: all correlation coefficients above 0.195) all substances except for SHBG and DHEAS correlated with with almost every other substance. However, the degree of correlation could only be considered at best as weak, probably representing the rise in levels with gestation, and therefore any attempt at interpretation would be meaningless.

The correlation coefficients for each 2 week gestation period were not very illuminating either. Consistent correlations throughout gestation were found between 'free' oestrone and 'free' oestradiol, 'free' oestrone and

Table 9.5 Previous longitudinal studies throughout pregnancy showing which steroid, protein and/or glycoprotein hormones were measured. Any conclusions which are relevant to the present study are also shown. [S=serial samples, C=cross-sectional samples, E1=oestrone, E2=oestradiol, E3=oestriol, P=progesterone, DHEAS=dehydroepiandrosterone, hPL=human placental lactogen, ß-hCG= ß-human chorionic gonadotropin, PRL=prolactin]

Author	Year	S/C	Gestation	E1	E2	E3	P	DHEAS	hPL	ß-hCG	PRL	Relevant conclusion
Tulchinsky et al	1972	C	8/52 - Del	x	x	x	x					
Loriaux et al	1972	C	10-38 weeks	x	x	x						
Lindberg et al	1974	C+S	22/52 - Del	x	x	x						
Allen & Lachelin	1978	S	21/52 - Del		x	x	x					
Buster et al	1979	S	26-40 weeks		x	x		x				
Mathur et al	1980	S	≈32/52-Del		x	x	x					
Braunstein et al	1980	C	4-40 weeks						x	x		No correlation between ß-hCG and hPL after 1st trimester Differences in secretion pattern may be due to inhibitors of hCG synthesis and secretion acting at cytoplasmic level.
Kletzky et al	1985	C	8-42 weeks							x	x	
Boroditsky et al	1975	C	25-41 weeks				x			x		No correlation between ß-hCG and P levels in maternal serum. ?ß-hCG lower in male pregnancies because of inhibitory effect of higher P levels in umbilical artery.
De Hertogh et al	1975	S	5-40 weeks	x	x	x			x			
Egyed et al	1978	S	6/52 - Del		x		x		x		x	No direct relationship between E2 and PRL levels.
Ottesen & Lebech	1979	S	32/52 - Del			x	x		x			
Yuen et al	1980	S	6/52 - Del		x		x			x	x	Negative correlation between ß-hCG and PRL. Positive correlation between PRL and E2. Suggested PRL involved in control of ß-hCG and oestrogen secretion.
Haning et al	1983	C	4-40 weeks		x	x	x			x		hCG has -ve regression with E3 and +ve regression with P hCG production inhibited by steroid from fetal adrenal
Aspillaga et al	1983	S	3-38 weeks		x		x		x	x	x	Only weak correlation between E2 and PRL, suggesting no direct influence of E2 on PRL.
Furuhashi et al	1984	S	32/52 - Del			x			x	x		Strong +ve correlation between ß-hCG and hPL. Significant but weak correlation of E3 with hPL and ß-hCG
Bremme et al	1990	S	20/52 - Del			x			x	x	x	No consistent correlations between E3 and PRL.

DHEAS, and during most of the third trimester between 'free' oestrone and ß-hCG. The only other significant correlations occurred at 38 weeks gestation, between oestradiol and oestriol, oestradiol and progesterone, and plasma unconjugated oestriol with plasma unconjugated progesterone.

Final Comment

From the data in this study, no further light was shed on the controlling factors modulating the changes in steroid production during pregnancy and prior to the onset of labour. Many suggestions have been made about possible interrelationships involving only the substances measured in this study, but the results did not provide supporting evidence for the suggestions.

A possible criticism of the study would be that maternal peripheral levels of the various substances are not a good reflection of what is happening in the fetus, and at a local or even cellular level in the placenta and the uterus *(Norman et al, 1989)*. Also, the controlling factors are diverse and complex, and inevitably include many substances not measured in this study. Nevertheless, this study was the first to include the simultaneous measurements of so many steroids and proteins throughout pregnancy, and so it was disappointing to find that the results did not allow the initiation of any new, or confirmation of previously suggested hypotheses.

10

Saliva and plasma cortisol levels in various groups of nonpregnant, pregnant and postpartum women - relationship of raised cortisol levels in pregnancy to corticosteroid binding globulin levels

Introduction

It has been known for many years that plasma total and unbound cortisol (F) levels are elevated in late pregnancy, but the reasons for this are still not clear. In pregnant sheep, the rise in cortisol levels is known to play an important role in the onset of parturition. However, in the human, the role of raised cortisol levels in pregnancy and particularly in relation to parturition is uncertain.

A recent study has reported a sharp rise in maternal plasma total cortisol levels during the last 2 weeks before the onset of spontaneous labour at term, and an inappropriate rise in total cortisol in women with idiopathic preterm labour and women with prolonged rupture of membranes *(Phocas et al, 1990).* However, previous studies did not find this surge in cortisol before the onset of labour, but only once labour had commenced *(Jolivet et al, 1974; Carr et al, 1981). In vitro* work has shown that cortisol can either stimulate or inhibit prostaglandin production in intrauterine tissues. One hypothesis is that human parturition may result from a positive cascade interaction of corticotrophin-releasing hormone (CRH), adrenocorticotrophic hormone (ACTH), cortisol and prostaglandins *(Challis et al, 1990).* Women who subsequently go into preterm labour have been found to have raised CRH levels for several weeks before the onset of labour *(Campbell et al, 1987).*

Certainly, cortisol seems likely to have an important role in pregnancy and in relation to parturition, and merits continuing investigation. This study was designed to re-examine the possible mechanisms involved in the rise in cortisol levels that occurs in pregnancy.

It was first suspected that there might be an increase in the activity of the maternal adrenal cortex during pregnancy when it was noted by Aschoff (1910) and Aschner (1912) that the adrenal glands of women dying at or soon after delivery weighed more and had a larger cortex than those of non-pregnant women *(Bayliss et al, 1955)*. It was then found that the urinary excretion of glucocorticoids was raised in pregnancy *(Venning, 1946)*, and by the early 1950's plasma 17-hydroxycorticosteroid levels were being measured and were also found to be higher in pregnancy, with a gradual return to approximately non-pregnant levels in the postpartum period *(Gemzell, 1953; Bayliss et al, 1955)*. Since then many studies have demonstrated a rise in total and unbound F in pregnancy, although relatively few have looked at levels earlier than the third trimester *(Brien and Dalrymple, 1976; Demey-Ponsart et al, 1982; Abou-Samra et al, 1984; Dörr et al, 1989)* or in the postpartum period *(Jolivet et al, 1974; Brien and Dalrymple, 1976; Demey-Ponsart et al, 1982; Allolio et al, 1989)* , and few have used saliva to study the unbound F levels *(Vining et al, 1983; Darné et al, 1989; Allolio et al, 1989)*.

A variety of causes have been suggested for the rise of cortisol in pregnancy:

1) The rise in F is due to a fetal contribution to maternal F levels *(Chattoraj et al, 1976)*

2) The rise in oestradiol in pregnancy is responsible for the rise in corticosteroid binding globulin (CBG) *(Doe et al, 1969; Moore et al, 1978; Smith et al, 1987)*, and in turn the raised CBG levels lead to a rise in total maternal F levels *(De Moor et al, 1966; Jolivet et al, 1974; Demey-Ponsart et al, 1982; Vining et al,1983)*
3) Increased levels of progesterone or 17-hydroxyprogesterone lead to elevation of 'free' plasma F levels by displacement of F from CBG binding sites *(Rosenthal et al, 1969; Dunn et al, 1981; Bustamente and Crabbé, 1984; Abou-Samra et al, 1984)*
4) The sensitivity of the hypothalamic-pituitary axis to 'free' F is altered by increased levels of oestrogen *(Doe et al, 1969; Burke and Roulet, 1970)* , or progesterone *(Demey-Ponsart et al, 1982; Abou-Samra et al, 1984; Nolten and Rueckert, 1981)* , or in some other way *(Nolten et al, 1980; Sasaki et al, 1987)* .
5) Placental production of CRH *(Goland et al, 1986; Sasaki et al, 1987; Petraglia et al, 1987)* and/or ACTH or an ACTH-like hormone stimulates the production of F *(Genazzani et al, 1975; Rees et al, 1975; Carr et al, 1981)*

The aim of this study was to attempt to further elucidate the mechanisms responsible for the increase in total and 'free' F levels in pregnancy by studying the diurnal variation of saliva F and alterations in plasma F, progesterone, oestrogen and CBG levels in various groups of non-pregnant, pregnant and puerperal women.

Materials and methods

Six groups of women were studied:

1)10 nonpregnant women with regular cycles (N)

2)8 women who had been taking a combined oral contraceptive pill (containing 35μg or less of oestrogen) cyclically for at least two months, and who had already taken a pill for more than 3 days in the study cycle (OC) (Table 10.1)

3)10 women who were in the mid-luteal phase of a cycle in which superovulation had been achieved following treatment with human menopausal gonadotrophin (S)

4)9 women in early pregnancy (12-16 weeks gestation) (EP)

5)9 women in late pregnancy (37-39 weeks gestation) 3 of whom provided saliva samples only (LP)

6)6 women from group 5 also provided samples daily for the first 5 days postpartum, and 4 of these women continued to provide samples weekly until 33 days postpartum. (PP1-33) All 6 women had vaginal deliveries of live healthy infants (3 males, 3 females, birthweights 3.07-3.60 kgs) following the spontaneous onset of labour, and were breast feeding. One patient (no. 2) in the postpartum group took a progestogen-only pill from day 7 postpartum onwards.¶

All of the subjects provided hourly saliva samples (2 ml) throughout the day, (groups 1-4 from 07.00h until 23.00h, groups 5 and 6 from 08.00h until 22.00h), and a peripheral venous blood sample was taken at 09.00 on each study day. Food intake was not standardized, although the subjects were asked not to eat breakfast or lunch until after 08.00 and 12.00

¶ A special tribute is due to these postpartum women who participated in a study, which was very demanding in the days immediately following the birth of their babies, and which provided no direct benefit to them or their babies.

Table 10.1 Oral contraceptive pills (and their constituents) taken by each subject in the OC group, together with the number of pills (nop) consumed in the study cycle, prior to the day of study.

Subject	OC taken (nop)	Constituents of OC	
OC1	Loestrin (9)	EE2 30μg	NA 1mg
OC2	Logynon (16)	EE2 30/40/30μg	LN 50/75/125μg
OC3	Brevinor (5)	EE2 35μg	NA 0.5mg
OC4	Microgynon (20)	EE2 30μg	LN 150μg
OC5	Logynon (3)	EE2 30/40/30μg	LN 50/75/125μg
OC6	Microgynon (15)	EE2 30μg	LN 150μg
OC7	Trinovum (11)	EE2 35μg	NA 0.5/0.75/1mg
OC8	Minulet (19)	EE2 30μg	G 75μg

(EE2...ethinyl oestradiol, NA...norethisterone acetate, LN... levonorgestrel, G...gestodene)

respectively. None of the women were hospitalized, (except for those who were in the early postpartum period), and all of them went about their normal routines on the days of the study.

Saliva and plasma specimens were stored at -40°C prior to assay. All saliva samples were assayed for F, and the saliva samples from the late pregnancy group were also assayed for progesterone. All plasma samples were assayed for F and CBG. Plasma samples from the superovulation group, the early pregnancy group and the late pregnancy group were also assayed for progesterone, oestradiol and unconjugated oestriol.

Statistical analyses were performed using Student's t test and Spearman correlations.

Results.

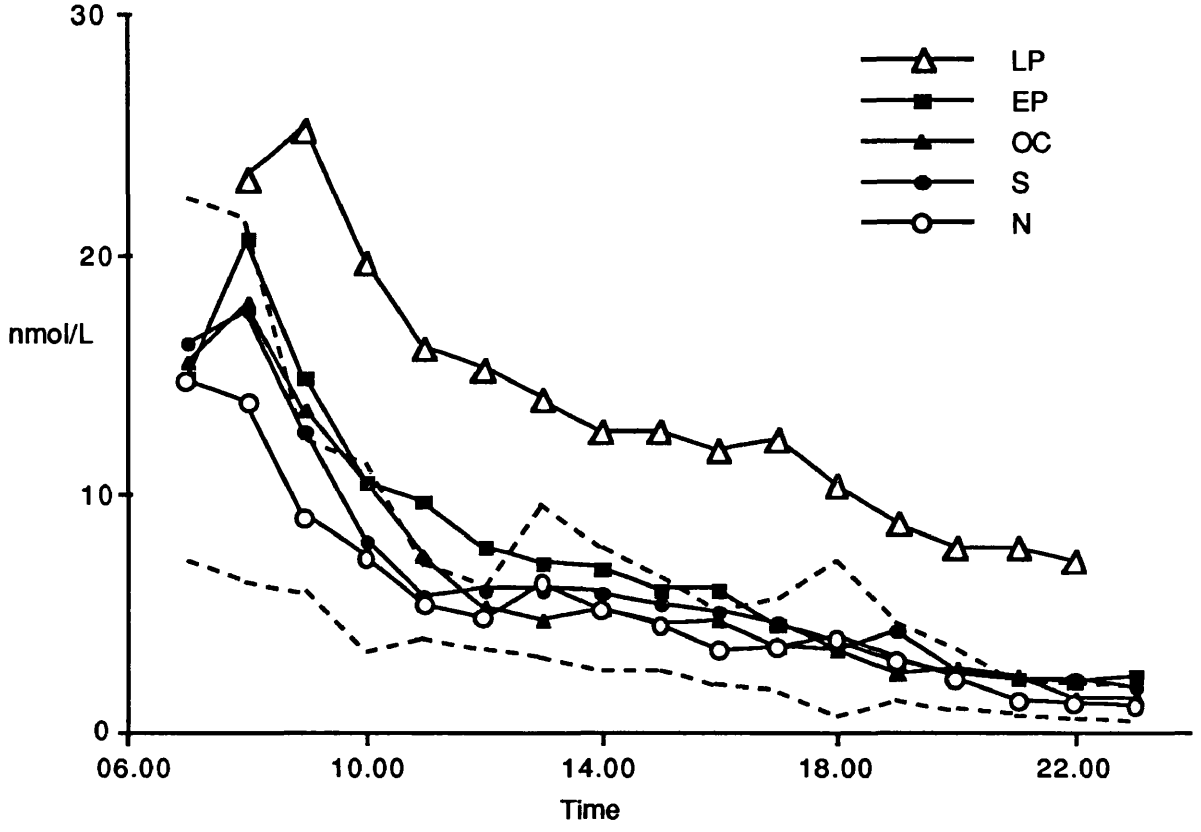
Saliva Cortisol

A clear diurnal variation in saliva F levels was present in each group, (Fig. 10.1). Saliva F levels in late pregnancy were significantly higher at all times of day than in normal, non-pregnant women, and mean levels were already slightly higher than in non-pregnant women by the beginning of the second trimester. There was a gradual decline in levels to normal during the puerperium. The saliva F levels were similar in the normal, oral contraceptive and superovulation groups.

The results were analysed in 2 ways:

¡) A mean daily saliva F score (= the mean of the hourly values from 08.00 until 22.00) was calculated for each individual and for each group (Table 10.2).

Figs. 10.1 Mean hourly saliva cortisol levels in different groups of women. [LP - late pregnancy (37-39 weeks gestation); EP - early pregnancy (12-16 weeks gestation); OC - taking combined oral contraceptive pill; S - superovulation following human menopausal gonadotropin; N - nonpregnant women with regular cycles; PP1-33 - postpartum days 1-33]
[The area between the dotted lines represents the 1-99% confidence limits of the normal mean]



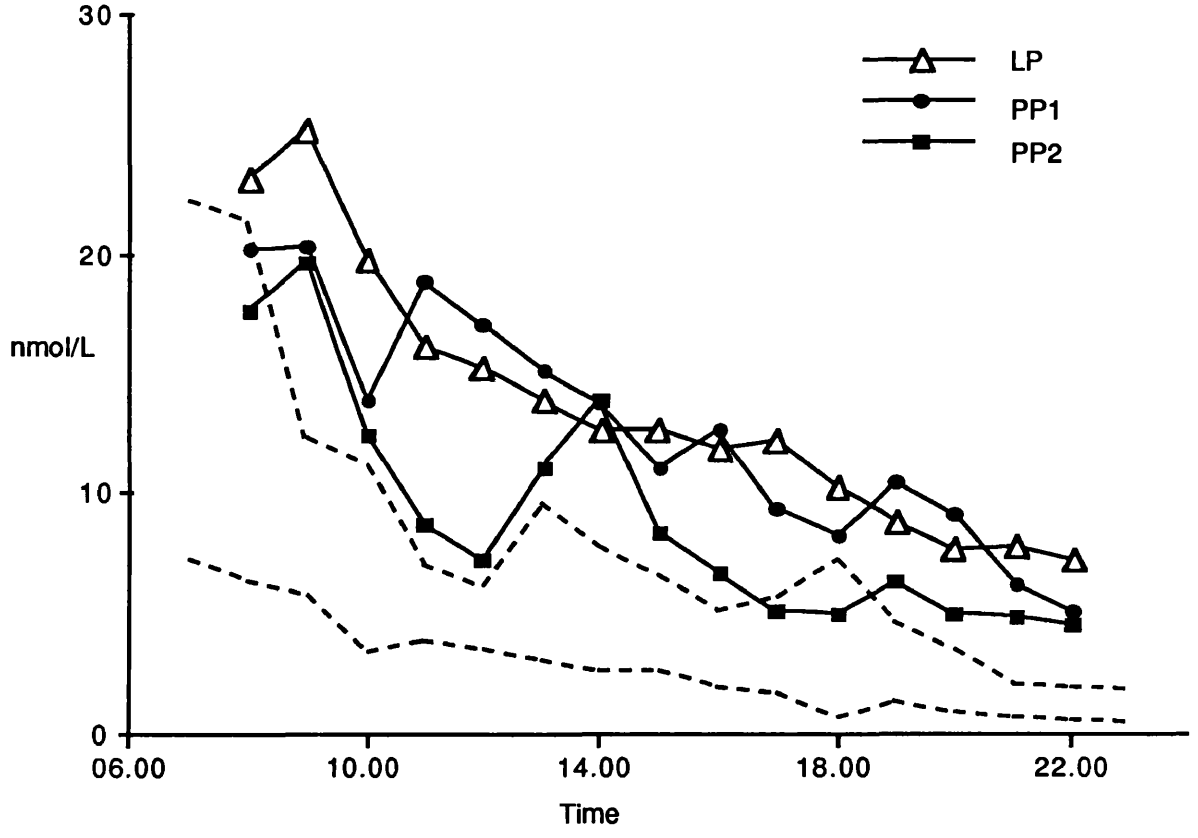
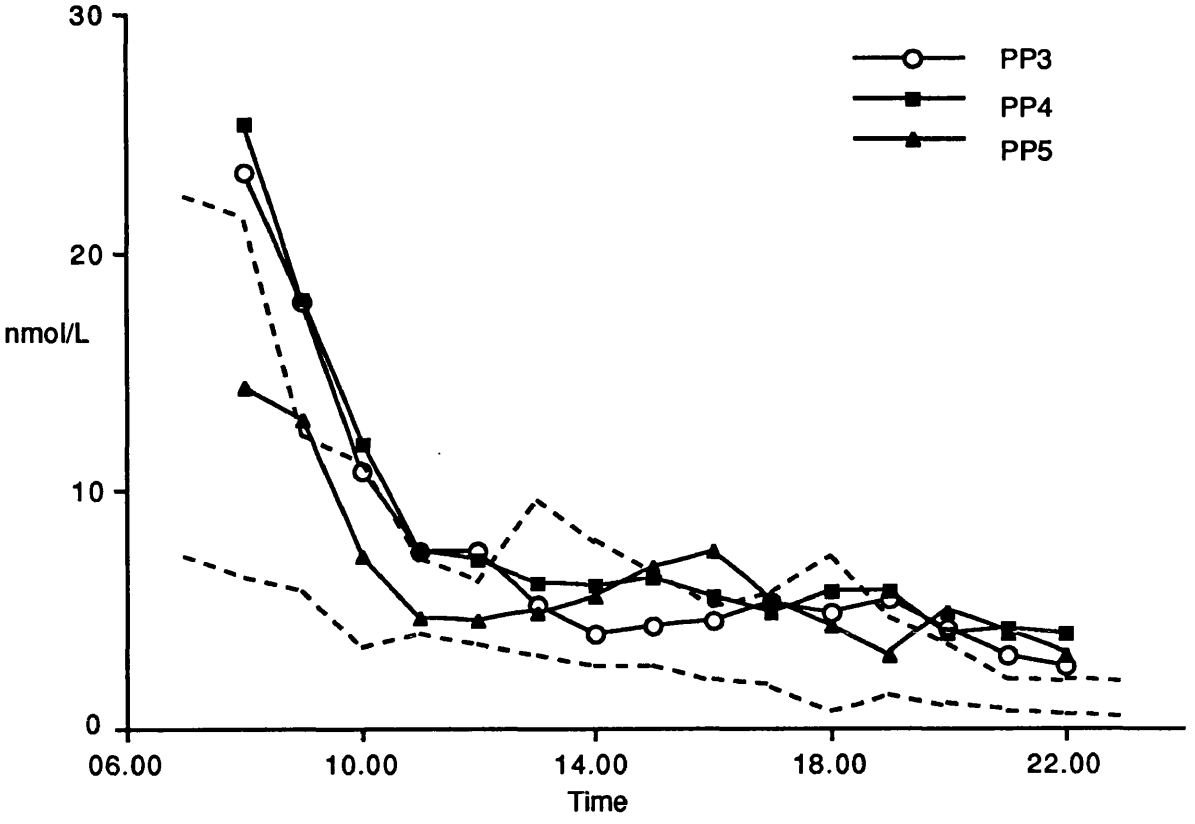


Fig. 10.1 cont. Mean hourly saliva cortisol levels in postpartum women



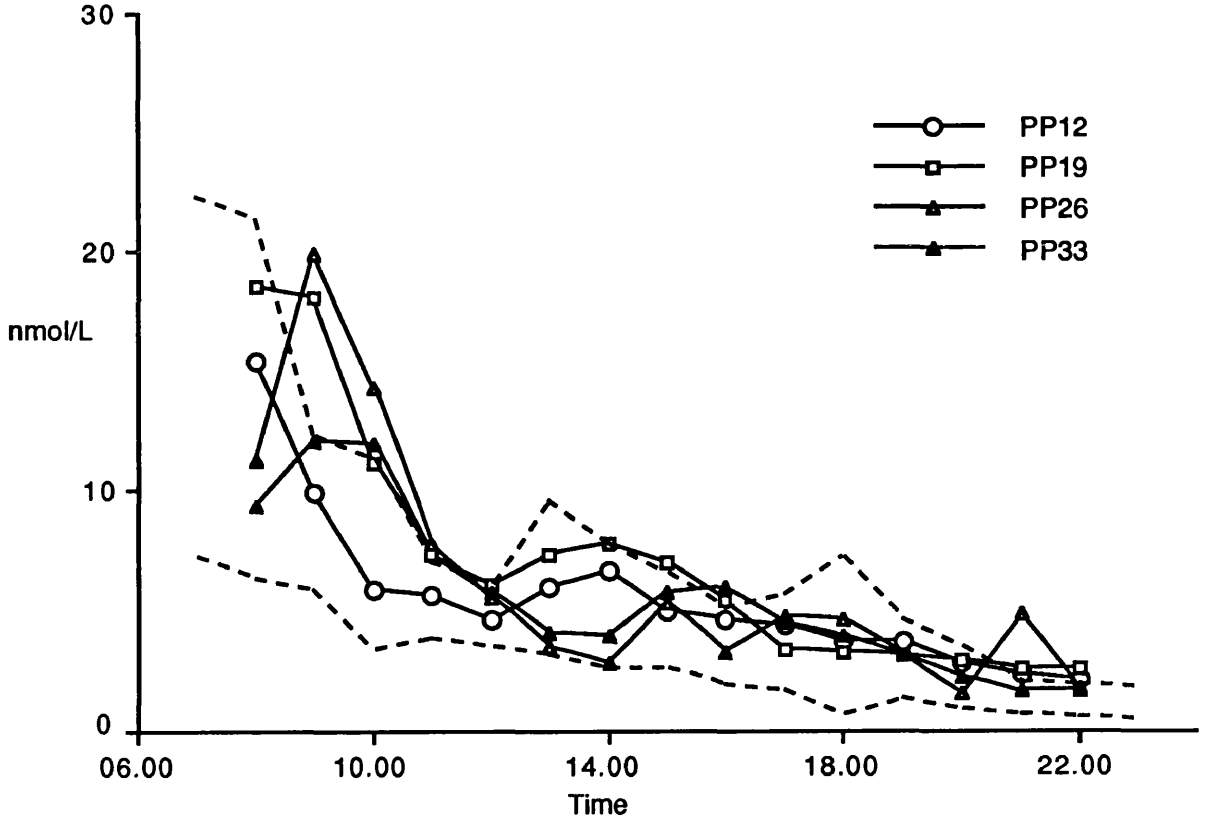


Table 10.2 Mean ± SD saliva F scores (hourly samples from 08.00-22.00h), CBG and plasma F (09.00h) levels (nmol/L) in different groups of nonpregnant and pregnant women. (Abbreviations are as for Fig. 10.1)

Group (n)	Saliva F	CBG	Plasma F
N (10)	5.0 ± 1.4	510 ± 106	285 ± 72
OC (8)	6.0 ± 1.4	1231 ± 117***	304 ± 97
S (10)	6.1 ± 0.9*	550 ± 83	404 ± 132*
EP (9)	7.2 ± 1.2**	929 ± 229***	520 ± 115***
LP (6)	13.6 ± 3.6***	1174 ± 193***	708 ± 183***
PP1 (4,6,6)¶	12.8 ± 5.3**	1024 ± 232***	913 ± 249***
PP2 (6)	9.1 ± 3.0*	934 ± 222***	736 ± 91***
PP3 (6)	9.0 ± 5.5*	905 ± 225**	657 ± 150***
PP4 (6)	8.1 ± 1.3**	867 ± 223**	569 ± 94***
PP5 (6)	6.2 ± 1.3	807 ± 171**	486 ± 63***
PP12 (4,6,6)¶	5.5 ± 1.7	678 ± 97*	382 ± 82*
PP19 (4,6,6)¶	7.1 ± 0.9*	539 ± 74	433 ± 147*
PP26 (4,6,6)¶	6.2 ± 1.6	511 ± 35	439 ± 181*
PP33 (4,6,6)¶	5.5 ± 1.1	502 ± 59	389 ± 126

*p≤0.05, **p≤0.002, ***p≤0.0001 - significantly different from normal nonpregnant group.

¶ Saliva samples were provided by only 4 subjects (the same 4) on each of these days.

¡¡) Within each group, the mean hourly saliva F score (= the mean of the scores within the group for each hour) was calculated (Table 10.3).

Daily saliva F scores

The superovulation group, and both the early and late pregnancy groups had significantly higher daily saliva F scores than the normal group ($p<0.05$, $p=0.002$ and $p<0.0001$ respectively) (Table 10.2). The scores in the early pregnancy group were significantly higher than in the superovulation group ($p<0.05$). The daily saliva scores fell gradually postpartum.

Hourly saliva F scores.

In late pregnancy, the F scores were significantly higher than in the normal group at every hour of the day, (Fig. 10.1, Table 10.3) In the superovulation and early pregnancy group the scores were significantly higher than in the normal group for 4 and 7 of the hours respectively, the higher values tending to be concentrated in the morning and evenings.

There was no significant fall in the hourly F scores on the first day postpartum compared to the late pregnancy group, although only 4 women managed to collect all the salivas on that day. However, by PP2 the F levels began to fall towards normal levels for some hours of the day and this trend continued over the following postpartum days. Again, the morning and evening values tended to remain high, whereas the afternoon values were not significantly different from normal from PP3 onwards. Unfortunately, only 4 women collected hourly samples from PP12 onwards, but in these women the F scores had returned to normal values at every hour (but one) of the day by PP33.

Table 10.3 Mean (SD) hourly saliva F scores in different groups of nonpregnant and pregnant women. (Abbreviations are as for Fig. 10.1) [* $p<0.05$, ** $p<0.002$, *** $p<0.0001$ significantly different from normal nonpregnant group]

Time	N	OC	S	EP	LP
07.00	14.7 (7.3)	15.5 (7.0)	16.3 (7.0)	14.8 (9.0)	
08.00	13.8 (7.3)	18.0 (4.3)	17.6 (3.2)	20.7 (4.4)*	23.1 (7.3)*
09.00	9.0 (3.0)	13.5 (5.7)*	12.6 (4.3)*	14.8 (5.7)*	25.2 (7.3)***
10.00	7.3 (3.7)	10.5 (4.0)	8.0 (1.7)	10.4 (3.6)	19.7 (3.2)***
11.00	5.4 (1.5)	7.4 (2.1)*	5.7 (1.5)	9.7 (3.0)**	16.1 (4.6)***
12.00	4.8 (1.4)	5.3 (1.8)	6.0 (1.4)	7.7 (1.8)**	15.2 (4.7)***
13.00	6.3 (3.0)	4.7 (1.5)	6.0 (2.1)	7.1 (3.5)	13.9 (3.8)***
14.00	5.2 (2.5)	5.2 (1.9)	5.8 (1.9)	6.8 (2.2)	12.6 (3.9)**
15.00	4.5 (2.0)	4.6 (1.7)	5.4 (2.1)	6.0 (1.1)	12.6 (4.4)**
16.00	3.5 (1.7)	4.7 (2.4)	5.1 (1.9)	5.9 (1.6)*	11.8 (4.4)***
17.00	3.6 (2.0)	3.6 (1.8)	4.6 (2.5)	4.5 (1.7)	12.2 (4.7)***
18.00	3.9 (3.3)	3.5 (2.5)	3.5 (1.1)	3.8 (1.9)	10.3 (2.5)**
19.00	3.0 (1.6)	2.5 (1.2)	4.3 (2.9)	2.9 (1.0)	8.8 (2.7)***
20.00	2.2 (1.3)	2.7 (1.8)	2.6 (1.3)	2.5 (0.9)	7.7 (2.2)***
21.00	1.3 (0.7)	2.4 (1.4)	2.2 (0.9)*	2.3 (0.7)*	7.8 (3.6)***
22.00	1.2 (0.5)	1.5 (1.1)	2.2 (0.7)**	2.1 (0.5)**	7.2 (3.6)***
23.00	1.1 (0.5)	1.5 (0.7)	1.9 (0.6)*	2.4 (1.6)*	

Time	PP1 (n4)	PP2 (n6)	PP3 (n6)	PP4 (n6)	PP5 (n6)
08.00	20.2 (4.5)	17.6 (5.3)	23.3 (8.0)*	25.4 (7.5)*	14.3 (6.2)
09.00	20.3 (12.5)*	19.6 (5.8)**	17.9 (6.8)*	18.0 (6.5)*	13.0 (3.9)*
10.00	13.9 (6.9)*	12.4 (3.2)*	10.8 (2.5)	11.9 (3.4)*	7.2 (2.2)
11.00	18.8 (11.0)**	8.7 (3.2)*	7.4 (1.7)*	7.4 (1.7)*	4.6 (1.6)
12.00	17.0 (9.9)**	7.2 (4.1)	7.4 (4.9)	7.1 (2.5)*	4.5 (1.1)
13.00	15.1 (13.2)	11.0 (7.0)	5.2 (5.9)	6.1 (2.1)	4.9 (1.6)
14.00	13.8 (11.7)*	13.9 (13.5)	3.9 (1.0)	6.0 (2.7)	5.5 (3.0)
15.00	11.1 (8.0)*	8.4 (3.7)*	4.3 (1.2)	6.3 (3.6)	6.8 (3.3)
16.00	12.6 (10.2)*	6.6 (1.4)*	4.5 (2.3)	5.5 (3.2)	7.5 (3.2)*
17.00	9.4 (3.6)**	5.1 (2.0)	5.3 (3.4)	4.9 (2.7)	5.4 (1.8)
18.00	8.2 (3.2)*	5.0 (3.0)	4.8 (3.0)	5.8 (3.2)	4.3 (1.7)
19.00	10.5 (7.2)*	6.3 (1.4)**	5.4 (2.4)*	5.7 (1.9)*	3.1 (1.3)
20.00	9.1 (3.8)**	5.0 (2.9)*	4.2 (2.1)*	3.9 (2.1)	4.9 (3.6)*
21.00	6.2 (2.8)**	4.8 (3.0)*	3.0 (1.3)*	4.2 (2.4)*	4.0 (1.9)**
22.00	5.1 (1.8)***	4.5 (2.4)**	2.6 (1.4)*	3.9 (2.3)*	3.1 (1.4)**

Time	PP12 (n4)	PP19 (n4)	PP26 (n4)	PP33 (n4)
08.00	15.4 (3.7)	18.5 (7.1)	11.2 (4.5)	9.3 (3.8)
09.00	9.9 (1.7)	18.1 (7.0)*	19.9 (8.8)*	12.0 (5.0)
10.00	5.8 (1.5)	11.1 (1.6)	14.3 (5.0)*	11.9 (7.7)
11.00	5.6 (1.7)	7.3 (0.9)*	7.7 (2.0)*	7.3 (4.6)
12.00	4.6 (2.4)	6.1 (1.8)	5.5 (2.8)	5.7 (2.1)
13.00	5.9 (4.6)	7.3 (2.2)	3.5 (2.0)	4.1 (0.9)
14.00	6.6 (6.7)	7.8 (4.2)	2.8 (0.8)	3.9 (0.5)
15.00	5.0 (1.6)	7.0 (3.6)	5.3 (1.5)	5.7 (2.1)
16.00	4.6 (1.6)	5.4 (2.4)	3.3 (1.0)	6.0 (2.3)*
17.00	4.4 (1.8)	3.4 (0.9)	4.7 (2.4)	4.5 (1.7)
18.00	3.7 (2.0)	3.3 (0.8)	4.6 (1.9)	3.9 (1.8)
19.00	3.7 (2.0)	3.1 (1.3)	3.2 (2.2)	3.2 (0.4)
20.00	2.8 (0.4)	2.9 (1.5)	1.6 (0.4)	2.2 (0.5)
21.00	2.4 (0.5)*	2.6 (1.0)*	4.8 (5.4)	1.7 (0.7)
22.00	2.1 (1.0)*	2.6 (1.0)*	1.8 (0.9)	1.7 (0.2)

Plasma CBG

Plasma CBG levels were significantly higher in early and late pregnancy ($p \leq 0.0001$) and in the oral contraception group ($p < 0.0001$) than in the normal nonpregnant group; whereas the levels in the superovulation group were not significantly different (Fig. 10.2, Table 10.2). CBG levels were also significantly higher in the early pregnancy than in the superovulation group ($p = 0.0001$). There was a gradual decline to non-pregnant levels by the third week postpartum. There was no correlation between CBG levels and plasma F levels in any group except for late pregnancy when the correlation was significant ($r = 0.94$, $p < 0.005$).

Plasma F

Plasma total F levels were significantly higher in early and late pregnancy ($p < 0.0001$) and in the superovulation group ($p < 0.05$) than in the non-pregnant group, (Fig. 10.3, Table 10.2). They were not significantly different from normal in the oral contraception group. Plasma F levels fell gradually towards normal by the fifth week postpartum.

Plasma and saliva progesterone, plasma oestradiol and oestriol levels

There was no diurnal variation in saliva progesterone levels in late pregnancy and there was no relationship between saliva F and progesterone in the hourly samples (Fig. 10.4). There was no significant difference between plasma progesterone levels in the superovulation (142 $\pm$ 38 nmol/L) and early pregnancy groups (114 $\pm$ 36 nmol/L). Mean plasma progesterone in the late pregnancy group was 357 $\pm$ 68 nmol/L. Mean plasma oestradiol levels were 2.3 $\pm$ 0.9, 10.3 $\pm$ 3.9 and 51.2 $\pm$ 16.6 nmol/L in the superovulation, early and late pregnancy groups respectively. Plasma unconjugated oestriol was not detectable in the superovulation group and

Fig. 10.2 Corticosteroid binding globulin (CBG) in different groups of women. The abbreviations used are the same as in Fig. 10.1
(Horizontal bar represents the mean for each group)

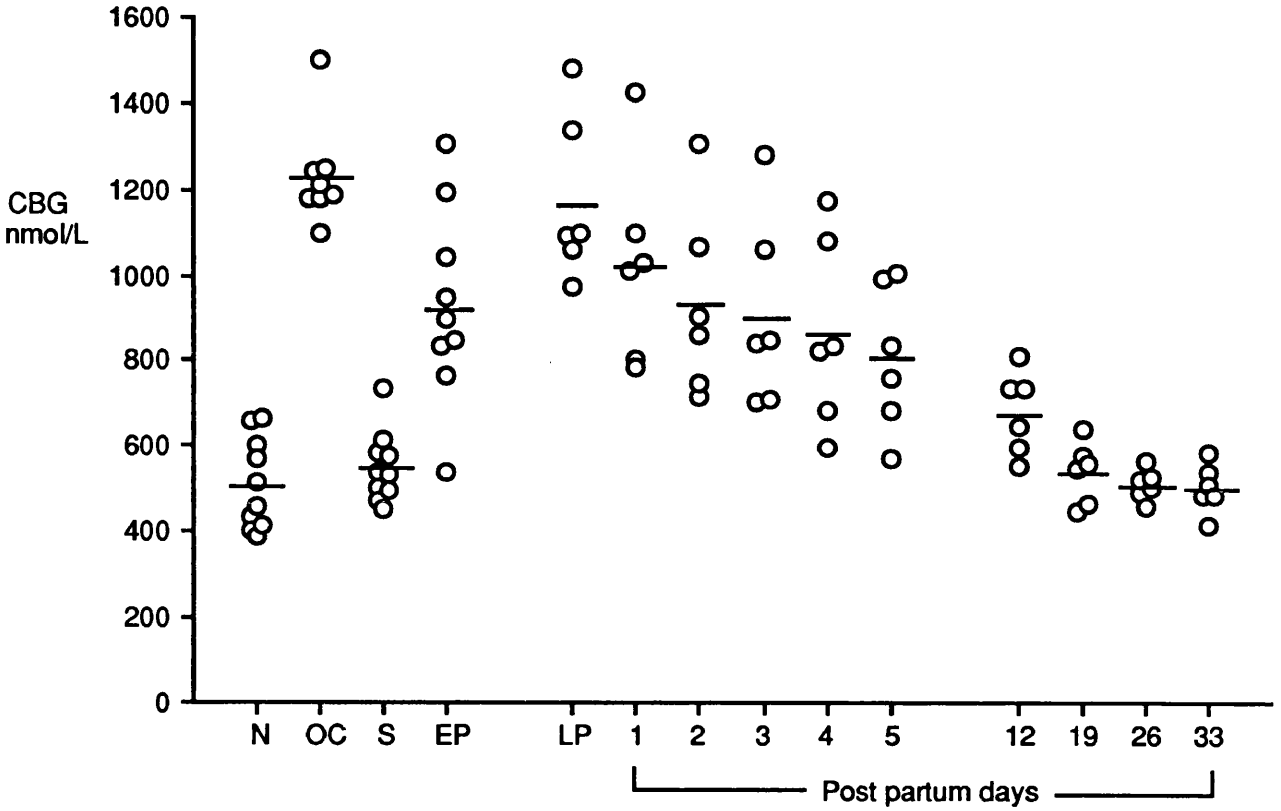


Fig. 10.3 Total plasma cortisol (F) levels in different groups of women. The abbreviations used are the same as in Fig. 10.1
(Horizontal bars represent the mean for each group)

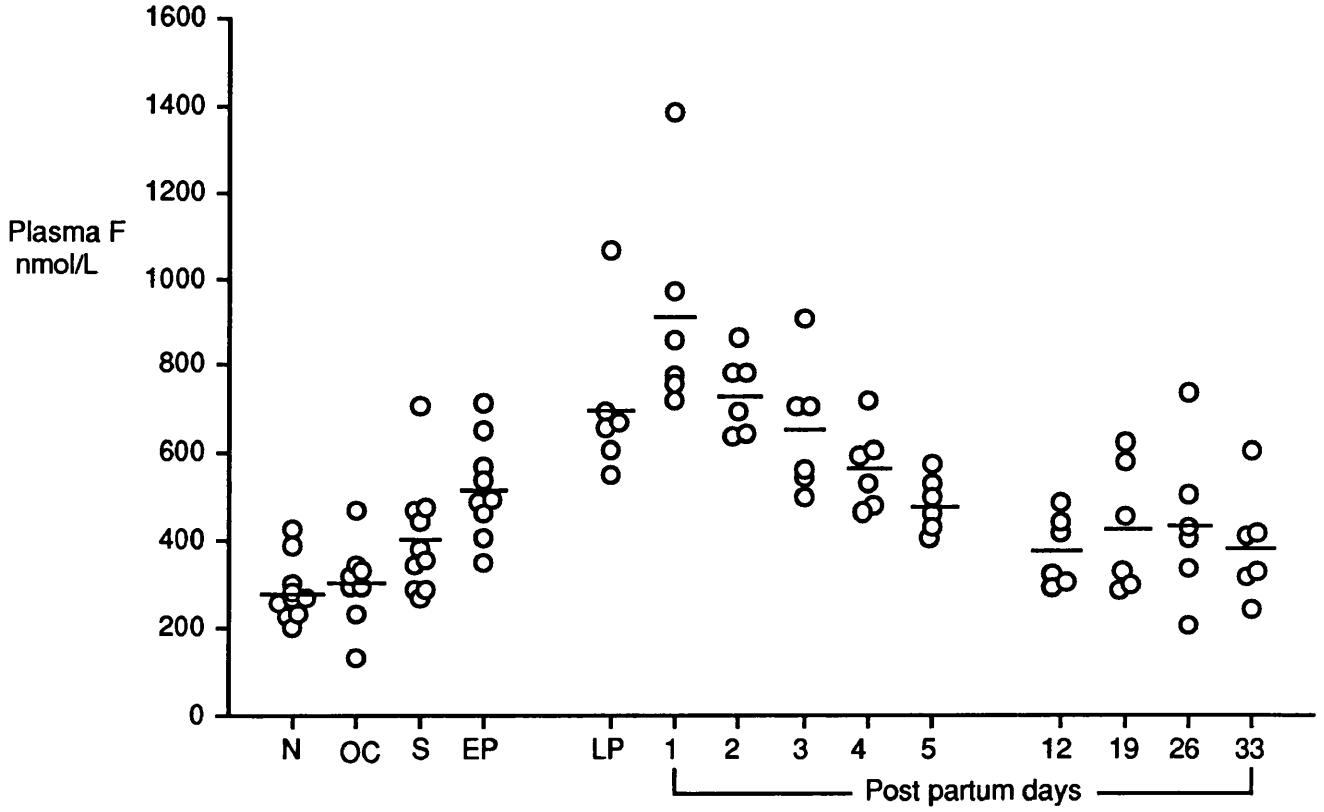
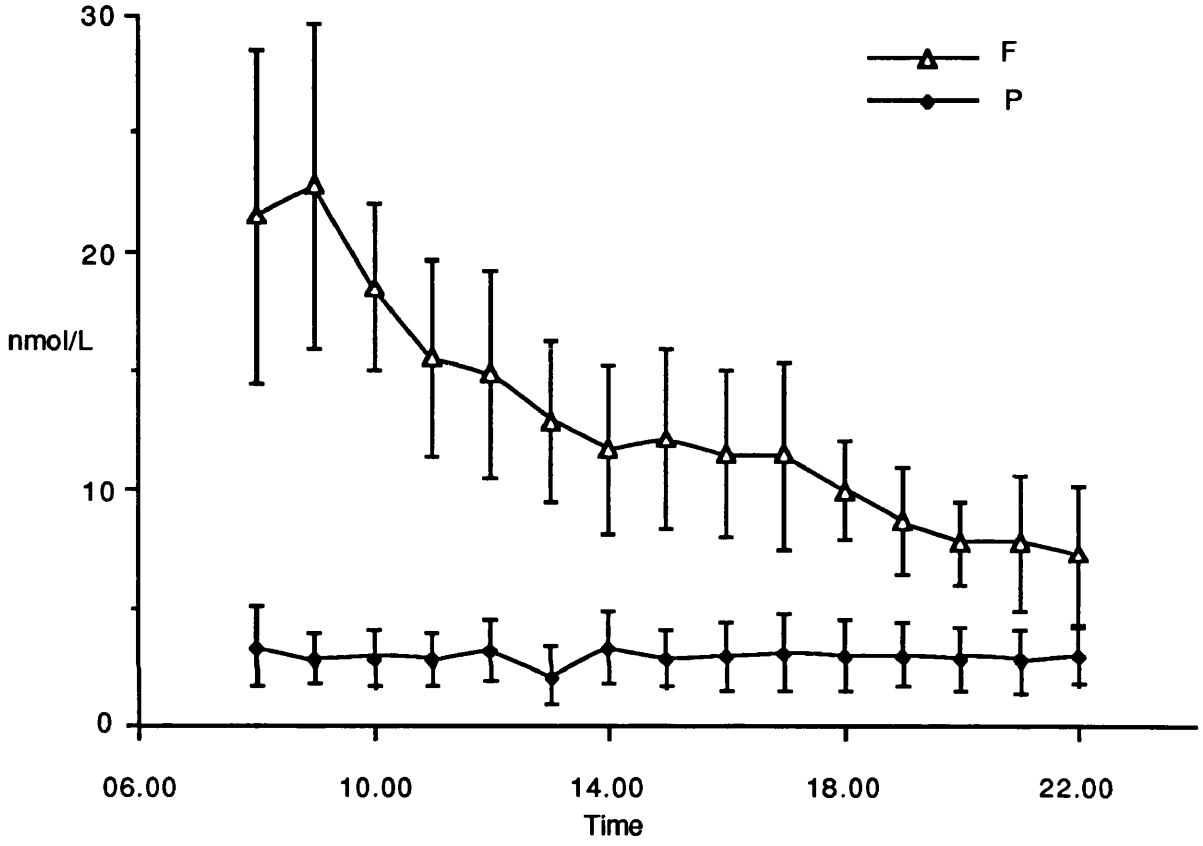


Fig. 10.4 Mean ± SD saliva cortisol (F) and progesterone (P) levels in nine women in late pregnancy.



the mean levels in the early and late pregnancy groups were 2.9 ± 1.0 and 39 ± 23.2 nmol/L respectively. There was no correlation between plasma F and unconjugated oestriol or between plasma F and oestradiol levels in any group. Neither was there any correlation between plasma CBG and plasma oestriol or oestradiol levels.

Discussion.

Saliva F levels have been found to be directly proportional to and about one third lower than plasma 'free' F levels *(Vining et al, 1983)*, and the use of saliva samples allows relatively easy assessment of circadian variations in 'free' F levels to be performed without the need for multiple blood sampling.

A normal pattern of diurnal variation in 'free' F was preserved in all of the groups studied but the baseline and mean saliva F levels differed from one group to another. The saliva F levels in the normal nonpregnant women and those in late pregnancy were similar to those described previously *(Vining et al, 1983; Darné et al, 1989; Allolio et al, 1989)*. Saliva F levels were significantly greater at all times studied in late pregnancy as compared to those in the normal nonpregnant women. An increase in mean saliva F levels was already apparent in the women in the early second trimester, in contrast to the findings of previous studies looking at unbound F in plasma at this gestation *(Brien and Dalyrmple, 1976; Demey-Ponsart et al, 1982)*.

The results are consistent with the findings in other studies of a normal pattern of diurnal variation but with significantly higher plasma 'free' F levels throughout the day resulting in a reduced percentage

morning/evening variation in levels in late pregnancy compared with the nonpregnant state *(Doe et al,1969; Burke and Roulet,1970; Nolten et al,1980; Vining et al,1983; Nolten and Rueckert, 1981; Allolio et al,1989).* The increased total F and CBG levels in pregnancy are also similar to those of other studies *(Nolten et al,1980; Demey-Ponsart et al,1982; Abou-Samra et al,1984).*

Current theories for the cause of elevated F levels in pregnancy were listed in the introduction, and will now be discussed in relation to the findings of this study where appropriate.

1) The rise in F is due to a fetal contribution to maternal F levels

Urinary 'free' F excretion was found to be increased in women with a normal pregnancy but lower in those with an anencephalic fetus, and it was postulated that there is a fetal contribution to maternal F *(Chattoraj et al, 1976).* However, Goldkrand et al *(1976)* found normal maternal plasma total F levels at delivery in two anencephalic pregnancies, although cord F levels were lower than normal. Further circumstantial evidence against the hypothesis is that maternal F levels in twin pregnancies are no higher than those for singleton pregnancies, and this should not be the case were both fetuses contributing to the maternal pool *(Goldkrand, 1978).* Furthermore, in the view of Nolten et al *(1980)* the steep maternal/fetal gradient would favour transfer of F from mother to fetus.

2) The rise in oestradiol in pregnancy is responsible for the rise in CBG, which in turn leads to a rise in total maternal F levels

It has been suggested that the increase in total F levels is due to the increase in CBG levels, but this would not explain the rise in 'free' F levels.

Previous studies have shown that the administration of various oestrogen and combined oestrogen/progestogen preparations results in considerable elevation of both total and 'free' F as well as CBG levels, *(Doe et al,1969; Burke,1969; Durber et al,1976).* However, using combined preparations, Burke *(1970)* demonstrated that plasma F levels were dependent on the dose of ethinyl oestradiol and that when less than 50μg oestrogen was taken both total and 'free' F remained in the normal range. Burke's findings were confirmed in this study, where the oral contraception group, (taking pills containing 35μg oestrogen or less), had plasma and saliva F levels which were normal in spite of greatly elevated CBG levels. Also, the % 'free' F was at the upper end of the range of all the groups in our study being 4.4% (range of all groups 2.2-4.5%). This suggests that increased CBG levels alone have little effect on plasma total or 'free' F levels.

3) Increased levels of progesterone or 17-hydroxyprogesterone lead to elevation of 'free' plasma F levels by displacement of F from CBG binding sites

One hypothesis for the increased 'free' F levels in pregnancy is that high levels of progesterone (or 17-hydroxyprogesterone) displace F from CBG. If such a displacement does occur during pregnancy, fluctuations in the concentration of saliva progesterone in a reverse circadian pattern to that of F might be expected. However, both this study (Fig 10.4) and a previous study (*Darné et al, 1987)* show that there is no significant change in saliva progesterone levels throughout the day in late pregnancy. Furthermore, it has been shown that although progesterone will displace F from purified CBG, when it is added to normal nonpregnant or pregnant serum at a concentration similar to that found in late pregnancy the displacement of F is only slight *(Doe et al, 1969).*

4) The sensitivity of the hypothalamic-pituitary axis to 'free' F is altered by increased levels of oestrogen, or progesterone, or in some other way

Nolten and Rueckert (1981) found that the 'free' F index was higher in pregnancy. They demonstrated that as pregnancy advanced, there was increasing responsiveness of the maternal adrenal glands to ACTH, as well as diminishing suppression of F levels by dexamethasone. They postulated that these changes are due to an increased sensitivity of the maternal adrenal to ACTH and also a resetting of the normal F feedback control mechanisms during pregnancy.

In one study involving the administration of high doses (200μg) of ethinyl oestradiol, 'free' F levels were found to be elevated at 09.00h but within the normal range at 21.00h *(Doe et al,1969)*, supporting a role for oestrogen in the elevation of cortisol levels in pregnancy. This contrasts with late pregnancy, when both morning and evening 'free' F levels are significantly higher than in nonpregnant women. It is possible that these changes are mediated via the hypothalamic-pituitary axis and that the difference in effect is due to the pharmacological action of ethinyl oestradiol as opposed to the physiological action of high levels of natural oestrogens, with possibly some additional effect from some other factor in late pregnancy.

Abou-Samra et al *(1984)* supported the hypothesis that a high progesterone concentration modifies the sensitivity of the hypothalamic-pituitary-adrenal axis, when they found that progesterone partly antagonized the negative feedback effect of corticosterone on ß-endorphin by rat anterior pituitary cells in primary culture.

It was also suggested that tissues may be refractory to the effects of F due to diminished binding of F to glucocorticoid receptors. Nolten and Rueckert *(1981)* did *in vitro* studies which indicated that specific binding of radioactively labelled dexamethasone to intact lymphocytes is significantly reduced in the presence of progesterone, presumably because of competitive binding of progesterone to glucocorticoid receptors.

To determine the effect of progesterone on plasma total and 'free' F levels, this study included women in whom superovulation was achieved with hMG. This provided a group with high progesterone levels combined with relatively low oestradiol levels as compared with early pregnancy. Saliva daily F means ($p<0.05$), and oestradiol levels ($p<0.0001$) were significantly higher in the early pregnancy group than in the superovulation group; whereas progesterone levels in the two groups were similar. Thus elevated progesterone levels do not appear to be the cause of the rise in 'free' F levels already found in early pregnancy. If increasing steroid levels are involved in the resetting of the sensitivity of the hypothalamic-pituitary-adrenal axis, this effect would appear to be more likely to be due to elevated oestrogen levels rather than to increased levels of progesterone.

5) Placental production of CRH and/or ACTH or an ACTH-like hormone stimulates the production of F

More recently, rising concentrations of CRH in the plasma of pregnant women have been reported *(Sasaki et al,1984; Goland et al,1986; Wolfe et al,1988)*. The CRH is thought to be placental in origin and CRH has been demonstrated in the placental cytotrophoblast cells *(Petraglia et al,1987)*. Further evidence supporting the placental production of CRH is the good correlation between maternal and fetal CRH concentrations, the fact that CRH is higher in the umbilical vein than umbilical artery, and that it

disappears within 15-24 hours after delivery *(Goland et al,1986; Sasaki et al,1987)*. It has been postulated that there is dual control of maternal ACTH secretion by hypothalamic and placental CRH in pregnancy *(Goland et al,1986; Sasaki et al,1987; Allolio et al,1989)*. Placental CRH does not have a diurnal variation *(Allolio et al,1989)*, and is not suppressed by dexamethasone *(Petraglia et al,1987)*.

Placental production of ACTH or an ACTH-like substance in pregnancy has also been demonstrated, and this placental production is another postulated cause of the raised F in pregnancy. However, conflicting data has been obtained about ACTH levels in pregnancy; and, although it seems that ACTH probably rises during pregnancy, in at least two studies the levels were still within the normal nonpregnant range *(Rees et al, 1975; Allolio et al,1989)* and in another study, the levels were lower than in nonpregnant women *(Carr et al,1981)*.

Although placental production of CRH and ACTH may lead to stimulation of F production by the maternal adrenals and it has been shown that the production rate of F is increased in pregnancy *(Nolten et al,1980)*, this cannot explain how a normal diurnal rhythm is maintained. Also, it has been shown that there is a carrier protein for CRH in the blood which may possibly mask its ACTH stimulating effects *(Linton et al,1988)*. Furthermore, our data show that both plasma and saliva F levels are already significantly elevated by the beginning of the second trimester, before the marked rise in CRH levels found in the second and third trimesters *(Sasaki et al,1987; Campbell et al,1987)*. Not surprisingly, there is no correlation between plasma CRH and F levels *(Campbell et al,1987)*.

It has been shown that arginine vasopressin augments the ACTH release that occurs in response to the administration of CRH *(Liu et al,1983)*. Recently it has been demonstrated that relatively small but significant changes in endogenous AVP levels, induced by infusion of hypertonic saline, are associated with a significant rise in ACTH and F levels *(Rittmaster et al,1987)*. Intravascular volume has increased by approximately 40% by the third trimester of pregnancy and as plasma AVP levels in the third trimester are similar to nonpregnant levels, this implies that the total amount of circulating AVP is increased during pregnancy *(Davison et al,1984; Brown and Gallery,1986)*. A further implication is that production of AVP must be significantly increased to maintain these peripheral levels if the metabolic clearance rate of AVP is unchanged. It is possible that such an increase might be sufficient to stimulate significant ACTH release from the anterior pituitary. It is also of interest that the enhanced F secretion stimulated by the administration of lysine vasopressin with CRH is not suppressed by pretreatment with dexamethasone *(Von Bardeleben et al,1985)*. An alteration in AVP secretion is therefore another possible mechanism by which the control of the hypothalamic-pituitary-adrenal axis might be modified in pregnancy.

Final Comment

Overall it seems most likely that there is a resetting of the hypothalamic-pituitary sensitivity to F feedback during pregnancy. This would be consistent with the finding of diminished F suppression by dexamethasone in the third trimester *(Nolten and Rueckert,1981; Rees et al,1975)*. The fact that the alteration in sensitivity to dexamethasone persists for several days postpartum *(Greenwood and Parker,1984; Smith et al,1987)* is also consistent with the finding in this study that F levels took several days

to return to normal after delivery. If the elevation in F was solely related to placental CRH and ACTH production it would be expected that F levels would return more rapidly to normal, as the half lives of CRH, ACTH and F are all short.

This study has shown clearly that the increase in 'free' and total F levels in pregnancy is not directly due to an increase in CBG levels. It seems likely that during pregnancy there is a resetting of the sensitivity of the hypothalamic-pituitary-adrenal axis probably under the influence of increasing oestrogen levels, and/or increased output of some other factor(s) such as AVP. Following delivery, the sensitivity of the hypothalamic-pituitary-adrenal axis slowly returns to normal.

11 Conclusions

1. A rise in the saliva oestriol:progesterone ratio prior to the onset of spontaneous labour at term was confirmed in the majority (68%) of the women studied. All of the women who went into preterm labour with intact membranes had saliva oestriol:progesterone ratios above the median for their gestation, and 47% had ratios above the 90th centile for their gestation. Women who laboured preterm following prolonged rupture of membranes had saliva oestriol:progesterone ratios which were evenly spread about the median. In the preterm group, if there was a rise in the ratio, it tended to occur 1 to 2 weeks prior to the onset of labour. As over half of the women who went into idiopathic preterm labour had ratios within the normal range, saliva oestriol:progesterone ratios are unlikely to be of use as a screening tool to predict women at risk of preterm labour in the general antenatal population. However, it might be a useful test in women who have recurrent preterm labours and who might benefit from progesterone treatment.

2. There is a linear increase in fetal adrenal size with increasing gestation, but there is no correlation between any adrenal parameter and plasma or saliva oestriol or progesterone levels at a given gestation. Therefore, even if saliva oestriol:progesterone levels had been found to be a good predictive test, fetal adrenal ultrasound measurements would not be helpful in the same circumstances. There was possibly a decrease in adrenal size in the weeks immediately prior to the spontaneous onset of labour, contrary to expectation, and this finding needs further investigation. As expected, the adrenal glands decreased markedly in size during the first 6 weeks of neonatal life, at a time when the kidneys were gradually increasing in size.

3. Progesterone can be administered conveniently by the oral or vaginal route. With pessaries, the absorption was slower, plasma peak values were lower and there was no correlation between plasma and saliva levels, with saliva levels actually exceeding plasma levels in some subjects. With oral progesterone plasma peak values were much higher and there was excellent correlation between plasma and saliva levels. The unpredictable and unphysiological levels of 'free' progesterone, which were found following 'Cyclogest' administration, require further investigation to determine whether it was a result of the route of administration or the pessary formulation.

4. Serial measurements of maternal plasma oestrone, oestradiol, oestriol, progesterone, dehydroepiandrosterone sulphate, human placental lactogen, ß-human chorionic gonadotrophin and prolactin and saliva oestrogens and progesterone throughout gestation did not shed any light on the controlling factors modulating the changes in steroid production during pregnancy and prior to the onset of labour.

5. The rise in 'free' and total cortisol levels in pregnancy is not due to the increase in corticosteroid binding globulin levels. The most plausible hypothesis seems to be a resetting of sensitivity of the hypothalamic-pituitary-adrenal axis, probably under the influence of increasing oestrogen levels, and/or increased levels of some other factor(s) such as AVP. Following delivery, the sensitivity of the hypothalamic-pituitary-adrenal axis slowly returns to normal.

A variety of possible studies, arising from the results in this thesis, could be undertaken in the future. Some of these have been mentioned above, and more are listed below:

1. Oestriol might be of use as a more physiological and gentle induction agent than those currently available. It would seem likely that the administration of oestriol for some days in late pregnancy would lead to the earlier onset of labour than would otherwise have occurred. Much larger doses of oestriol than are currently available would be required to cause an appropriate rise in the 'free' oestriol:progesterone ratio. Clearly, the first step would be to study the absorption of oestriol in pregnant women in order to determine the optimum dose and route of administration. This could then be followed by a double blind, controlled clinical trial to determine the efficacy of oestriol as an induction agent.

2. Whilst fetal adrenal ultrasonography is unlikely to be a useful clinical tool, it would be interesting to perform more frequent serial scans from 36 weeks gestation onwards in order to confirm the decrease in fetal adrenal size in the weeks immediately preceding the onset of labour that was found in this study. It would also be of interest to perform fetal adrenal ultrasonography on women admitted in preterm labour to see if the adrenal size fell within the normal range for the gestation.

3. Further work is necessary to determine the optimum dose and route of administration for progesterone in pregnancy. Once this has been achieved, the ideal way to assess the efficacy of progesterone as a treatment for women in preterm labour would be to carry out a multicentre trial. It would be preferable to include in the study design the collection of serial pretreatment and posttreatment saliva sample(s), which would enable the

oestriol:progesterone ratios of the women to be calculated subsequently. This would be important, as it is possible that only those women with a raised oestriol:progesterone ratio prior to treatment will respond to progesterone therapy.

4. It might be instructive to carry out a serial study from very early gestation, which included measurement of plasma and saliva oestrogens, progesterone and cortisol and plasma DHEAS, ACTH, CRH and if possible aVP, in order to study further the mechanisms and role of raised cortisol in pregnancy. Measurement of oestriol, progesterone, cortisol, ACTH and CRH in plasma and saliva where appropriate, in women who go into preterm labour might also be worthwhile, particularly as it has been suggested that cortisol and CRH levels are particularly elevated in preterm labour. It is possible that there may be an interrelationship between these findings, and the findings of an inappropriately raised oestriol:progesterone ratio in a proportion of women in preterm labour.

Work on some of these studies is already in progress at University College Hospital.

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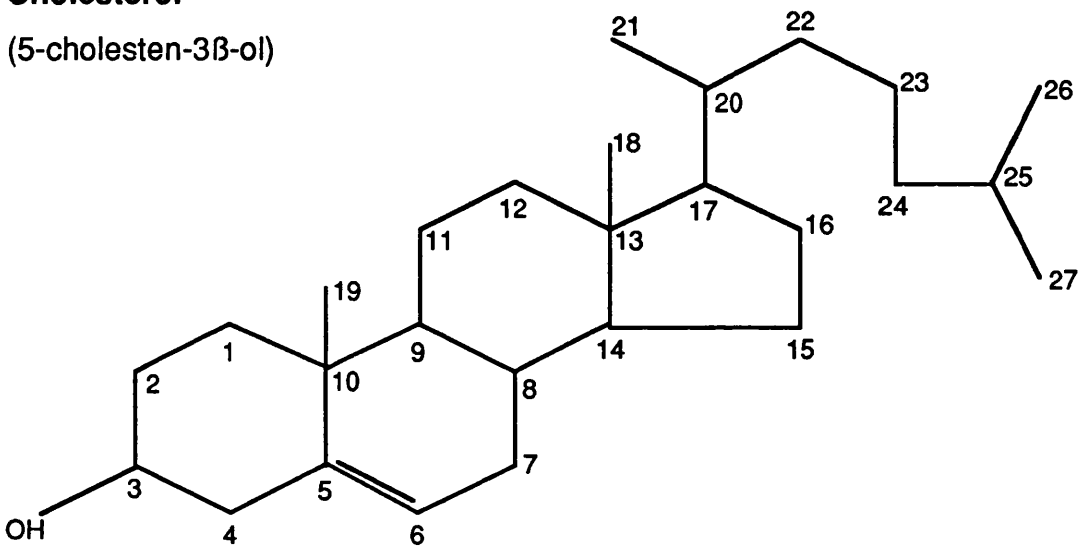
Appendix 1 Steroid Nomenclature

All steroid hormones are of basically similar structure with relatively minor chemical differences leading to striking alterations in biological activity. The basic structure is the perhydrocyclopentanephenanthrene molecule. It is composed of three 6 carbon rings and one 5 carbon ring. The convention for the numbering of the carbon atoms is shown below, using cholesterol as an example.

Cholesterol

(5-cholesten-3ß-ol)



Steroids are divided into three main groups according to the number of carbon atoms in the molecule:

21 carbon	Pregnane nucleus	Progestins and Corticoids
19 carbon	Androstane nucleus	Androgens
18 carbon	Oestrane nucleus	Oestrogens

Almost all naturally occurring and active steroids are nearly flat. Substituents below and above the plane of the ring are designated alpha (α) and beta (ß) respectively.

The basic name of a steroid is designated by the number of carbon atoms (ie. pregnane, androstane, oestrane). Numbers are used to indicate the positions of double bonds or extra groups on the molecule.

1,2 or 3 double bonds are described by -ene, -diene or -triene

1,2 or 3 hydroxyl groups " " " -ol, -diol or -triol

1,2 or 3 ketone groups " " " -one, -dione or -trione

Other designations include:

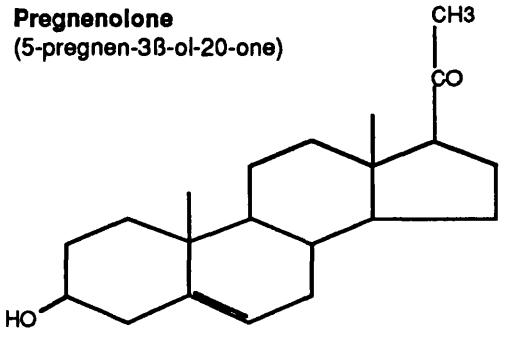
dehydro	elimination of 2 hydrogen atoms
deoxy	elimination of oxygen
nor	elimination of carbon
delta (Δ)	location of double bond

During steroidogenesis, the number of carbon atoms in cholesterol or any other steroid molecule may be reduced but never increased. The following reactions may take place:

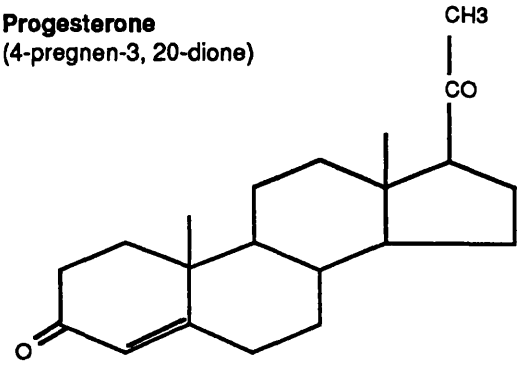
Cleavage of a side chain.....	desmolase reaction
Conversion of hydroxyl groups into ketones or vice versa.....	dehydrogenase reaction
Addition of an OH group.....	hydroxylation reaction
Creation of double bonds.....	removal of hydrogen
Addition of hydrogen to reduce double bonds...	saturation

The trivial and biochemical names of various steroids together with their structures are shown on the following pages.

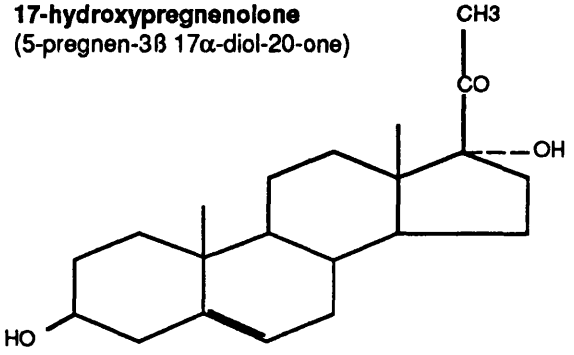
Pregnenolone
(5-pregnen-3ß-ol-20-one)



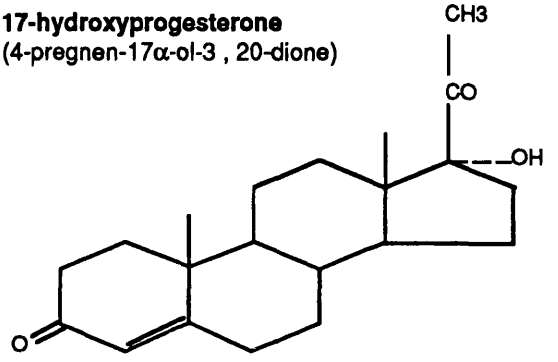
Progesterone
(4-pregnen-3, 20-dione)



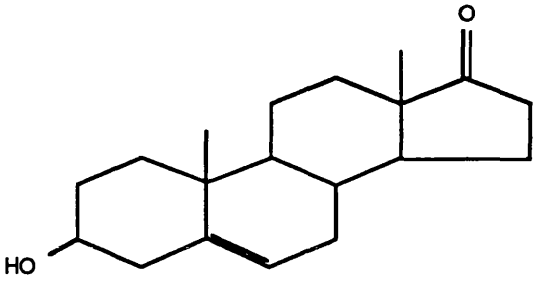
17-hydroxypregnenolone
(5-pregnen-3ß 17α-diol-20-one)



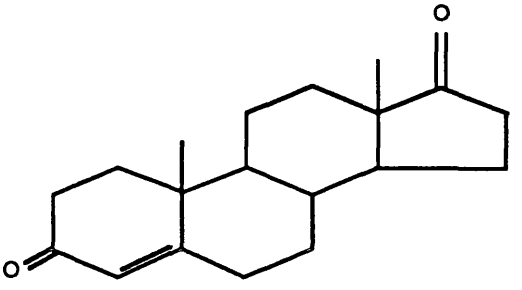
17-hydroxyprogesterone
(4-pregnen-17α-ol-3 , 20-dione)



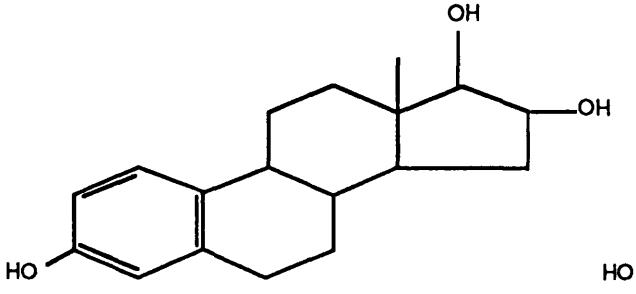
Dehydroepiandrosterone
(5-androsten-3ß-ol-17-one)



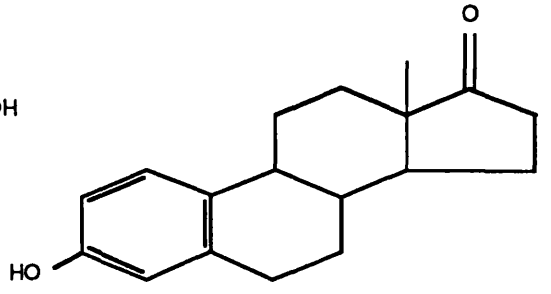
Androstenedione
(4-androsten-3, 17-dione)



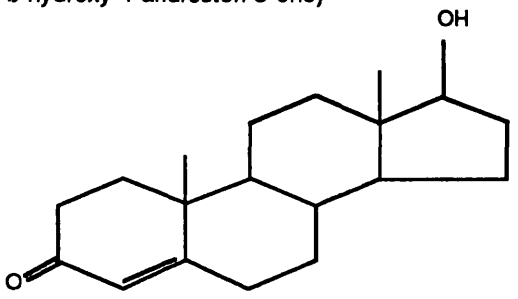
Oestriol
(1,3,5(10)-oestratrien-3,16α,17ß-triol)



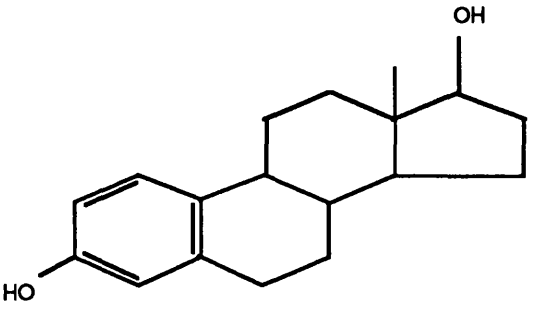
Oestrone
(1,3,5(10)-oestratrien-3-ol-17-one)



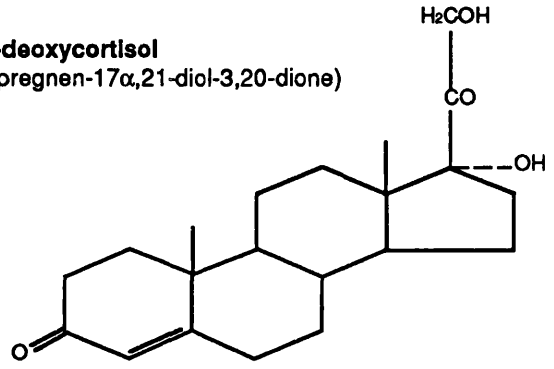
Testosterone
(17β-hydroxy-4-androsten-3-one)



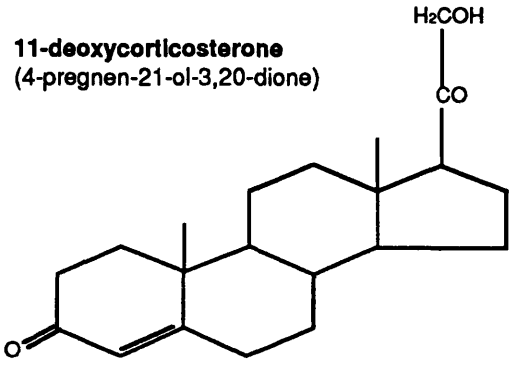
Oestradiol
(1,3,5(10)-oestratrien-3,17β-diol)



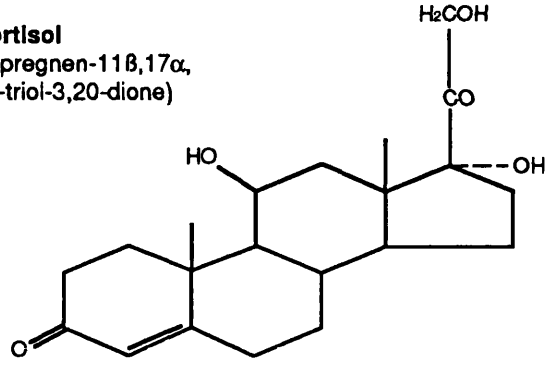
11-deoxycortisol
(4-pregnen-17α,21-diol-3,20-dione)



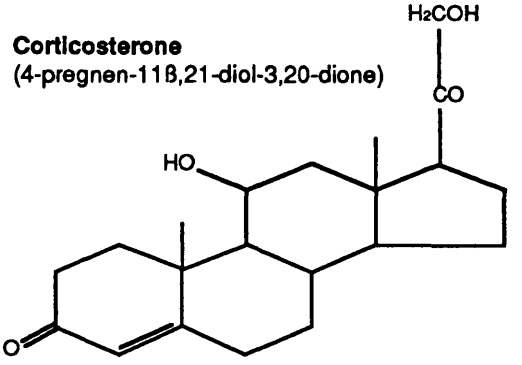
11-deoxycorticosterone
(4-pregnen-21-ol-3,20-dione)



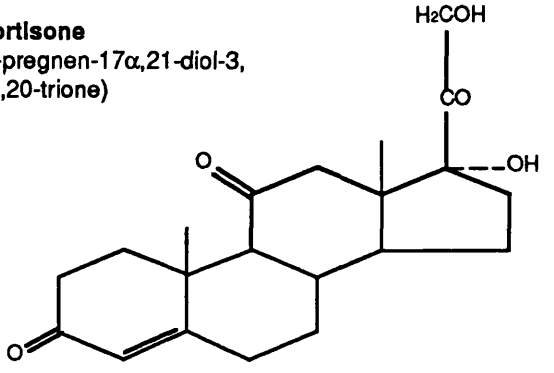
Cortisol
(4-pregnen-11β,17α,
21-triol-3,20-dione)



Corticosterone
(4-pregnen-11β,21-diol-3,20-dione)



Cortisone
(4-pregnen-17α,21-diol-3,
11,20-trione)



Aldosterone
(4-pregnen-11β,
21-diol-3,
18,20-trione)

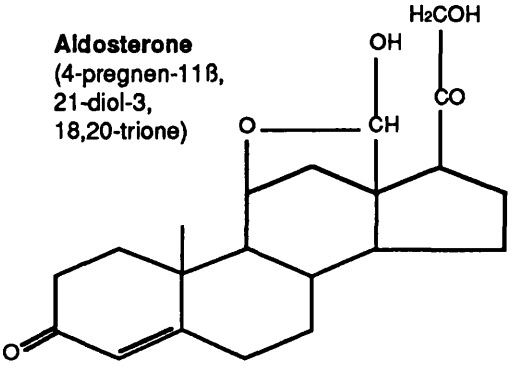


Table A1.1 The molecular weights of oestrone (E1), oestradiol (E2), oestriol (E3) and progesterone (P) together with the conversion factor from ng/ml to nmol/L for each steroid.

Steroid	MW	Conversion factor
E1	270.4	1 ng/ml = 3.698 nmol/L
E2	272.4	1 ng/ml = 3.671 nmol/L
E3	288.4	1 ng/ml = 3.467 nmol/L
P	314.5	1 ng/ml = 3.180 nmol/L
DHEAS	368.5	1 ng/ml = 2.714 nmol/L

Appendix 2

Raw Data

Table R.6.1 Saliva oestriol (E3) and saliva progesterone (P) levels in nmol/L throughout gestation (G, in days) in normal subjects (S1 to 28). The number of days before delivery (Dbd) and the oestriol:progesterone ratios (E3/P) are also shown.

S	G	Dbd	E3	P	E3/P
1	137	131	0.9	1.4	0.64
1	142	126	0.7	1.4	0.50
1	145	123	0.9	1.8	0.50
1	147	121	1.0	1.5	0.67
1	149	119	1.1	1.6	0.69
1	156	112	1.1	1.6	0.69
1	158	110	1.1	1.6	0.69
1	161	107	1.2	1.4	0.86
1	163	105	1.4	1.7	0.82
1	166	102	1.2	1.6	0.75
1	168	100	1.2	1.6	0.75
1	170	98	1.2	1.7	0.71
1	172	96	1.3	1.5	0.87
1	175	93	1.2	1.4	0.86
1	178	90	1.2	1.8	0.67
1	179	89	1.2	1.3	0.92
1	182	86	0.9	1.5	0.60
1	184	84	1.4	1.5	0.93
1	186	82	1.3	2.0	0.65
1	189	79	1.1	1.6	0.69
1	192	76	0.7	1.6	0.44
1	193	75	1.6	2.2	0.73
1	198	70	1.6	2.0	0.80
1	200	68	1.9	2.4	0.79
1	203	65	1.6	1.9	0.84
1	205	63	1.5	2.3	0.65
1	207	61	1.4	2.5	0.56
1	210	58	1.5	2.4	0.63
1	212	56	1.7	2.9	0.59
1	214	54	1.9	2.7	0.70
1	218	50	1.3	2.5	0.52
1	219	49	1.6	2.9	0.55
1	221	47	1.6	2.8	0.57
1	224	44	1.5	3.0	0.50
1	225	43	1.2	3.3	0.36
1	228	40	1.4	2.7	0.52
1	231	37	1.5	3.0	0.50
1	233	35	1.5	3.2	0.47
1	235	33	2.0	3.6	0.56
1	238	30	2.0	3.6	0.56
1	240	28	2.2	3.6	0.61
1	242	26	2.4	3.3	0.73
1	246	22	2.5	3.3	0.76
1	247	21	2.9	3.1	0.94
1	249	19	2.3	3.0	0.77
1	253	15	3.2	3.6	0.89
1	254	14	3.9	3.7	1.05
1	256	12	3.8	3.7	1.03
1	259	9	4.0	2.6	1.54
1	261	7	4.2	3.0	1.40
1	263	5	5.3	4.1	1.29
1	266	2	5.4	3.5	1.54

S	G	Dbd	E3	P	E3/P
2	147	144	0.6	1.1	0.55
2	151	140	0.7	1.6	0.44
2	152	139	0.8	1.9	0.42
2	154	137	1.0	1.6	0.63
2	157	134	0.8	1.7	0.47
2	159	132	0.8	1.5	0.53
2	162	129	1.0	1.8	0.56
2	164	127	0.9	2.0	0.45
2	168	123	1.1	2.3	0.48
2	171	120	1.2	1.2	1.00
2	174	117	1.1	1.1	1.00
2	178	113	1.2	1.3	0.92
2	180	111	1.2	1.6	0.75
2	182	109	1.6	1.7	0.94
2	185	106	1.6	1.9	0.84
2	187	104	1.4	2.0	0.70
2	189	102	1.7	2.0	0.85
2	192	99	1.6	2.5	0.64
2	194	97	1.2	2.0	0.60
2	196	95	1.4	2.3	0.61
2	203	88	1.9	2.5	0.76
2	206	85	1.7	2.4	0.71
2	208	83	1.3	2.3	0.57
2	210	81	1.5	2.2	0.68
2	213	78	1.9	2.2	0.86
2	215	76	1.5	2.3	0.65
2	217	74	2.2	2.6	0.85
2	221	70	1.7	2.7	0.63
2	222	69	1.4	2.5	0.56
2	224	67	2.2	2.5	0.88
2	226	65	2.1	3.6	0.58
2	231	60	1.9	2.5	0.76
2	233	58	2.1	2.0	1.05
2	234	57	2.9	2.7	1.07
2	236	55	2.7	2.4	1.13
2	239	52	2.7	2.6	1.04
2	241	50	2.8	3.0	0.93
2	245	46	3.1	3.2	0.97
2	249	42	2.7	2.8	0.96
2	252	39	3.4	3.0	1.13
2	255	36	4.7	3.5	1.34
2	257	34	4.0	2.9	1.38
2	259	32	5.3	3.1	1.71
2	263	28	4.1	3.0	1.37
2	264	27	4.3	3.2	1.34
2	266	25	4.1	3.0	1.37
2	269	22	4.5	3.7	1.22
2	272	19	4.7	3.0	1.57
2	273	18	4.8	3.3	1.45
2	276	15	5.4	3.6	1.50
2	278	13	5.3	3.1	1.71
2	281	10	5.5	3.1	1.77
2	283	8	7.1	4.0	1.78

S	G	Dbd	E3	P	E3/P
3	146	142	0.4	1.0	0.40
3	148	140	0.3	0.4	0.75
3	151	137	0.4	0.4	1.00
3	153	135	0.4	0.5	0.80
3	155	133	0.5	1.0	0.50
3	157	131	0.5	0.7	0.71
3	160	128	0.6	0.9	0.67
3	167	121	0.6	0.6	1.00
3	169	119	0.4	0.6	0.67
3	171	117	0.6	0.4	1.50
3	174	114	0.6	0.4	1.50
3	176	112	0.5	0.4	1.25
3	179	109	0.8	0.5	1.60
3	182	106	0.6	0.5	1.20
3	183	105	0.6	0.7	0.86
3	186	102	0.6	0.6	1.00
3	189	99	0.6	0.5	1.20
3	191	97	0.9	0.7	1.29
3	193	95	1.0	0.4	2.50
3	195	93	0.8	0.8	1.00
3	197	91	1.1	1.6	0.69
3	199	89	1.1	1.0	1.10
3	202	86	1.0	0.8	1.25
3	204	84	1.2	0.9	1.33
3	207	81	0.8	1.1	0.73
3	209	79	1.1	1.2	0.92
3	212	76	1.1	1.2	0.92
3	214	74	0.9	1.4	0.64
3	217	71	1.3	1.3	1.00
3	218	70	1.4	1.3	1.08
3	223	65	1.0	1.3	0.77
3	230	58	0.9	1.3	0.69
3	232	56	1.1	1.2	0.92
3	234	54	1.2	1.3	0.92
3	238	50	1.2	1.7	0.71
3	240	48	1.4	1.5	0.93
3	242	46	1.8	1.2	1.50
3	246	42	1.6	1.5	1.07
3	249	39	1.4	1.3	1.08
3	253	35	1.3	1.6	0.81
3	255	33	1.6	1.7	0.94
3	258	30	1.8	2.0	0.90
3	262	26	1.4	1.4	1.00
3	265	23	1.3	1.7	0.76
3	268	20	1.7	1.7	1.00
3	271	17	1.7	1.5	1.13
3	273	15	2.0	1.3	1.54
3	277	11	3.0	2.3	1.30
3	279	9	2.9	2.0	1.45
3	282	6	3.1	2.3	1.35
3	285	3	3.3	1.7	1.94

S	G	Dbd	E3	P	E3/P
4	137	150	0.7	1.1	0.64
4	139	148	0.6	1.0	0.60
4	141	146	1.1	1.2	0.92
4	144	143	1.0	1.3	0.77
4	147	140	1.6	1.3	1.23
4	149	138	0.8	0.8	1.00
4	151	136	0.3	1.3	0.23
4	153	134	1.0	1.5	0.67
4	155	132	1.3	1.3	1.00
4	158	129	1.1	1.4	0.79
4	161	126	1.5	1.3	1.15
4	165	122	1.1	0.9	1.22
4	167	120	1.2	1.1	1.09
4	169	118	1.5	1.4	1.07
4	172	115	1.5	1.2	1.25
4	174	113	0.6	1.3	0.46
4	177	110	1.3	1.3	1.00
4	179	108	1.5	1.3	1.15
4	181	106	1.8	1.1	1.64
4	183	104	1.2	1.0	1.20
4	186	101	1.6	1.4	1.14
4	188	99	1.3	1.3	1.00
4	190	97	1.5	1.3	1.15
4	193	94	2.1	1.2	1.75
4	197	90	1.6	1.4	1.14
4	200	87	2.0	1.7	1.18
4	203	84	1.2	1.8	0.67
4	209	78	1.7	1.6	1.06
4	211	76	1.1	2.6	0.42
4	215	72	2.1	2.7	0.78
4	220	67	2.0	2.5	0.80
4	224	63	2.1	2.5	0.84
4	226	61	2.2	2.6	0.85
4	229	58	1.7	2.4	0.71
4	232	55	2.3	2.6	0.88
4	234	53	2.3	1.7	1.35
4	237	50	2.9	2.7	1.07
4	240	47	3.5	2.6	1.35
4	244	43	3.5	2.7	1.30
4	246	41	2.8	1.6	1.75
4	247	40	2.9	2.1	1.38
4	249	38	4.7	2.2	2.14
4	251	36	3.9	2.9	1.34
4	253	34	3.9	2.8	1.39
4	256	31	5.2	2.5	2.08
4	258	29	4.4	2.0	2.20
4	260	27	5.3	2.2	2.41
4	265	22	4.6	2.8	1.64
4	268	19	5.2	2.5	2.08
4	270	17	4.2	2.1	2.00
4	272	15	5.8	2.7	2.15
4	275	12	7.1	2.7	2.63
4	278	9	6.4	3.0	2.13

S	G	Dbd	E3	P	E3/P
5	140	128	0.5	0.8	0.63
5	145	123	0.5	1.0	0.50
5	147	121	0.9	1.1	0.82
5	150	118	0.8	1.6	0.50
5	152	116	0.8	1.3	0.62
5	154	114	0.9	1.4	0.64
5	157	111	0.8	1.5	0.53
5	159	109	0.5	1.4	0.36
5	161	107	0.8	1.4	0.57
5	164	104	0.8	1.3	0.62
5	166	102	0.7	1.2	0.58
5	168	100	0.8	1.4	0.57
5	171	97	0.8	1.5	0.53
5	173	95	0.9	1.3	0.69
5	175	93	0.6	1.2	0.50
5	178	90	0.9	1.2	0.75
5	180	88	0.9	1.4	0.64
5	182	86	0.9	1.5	0.60
5	185	83	0.9	1.6	0.56
5	187	81	1.0	1.6	0.63
5	202	66	1.2	1.8	0.67
5	203	65	0.9	1.9	0.47
5	206	62	1.1	1.3	0.85
5	208	60	1.1	1.2	0.92
5	210	58	2.2	1.8	1.22
5	213	55	1.5	1.5	1.00
5	215	53	1.8	1.6	1.13
5	217	51	1.9	2.2	0.86
5	220	48	1.4	2.1	0.67
5	222	46	1.4	2.2	0.64
5	224	44	1.6	2.0	0.80
5	231	37	1.7	1.6	1.06
5	234	34	2.0	2.3	0.87
5	236	32	1.3	2.2	0.59
5	238	30	1.9	1.8	1.06
5	241	27	2.3	2.2	1.05
5	243	25	1.4	2.0	0.70
5	245	23	1.4	1.4	1.00
5	248	20	2.1	1.3	1.62
5	250	18	1.8	1.4	1.29
5	252	16	2.1	1.5	1.40
5	255	13	2.2	1.6	1.38
5	257	11	2.2	2.3	0.96
5	259	9	2.4	2.2	1.09
5	264	4	2.7	2.9	0.93
5	266	2	2.6	1.6	1.63
6	131	150	0.3	1.3	0.23
6	143	138	0.4	0.9	0.44
6	146	135	0.3	0.7	0.43
6	148	133	0.7	1.1	0.64
6	150	131	0.5	1.9	0.26
6	152	129	0.4	0.9	0.44
6	156	125	0.5	0.8	0.63

S	G	Dbd	E3	P	E3/P
6	157	124	0.5	0.9	0.56
6	160	121	0.5	1.0	0.50
6	162	119	0.5	0.9	0.56
6	165	116	0.6	1.1	0.55
6	166	115	0.4	1.0	0.40
6	170	111	0.5	1.1	0.45
6	171	110	0.5	0.6	0.83
6	174	107	0.6	0.9	0.67
6	178	103	0.7	1.2	0.58
6	179	102	0.5	1.4	0.36
6	181	100	0.5	1.4	0.36
6	184	97	0.6	1.7	0.35
6	186	95	0.6	1.9	0.32
6	189	92	0.6	1.8	0.33
6	191	90	0.5	1.7	0.29
6	193	88	0.8	1.4	0.57
6	194	87	0.6	1.5	0.40
6	197	84	0.7	1.6	0.44
6	199	82	0.8	1.5	0.53
6	201	80	0.8	1.9	0.42
6	204	77	0.7	1.9	0.37
6	206	75	0.8	1.6	0.50
6	208	73	0.9	1.5	0.60
6	212	69	0.7	1.7	0.41
6	213	68	0.9	1.7	0.53
6	217	64	0.9	1.7	0.53
6	218	63	0.9	1.7	0.53
6	220	61	0.9	1.7	0.53
6	222	59	0.8	1.9	0.42
6	226	55	0.9	2.1	0.43
6	228	53	1.0	2.2	0.45
6	229	52	1.4	2.4	0.58
6	232	49	1.4	2.3	0.61
6	234	47	1.4	2.1	0.67
6	236	45	1.2	2.4	0.50
6	240	41	2.1	2.7	0.78
6	241	40	2.2	2.8	0.79
6	243	38	1.6	2.7	0.59
6	246	35	1.6	2.0	0.80
6	248	33	1.6	2.1	0.76
6	251	30	1.7	2.0	0.85
6	254	27	2.2	3.6	0.61
6	255	26	1.8	2.7	0.67
6	257	24	2.2	3.5	0.63
6	261	20	2.7	3.3	0.82
6	262	19	2.8	3.2	0.88
6	265	16	2.4	3.6	0.67
6	267	14	3.7	3.5	1.06
6	269	12	4.7	3.6	1.31
6	271	10	5.3	3.5	1.51
6	275	6	6.0	4.0	1.50
6	276	5	6.4	3.7	1.73
6	278	3	6.0	3.7	1.62

S	G	Dbd	E3	P	E3/P
7	144	136	0.2	0.5	0.40
7	146	134	0.4	0.7	0.57
7	148	132	0.4	0.9	0.44
7	151	129	0.5	0.9	0.56
7	154	126	0.4	0.7	0.57
7	156	124	0.4	0.9	0.44
7	158	122	0.4	0.7	0.57
7	160	120	0.3	0.7	0.43
7	161	119	0.4	0.6	0.67
7	163	117	0.4	0.6	0.67
7	172	108	0.6	0.7	0.86
7	174	106	1.0	1.6	0.63
7	175	105	0.5	0.6	0.83
7	177	103	0.5	0.7	0.71
7	179	101	0.5	0.6	0.83
7	182	98	0.7	0.4	1.75
7	184	96	1.3	1.0	1.30
7	187	93	0.6	1.1	0.55
7	189	91	0.6	0.6	1.00
7	191	89	0.5	0.6	0.83
7	193	87	0.5	0.7	0.71
7	196	84	0.7	0.5	1.40
7	199	81	1.0	1.0	1.00
7	200	80	0.8	0.9	0.89
7	205	75	0.9	1.6	0.56
7	207	73	1.0	1.3	0.77
7	209	71	0.9	1.2	0.75
7	213	67	1.3	6.5	0.20
7	215	65	1.0	1.4	0.71
7	217	63	0.9	1.4	0.64
7	219	61	1.0	1.4	0.71
7	221	59	1.4	1.1	1.27
7	223	57	1.1	0.9	1.22
7	225	55	1.5	1.2	1.25
7	228	52	1.5	1.5	1.00
7	230	50	1.3	1.2	1.08
7	232	48	1.3	1.5	0.87
7	235	45	16.5	2.6	6.35
7	237	43	5.7	1.5	3.80
7	241	39	2.0	2.0	1.00
7	242	38	2.1	2.0	1.05
7	246	34	1.9	1.5	1.27
7	250	30	1.7	2.0	0.85
7	253	27	2.7	1.8	1.50
7	255	25	1.9	1.7	1.12
7	258	22	2.5	2.8	0.89
7	261	19	2.2	3.0	0.73
7	264	16	3.2	3.2	1.00
7	267	13	3.2	3.5	0.91
7	271	9	2.9	2.7	1.07
7	273	7	2.9	2.4	1.21
7	276	4	2.4	1.9	1.26
8	141	143	1.1	0.7	1.57
8	144	140	0.8	0.6	1.33
8	146	138	1.3	0.8	1.63
8	147	137	1.2	0.6	2.00
8	149	135	1.1	0.5	2.20

S	G	Dbd	E3	P	E3/P
8	152	132	1.3	0.8	1.63
8	155	129	1.3	0.8	1.63
8	157	127	1.4	0.8	1.75
8	159	125	1.9	0.9	2.11
8	163	121	1.2	0.8	1.50
8	165	119	1.2	0.8	1.50
8	167	117	1.5	1.0	1.50
8	169	115	1.9	0.8	2.38
8	173	111	1.6	0.7	2.29
8	175	109	1.7	0.9	1.89
8	177	107	2.5	1.0	2.50
8	178	106	2.3	0.9	2.56
8	181	103	2.3	1.3	1.77
8	183	101	2.3	1.0	2.30
8	185	99	2.6	1.0	2.60
8	188	96	2.2	1.0	2.20
8	190	94	2.1	0.8	2.63
8	192	92	2.8	1.0	2.80
8	194	90	2.4	1.1	2.18
8	197	87	2.5	1.0	2.50
8	199	85	2.9	1.4	2.07
8	202	82	2.9	1.2	2.42
8	204	80	2.9	1.5	1.93
8	206	78	3.4	1.6	2.13
8	210	74	2.3	1.2	1.92
8	212	72	3.0	1.5	2.00
8	214	70	2.6	1.3	2.00
8	216	68	3.0	1.4	2.14
8	218	66	2.9	1.4	2.07
8	220	64	3.2	1.4	2.29
8	222	62	2.9	1.4	2.07
8	225	59	3.5	1.8	1.94
8	227	57	3.7	2.2	1.68
8	231	53	4.8	1.7	2.82
8	234	50	5.4	1.9	2.84
8	236	48	3.5	1.8	1.94
8	238	46	4.3	2.1	2.05
8	240	44	4.4	2.5	1.76
8	242	42	5.5	2.1	2.62
8	247	37	2.0	1.5	1.33
8	251	33	4.2	1.8	2.33
8	253	31	4.5	2.1	2.14
8	255	29	6.1	2.6	2.35
8	258	26	5.9	2.1	2.81
8	261	23	8.9	2.3	3.87
8	265	19	9.4	2.6	3.62
8	268	16	5.4	2.0	2.70
8	270	14	8.2	2.4	3.42
8	272	12	10.6	3.0	3.53
8	274	10	10.7	2.6	4.12
8	276	8	11.1	2.6	4.27
8	278	6	9.1	2.0	4.55
8	279	5	7.2	2.3	3.13
8	280	4	10.9	2.8	3.89
8	281	3	7.5	1.4	5.36
8	282	2	11.1	2.4	4.63

S	G	Dbd	E3	P	E3/P
9	139	141	0.6	0.6	1.00
9	141	139	0.7	0.8	0.88
9	144	136	0.5	0.9	0.56
9	148	132	1.0	0.9	1.11
9	153	127	1.1	1.0	1.10
9	156	124	1.1	1.1	1.00
9	159	121	0.9	0.8	1.13
9	161	119	1.1	0.8	1.38
9	163	117	1.0	0.8	1.25
9	166	114	0.8	1.0	0.80
9	168	112	0.9	0.9	1.00
9	170	110	1.2	1.1	1.09
9	173	107	1.2	1.5	0.80
9	175	105	1.3	1.2	1.08
9	177	103	1.2	1.1	1.09
9	180	100	1.3	1.3	1.00
9	182	98	1.2	1.2	1.00
9	184	96	1.3	1.5	0.87
9	189	91	1.1	1.2	0.92
9	191	89	1.5	1.5	1.00
9	194	86	1.5	1.1	1.36
9	196	84	1.4	1.2	1.17
9	198	82	2.0	2.0	1.00
9	201	79	1.3	1.1	1.18
9	203	77	1.6	1.0	1.60
9	206	74	1.2	1.0	1.20
9	216	64	2.0	1.7	1.18
9	218	62	1.5	1.5	1.00
9	219	61	1.7	1.5	1.13
9	222	58	2.1	1.6	1.31
9	224	56	2.1	1.9	1.11
9	226	54	1.8	1.5	1.20
9	229	51	1.7	1.4	1.21
9	231	49	1.8	1.9	0.95
9	233	47	2.3	1.8	1.28
9	236	44	2.1	1.8	1.17
9	238	42	2.0	1.6	1.25
9	240	40	2.2	1.8	1.22
9	243	37	2.0	1.8	1.11
9	248	32	2.0	1.9	1.05
9	250	30	2.6	2.3	1.13
9	252	28	3.7	3.1	1.19
9	255	25	2.9	2.4	1.21
9	257	23	2.8	2.0	1.40
9	259	21	3.7	2.2	1.68
9	261	19	5.2	2.4	2.17
9	265	15	3.9	2.6	1.50
9	267	13	4.0	2.2	1.82
9	269	11	3.7	2.2	1.68
9	271	9	4.4	2.4	1.83
9	273	7	4.0	2.1	1.90
9	275	5	4.6	2.1	2.19
10	145	137	0.7	0.7	1.00
10	147	135	0.7	0.9	0.78
10	149	133	0.9	1.0	0.90
10	152	130	0.6	0.8	0.75

S	G	Dbd	E3	P	E3/P
10	154	128	0.8	0.9	0.89
10	156	125	1.1	0.8	1.38
10	159	122	1.0	0.9	1.11
10	161	120	1.1	1.0	1.10
10	163	118	1.2	0.9	1.33
10	165	116	1.0	0.7	1.43
10	167	114	1.0	0.8	1.25
10	169	112	1.0	0.9	1.11
10	171	110	1.2	1.1	1.09
10	173	108	1.0	0.8	1.25
10	175	106	1.2	1.1	1.09
10	177	104	1.4	1.0	1.40
10	180	101	1.3	1.3	1.00
10	182	99	1.3	0.9	1.44
10	185	96	1.2	1.1	1.09
10	187	94	1.3	1.3	1.00
10	189	92	1.1	1.2	0.92
10	190	91	1.2	1.1	1.09
10	194	87	1.1	1.3	0.85
10	197	85	1.2	1.3	0.92
10	199	83	1.4	1.9	0.74
10	201	81	1.6	1.7	0.94
10	203	79	1.6	1.4	1.14
10	205	77	1.4	1.6	0.88
10	208	74	1.5	1.9	0.79
10	210	72	1.4	1.3	1.08
10	212	70	1.6	1.3	1.23
10	215	67	1.7	1.1	1.55
10	217	65	1.6	1.2	1.33
10	219	63	1.6	1.2	1.33
10	222	60	2.1	1.2	1.75
10	224	58	1.6	1.2	1.33
10	226	56	2.0	1.6	1.25
10	229	53	1.7	1.5	1.13
10	231	51	1.8	1.7	1.06
10	233	49	1.9	1.2	1.58
10	236	46	2.2	1.4	1.57
10	238	44	2.5	1.6	1.56
10	240	42	2.5	1.5	1.67
10	243	39	2.7	1.7	1.59
10	245	37	3.2	2.3	1.39
10	247	35	3.0	2.3	1.30
10	250	32	2.4	2.3	1.04
10	252	30	3.0	2.1	1.43
10	254	28	2.8	2.2	1.27
10	257	25	3.2	2.9	1.10
10	259	23	2.6	2.3	1.13
10	261	21	2.5	2.3	1.09
10	264	18	3.4	2.3	1.48
10	266	16	3.3	2.7	1.22
10	268	14	2.9	2.2	1.32
10	271	11	4.9	2.6	1.88
10	273	9	4.8	2.3	2.09
10	275	7	5.1	2.9	1.76
10	278	4	3.6	2.6	1.38
10	280	2	3.5	1.8	1.94

S	G	Dbd	E3	P	E3/P
11	140	138	0.9	1.5	0.60
11	142	136	0.9	1.6	0.56
11	144	134	1.0	1.9	0.53
11	147	131	0.7	1.3	0.54
11	149	129	1.0	1.7	0.59
11	151	127	0.9	1.8	0.50
11	154	124	1.1	2.1	0.52
11	156	122	1.3	1.7	0.76
11	158	120	0.8	1.5	0.53
11	161	117	1.0	1.5	0.67
11	163	115	0.2	1.7	0.12
11	165	113	0.3	1.6	0.19
11	168	110	0.5	2.2	0.23
11	170	108	0.5	1.8	0.28
11	172	106	1.1	1.7	0.65
11	175	103	1.4	1.5	0.93
11	177	101	1.3	1.5	0.87
11	179	99	1.2	1.7	0.71
11	182	96	1.7	1.7	1.00
11	184	94	1.8	1.5	1.20
11	186	92	1.7	1.5	1.13
11	189	89	1.7	1.7	1.00
11	191	87	1.9	1.6	1.19
11	193	85	2.0	2.3	0.87
11	196	82	1.5	1.8	0.83
11	198	80	2.2	1.9	1.16
11	200	78	1.7	1.5	1.13
11	205	73	2.1	1.6	1.31
11	207	71	2.0	1.6	1.25
11	210	68	2.0	2.4	0.83
11	212	66	2.1	1.7	1.24
11	214	64	2.6	1.8	1.44
11	219	59	1.7	1.7	1.00
11	221	57	2.4	2.0	1.20
11	224	54	2.1	1.7	1.24
11	226	52	2.4	1.7	1.41
11	228	50	2.4	2.4	1.00
11	233	45	3.0	2.2	1.36
11	235	43	3.0	2.3	1.30
11	238	40	3.4	2.5	1.36
11	240	38	4.4	2.7	1.63
11	242	36	4.9	2.2	2.23
11	245	33	3.9	2.6	1.50
11	247	31	5.1	2.5	2.04
11	249	29	4.5	2.6	1.73
11	252	26	5.5	2.9	1.90
11	254	24	4.8	2.4	2.00
11	256	22	6.1	2.8	2.18
11	259	19	4.6	2.0	2.30
11	261	17	8.1	3.2	2.53
11	263	15	6.8	2.6	2.62
11	266	12	7.4	2.7	2.74
11	268	10	6.9	3.1	2.23
11	270	8	7.3	3.5	2.09
11	273	5	9.4	3.9	2.41
11	274	4	5.9	3.2	1.84
11	275	3	6.0	3.4	1.76

S	G	Dbd	E3	P	E3/P
12	143	125	0.5	0.2	2.50
12	145	123	0.3	0.3	1.00
12	147	121	0.3	0.4	0.75
12	149	119	0.3	0.3	1.00
12	150	118	0.5	0.5	1.00
12	154	114	0.4	0.3	1.33
12	156	112	0.4	0.4	1.00
12	158	110	0.5	0.4	1.25
12	160	108	0.5	0.4	1.25
12	163	105	0.5	0.4	1.25
12	166	102	0.5	0.6	0.83
12	167	101	0.4	0.5	0.80
12	171	97	0.6	0.7	0.86
12	172	96	0.7	0.5	1.40
12	174	94	0.7	0.6	1.17
12	177	91	0.6	0.8	0.75
12	179	89	0.7	0.4	1.75
12	181	87	0.6	0.4	1.50
12	184	84	0.7	0.6	1.17
12	187	81	0.6	0.4	1.50
12	188	80	0.6	0.5	1.20
12	191	77	0.6	0.5	1.20
12	193	75	0.7	0.6	1.17
12	195	73	0.6	0.7	0.86
12	199	69	0.7	0.6	1.17
12	201	67	0.9	0.9	1.00
12	204	64	0.8	0.9	0.89
12	206	62	0.9	1.2	0.75
12	208	60	0.7	0.7	1.00
12	211	57	0.7	0.8	0.88
12	213	55	0.9	0.6	1.50
12	215	53	0.9	1.1	0.82
12	218	50	0.8	0.8	1.00
12	220	48	0.8	0.6	1.33
12	225	43	1.0	1.2	0.83
12	229	39	0.8	0.9	0.89
12	232	36	1.0	1.0	1.00
12	234	34	0.8	0.8	1.00
12	236	32	1.0	0.7	1.43
12	239	29	1.3	1.1	1.18
12	241	27	1.3	1.0	1.30
12	243	25	1.2	1.1	1.09
12	246	22	1.3	1.4	0.93
12	248	20	1.4	1.4	1.00
12	250	18	1.6	1.5	1.07
12	254	14	1.7	1.4	1.21
12	255	13	1.8	1.5	1.20
12	257	11	2.0	1.5	1.33
12	260	8	1.6	1.3	1.23
12	262	6	1.7	1.4	1.21
12	264	4	2.1	1.5	1.40
12	267	1	2.5	1.6	1.56

S	G	Dbd	E3	P	E3/P
13	151	128	0.6	0.8	0.75
13	153	126	0.7	0.6	1.17
13	155	124	0.6	0.7	0.86
13	158	121	0.6	0.6	1.00
13	160	119	0.6	0.9	0.67
13	162	117	1.1	1.4	0.79
13	165	114	0.7	0.8	0.88
13	167	112	0.6	0.9	0.67
13	169	110	0.8	0.9	0.89
13	172	107	1.1	0.6	1.83
13	174	105	1.0	0.6	1.67
13	176	103	0.9	1.0	0.90
13	179	100	0.6	1.0	0.60
13	181	98	1.0	0.8	1.25
13	183	96	0.9	1.3	0.69
13	186	93	0.9	1.1	0.82
13	188	91	0.8	1.2	0.67
13	190	89	1.0	1.0	1.00
13	193	86	1.1	1.1	1.00
13	195	84	1.6	1.1	1.45
13	197	82	1.3	1.3	1.00
13	200	79	1.2	1.1	1.09
13	202	77	1.0	0.6	1.67
13	204	75	1.2	1.2	1.00
13	207	72	1.2	1.5	0.80
13	209	70	1.4	1.7	0.82
13	211	68	1.2	1.8	0.67
13	214	65	1.7	2.1	0.81
13	216	63	1.8	2.2	0.82
13	218	61	1.3	1.7	0.76
13	221	58	1.0	1.7	0.59
13	223	56	1.6	1.6	1.00
13	225	54	1.3	1.5	0.87
13	228	51	1.9	2.6	0.73
13	230	49	1.3	1.9	0.68
13	232	47	1.7	2.6	0.65
13	235	44	2.1	2.1	1.00
13	237	42	1.6	1.8	0.89
13	239	40	1.6	1.7	0.94
13	242	37	1.7	1.5	1.13
13	244	35	1.7	1.1	1.55
13	246	33	1.4	1.6	0.88
13	249	30	2.0	1.4	1.43
13	251	28	2.6	1.2	2.17
13	253	26	1.8	1.0	1.80
13	256	23	2.3	1.8	1.28
13	259	20	2.7	1.8	1.50
13	260	19	3.9	2.0	1.95
13	263	16	3.8	1.4	2.71
13	266	13	3.6	1.6	2.25
13	267	12	4.5	1.6	2.81
13	270	9	4.4	1.5	2.93
13	273	6	3.7	1.9	1.95

S	G	Dbd	E3	P	E3/P
14	147	135	0.4	1.0	0.40
14	150	132	0.4	1.2	0.33
14	152	130	0.8	1.0	0.80
14	154	128	0.6	1.0	0.60
14	156	126	0.5	0.8	0.63
14	159	123	0.4	0.7	0.57
14	162	120	0.4	0.9	0.44
14	165	117	0.5	0.9	0.56
14	169	113	0.5	0.7	0.71
14	171	111	0.5	0.8	0.63
14	173	109	0.6	0.8	0.75
14	176	106	0.6	0.9	0.67
14	181	101	0.6	0.6	1.00
14	183	99	0.7	0.8	0.88
14	185	97	0.5	0.6	0.83
14	188	94	0.5	0.8	0.63
14	192	90	0.5	0.8	0.63
14	197	85	0.6	1.1	0.55
14	199	83	0.7	1.0	0.70
14	201	81	0.6	1.1	0.55
14	204	78	0.8	1.1	0.73
14	206	76	0.8	1.0	0.80
14	209	73	1.1	1.6	0.69
14	211	71	0.8	1.4	0.57
14	214	68	0.8	1.3	0.62
14	216	66	0.7	1.0	0.70
14	219	63	0.8	1.3	0.62
14	223	59	0.7	1.0	0.70
14	225	57	0.8	1.3	0.62
14	228	54	0.9	1.5	0.60
14	229	53	0.9	1.5	0.60
14	232	50	0.7	2.2	0.32
14	234	48	0.9	1.8	0.50
14	236	46	0.8	1.7	0.47
14	243	39	0.8	1.4	0.57
14	246	36	1.2	2.1	0.57
14	251	31	1.3	1.9	0.68
14	253	29	1.2	2.4	0.50
14	255	27	1.3	2.1	0.62
14	260	22	1.6	1.9	0.84
14	261	21	1.3	2.2	0.59
14	262	20	1.6	1.6	1.00
14	264	18	1.5	2.0	0.75
14	267	15	1.4	1.4	1.00
14	269	13	1.3	1.6	0.81
14	274	8	1.5	1.3	1.15
14	275	7	2.0	1.8	1.11
14	277	5	1.6	1.5	1.07
14	279	3	1.8	1.8	1.00
14	281	1	2.5	2.6	0.96

S	G	Dbd	E3	P	E3/P
15	144	143	0.7	0.7	1.00
15	146	141	0.7	0.7	1.00
15	148	139	0.9	0.7	1.29
15	151	136	0.6	0.7	0.86
15	154	133	0.7	0.8	0.88
15	156	131	0.9	0.9	1.00
15	160	127	1.0	0.8	1.25
15	161	126	0.7	0.8	0.88
15	168	119	1.1	0.8	1.38
15	171	116	0.9	1.0	0.90
15	173	114	1.0	0.8	1.25
15	175	112	0.9	0.8	1.13
15	177	110	0.8	1.0	0.80
15	179	108	1.0	0.8	1.25
15	181	106	1.1	0.9	1.22
15	183	104	1.1	0.9	1.22
15	186	101	1.1	0.8	1.38
15	188	99	1.3	0.8	1.63
15	190	97	0.9	0.8	1.13
15	193	94	1.0	0.8	1.25
15	195	92	1.2	0.9	1.33
15	197	90	1.1	1.0	1.10
15	201	86	1.1	1.0	1.10
15	202	85	0.9	0.8	1.13
15	204	83	1.2	1.0	1.20
15	207	80	1.3	0.9	1.44
15	209	78	1.3	1.0	1.30
15	211	76	1.8	1.0	1.80
15	214	73	1.6	1.2	1.33
15	216	71	1.5	0.9	1.67
15	218	69	1.6	0.7	2.29
15	221	66	1.4	1.0	1.40
15	223	64	1.8	1.2	1.50
15	228	59	1.8	1.0	1.80
15	230	57	2.0	1.0	2.00
15	232	55	2.5	0.8	3.13
15	235	52	2.1	1.0	2.10
15	237	50	1.8	1.0	1.80
15	242	45	2.4	0.9	2.67
15	245	42	1.9	0.8	2.38
15	246	41	2.5	0.9	2.78
15	249	38	3.0	1.0	3.00
15	251	36	3.3	1.0	3.30
15	254	33	3.9	1.1	3.55
15	256	31	3.6	0.9	4.00
15	258	29	3.6	0.9	4.00
15	260	27	3.4	1.0	3.40
15	263	24	5.3	0.9	5.89
15	265	22	4.7	0.9	5.22
15	267	20	5.3	1.3	4.08
15	270	17	4.9	1.1	4.45
15	273	14	3.7	0.9	4.11
15	274	13	6.0	0.8	7.50
15	277	10	6.3	1.1	5.73
15	279	8	7.0	1.2	5.83
15	281	6	7.3	1.0	7.30
15	284	3	7.3	1.0	7.30
15	286	1	6.4	1.1	5.82

S	G	Dbd	E3	P	E3/P
16	143	142	1.2	1.1	1.09
16	145	140	1.0	1.3	0.77
16	148	137	1.0	1.2	0.83
16	150	135	1.3	1.6	0.81
16	152	133	1.2	1.7	0.71
16	155	130	1.3	1.5	0.87
16	157	128	1.0	1.4	0.71
16	159	126	1.2	1.9	0.63
16	162	123	1.4	1.6	0.88
16	164	121	1.6	1.9	0.84
16	166	119	1.2	1.7	0.71
16	169	116	1.5	1.9	0.79
16	171	114	1.4	1.9	0.74
16	173	112	1.4	1.9	0.74
16	176	109	1.7	2.2	0.77
16	178	107	1.7	1.9	0.89
16	181	104	1.5	2.1	0.71
16	183	102	1.7	1.7	1.00
16	185	100	1.7	2.3	0.74
16	187	98	1.9	2.8	0.68
16	190	95	1.8	2.2	0.82
16	192	93	1.8	2.7	0.67
16	194	91	1.9	3.1	0.61
16	197	88	2.4	3.8	0.63
16	199	86	1.9	3.2	0.59
16	201	84	2.3	2.8	0.82
16	204	81	2.3	3.2	0.72
16	206	79	2.1	2.9	0.72
16	208	77	1.8	2.7	0.67
16	211	74	1.6	2.2	0.73
16	213	72	2.0	3.0	0.67
16	215	70	2.5	3.5	0.71
16	218	67	2.6	3.9	0.67
16	221	64	3.2	4.4	0.73
16	222	63	1.6	4.2	0.38
16	225	60	3.0	3.5	0.86
16	227	58	2.8	4.1	0.68
16	229	56	2.7	4.0	0.68
16	232	53	3.4	4.7	0.72
16	234	51	3.4	4.7	0.72
16	236	49	3.8	4.0	0.95
16	239	46	3.5	4.6	0.76
16	240	45	3.1	4.0	0.78
16	243	42	3.7	5.0	0.74
16	246	39	4.8	4.8	1.00
16	248	37	3.3	3.5	0.94
16	250	35	5.1	5.4	0.94
16	253	32	5.0	5.4	0.93
16	255	30	3.0	4.4	0.68
16	257	28	5.8	4.8	1.21
16	260	25	5.5	5.3	1.04
16	262	23	5.4	4.5	1.20
16	264	21	6.6	4.6	1.43
16	267	18	7.1	6.1	1.16
16	270	15	7.7	3.8	2.03
16	271	14	6.2	3.4	1.82
16	274	11	3.4	4.2	0.81
16	276	9	5.6	3.8	1.47

S	G	Dbd	E3	P	E3/P
16	278	7	7.4	3.6	2.06
16	281	4	7.8	3.3	2.36
16	283	2	6.9	3.4	2.03
17	144	145	1.3	1.1	1.18
17	145	144	0.7	0.7	1.00
17	146	143	0.7	0.7	1.00
17	151	138	1.2	1.2	1.00
17	152	137	0.8	0.7	1.14
17	153	136	1.2	1.0	1.20
17	158	131	1.1	1.1	1.00
17	159	130	1.5	1.4	1.07
17	160	129	1.1	1.1	1.00
17	165	124	1.2	0.8	1.50
17	166	123	1.1	1.0	1.10
17	167	122	1.3	1.1	1.18
17	172	117	1.0	1.0	1.00
17	173	116	0.3	1.0	0.30
17	174	115	1.1	1.1	1.00
17	180	109	1.0	1.0	1.00
17	181	108	0.9	0.7	1.29
17	182	107	1.5	1.2	1.25
17	186	103	1.3	1.2	1.08
17	187	102	1.4	1.1	1.27
17	188	101	1.4	1.1	1.27
17	193	96	1.4	1.4	1.00
17	194	95	1.3	1.2	1.08
17	195	94	1.1	1.1	1.00
17	200	89	1.0	0.8	1.25
17	201	88	1.1	1.3	0.85
17	202	87	1.3	1.2	1.08
17	209	80	1.2	1.1	1.09
17	210	79	0.9	1.1	0.82
17	215	74	0.9	1.1	0.82
17	216	73	1.4	1.6	0.88
17	217	72	0.9	1.1	0.82
17	218	71	2.0	2.4	0.83
17	221	68	1.5	1.5	1.00
17	222	67	1.4	1.7	0.82
17	223	66	0.9	1.0	0.90
17	229	60	0.8	1.3	0.62
17	230	59	1.8	2.0	0.90
17	231	58	1.3	1.1	1.18
17	235	54	1.4	1.7	0.82
17	236	53	0.9	1.0	0.90
17	237	52	2.0	3.0	0.67
17	243	46	1.0	1.6	0.63
17	244	45	1.4	1.8	0.78
17	245	44	1.2	1.4	0.86
17	249	40	2.4	4.6	0.52
17	250	39	1.4	1.7	0.82
17	251	38	2.1	2.2	0.95
17	256	33	1.4	1.6	0.88
17	257	32	2.0	3.1	0.65
17	258	31	1.8	1.7	1.06
17	264	25	2.5	2.7	0.93
17	265	24	2.0	2.3	0.87
17	266	23	2.5	2.4	1.04
17	270	19	4.9	2.6	1.88

S	G	Dbd	E3	P	E3/P
17	271	18	2.4	2.2	1.09
17	272	17	3.6	2.7	1.33
17	278	11	4.0	2.6	1.54
17	279	10	3.2	1.8	1.78
17	280	9	4.3	2.5	1.72
17	284	5	4.3	3.5	1.23
17	285	4	3.9	2.3	1.70
17	286	3	4.7	3.9	1.21
18	140	150	0.7	1.2	0.58
18	142	148	0.7	1.3	0.54
18	144	146	1.1	1.2	0.92
18	146	144	1.1	1.2	0.92
18	149	141	0.8	1.0	0.80
18	151	139	0.9	1.5	0.60
18	154	136	1.0	1.6	0.63
18	157	133	0.9	1.5	0.60
18	158	132	1.0	1.3	0.77
18	161	129	0.9	1.9	0.47
18	163	127	1.2	1.9	0.63
18	165	125	1.2	1.2	1.00
18	168	122	1.0	1.2	0.83
18	170	120	1.1	1.3	0.85
18	172	118	1.2	1.9	0.63
18	177	113	1.3	1.1	1.18
18	179	111	0.9	1.3	0.69
18	182	108	1.4	1.3	1.08
18	184	106	1.5	1.3	1.15
18	186	104	1.4	1.4	1.00
18	188	102	1.2	1.3	0.92
18	191	99	1.6	1.4	1.14
18	193	97	1.4	1.3	1.08
18	198	92	1.0	2.0	0.50
18	200	90	1.6	1.8	0.89
18	203	87	1.1	1.4	0.79
18	205	85	1.3	1.2	1.08
18	207	83	1.2	1.6	0.75
18	211	79	1.4	1.6	0.88
18	212	78	1.8	2.1	0.86
18	214	76	1.7	2.1	0.81
18	217	73	1.1	2.2	0.50
18	219	71	1.5	2.1	0.71
18	221	69	1.6	2.2	0.73
18	223	67	1.6	2.4	0.67
18	225	65	1.6	2.3	0.70
18	227	63	2.0	2.5	0.80
18	230	60	2.0	2.8	0.71
18	232	58	1.6	2.5	0.64
18	234	56	1.7	2.5	0.68
18	237	53	2.0	2.2	0.91
18	239	51	2.0	2.2	0.91
18	241	49	2.3	2.6	0.88
18	244	46	1.8	2.5	0.72
18	246	44	2.4	2.4	1.00
18	248	42	2.6	3.0	0.87
18	251	39	2.2	2.5	0.88
18	253	37	2.9	2.5	1.16
18	255	35	2.3	3.0	0.77
18	258	32	2.6	2.8	0.93

S	G	Dbd	E3	P	E3/P
18	260	30	2.5	2.8	0.89
18	262	28	3.0	3.0	1.00
18	265	25	2.8	2.3	1.22
18	267	23	2.7	2.7	1.00
18	269	21	2.3	1.8	1.28
18	273	17	3.9	2.8	1.39
18	275	15	4.1	2.9	1.41
18	276	14	5.2	5.1	1.02
18	280	10	5.3	4.8	1.10
18	282	8	4.1	3.3	1.24
18	284	6	4.3	3.0	1.43
18	287	3	5.3	3.6	1.47
18	289	1	5.5	2.8	1.96
19	143	152	0.6	3.3	0.18
19	145	150	0.5	1.2	0.42
19	147	148	1.2	1.4	0.86
19	150	145	0.5	0.9	0.56
19	152	143	0.9	1.1	0.82
19	154	141	0.7	3.5	0.20
19	157	138	1.0	1.0	1.00
19	159	136	1.1	2.6	0.42
19	161	134	0.6	1.3	0.46
19	164	131	1.5	1.2	1.25
19	166	129	1.4	1.0	1.40
19	168	127	1.2	1.0	1.20
19	171	124	1.1	0.5	2.20
19	173	122	1.2	1.1	1.09
19	175	120	1.5	1.0	1.50
19	178	117	1.1	1.0	1.10
19	180	115	1.1	1.2	0.92
19	182	113	1.2	1.9	0.63
19	185	110	1.0	1.3	0.77
19	189	106	1.3	1.1	1.18
19	192	103	1.4	1.3	1.08
19	194	101	1.5	1.4	1.07
19	195	100	1.1	1.6	0.69
19	199	96	1.5	1.1	1.36
19	201	94	1.4	2.4	0.58
19	203	92	1.4	2.6	0.54
19	206	89	1.1	1.4	0.79
19	208	87	1.3	3.1	0.42
19	210	85	1.6	1.5	1.07
19	213	82	1.8	1.4	1.29
19	215	80	1.3	2.0	0.65
19	217	78	1.2	1.5	0.80
19	220	75	1.4	3.2	0.44
19	223	72	2.0	1.9	1.05
19	224	71	1.9	1.2	1.58
19	227	68	1.8	3.6	0.50
19	229	66	2.0	1.5	1.33
19	231	64	2.8	2.6	1.08
19	234	61	1.6	1.4	1.14
19	236	59	1.9	1.6	1.19
19	238	57	1.5	1.4	1.07
19	241	54	1.8	1.4	1.29
19	243	52	2.4	1.5	1.60
19	245	50	1.6	1.7	0.94
19	248	47	1.9	2.4	0.79

S	G	Dbd	E3	P	E3/P
19	250	45	1.8	3.1	0.58
19	252	43	2.4	2.1	1.14
19	255	40	2.1	2.8	0.75
19	257	38	2.6	2.2	1.18
19	259	36	3.0	2.0	1.50
19	262	33	2.6	1.3	2.00
19	264	31	3.4	1.5	2.27
19	266	29	2.8	2.0	1.40
19	269	26	2.4	2.5	0.96
19	271	24	4.1	2.6	1.58
19	274	21	3.5	1.5	2.33
19	276	19	4.4	2.6	1.69
19	278	17	4.6	1.6	2.88
19	280	15	3.2	1.3	2.46
19	283	12	4.3	1.2	3.58
19	285	10	4.0	3.1	1.29
19	287	8	4.6	2.2	2.09
19	290	5	4.3	2.2	1.95
19	292	3	4.7	1.8	2.61
20	144	126	1.6	1.3	1.23
20	146	124	1.7	1.1	1.55
20	148	122	1.6	1.1	1.45
20	151	119	1.5	1.6	0.94
20	153	117	2.0	1.3	1.54
20	155	115	1.4	1.2	1.17
20	158	112	1.9	1.5	1.27
20	160	110	1.9	1.2	1.58
20	162	108	1.7	1.2	1.42
20	167	103	2.0	1.5	1.33
20	169	101	2.2	1.4	1.57
20	172	98	1.9	1.7	1.12
20	174	96	2.1	1.5	1.40
20	176	94	2.0	1.4	1.43
20	179	91	2.3	1.6	1.44
20	181	89	2.0	1.4	1.43
20	183	87	1.9	1.7	1.12
20	186	84	2.6	1.8	1.44
20	188	82	1.5	2.5	0.60
20	193	77	2.8	2.1	1.33
20	195	75	2.6	2.1	1.24
20	197	73	2.6	2.0	1.30
20	201	69	2.8	2.1	1.33
20	203	67	2.6	2.8	0.93
20	204	66	2.5	2.3	1.09
20	207	63	2.6	2.6	1.00
20	210	60	2.6	2.4	1.08
20	212	58	2.2	2.3	0.96
20	215	55	2.6	2.4	1.08
20	218	52	2.3	2.1	1.10
20	221	49	2.5	3.1	0.81
20	223	47	2.5	3.1	0.81
20	225	45	2.6	2.7	0.96
20	228	42	2.7	3.2	0.84
20	230	40	2.9	3.4	0.85
20	233	37	3.7	4.8	0.77
20	235	35	2.9	3.2	0.91
20	237	33	3.3	3.5	0.94
20	239	31	3.4	3.0	1.13

S	G	Dbd	E3	P	E3/P
20	243	27	4.0	3.8	1.05
20	244	26	4.3	3.0	1.43
20	247	23	4.2	3.3	1.27
20	249	21	4.6	4.5	1.02
20	251	19	4.6	4.0	1.15
20	254	16	5.8	4.7	1.23
20	258	12	4.8	4.7	1.02
20	261	9	4.7	4.6	1.02
20	263	7	6.9	5.1	1.35
20	265	5	6.8	4.2	1.62
20	268	2	6.6	5.4	1.22
20	270	0	5.8	4.8	1.21
21	153	123	1.0	0.9	1.11
21	156	120	1.2	1.3	0.92
21	157	119	0.7	1.1	0.64
21	161	115	1.1	1.5	0.73
21	163	113	1.6	1.5	1.07
21	164	112	1.5	1.4	1.07
21	167	109	1.3	1.2	1.08
21	169	107	0.9	0.9	1.00
21	170	106	1.3	1.2	1.08
21	174	102	1.5	1.5	1.00
21	175	101	1.3	1.6	0.81
21	179	97	1.2	1.3	0.92
21	188	88	1.5	1.6	0.94
21	190	86	1.4	1.8	0.78
21	193	83	1.5	1.4	1.07
21	195	81	1.8	1.5	1.20
21	198	78	2.5	1.2	2.08
21	203	73	2.2	2.0	1.10
21	204	72	2.4	1.8	1.33
21	207	69	2.1	1.7	1.24
21	210	66	1.8	2.1	0.86
21	216	60	2.1	1.7	1.24
21	219	57	2.3	1.9	1.21
21	224	52	2.0	2.2	0.91
21	226	50	2.5	1.8	1.39
21	228	48	2.8	2.5	1.12
21	230	46	2.2	3.1	0.71
21	232	44	2.9	2.5	1.16
21	238	38	3.7	2.4	1.54
21	240	36	3.5	1.9	1.84
21	242	34	2.9	1.8	1.61
21	247	29	4.2	2.1	2.00
21	248	28	3.7	1.7	2.18
21	250	26	3.2	1.9	1.68
21	252	24	3.6	1.8	2.00
21	254	22	4.3	2.6	1.65
21	256	20	4.2	2.2	1.91
21	257	19	5.7	2.3	2.48
21	258	18	4.6	2.3	2.00
21	266	10	5.2	2.1	2.48
21	267	9	4.7	1.8	2.61
21	269	7	4.0	1.7	2.35
21	274	2	3.6	2.3	1.57
21	275	1	5.2	2.3	2.26

S	G	Dbd	E3	P	E3/P
22	139	148	0.8	1.0	0.80
22	141	146	0.8	0.9	0.89
22	143	144	0.7	0.6	1.17
22	146	141	0.9	0.9	1.00
22	148	139	0.9	1.0	0.90
22	150	137	0.8	1.1	0.73
22	153	134	0.6	0.7	0.86
22	155	132	0.7	0.9	0.78
22	157	130	0.5	0.6	0.83
22	160	127	0.9	0.7	1.29
22	162	125	0.6	0.8	0.75
22	164	123	0.9	0.7	1.29
22	174	113	1.0	0.7	1.43
22	176	111	1.2	0.9	1.33
22	179	108	1.5	0.9	1.67
22	181	106	1.3	1.0	1.30
22	186	101	1.3	1.0	1.30
22	188	99	1.4	1.3	1.08
22	190	97	1.4	1.1	1.27
22	192	95	1.5	1.0	1.50
22	195	92	1.2	0.9	1.33
22	197	90	1.3	0.8	1.63
22	199	88	1.7	0.8	2.13
22	202	85	2.0	1.0	2.00
22	204	83	1.6	1.1	1.45
22	209	78	1.4	0.9	1.56
22	210	77	1.1	0.9	1.22
22	213	74	0.9	0.9	1.00
22	216	71	1.7	0.6	2.83
22	218	69	1.0	0.5	2.00
22	220	67	1.5	0.4	3.75
22	223	64	2.2	0.7	3.14
22	225	62	1.5	0.7	2.14
22	227	60	1.7	1.2	1.42
22	230	57	1.0	1.1	0.91
22	233	54	1.4	0.6	2.33
22	234	53	2.1	1.0	2.10
22	238	49	0.8	1.0	0.80
22	239	48	2.1	1.1	1.91
22	244	43	2.0	1.3	1.54
22	246	41	2.6	1.0	2.60
22	248	39	2.6	1.5	1.73
22	251	36	1.9	1.9	1.00
22	253	34	2.4	1.4	1.71
22	254	33	2.5	1.5	1.67
22	258	29	3.3	1.6	2.06
22	262	25	2.5	2.2	1.14
22	266	21	3.0	1.7	1.76
22	267	20	3.4	2.0	1.70
22	269	18	3.4	2.3	1.48
22	272	15	4.0	2.2	1.82
22	274	13	3.1	2.0	1.55
22	275	12	2.1	2.3	0.91
22	280	7	2.5	2.0	1.25
22	282	5	3.3	2.0	1.65
22	287	0	4.0	2.3	1.74

S	G	Dbd	E3	P	E3/P
23	133	154	0.5	0.5	1.00
23	137	150	0.5	0.5	1.00
23	139	148	0.5	0.5	1.00
23	144	143	0.9	1.1	0.82
23	146	141	0.9	0.7	1.29
23	158	129	1.0	0.8	1.25
23	161	126	0.8	0.7	1.14
23	165	122	0.9	0.8	1.13
23	168	119	1.2	0.9	1.33
23	170	117	1.2	1.0	1.20
23	174	113	1.4	1.1	1.27
23	177	110	1.3	1.1	1.18
23	180	107	1.5	1.3	1.15
23	181	106	1.4	1.4	1.00
23	185	102	1.3	1.1	1.18
23	186	101	1.4	1.3	1.08
23	188	99	1.5	1.1	1.36
23	189	98	1.3	1.4	0.93
23	192	95	1.3	1.6	0.81
23	196	91	1.4	1.3	1.08
23	199	88	1.4	1.3	1.08
23	205	82	1.4	1.4	1.00
23	210	77	1.7	1.6	1.06
23	215	72	1.5	1.7	0.88
23	220	67	1.8	1.7	1.06
23	225	62	2.0	1.6	1.25
23	227	60	1.9	1.9	1.00
23	229	59	2.0	1.9	1.05
23	232	55	1.6	1.5	1.07
23	234	53	1.2	1.5	0.80
23	238	49	2.0	1.8	1.11
23	241	46	2.3	1.7	1.35
23	244	43	2.7	1.7	1.59
23	248	39	2.1	1.7	1.24
23	250	37	3.2	1.5	2.13
23	253	34	1.8	1.8	1.00
23	256	31	2.6	2.0	1.30
23	260	27	3.4	1.9	1.79
23	263	24	3.1	1.7	1.82
23	266	21	3.8	2.4	1.58
23	271	16	5.0	2.2	2.27
23	274	13	4.2	2.2	1.91
23	277	10	4.8	2.3	2.09
23	279	8	4.4	1.9	2.32
23	281	6	4.7	2.5	1.88
23	283	4	4.4	2.1	2.10
23	286	1	4.8	2.4	2.00

S	G	Dbd	E3	P	E3/P
24	142	132	1.4	1.1	1.27
24	145	129	1.5	1.2	1.25
24	146	128	0.8	0.9	0.89
24	148	126	1.3	1.1	1.18
24	152	122	1.4	1.2	1.17
24	154	120	1.6	1.4	1.14
24	155	119	1.5	1.2	1.25
24	158	116	1.7	1.1	1.55
24	162	112	1.3	0.9	1.44
24	164	110	1.5	1.2	1.25
24	166	108	1.0	1.0	1.00
24	169	105	1.5	1.4	1.07
24	171	103	1.1	1.0	1.10
24	176	98	1.2	1.1	1.09
24	177	97	1.6	1.1	1.45
24	183	91	1.3	1.2	1.08
24	187	87	2.0	1.3	1.54
24	192	82	1.3	1.2	1.08
24	196	78	1.9	1.6	1.19
24	198	76	1.5	1.1	1.36
24	203	71	2.0	1.5	1.33
24	205	69	2.4	2.5	0.96
24	207	67	2.1	2.2	0.95
24	210	64	2.3	2.0	1.15
24	211	63	1.8	1.2	1.50
24	213	61	1.9	1.8	1.06
24	217	57	1.4	1.8	0.78
24	220	54	1.3	1.3	1.00
24	221	53	1.4	1.5	0.93
24	225	49	1.4	1.5	0.93
24	229	45	1.5	1.3	1.15
24	231	43	1.4	1.4	1.00
24	233	41	1.9	2.1	0.90
24	235	39	3.9	4.0	0.98
24	237	37	2.0	2.1	0.95
24	239	35	2.9	3.4	0.85
24	241	33	2.3	2.2	1.05
24	243	31	2.1	2.3	0.91
24	245	29	1.8	2.1	0.86
24	247	27	2.8	2.3	1.22
24	250	24	2.5	2.4	1.04
24	253	21	2.3	2.2	1.05
24	255	19	2.0	2.2	0.91
24	257	17	4.3	3.1	1.39
24	261	13	2.4	1.9	1.26
24	263	11	4.8	2.7	1.78
24	265	9	7.2	3.4	2.12
24	267	7	6.1	3.7	1.65
24	270	4	6.2	3.0	2.07
24	272	2	5.7	2.8	2.04

S	G	Dbd	E3	P	E3/P
25	140	126	0.7	0.9	0.78
25	142	124	0.7	1.1	0.64
25	144	122	0.7	1.2	0.58
25	148	118	0.8	1.0	0.80
25	149	117	0.5	0.9	0.56
25	151	115	0.8	1.1	0.73
25	156	110	0.8	0.7	1.14
25	158	108	1.3	1.2	1.08
25	160	106	1.2	1.2	1.00
25	161	105	1.4	1.2	1.17
25	164	102	1.4	1.2	1.17
25	166	100	1.5	1.2	1.25
25	169	97	1.6	1.8	0.89
25	172	94	1.1	1.0	1.10
25	175	91	1.5	1.4	1.07
25	178	88	1.4	1.8	0.78
25	180	86	1.3	1.5	0.87
25	182	84	1.5	1.3	1.15
25	186	80	1.3	1.1	1.18
25	190	76	1.7	1.6	1.06
25	192	74	1.8	1.8	1.00
25	196	70	1.9	1.7	1.12
25	198	68	2.0	1.6	1.25
25	200	66	2.0	1.6	1.25
25	203	63	1.6	1.5	1.07
25	206	60	2.6	2.0	1.30
25	210	56	2.6	2.5	1.04
25	213	53	3.0	2.4	1.25
25	215	51	2.8	2.7	1.04
25	217	49	2.7	2.3	1.17
25	220	46	3.3	2.5	1.32
25	224	42	2.9	2.6	1.12
25	226	40	3.5	3.0	1.17
25	228	38	3.4	2.3	1.48
25	233	33	3.4	2.2	1.55
25	236	30	3.7	3.0	1.23
25	238	28	3.8	3.6	1.06
25	242	24	5.1	3.4	1.50
25	247	19	5.6	4.1	1.37
25	249	17	5.6	3.7	1.51
25	252	14	7.3	4.8	1.52
25	256	10	10.0	4.8	2.08
25	261	5	9.2	4.6	2.00
25	263	3	8.9	5.0	1.78

S	G	Dbd	E3	P	E3/P
26	135	138	1.4	0.9	1.56
26	137	136	1.1	0.7	1.57
26	139	134	1.4	1.1	1.27
26	142	131	1.5	1.2	1.25
26	146	127	1.2	1.0	1.20
26	149	124	0.9	1.3	0.69
26	151	122	0.7	0.5	1.40
26	153	120	0.5	0.6	0.83
26	156	117	1.0	0.8	1.25
26	159	114	1.1	1.0	1.10
26	160	113	0.4	0.5	0.80
26	163	110	0.6	0.5	1.20
26	165	108	0.5	0.4	1.25
26	170	103	1.2	1.0	1.20
26	172	101	1.5	0.8	1.88
26	174	99	0.7	0.9	0.78
26	177	96	0.9	1.0	0.90
26	179	94	0.9	0.8	1.13
26	181	92	1.0	1.3	0.77
26	184	89	0.6	0.6	1.00
26	186	87	0.9	1.0	0.90
26	188	85	1.4	1.1	1.27
26	191	82	0.7	0.6	1.17
26	193	80	0.6	0.8	0.75
26	198	75	0.7	0.4	1.75
26	205	68	0.8	0.4	2.00
26	208	65	1.3	2.3	0.57
26	211	62	0.8	0.8	1.00
26	220	53	0.9	0.6	1.50
26	222	51	3.0	1.6	1.88
26	236	37	1.4	0.9	1.56
26	241	32	3.2	1.4	2.29
26	247	26	2.8	1.4	2.00
26	249	24	1.3	0.4	3.25
26	250	23	0.9	0.4	2.25
26	257	16	3.9	1.6	2.44
26	264	9	2.3	1.1	2.09
26	265	8	1.2	0.7	1.71
26	268	5	1.9	0.8	2.38
26	271	2	0.8	0.8	1.00
27	143	148	0.8	1.2	0.67
27	145	146	0.8	1.1	0.73

S	G	Dbd	E3	P	E3/P
27	147	144	0.7	1.3	0.54
27	150	141	0.9	1.1	0.82
27	152	139	0.9	1.4	0.64
27	155	136	1.1	1.0	1.10
27	157	134	0.9	0.9	1.00
27	160	131	1.1	1.1	1.00
27	162	129	1.2	1.2	1.00
27	165	126	1.2	1.0	1.20
27	167	124	1.1	0.8	1.38
27	169	122	1.4	1.0	1.40
27	172	119	1.3	0.9	1.44
27	174	117	1.6	1.1	1.45
27	176	115	1.3	1.0	1.30
27	182	109	1.3	1.1	1.18
27	186	105	1.2	0.9	1.33
27	187	104	1.4	1.1	1.27
27	189	102	1.2	1.0	1.20
27	193	98	1.8	1.3	1.38
27	195	96	1.8	1.1	1.64
27	197	94	1.6	1.2	1.33
27	200	91	1.4	2.0	0.70
27	202	89	1.6	1.5	1.07
27	204	87	1.8	1.5	1.20
27	208	83	1.9	1.3	1.46
27	214	77	2.1	1.5	1.40
27	225	66	2.3	1.4	1.64
27	227	64	2.1	1.7	1.24
27	230	61	1.9	2.0	0.95
27	236	55	2.3	1.6	1.44
27	237	54	2.1	2.1	1.00
27	238	53	1.7	2.2	0.77
27	241	50	2.4	2.4	1.00
27	243	48	2.6	1.8	1.44
27	244	47	2.5	2.3	1.09
27	249	42	2.8	1.8	1.56
27	252	39	2.5	2.6	0.96
27	253	38	3.1	2.5	1.24
27	258	33	3.1	2.9	1.07
27	261	30	3.0	2.7	1.11
27	264	27	4.9	4.4	1.11
27	265	26	5.0	4.0	1.25
27	268	23	5.5	5.0	1.10
27	269	22	5.3	5.2	1.02
27	270	21	5.2	3.7	1.41
27	271	20	5.5	5.7	0.96
27	272	19	5.2	5.9	0.88
27	273	18	6.5	5.9	1.10
27	275	16	4.9	5.5	0.89
27	276	15	5.8	5.8	1.00
27	278	13	5.3	4.3	1.23
27	279	12	6.0	4.2	1.43
27	280	11	5.4	3.2	1.69
27	282	9	5.7	4.1	1.39
27	285	6	6.9	3.5	1.97
27	286	5	3.1	2.8	1.11
27	288	3	5.6	4.1	1.37
27	290	1	7.1	2.9	2.45

S	G	Dbd	E3	P	E3/P
28	139	143	0.6	0.9	0.67
28	143	139	0.6	1.2	0.50
28	146	136	0.8	1.1	0.73
28	150	132	0.9	1.3	0.69
28	153	129	1.0	1.2	0.83
28	155	127	0.9	1.0	0.90
28	160	122	0.9	1.4	0.64
28	162	120	1.5	1.0	1.50
28	164	118	1.0	1.1	0.91
28	167	115	1.6	1.0	1.60
28	169	113	1.5	1.4	1.07
28	171	111	1.2	1.2	1.00
28	174	108	1.0	1.2	0.83
28	176	106	1.0	1.3	0.77
28	178	104	1.2	1.1	1.09
28	181	101	1.1	1.2	0.92
28	188	94	1.4	1.5	0.93
28	190	92	1.1	1.1	1.00
28	192	90	1.0	1.2	0.83
28	195	87	1.3	2.4	0.54
28	197	85	1.8	1.9	0.95
28	199	83	1.3	1.3	1.00
28	202	80	1.5	1.2	1.25
28	204	78	1.6	1.7	0.94
28	206	76	1.6	1.7	0.94
28	209	73	1.4	1.6	0.88
28	211	71	1.6	1.9	0.84
28	213	69	2.0	2.2	0.91
28	216	66	1.7	2.2	0.77
28	218	64	1.3	1.9	0.68
28	220	62	1.3	1.8	0.72
28	223	59	1.4	1.8	0.78
28	225	57	1.1	1.9	0.58
28	227	55	1.2	1.3	0.92
28	230	52	1.9	2.2	0.86
28	232	50	1.4	2.1	0.67
28	234	48	1.7	2.2	0.77
28	237	45	1.5	2.1	0.71
28	239	43	1.6	2.3	0.70
28	241	41	1.5	2.1	0.71
28	244	38	2.0	2.8	0.71
28	246	36	1.7	2.4	0.71
28	248	34	1.5	1.9	0.79
28	251	31	2.2	2.4	0.92
28	253	29	1.8	1.6	1.13
28	255	27	2.3	2.6	0.88
28	258	24	2.7	2.6	1.04
28	260	22	3.0	2.8	1.07
28	262	20	2.5	2.8	0.89
28	265	17	2.4	2.0	1.20
28	267	15	4.0	2.3	1.74
28	269	13	3.9	2.7	1.44
28	272	10	3.2	2.7	1.19
28	274	8	3.5	2.5	1.40
28	276	6	3.8	2.4	1.58
28	279	3	4.9	2.6	1.88
28	281	1	5.3	2.5	2.12

Table R.6.2 Saliva oestriol (E3) and saliva progesterone (P) levels in nmol/L during the days before delivery (Dbd) in individual subjects (1 to 31), who had spontaneous preterm labours. The gestation (G) and the oestriol:progesterone (E3/P) ratios are also shown.

S	G	Dbd	E3	P	E3/P
1	150	102	0.42	0.90	0.47
1	153	99	0.43	1.00	0.43
1	157	95	0.84	1.11	0.76
1	158	94	0.78	1.15	0.68
1	159	93	0.63	1.33	0.47
1	161	91	0.87	1.09	0.80
1	164	88	1.02	1.17	0.87
1	166	86	0.89	1.13	0.79
1	168	84	1.03	1.08	0.95
1	171	81	1.14	0.95	1.20
1	173	79	0.83	1.64	0.51
1	175	77	0.79	1.92	0.41
1	178	74	0.82	1.83	0.45
1	179	73	1.01	1.87	0.54
1	182	70	0.87	1.94	0.45
1	185	67	1.11	1.01	1.10
1	187	65	0.65	1.02	0.64
1	189	63	1.09	0.83	1.31
1	192	60	1.37	0.98	1.40
1	194	58	1.23	1.13	1.09
1	196	56	1.36	1.01	1.35
1	199	53	1.04	0.95	1.09
1	201	51	1.45	0.96	1.51
1	208	44	1.43	0.78	1.83
1	210	42	1.33	0.99	1.34
1	213	39	1.45	1.08	1.34
1	215	37	1.44	0.94	1.53
1	217	35	1.65	1.01	1.63
1	220	32	1.71	1.49	1.15
1	222	30	1.59	2.86	0.56
1	224	28	1.67	1.73	0.97
1	227	25	1.96	1.86	1.05
1	229	23	3.31	1.96	1.69
1	231	21	2.95	2.21	1.33
1	234	18	2.50	1.83	1.37
1	236	16	2.98	1.83	1.63
1	239	13	2.98	1.60	1.86
1	241	11	3.15	2.08	1.51
1	243	9	2.61	1.93	1.35
1	248	4	5.97	2.29	2.61
1	250	2	6.61	1.86	3.55

S	G	Dbd	E3	P	E3/P
2	149	96	0.62	0.53	1.17
2	151	94	0.67	0.82	0.82
2	153	92	0.40	0.60	0.67
2	155	90	0.74	0.65	1.14
2	157	88	0.78	0.93	0.84
2	159	86	0.81	1.03	0.79
2	161	84	0.74	1.06	0.70
2	163	82	0.69	0.95	0.73
2	166	79	0.89	0.86	1.03
2	168	77	0.83	0.80	1.04
2	170	75	0.74	0.62	1.19
2	172	73	0.70	0.65	1.08
2	175	70	0.87	0.82	1.06
2	177	68	0.93	0.99	0.94
2	180	65	0.97	0.88	1.10
2	194	51	1.17	1.47	0.80
2	198	47	1.06	1.33	0.80
2	200	45	1.20	1.07	1.12
2	202	43	1.07	0.90	1.19
2	205	40	1.43	1.68	0.85
2	207	38	1.05	1.22	0.86
2	209	36	1.34	1.19	1.13
2	212	33	0.85	1.20	0.71
2	214	31	0.79	0.67	1.18
2	217	28	0.99	0.92	1.08
2	219	26	1.68	1.36	1.24
2	222	23	1.07	1.15	0.93
2	229	16	1.86	1.45	1.28
2	233	12	1.70	1.30	1.31
2	235	10	1.68	1.14	1.47
2	237	8	2.16	1.41	1.53
2	240	5	2.25	1.43	1.57

S	G	Dbd	E3	P	E3/P
3	136	118	2.00	1.80	1.11
3	138	116	1.90	1.53	1.24
3	139	115	1.56	1.37	1.14
3	142	112	1.60	1.57	1.02
3	144	110	1.59	1.53	1.04
3	146	108	1.05	1.46	0.72
3	149	105	2.09	1.73	1.21
3	151	103	1.60	2.36	0.68
3	152	102	1.60	2.98	0.54
3	154	100	1.75	1.65	1.06
3	156	98	2.13	1.92	1.11
3	158	96	1.57	2.34	0.67
3	160	94	1.67	1.76	0.95
3	162	92	1.10	1.56	0.71
3	165	89	1.51	1.84	0.82
3	167	87	1.58	1.98	0.80
3	170	84	1.15	1.57	0.73
3	172	82	1.56	1.89	0.83
3	174	80	1.28	1.71	0.75
3	176	78	1.13	1.56	0.72
3	182	72	1.48	1.88	0.79
3	183	71	1.90	1.92	0.99
3	187	67	2.15	2.12	1.01
3	188	66	1.65	1.63	1.01
3	190	64	1.47	2.32	0.63
3	192	62	1.39	1.61	0.86
3	195	59	1.65	1.88	0.88
3	197	57	1.85	2.39	0.77
3	199	55	1.50	2.03	0.74
3	202	52	1.57	2.11	0.74
3	205	49	1.57	2.25	0.70
3	206	48	1.63	2.55	0.64
3	207	47	1.73	2.32	0.75
3	213	41	1.76	2.50	0.70
3	215	39	1.54	2.47	0.62
3	218	36	1.59	2.58	0.62
3	220	34	1.55	2.08	0.75
3	222	32	1.46	1.96	0.74
3	225	29	1.60	2.36	0.68
3	229	25	1.63	1.91	0.85
3	230	24	1.97	2.15	0.92
3	232	22	2.29	2.25	1.02
3	234	20	1.75	2.26	0.77
3	239	15	3.05	3.15	0.97
3	241	13	2.91	3.32	0.88
3	243	11	2.77	3.65	0.76
3	246	8	3.82	4.48	0.85
3	249	5	3.91	3.56	1.10
3	250	4	5.35	4.08	1.31

S	G	Dbd	E3	P	E3/P
4	152	101	0.95	0.57	1.67
4	154	99	1.23	0.59	2.08
4	157	96	1.34	0.54	2.48
4	159	94	1.35	0.61	2.21
4	161	92	1.99	0.63	3.16
4	164	89	1.92	0.59	3.25
4	166	87	1.19	0.44	2.70
4	168	85	1.32	0.40	3.30
4	171	82	1.42	0.44	3.23
4	173	80	1.52	0.40	3.80
4	175	78	1.92	0.42	4.57
4	178	75	1.81	0.41	4.41
4	180	73	1.29	0.46	2.80
4	182	71	1.48	0.45	3.29
4	185	68	1.58	0.69	2.29
4	187	66	1.92	0.35	5.49
4	189	64	1.63	0.27	6.04
4	192	61	1.51	0.33	4.58
4	194	59	1.37	0.39	3.51
4	196	57	1.88	0.50	3.76
4	199	54	1.62	0.36	4.50
4	201	52	1.52	0.33	4.61
4	203	50	2.09	0.43	4.86
4	206	47	1.76	0.48	3.67
4	208	45	1.71	0.42	4.07
4	210	43	1.57	0.54	2.91
4	217	36	1.94	0.50	3.88
4	220	33	2.23	0.59	3.78
4	222	31	1.87	0.61	3.07
4	224	29	1.31	0.64	2.05
4	227	26	1.32	0.94	1.40
4	229	24	2.41	0.75	3.21
4	231	22	1.82	0.82	2.22
4	234	19	2.00	0.84	2.38
4	236	17	2.16	0.75	2.88
4	238	15	1.62	1.09	1.49
4	241	12	3.43	1.01	3.40
4	243	10	2.89	0.83	3.48
4	245	8	3.53	0.98	3.60
4	248	5	4.82	0.95	5.07
4	250	3	5.15	1.31	3.93
4	252	1	4.13	1.05	3.93

S	G	Dbd	E3	P	E3/P
5	147	72	0.70	1.20	0.58
5	152	67	0.80	1.30	0.62
5	153	66	0.90	1.20	0.75
5	154	65	0.90	1.00	0.90
5	156	63	0.60	0.90	0.67
5	159	60	1.00	1.30	0.77
5	172	47	1.40	1.60	0.88
5	174	45	1.00	1.40	0.71
5	177	42	1.10	1.60	0.69
5	182	37	1.10	1.30	0.85
5	187	32	2.20	1.90	1.16
5	189	30	2.10	2.10	1.00
5	191	28	2.00	2.00	1.00
5	195	24	1.20	1.50	0.80
5	196	23	1.70	1.80	0.94
5	201	18	1.30	1.70	0.76
5	203	16	1.20	1.20	1.00
5	205	14	1.50	1.30	1.15
5	210	9	1.60	1.40	1.14
5	212	7	1.40	1.20	1.17
5	216	3	1.70	1.40	1.21
5	217	2	1.70	1.50	1.13

S	G	Dbd	E3	P	E3/P
6	146	106	0.91	1.35	0.67
6	148	104	0.78	1.43	0.55
6	151	101	0.61	0.80	0.76
6	153	99	0.79	0.61	1.30
6	155	97	0.92	0.83	1.11
6	158	94	0.87	1.27	0.69
6	160	92	0.91	1.07	0.85
6	162	90	1.03	0.83	1.24
6	165	87	1.05	1.13	0.93
6	167	85	0.97	1.20	0.81
6	169	83	1.08	1.17	0.92
6	172	80	1.03	1.07	0.96
6	174	78	0.97	0.98	0.99
6	176	76	0.95	1.65	0.58
6	179	73	1.22	1.05	1.16
6	181	71	0.91	1.17	0.78
6	183	69	1.02	1.31	0.78
6	186	66	1.01	1.14	0.89
6	188	64	1.01	1.57	0.64
6	190	62	0.86	1.12	0.77
6	193	59	1.17	1.66	0.70
6	195	57	1.08	1.22	0.89
6	197	55	1.14	1.32	0.86
6	200	52	1.03	1.69	0.61
6	202	50	1.30	1.43	0.91
6	204	48	1.15	2.07	0.56
6	209	43	1.15	1.66	0.69
6	211	41	1.04	1.62	0.64
6	214	38	1.20	1.82	0.66
6	216	36	1.68	1.80	0.93
6	218	34	1.21	1.78	0.68
6	220	32	1.55	1.85	0.84
6	223	29	1.69	1.85	0.91
6	225	27	1.86	2.06	0.90
6	228	24	2.42	1.77	1.37
6	230	22	2.22	1.96	1.13
6	232	20	2.26	1.93	1.17
6	235	17	1.18	2.39	0.49
6	237	15	2.61	1.96	1.33
6	239	13	2.72	3.47	0.78
6	242	10	2.79	2.53	1.10
6	246	6	2.68	2.11	1.27
6	251	1	3.68	1.73	2.13

S	G	Dbd	E3	P	E3/P
7	140	106	0.71	0.86	0.83
7	142	104	0.68	1.34	0.51
7	144	102	0.73	0.97	0.75
7	147	99	0.88	1.03	0.85
7	149	97	0.87	1.02	0.85
7	151	95	0.67	1.67	0.40
7	154	92	0.97	0.92	1.05
7	156	90	1.12	0.69	1.62
7	158	88	0.88	0.90	0.98
7	161	85	0.96	1.54	0.62
7	163	83	1.16	1.06	1.09
7	165	81	1.14	0.95	1.20
7	168	78	1.42	1.23	1.15
7	170	76	1.18	1.75	0.67
7	172	74	1.17	0.94	1.24
7	175	71	1.47	1.36	1.08
7	177	69	1.56	1.46	1.07
7	179	67	1.14	1.13	1.01
7	182	64	1.47	1.44	1.02
7	184	62	1.25	1.00	1.25
7	189	57	1.67	1.17	1.43
7	190	56	2.15	1.98	1.09
7	191	55	1.57	1.65	0.95
7	193	53	1.63	0.91	1.79
7	195	51	1.64	1.45	1.13
7	197	49	1.43	1.27	1.13
7	198	48	1.72	1.62	1.06
7	202	44	3.27	2.85	1.15
7	205	41	1.78	1.46	1.22
7	206	40	1.90	1.34	1.42
7	207	39	1.75	1.43	1.22
7	208	38	2.16	1.66	1.30
7	209	37	1.86	1.79	1.04
7	211	35	1.59	1.49	1.07
7	212	34	1.93	1.72	1.12
7	213	33	2.26	1.48	1.53
7	215	31	2.07	2.01	1.03
7	216	30	2.60	2.55	1.02
7	217	29	2.34	1.92	1.22
7	219	27	1.78	1.82	0.98
7	220	26	1.78	1.82	0.98
7	221	25	2.39	2.06	1.16
7	222	24	2.54	2.71	0.94
7	223	23	2.85	2.49	1.14
7	225	21	3.23	2.16	1.50
7	226	20	2.42	2.29	1.06

S	G	Dbd	E3	P	E3/P
7	228	18	2.18	1.85	1.18
7	229	17	2.49	2.24	1.11
7	231	15	3.26	2.25	1.45
7	232	14	2.82	1.79	1.58
7	235	11	3.76	1.61	2.34
7	236	10	3.61	2.00	1.81
7	237	9	3.85	2.10	1.83
7	239	7	4.24	2.45	1.73
7	241	5	4.10	2.61	1.57
7	242	4	4.41	2.82	1.56
7	243	3	5.41	2.97	1.82
7	245	1	4.91	2.81	1.75
8	149	82	0.70	0.70	1.00
8	150	81	0.62	0.88	0.70
8	153	78	0.62	0.95	0.65
8	155	76	0.57	0.89	0.64
8	157	74	0.53	1.04	0.51
8	160	71	0.63	1.21	0.52
8	162	69	0.73	1.20	0.61
8	164	67	0.57	1.18	0.48
8	167	64	0.48	1.45	0.33
8	169	62	0.63	1.42	0.44
8	172	59	0.82	1.43	0.57
8	174	57	0.55	1.63	0.34
8	183	48	0.57	2.10	0.27
8	184	47	0.86	2.02	0.43
8	188	43	0.47	1.89	0.25
8	190	41	0.54	1.90	0.28
8	195	36	0.64	2.13	0.30
8	197	34	0.46	1.86	0.25
8	202	29	0.72	2.22	0.32
8	204	27	0.47	1.82	0.26
8	209	22	0.56	2.16	0.26
8	215	16	0.60	2.36	0.25
8	218	13	0.61	2.07	0.29
8	220	11	0.56	2.06	0.27
8	224	7	0.60	1.74	0.34
8	228	3	0.58	1.93	0.30
8	230	1	1.02	3.03	0.34

S	G	Dbd	E3	P	E3/P
9	196	29	1.71	0.76	2.25
9	198	27	2.10	0.81	2.59
9	197	26	1.30	0.87	1.49
9	194	23	1.75	0.75	2.33
9	196	21	1.71	0.73	2.34
9	198	19	1.95	0.90	2.17
9	200	17	1.63	0.68	2.40
9	203	14	1.85	0.83	2.23
9	206	11	2.00	0.55	3.64
9	208	9	1.71	0.63	2.71
9	210	7	1.92	0.56	3.43
9	212	5	2.03	0.76	2.67
9	217	0	5.36	1.10	4.87
10	248	1	3.29	2.38	1.38
11	173	32	0.99	0.54	1.83
11	174	31	0.80	0.49	1.63
11	177	28	1.16	0.54	2.15
11	184	21	1.08	1.50	0.72
11	186	19	1.39	2.01	0.69
11	189	16	1.27	1.45	0.88
11	190	15	1.26	1.48	0.85
11	200	5	1.24	2.20	0.56
11	202	3	1.16	0.83	1.40
11	203	2	1.25	1.78	0.70
12	222	17	1.08	1.80	0.60
12	223	16	0.51	1.07	0.48
12	224	15	0.65	1.54	0.42
12	226	13	0.94	1.76	0.53
12	227	12	1.11	2.31	0.48
12	228	11	1.20	2.21	0.54
12	229	10	0.43	2.35	0.18
12	231	8	0.10	1.73	0.06
12	232	7	1.23	1.75	0.70
12	234	5	1.51	2.13	0.71
12	235	4	1.55	2.22	0.70
12	236	3	0.44	3.35	0.13
13	176	4	1.14	1.03	1.11
14	191	23	0.71	0.74	0.96
14	194	20	1.10	0.85	1.29
14	197	17	1.05	0.43	2.44
14	199	15	1.29	0.90	1.43
14	201	13	1.02	1.25	0.82
14	212	2	1.71	2.25	0.76

S	G	Dbd	E3	P	E3/P
15	169	8	1.36	0.82	1.66
15	170	7	1.32	0.51	2.59
15	171	6	1.60	0.99	1.62
15	172	5	1.23	0.64	1.92
16	192	13	2.23	1.85	1.21
16	193	12	1.75	1.71	1.02
16	194	11	1.77	1.35	1.31
16	195	10	1.33	0.97	1.37
16	196	9	1.32	0.88	1.50
16	197	8	1.76	1.00	1.76
16	198	7	1.62	1.00	1.62
16	199	6	1.30	1.12	1.16
16	200	5	1.49	1.37	1.09
16	201	4	1.39	1.41	0.99
16	202	3	1.47	1.66	0.89
16	203	2	1.38	1.23	1.12
16	204	1	0.61	1.83	0.33
17	250	0	5.26	1.44	3.65
18	171	8	1.52	0.44	3.45
18	174	5	2.19	0.47	4.66
18	176	3	1.67	0.37	4.51
18	177	2	2.01	0.49	4.10
18	178	1	1.71	0.56	3.05
19	249	0	1.98	0.34	5.82
20	231	0	11.12	3.91	2.84
21	239	0	1.00	1.12	0.89
22	237	4	2.54	1.31	1.94
22	238	3	7.35	1.31	5.61
23	236	2	2.00	1.20	1.67
23	238	0	2.52	1.66	1.52
24	184	0	2.60	2.10	1.24
25	245	1	3.50	1.90	1.84
26	246	0	6.50	3.30	1.97
27	241	0	2.90	1.60	1.81
28	193	0	2.70	1.00	2.70

S	G	Dbd	E3	P	E3/P
29	234	19	1.90	1.40	1.36
29	243	10	3.10	1.50	2.07
29	246	7	3.20	1.80	1.78
29	248	5	5.00	2.20	2.27
29	250	3	4.20	2.40	1.75
29	252	1	4.70	2.90	1.62
30	212	6	0.86	0.78	1.10
30	214	4	0.94	0.90	1.04
30	215	3	0.91	1.28	0.71
30	216	2	0.84	1.36	0.62
30	217	1	0.67	1.32	0.51
31	193	58	1.84	1.06	1.74
31	194	57	1.26	1.31	0.96
31	195	56	1.52	1.11	1.37
31	196	55	1.54	1.36	1.13
31	205	46	2.25	1.34	1.68
31	206	45	2.01	1.16	1.73
31	207	44	2.15	1.42	1.51
31	209	42	1.81	1.59	1.14
31	210	41	2.65	1.56	1.70
31	212	39	3.65	2.61	1.40
31	218	33	1.96	1.58	1.24
31	219	32	1.79	1.75	1.02
31	220	31	1.79	1.91	0.94
31	221	30	2.03	2.10	0.97
31	222	29	1.85	1.30	1.42
31	224	27	3.12	1.33	2.35
31	226	25	2.17	1.05	2.07
31	227	24	2.19	1.09	2.01
31	228	23	2.02	1.14	1.77
31	232	19	2.14	1.24	1.73
31	235	16	2.93	1.57	1.87
31	236	15	2.71	1.17	2.32
31	238	13	3.51	1.89	1.86
31	240	11	3.09	1.95	1.58
31	242	9	2.80	1.94	1.44
31	243	8	2.33	1.53	1.52
31	244	7	1.87	1.40	1.34
31	245	6	2.36	1.38	1.71
31	246	5	3.14	1.83	1.72

Table R.7.1 Fetal adrenal measurements in individual subjects (S) throughout gestation (in days). [RT - right transverse, LT - left transverse, RAP- right anteroposterior, LAP - left anteroposterior, RC - right circumference, LC - left circumference, RA - right area, LA - left area, RL - right length, LL - left length]

S	Gest.	RT	LT	RAP	LAP	RC	LC	RA	LA	RL	LL
1	161		11		6		27		0.5		
1	190	13		9		43		1.3			
1	222	19		9		51		1.7			
1	251	25		15		72		3.9			
1	278	30		17		82		4.7		25	
2	171	17	15	11	8	47	39	1.5	1.1	15	
2	199	16		11		43		1.4			16
2	227	25		13		62		2.7			
2	262		27		16		66		2.9		
2	283		26		13		64		2.9		
3	168	12		5		31		0.6			
3	196	13		7		36		0.9			
3	224		21		9		47		1.5		
3	252	21	19	11	12	51	51	1.8	1.9		
4	173	11		6		30		0.6			
4	196	13		8		35		0.9			
4	224		18		11		48		1.6		
4	238	23		12		59		2.4			
4	252	25		11		62		2.6			
4	280	20		12		53		2.1			
5	154	13		8		34		0.8			
5	179	15		8		37		1.0			
5	193	18		11		46		1.5			
5	219	20		12		52		2.0			
5	249		26		14		74		4.0		
6	168		12		7		39		1.1		
6	197		14		7		37		1.0		
6	225	22		13		59		2.7			
6	254		26		13		72		3.6		
7	169		17		9		41		1.2		
7	196		19		8		48		1.4		
7	225		22		12		54		2.0		
7	247		25		10		59		2.2		
7	274	32		17		84		4.9			
8	168	12		6		35		0.8			
8	189		15		9		40		1.2		
8	224	20		12		51		1.8		17	
8	251		20		12		51		1.9		21
9	167	15		8		38		1.1			
9	195		17		9		46		1.6		
9	209	20		7		50		1.5			
9	223	23		11		53		1.8		15	16
9	251	31		17		82		4.5		21	

S	Gest.	RT	LT	RAP	LAP	RC	LC	RA	LA	RL	LL
10	168	14		6		32		0.6			
10	196		20		11		54		1.9		
10	227	24	22	14	14	61	61	2.7	2.8		
10	251	29	29	18	18	77	76	4.5	4.3		
11	165	16	14	8	6	41	34	1.2	0.7		
11	193	18	17	12	13	53	50	2.0	2.0		17
11	221	24		10		54		1.7			
11	258	26		14		68		3.3			
11	277	34		13		81		4.2			
12	167	15		6		37		0.8			
12	200		18		9		51		1.8		
12	225		20		9		48		1.4		
12	249		26		12		62		2.5		
12	278	29	30	16	18	73	83	3.6	4.8		
13	213	14		8		36		0.9		11	
13	228		13		8		54		2.2		
13	252	20		9		48		1.7			
13	259	23		10		59		2.4			
14	166	14	13	8	6	37	34	1.0	0.7		
14	194	19	17	10	9	49	47	1.8	1.4		
14	222	23	25	14	18	60	64	2.7	3.1		
14	245	26		17		68		3.4		22	
14	278	26		18		70		3.7			
15	178	17	16	9	8	44	42	1.4	1.2		
15	210	19	18	11	9	52	44	2.0	1.2		
15	230		23		13		57		2.2		
15	244	28		16		72		3.5			
15	258	27		15		69		3.3			
16	169		17		8		40		1.1		
16	197	14		8		38		1.0			14
16	225	20		9		46		1.3			16
16	255	23		15		58		2.5			
16	283	31		14		79		4.2			
17	168		12		7		33		0.7		
17	196	22		14		57		2.5			
17	230	25		13		60		2.5			
17	252		29		13		68		3.0		
18	168	15		5		33		0.7			
18	199	16		10		43		1.3			
18	223	17		9		48		1.6			
18	251	27	24	13	13	62	62	2.4	2.7		
18	279	28		17		71		3.5			19
19	162	16	17	8	9	44	42	1.3	1.2		
19	194		16		7		35		0.8		
19	222		25		12		59		2.3		
19	244		28		19		73		4.1		
19	278		23		15		57		2.2		
20	166	14		7		35		0.7			
20	194		16		8		39		1.0		
20	222		21		10		50		1.7		
20	250	22	23	13	15	57	63	2.3	2.9		

S	Gest.	RT	LT	RAP	LAP	RC	LC	RA	LA	RL	LL
21	175	14	16	6	7	35	41	0.7	1.1		12
21	203	16		11		44		1.5			
21	231	22		12		55		2.3			
21	257	24		11		62		2.7			13
22	168		13		6		35		0.8		
22	198	21		9		52		1.8			
22	225	22		14		55		2.2			
22	254	26		13		64		2.9			
23	168	15	15	6	6	35	39	0.7	1.0	13	14
23	196		17		10		44		1.4		
23	226	21		10		49		1.7			
23	252	25		14		62		2.6			
23	273		27		16		74		3.9		
24	176	12		8		33		0.8			
24	198	16		8		42		1.2			
24	211	18		9		46		1.5			
24	225		19		12		54		2.2		
24	239		25		16		69		3.6		
25	163		15		8		39		1.0		11
25	190		17		8		40		1.1		
25	219	19		12		51		1.9			
25	247		30		13		69		3.0		
25	280	26		17		70		3.5			
26	168	13		8		37		1.0			
26	195		16		11		45		1.5		
26	224	23		10		58		2.5			
26	252		26		15		70		3.6		
26	280	26		16		73		3.8			
27	167	15		8		37		1.0			
27	195		17		10		44		1.5		
27	223	20		13		56		2.5			
27	251	27		16		65		3.1			
27	279	26		12		65		2.7			
28	166	14	16	7	7	34	37	0.8	0.9		
28	194	22		9		49		1.4		17	
28	222		27		15		67		3.2		
28	236	22		11		56		2.1			
28	256	28		15		68		3.1			
28	278	29		13		70		3.1			
29	168	17		8		43		1.2			
29	201	18		10		47		1.6			18
29	224	25		13		57		2.2			
29	251	24		11		59		2.0			
30	195	15		8		32		0.8			
30	222		20		14		59		2.8		
30	251	23		12		60		2.7			
30	266	24		14		69		3.7			
30	280	24		14		69		3.7			

S	Gest.	RT	LT	RAP	LAP	RC	LC	RA	LA	RL	LL
31	164	10	12	6	7	28	32	0.5	0.7		
31	191	17	18	9	9	43	49	1.3	1.6		
31	219	25		13		61		2.5			
31	253	24		13		61		2.6			
31	281	33		19		82		4.7			
32	175	11	14	8	7	30	34	0.7	0.8	14.1	14.6
32	196		14		9		40		1.2		
32	222	18		10		48		1.7			
32	247	25	22	10	13	57	57	2.1	2.4		
32	278	30		14		78		4.2			
33	169	12		6		33		0.7			
33	198		22		13		62		3.0		
33	226		23		11		56		1.9		18
33	254	29		18		75		4.2			
33	255		30		15		79		3.9		
34	164		15		11		44		1.4		
34	192		24		11		57		2.0		
34	227		23		11		55		2.1	14	
34	248		28		11		64		2.4		
35	166		13		7		35		0.9		
35	194	19	20	10	9	50	48	1.8	1.5		
35	223		24		12		59		2.4		
35	252	21		15		59		2.8			25
35	275	24		14		59		2.7			
36	170	13		7		36		1.0			
36	196	17	18	10	11	46	47	1.6	1.6		
36	224		23		14		59		2.6	19	
36	252	24		18		64		2.9			
37	166		16		11		44		1.4		
37	194	21	21	9	10	48	51	1.4	1.6		
37	229	22		13		56		2.2			
37	250	25		12		57		2.1			
38	170	14		8		40		1.2			
38	198	15		8		37		0.9			
38	226	19		11		48		1.6			
38	250	28	26	16	14	72	66	3.9	3.1	18	19
38	271	33		18		79		4.3		26	
39	168		16		9		41		1.2		
39	196	20		12		48		1.6			
39	224	24		14		65		3.1		16	
39	252	27		16		67		3.7			
40	171		15		6		38		1.0		
40	200		18		9		45		1.4		
40	221	22		8		54		1.6		13	
40	249	26		11		59		2.1		20	
41	171	12		7		34		0.9			
41	198	16		9		42		1.2			
41	219	24		11		65		2.7			
41	254	28		11		72		3.2		21	23

S	Gest.	RT	LT	RAP	LAP	RC	LC	RA	LA	RL	LL
42	168	14		7		37		0.8			
42	196	16		9		44		1.3		14	
42	224		20		11		52		1.8		
42	260		30		18		73		3.9		
42	280		31		19		83		5.3		
43	167	16	16	9	8	39	39	1.1	1.0		
43	192	15	18	9	10	40	47	1.2	1.5	15	
43	228		24		13		58		2.4		
43	248	25		16		66		3.3			
43	276		27		15		68		3.2		
44	168	16		8		35		0.7			
44	196	18	17	9	10	44	44	1.4	1.3		
44	224		26		15		65		3.2		
44	250	28	25	15	14	67	64	3.1	3.0		
45	166	15	12	7	5	38	30	0.9	0.6		
45	194		20		12		49		1.7		
45	222		27		14		63		2.9		
45	236	30		17		75		4.1			
45	258	35		21		87		5.8			

Table R.7.2 Fetal kidney and growth measurements in individual subjects (S) throughout gestation (in days). [RT - right transverse, LT - left transverse, RAP - right anteroposterior, LAP - left anteroposterior, RC - right circumference, LC - left circumference, RA - left area, LA - left area, HC - head circumference, AC - abdominal circumference, FL - femur length]

S	Gest.	RT	LT	RAP	LAP	RC	LC	RA	LA	HC	AC	FL
1	161		16		12		43		1.4	217	175	43
1	190	22		17		66		3.4		263	231	53
1	222	27		24		92		6.5		297	272	60
1	251	43		26		108		8.9			324	67
1	278	39		31		110		9.4			358	69
2	171	21		13		57		2.4		233	216	43
2	199	25	27	16	19	62	75	3	4.4	272	252	54
2	227	32		25		91		6.5		290	286	62
2	262		38		28		99		7.7	331	329	70
2	283	36	36	25	20	95	89	6.8	5.8	328	343	72
3	168	16		13		50		2		219	178	40
3	196	22		16		66		3.4			223	49
3	224		28		21		75		4.5	295	283	58
3	252	34	33	23	24	86	90	5.6	6.2		311	67
4	173	19		15		53		2.2		222	190	42
4	196	26		16		67		3.5		263	221	49
4	224		31		22		84		5.5	300	252	60
4	238	31		22		87		5.8		320	277	62
4	252	34		23		88		5.8		323	289	60
4	280	35		27		98		7.3			299	68
5	154											
5	179	23.9		17.9		66		3.36		245	234	49
5	193	27		17		70		3.59		273	255	57
5	219	30		20		86		5.5		306	299	61
5	249		35		25		101		7.3	317	336	69
6	168		21		13		56		2.4	228	204	44
6	197		27		16		72		3.7	278	246	51
6	225	35		19		98		3.7		312	274	61
6	254		39		28		108		8.5	320	309	68
7	169		25		19		69		3.7	229	209	42
7	196		28		19		73		4.0	278	252	50
7	225		32		24		87		6.0	304	282	63
7	247		33		25		92		6.5	323	324	66
7	274	41		31		108		9		343	353	71
8	168	18		17		57		2.7		224	193	39
8	189		24		14		61		2.9	271	233	49
8	224	27		19		74		4.2		304	263	60
8	251		36		25		99		7.1	330	302	75
9	167	23		14		63		3.1		218	193	42
9	195		28		17		73		4.0	269	247	53
9	209	25		20		78		4.7		296	263	57
9	223	37	34	26	20	101	90	7.5	6.0		294	64
9	251	51		31		130		12.5			329	70

S	Gest.	RT	LT	RAP	LAP	RC	LC	RA	LA	HC	AC	FL
10	168	20		14		53		2.1			198	43
10	196		26		17		73		3.9	297	262	52
10	227	30	35	22	25	88	97	6.2	7.5	325	314	62
10	251	34	41	28	25	94	107	7.0	8.6		339	66
11	165	22		15		62		2.8		231	214	44
11	193	32	22	24	20	87	68	5.8	3.7		248	55
11	221	35	35	25	24	96	91	7.1	6.2		308	62
11	258	38		26		106		8.6		354	339	69
11	277	38		26		105		8.6		366	361	77
12	167	19		16		62		3.1		223	205	38
12	200		22		17		65		3.2	286	244	53
12	225		20		20		82		5.3		288	61
12	249		35		24		103		7.3		320	70
12	278	39	37	26	29	104	107	8.2	9.1		367	75
13	213	23		11		65		2.8		259	222	50
13	228		26		19		74		4.2	286	252	60
13	252	28		24		88		6.2		304	284	64
13	259	32		25		93		6.9		304	295	65
14	166	24	24	18	17	69	64	3.6	2.9	223	191	44
14	194	26	26	20	22	77	71	4.6	4.0	256	248	54
14	222	35	30	27	22	100	83	7.7	5.2	285	289	62
14	245	40		27		101		7.5			337	68
14	278	41		29		124		11.8		330	367	73
15	178	21	23	16	23	58	67	2.7	3.4	230	204	44
15	210	28	29	20	17	78	77	4.7	4.5	289	278	58
15	230		33		27		97		7.5	305	309	66
15	244	40		31		109		9.5		328	315	64
15	258	43		31		116		10.2		334	325	70
16	169		17		14		49		1.9	214	176	46
16	197	22		18		64		3.3		252	228	55
16	225	30		25		89		6.2		294	273	59
16	255	31	31	28	23	93	87	6.3	5.7	333	318	72
16	283	46		31		126		12.3		335	329	76
17	168		19		13		55		2.3	228	205	42
17	196	29	28	23	21	88	80	6.1	5.0	269	250	53
17	230	31	33	24	21	88	83	6.1	5.3	306	292	63
17	252	40	39	28	22	109	96	9.0	6.6	323	324	70
18	168	20		15		63		2.9		215	186	42
18	199	25		17		67		3.5			251	57
18	223	29		22		85		5.5			274	61
18	251	35	20	22	31	92	80	6.2	4.9		294	72
18	279	37	32	27	23	98	90	7.4	6.1		328	74
19	162		23		18		67		3.6	220	195	43
19	194		19		16		53		2.2	266	246	55
19	222		29		11		78		4.6	294	294	63
19	244		34		25		92		6.5	313	320	68
19	278		36		24		101		7.5		359	76
20	166	23		16		63		3.2		228	207	46
20	194		29		18		74		4.1	266	254	54
20	222		36		23		95		6.7	301	285	62
20	250	36	33	26	21	102	85	8.1	5.2	315	326	

S	Gest.	RT	LT	RAP	LAP	RC	LC	RA	LA	HC	AC	FL
21	175	21		14		58		2.5		234	203	46
21	203	27	24	21	24	77	74	4.6	4.3	278	246	57
21	231	33	29	19	22	85	78	5.2	4.9		261	63
21	257	31	31	24	23	91	91	6.3	6.3		314	69
22	168		22		12		55		2.3	223	205	47
22	198	36		21		86		5.4		268	239	
22	225	34		25		93		6.7		297	265	63
22	254	38		26		97		7.3		327	299	71
23	168	21	25	17	13	59	61	2.7	2.7	220	210	43
23	196		27		19		73		4.2	264	250	50
23	226	32	28	21	21	82	78	5.2	4.7	295	293	62
23	252	39	35	30	28	108	97	9.0	7.4	321	339	65
23	273		35		26		101		8.0	336	343	72
24	176	16		17		56		2.5		247	200	49
24	198	35		19		83		4.6		174	242	50
24	211	26		19		77		4.6		307	284	62
24	225		26		24		80		5.0	322	300	63
24	239		28		22		92		6.6	340	338	68
25	163		25		17		75		4.3	223	188	41
25	190		27		16		68		3.4	258	228	50
25	219	29		22		82		5.2			277	62
25	247		37		29		104		8.6	310	307	67
25	280	36		27		97		7.2		329	338	75
26	168	12		11		50		2.0		221	207	41
26	195		23		13		60		2.7	270	253	51
26	224	32		18		82		4.5		313	295	64
26	252		32		24		100		7.4	333	344	68
26	280	37		35		107		8.5		351	388	78
27	167	22		19		65		3.3		227	200	43
27	195		23		19		68		3.5	270	255	52
27	223	38		22		96		6.9		310	285	62
27	251	35		27		105		8.5		332	334	69
27	279	44		29		114		9.8			349	74
28	166		18		15		51		2.1	228	196	40
28	194	31		20		80		4.8		263	238	51
28	222		34		25		95		7.0	297	265	59
28	236	32		24		87		5.8			278	
28	256	35		25		102		7.9			315	65
28	278	39		25		103		7.8			323	71
29	168	22		15		60		2.7		237	196	45
29	201	30	26	15	21	75	74	4.1	4.4	286	235	56
29	224	32	30	20	21	83	84	5.2	5.2	311	276	62
29	251	43		30		112		9.6		337	307	69
30	195	19		11		72		3.2		259	250	55
30	222		29		20		85		5.3	296	282	64
30	251	38		26		107		8.8		319	317	71
30	266	30		31		101		7.8		337	334	75
30	280	43		31		124		11.7		337	352	76

S	Gest.	RT	LT	RAP	LAP	RC	LC	RA	LA	HC	AC	FL
31	164		18		11		48		1.7	211	168	38
31	191	28	20	16	19	74	64	4.0	3.3	250	221	49
31	219	30		21		82		5.2		293	285	55
31	253	38		25		104		8.3		322	312	64
31	281	39		29		105		8.5		338	343	72
32	175	25	23	17	15	52	49	2.1	1.9	232	198	
32	196		21		15		64		3.2	257	243	49
32	222	28		21		78		4.6		310	265	60
32	247	36	31	26	20	99	85	7.6	5.4	330	302	64
32	278	45		28		116		10.3		344	354	72
33	169	17		17		58		2.6		244		42
33	198		27		25		82		5.4	286	277	52
33	226		33		20		80		4.8	326	308	63
33	254	32	34	25	22	90	91	6.4	6.6	348	361	67
33	255		43		26		111		8.9			
34	164		21		14		58		2.4	231	207	44
34	192		28		18		72		3.9	281	251	58
34	227	34		24		96		7.3		324	316	68
34	248		44		24		114		9.1	346	322	69
35	166		24		14		63		2.9	227	190	43
35	194	27	27	18	18	75	74	4.3	4.2	260	243	53
35	223		31		25		93		6.7	304	276	59
35	252	36	38	26	26	97	105	7.2	8.3	315	325	68
35	275	39		27		104		8.2		342	338	75
36	170	18		11		51		1.8		247	224	47
36	196	25	27	23	23	83	82	5.5	5.4	285	258	56
36	224	34	35	24	21	100	91	7.7	6.3		316	63
36	252	41		24		107		8.4			318	69
37	166	26	21	16	16	69	62	3.5	3.0	229	217	43
37	194	27	26	23	22	76	73	4.5	4.1	279	252	55
37	229	32		21		84		5.4		314	297	65
37	250	36		27		102		7.5		334	330	67
38	170	18		16		57		2.6		250	224	43
38	198	26		13		66		3.2		281	260	52
38	226	30		27		98		7.6			287	62
38	250	37	39	22	24	94	100	6.6	6.9		328	69
38	271	41		25		109		8.5			360	75
39	168		24		18		70		3.8	225	201	44
39	196	29		18		76		4.2		272	269	50
39	224	33		22		90		5.9		296	283	63
39	252	37		26		99		7.5		320	339	68
40	171		21		18		67		3.3	231	208	43
40	200		21		20		67		3.6	278	243	52
40	221	32		20		94		6.6		313	281	59
40	249	39	29	26	23	103	83	7.9	5.3	322	310	65
41	171	23		17		73		3.9		241	205	48
41	198	28		23		80		5.1			249	61
41	219	35		27		96		7.1		312	276	63
41	254	37	41	27	29	103	117	8.2	10.7	333	347	74

S	Gest.	RT	LT	RAP	LAP	RC	LC	RA	LA	HC	AC	FL
42	168	19		16		59		2.7		209	198	41
42	196	27	23	20	17	75	60	4.3	2.8	252	244	51
42	224		33		21		89		5.9	289	295	62
42	260		44		29		116		10.1	323	312	68
42	280	35	38	27	28	102	108	8.1	9.0	332	328	73
43	167		22		16		64		3.1	236	198	42
43	192	22	22	19	18	65	62	3.3	3.0	273	239	53
43	228		31		23		85		5.6	317	308	67
43	248	32	30	21	23	79	86	4.7	5.7	350	317	68
43	276		37		31		110		9.4	345	335	76
44	168	20		19		70		3.7		234	199	42
44	196	32	26	24	22	88	77	6.0	4.6	266	238	54
44	224	30	39	29	24	91	102	6.7	7.5	310	289	65
44	250	38	34	27	24	101	101	7.9	7.9	330	315	68
45	166	18	18	11	14	49	53	1.8	2.2	237	201	
45	194		28		22		82		5.2	286	271	55
45	222		43		25		109		8.8	313	327	67
45	236	36		25		101		7.9		325	329	71
45	258	39		28		116		10.5		342	353	76

Table R.7.3 Saliva oestriol (E3), saliva progesterone (P), plasma oestriol and plasma progesterone levels throughout gestation (in days) in individual subjects (S).

S	Gest.	Saliva E3	Saliva P	Saliva E3/P	Plasma E3	Plasma P
1	161	1.27	1.10	1.15	15.7	96
1	190	1.56	1.25	1.25	21.8	115
1	222	2.78	1.71	1.63	32.6	192
1	251	3.71	1.79	2.07	39.4	212
1	278	5.95	2.01	2.96	56.8	265
2	171	1.74	1.97	0.88	22.3	251
2	199	2.20	1.89	1.16	35.1	374
2	227	2.92	2.76	1.06	39.0	437
2	262				56.6	494
2	283	5.36	2.75	1.95	67.6	506
3	168	1.25	0.90	1.39	22.4	156
3	196	1.48	0.91	1.63	27.5	157
3	224	2.22	1.01	2.20	32.1	167
3	252	4.17	1.30	3.21	38.1	201
4	173	0.75	1.16	0.65	14.9	154
4	196	0.81	1.15	0.70	15.2	211
4	224	1.02	1.76	0.58	17.7	298
4	238					
4	252	0.80	1.62	0.49	13.3	239
4	280	0.96	1.97	0.49	19.4	353
5	154	1.14	1.51	0.75	14.2	155
5	179	1.48	1.84	0.80	16.1	148
5	193	1.69	2.10	0.80	18.9	150
5	219	2.55	2.82	0.90	28.6	296
5	249	6.05	3.27	1.85	58.4	362
6	168	0.59	0.82	0.72	15.4	158
6	197	0.81	0.97	0.84	20.7	216
6	225	0.87	1.30	0.67	21.6	248
6	254	1.57	1.51	1.04	29.5	291
7	169	1.66	1.72	0.97	15.5	174
7	196	2.21	2.23	0.99	16.7	255
7	225	2.48	2.57	0.96	23.4	
7	247	2.51	3.04	0.83	24.7	411
7	274	6.95	3.56	1.95	65.6	457
8	168	1.03	1.89	0.54	12.8	184
8	189	1.19	1.40	0.85	14.5	165
8	224	1.37	2.13	0.64	15.4	257
8	251	2.56	2.56	1.00	29.9	304
9	167	2.09	1.40	1.49	13.3	143
9	195	1.90	1.20	1.58	17.4	176
9	209	1.90	1.20	1.58	17.4	176
9	223	2.75	1.70	1.62	22.7	194
9	251	3.01	3.25	0.93	26.5	295

S	Gest.	Saliva E3	Saliva P	Saliva E3/P	Plasma E3	Plasma P
10	168	1.48	1.85	0.80	16.5	227
10	196	1.65	1.45	1.14	18.5	286
10	227	2.76	2.79	0.99	23.7	456
10	251	4.79	2.64	1.81	41.0	428
11	165	0.75	1.42	0.53	9.3	149
11	193	0.77	1.67	0.46	8.2	172
11	221	0.78	2.54	0.31	12.1	301
11	258	2.53	2.41	1.05	29.2	402
11	277	3.31	3.52	0.94	36.4	424
12	167	1.72	1.70	1.01	14.5	174
12	200	1.96	1.69	1.16	18.9	221
12	225	2.41	2.67	0.90	20.6	299
12	249	4.13	3.42	1.21	30.9	452
12	278	7.78	2.73	2.85	54.3	381
13	213	1.42	1.31	1.08	15.4	155
13	228	1.32	1.96	0.67	13.3	187
13	252	2.21	2.43	0.91	17.4	190
13	259	2.52	2.86	0.88	25.9	264
14	166	1.07	1.14	0.94	18.2	162
14	194	1.26	1.22	1.03	20.4	146
14	222	1.72	2.41	0.71	22.2	265
14	245	2.11	3.01	0.70	30.7	366
14	278	4.51	5.09	0.89	50.1	613
15	178	1.29	1.67	0.77	11.3	173
15	210	1.44	1.55	0.93	14.4	223
15	230	1.97	2.21	0.89	20.3	320
15	244					
15	258	2.12	2.46	0.86	19.9	350
16	169	0.96	0.95	1.01	11.8	142
16	197	1.29	1.10	1.17	12.4	168
16	225	1.29	1.31	0.98	13.2	184
16	255	3.25	2.42	1.34	31.5	309
16	283	5.56	2.85	1.95	54.6	367
17	168	1.08	0.89	1.21	9.2	148
17	196	1.01	1.10	0.92	10.8	161
17	230	1.41	1.85	0.76	14.1	299
17	252	2.77	2.52	1.10	34.2	377
18	168	0.90	1.51	0.60	11.6	206
18	199	1.46	1.82	0.80	14.5	260
18	223	1.84	2.86	0.64	17.7	282
18	251	1.97	3.80	0.52	20.2	333
18	279	4.32	4.74	0.91	41.4	429
19	162	1.46	0.97	1.51	16.3	138
19	194	1.98	1.39	1.42	21.1	171
19	222	2.10	1.67	1.26	25.8	215
19	244	3.12	1.89	1.65	44.1	259
19	278	6.26	2.91	2.15	63.4	383
20	166	1.15	0.91	1.26	15.7	112
20	194	1.64	1.00	1.64	26.9	131
20	222	2.19	1.19	1.84	27.2	143
20	250	2.97	1.53	1.94	36.7	208

S	Gest.	Saliva E3	Saliva P	Saliva E3/P	Plasma E3	Plasma P
21	175	2.25	2.16	1.04	21.9	145
21	203	4.26	2.43	1.75	41.8	254
21	231	5.33	2.86	1.86	49.3	303
21	257	8.10	5.42	1.49	78.5	331
22	168	0.74	1.43	0.52		166
22	198	0.89	1.98	0.45	14.2	225
22	225	1.31	2.15	0.61	18.7	302
22	254	2.65	2.43	1.09	29.3	333
23	168	1.18	1.41	0.84	11.3	174
23	196	1.16	2.14	0.54	14.6	221
23	226	1.57	2.62	0.60	16.4	253
23	252	2.01	2.95	0.68	30.9	309
23	273	5.56	4.94	1.13	55.2	385
24	176	0.99	0.85	1.16	12.1	118
24	198	1.18	1.26	0.94	12.8	149
24	211					
24	225	1.38	1.51	0.91	14.2	166
24	239	1.43	1.57	0.91	17.3	198
25	163	0.83	1.13	0.73	12.1	166
25	190	1.29	1.33	0.97	16.4	173
25	219	1.34	2.64	0.51	19.7	213
25	247	2.65	2.39	1.11	29.1	338
25	280	5.11	1.29	3.96	56.2	424
26	168	1.22	0.90	1.36	13.9	164
26	195	1.76	1.37	1.28	18.7	206
26	224	2.01	1.54	1.31	21.7	252
26	252	2.62	2.39	1.10	28.6	381
26	280	4.01	2.92	1.37	35.2	351
27	167	0.84	0.83	1.01	13.1	131
27	195	0.91	1.11	0.82	14.7	148
27	223	1.46	2.00	0.73	24.0	288
27	251	2.42	2.92	0.83	32.6	351
27	279	2.32	3.73	0.62	31.6	419
28	166	1.18	1.03	1.15	8.7	90
28	194					
28	222	1.53	1.17	1.31	12.4	202
28	236					
28	256	3.61	1.97	1.83	34.3	353
28	278	4.69	2.29	2.05	40.5	348
29	168	1.52	1.25	1.22	16.1	206
29	201	2.61	2.58	1.01	19.4	299
29	224	2.95	3.36	0.88	24.4	317
29	251	3.93	3.95	0.99	26.6	345
30	195	1.47	1.34	1.10	12.2	129
30	222	1.89	1.36	1.39	16.2	265
30	251	2.36	2.71	0.87	24.8	327
30	266	4.85	3.32	1.46	41.6	472
30	280	4.68	2.90	1.61	40.1	348

S	Gest.	Saliva E3	Saliva P	Saliva E3/P	Plasma E3	Plasma P
31	164	1.35	1.20	1.13	14.6	136
31	191	1.60	1.52	1.05	18.1	216
31	219	1.81	1.98	0.91	19.8	258
31	253	4.90	3.65	1.34	50.1	528
31	281	7.53	4.04	1.86	71.8	550
32	175	0.86	1.15	0.75	17.0	186
32	196	1.27	1.35	0.94	15.8	222
32	222	1.59	1.12	1.42	18.9	201
32	247	2.07	1.90	1.09	30.9	328
32	278	4.87	3.01	1.62	52.1	376
33	169	0.92	1.30	0.71	11.5	217
33	198	0.78	1.40	0.56	9.6	258
33	226	1.54	1.74	0.89	14.5	325
33	254	3.22	3.81	0.85	33.5	460
33	255					
34	164	1.28	0.93	1.38	14.9	142
34	192	1.54	1.48	1.04	13.8	167
34	227	2.36	2.04	1.16	22.6	276
34	248	3.95	2.29	1.72	37.8	348
35	166	0.81	1.12	0.72	12.2	84
35	194	0.90	1.93	0.47	17.0	164
35	223	1.18	1.86	0.63	18.6	178
35	252	1.82	2.95	0.62	30.6	275
35	275	5.85	2.46	2.38	61.5	312
36	170	1.06	1.20	0.88	14.0	120
36	196	1.00	1.35	0.74	14.9	119
36	224	1.95	1.76	1.11	21.8	182
36	252	4.24	2.68	1.58	46.5	294
37	166	1.85	2.25	0.82	10.3	105
37	194	2.06	3.12	0.66	17.4	124
37	229	2.69	3.52	0.76	24.6	188
37	250	3.82	3.60	1.06	31.5	263
38	170	1.72	2.93	0.59	15.3	205
38	198	2.52	3.28	0.77	22.8	396
38	226	3.81	5.07	0.75	37.0	564
38	250					
38	271	11.00	8.99	1.22	96.5	1118
39	168	2.34	1.56	1.50	22.5	105
39	196	2.41	1.88	1.28	24.3	132
39	224	3.21	1.84	1.74	31.6	183
39	252	7.65	2.30	3.33	52.3	222
40	171	2.00	1.23	1.63	15.6	113
40	200	2.25	1.60	1.41	14.6	130
40	221	2.69	1.81	1.49	18.2	165
40	249	2.95	2.30	1.28	28.6	223
41	171	1.53	2.06	0.74	10.5	142
41	198	1.87	2.72	0.69	16.6	197
41	219	1.90	3.09	0.61	17.2	271
41	254	5.47	4.72	1.16	43.8	397

S	Gest.	Saliva E3	Saliva P	Saliva E3/P	Plasma E3	Plasma P
42	168	1.21	1.08	1.12	12.7	137
42	196	1.94	1.78	1.09	19.2	203
42	224	1.86	2.60	0.72	23.5	226
42	260	4.03	3.45	1.17	33.6	346
42	280	6.76	4.83	1.40	64.2	455
43	167	1.06	1.20	0.88	13.0	109
43	192	1.13	1.10	1.03	15.1	128
43	228	1.63	2.03	0.80	19.7	201
43	248	2.09	2.02	1.03	29.9	210
43	276	2.39	2.19	1.09	25.6	253
44	168	1.15	1.12	1.03	11.7	166
44	196	1.77	2.21	0.80	17.4	290
44	224	1.89	2.26	0.84	20.2	253
44	250	2.86	3.50	0.82	30.8	447
45	166	1.22	1.60	0.76	23.3	161
45	194	1.88	2.30	0.82	25.6	208
45	222	2.55	4.09	0.62	28.1	303
45	236					
45	258	5.99	5.50	1.09	72.4	534

Table R.7.4 Consecutive adrenal and kidney measurements for calculation of coefficients of variation for ultrasound measurement of these organs at 24 - 26 weeks gestation. [AT & KT - adrenal & kidney transverse, AAP & KAP - adrenal & kidney anteroposterior, AC & KC - adrenal & kidney circumference, AA & KA - adrenal and kidney area respectively]

M	AT	AAP	AC	AA	KT	KAP	KC	KA
1	13	8	33	0.9	20	14	53	2.2
2	12	7	32	0.7	21	14	57	2.4
3	13	8	33	0.8	21	17	62	3.0
4	11	8	32	0.8	19	11	57	2.6
5	13	7	30	0.6	19	14	55	2.3
1	12	6	31	0.7	21	16	59	2.6
2	12	6	30	0.6	18	15	49	1.9
3	12	8	31	0.7	20	15	56	2.5
4	13	7	32	0.7	21	16	59	2.7
5	13	5	31	0.6	21	15	59	2.6
1	11	5	28	0.5	23	16	65	3.2
2	11	5	28	0.5	22	15	61	2.7
3	11	5	29	0.5	21	16	59	2.7
4	12	5	30	0.5	19	14	54	2.2
5	13	7	33	0.5	21	14	57	2.4
1	16	8	38	0.8	31	21	87	5.7
2	15	8	35	0.8	30	23	82	5.3
3	14	7	36	0.8	30	20	82	5.2
4	15	7	36	0.9	31	22	83	5.3
5	14	7	37	0.9	31	22	84	5.5
1	12	7	31	0.7	23	17	62	3.0
2	12	8	31	0.6	21	18	64	3.3
3	12	6	31	0.6	22	22	69	3.8
4	13	7	31	0.7	24	19	68	3.7
5	13	7	33	0.8	24	18	68	3.7
1	16	7	39	1.0	25	20	74	4.2
2	16	8	38	1.0	24	22	73	4.3
3	13	6	32	0.7	22	19	66	3.3
4	14	7	35	0.9	24	18	68	3.5
5	15	8	37	1.0	24	19	67	3.6

M	AT	AAP	AC	AA	KT	KAP	KC	KA
1	11	7	28	0.6	20	17	61	3.0
2	11	6	29	0.5	18	13	50	2.0
3	10	6	25	0.4	20	16	60	2.9
4	10	7	25	0.5	18	15	54	2.3
5	11	6	29	0.5	18	13	49	1.8
1	11	6	30	0.6	16	13	48	1.8
2	11	7	30	0.7	17	13	50	1.9
3	12	7	35	0.9	16	14	50	2.0
4	11	6	30	0.6	18	14	50	1.9
5	10	6	28	0.6	18	14	54	2.3
1	13	6	32	0.7	21	18	60	2.8
2	13	6	32	0.7	20	17	60	2.9
3	13	7	32	0.7	20	16	54	2.3
4	12	6	30	0.5	21	17	58	2.6
5	12	7	31	0.7	20	17	60	2.9
1	14	9	38	1.0	21	17	60	2.9
2	12	7	34	0.8	21	17	59	2.7
3	14	8	36	0.9	21	18	65	3.4
4	14	7	36	1.0	21	17	64	3.2
5	14	8	36	0.9	19	17	60	2.9
1	15	8	36	0.9	23	19	69	3.8
2	14	8	36	0.9	21	17	62	3.1
3	13	8	37	1.0	21	23	67	3.6
4	14	9	37	1.0	22	23	73	4.3
5	13	9	35	1.0	23	19	64	3.4
1	14	7	34	0.8	24	18	62	2.8
2	15	7	36	0.8	22	15	63	2.8
3	13	6	33	0.7	21	17	61	3.0
4	12	7	33	0.8	21	19	65	3.4
5	13	7	35	0.8	21	17	60	2.7

Table R.7.5 Consecutive adrenal and kidney measurements taken for calculation of coefficients of variation for ultrasound measurement of these organs at 36 - 40 weeks gestation. [Abbreviations as for Table R.7.4]

M	AT	AAP	AC	AA	KT	KAP	KC	KA
1	23	15	57	2.2	36	26	101	7.9
2	21	13	53	2.1	33	26	94	6.6
3	21	13	55	2.1	36	26	96	7.1
4	21	14	54	2.1	36	25	100	7.4
5	24	11	58	2.1	36	24	101	7.5
1	29	20	81	4.9	36	25	95	6.8
2	26	13	64	2.9	34	24	91	6.4
3	24	14	56	2.2	35	26	96	7.2
4	21	14	55	2.2	33	27	91	6.5
5	24	13	57	2.3	36	26	91	6.4
1	29	13	70	3.1	39	25	103	7.8
2	28	12	67	3.0	35	27	97	7.1
3	26	13	63	2.5	36	25	98	7.1
4	26	13	64	2.8	37	29	102	8.4
5	26	12	68	3.1	36	23	92	6.4
1	27	15	63	2.7	36	20	89	5.8
2	26	16	65	3.1	36	24	92	6.2
3	24	15	64	3.0	33	20	87	5.7
4	23	14	59	2.5	34	21	88	5.7
5	27	15	71	3.6	34	24	92	6.4
1	23	10	56	2.1	39	25	104	7.9
2	22	11	55	2.1	39	29	111	9.2
3	23	11	54	2.0	40	29	109	9.0
4	24	10	59	2.3	41	28	111	9.5
5	21	11	51	1.8	43	28	114	9.6
1	20	9	45	1.3	29	26	95	7.2
2	21	10	49	1.5	32	23	85	5.5
3	19	9	45	1.3	28	24	84	5.7
4	19	10	45	1.4	31	24	85	5.5
5	18	9	45	1.3	32	24	87	5.8

M	AT	AAP	AC	AA	KT	KAP	KC	KA
1	27	14	68	3.4	40	26	107	8.7
2	28	14	68	3.4	39	26	104	8.2
3	25	12	63	2.6	35	29	103	8.3
4	26	13	65	2.7	37	26	100	7.6
5	26	14	65	3.1	38	28	102	8.2
1	24	14	64	3.0	40	29	112	9.8
2	19	12	50	1.9	40	29	108	8.9
3	19	9	50	1.7	40	33	113	10.2
4	19	12	49	1.7	41	35	116	10.8
5	20	11	51	1.8	44	31	115	10.1
1	25	15	61	2.8	33	23	90	6.2
2	24	12	56	2.1	32	27	89	6.1
3	23	13	57	2.4	29	26	83	5.4
4	22	13	55	2.1	31	23	86	5.7
5	20	10	50	1.7	33	24	91	6.5
1	21	9	50	1.5	37	25	97	7.3
2	23	10	54	1.8	33	26	87	6.1
3	22	11	50	1.8	33	32	104	8.9
4	23	10	52	1.8	36	29	97	7.4
5	22	10	52	1.7	33	29	96	7.4
1	22	10	50	1.5	35	29	99	7.8
2	21	9	49	1.5	37	29	103	8.1
3	20	11	49	1.6	38	26	103	8.2
4	20	8	44	1.1	35	29	101	8.1
5	20	8	46	1.3	39	31	111	9.6
1	22	13	55	2.2	36	24	103	7.9
2	21	11	49	1.6	34	27	96	7.4
3	19	11	47	1.6	34	25	96	7.1
4	19	11	48	1.5	34	27	96	7.0
5	19	10	46	1.4	36	25	96	6.9

Table R.7.6 Fetal and neonatal right adrenal and kidney measurements in subjects P1 to P9. [Abbreviations as for Table R.7.4]

S	Days	AT	AAP	AC	AA	KT	KAP	KC	KA
P1	-23	27.0	15.0	66.0	3.30	47.0	27.0	116.0	9.8
	1	19.3	11.1	49.9	1.75	37.0	22.0	95.0	6.6
P2	-19	20.0	10.0	52.0	1.80	40.0	23.0	99.0	7.1
	1	17.6	13.5	52.4	2.08	34.2	18.1	85.3	5.0
P3	-19	26.0	15.0	72.0	3.40	36.0	23.0	98.0	7.0
	1	21.0	11.2	51.6	1.85	42.5	26.0	111.8	9.4
P4	-13	29.0	18.0	77.0	4.50	35.0	28.0	100.0	7.9
	1	28.9	13.9	70.0	3.20	29.0	28.9	81.0	5.0
P5	-30	33.0	21.0	76.0	4.50	36.0	23.0	101.0	7.9
	1	22.2	11.1	51.6	1.83	40.6	26.3	107.1	8.5
P6	-14	33.0	18.0	83.0	4.60	41.0	25.0	106.0	8.0
	1	25.2	12.7	64.0	2.90	39.8	28.5	107.0	8.8
P7	-18	28.0	17.0	75.0	4.00	42.0	24.0	108.0	8.2
	1	21.7	12.7	56.9	2.27	32.7	20.4	85.0	5.2
P8	-20	23.0	14.0	62.0	3.00	37.0	27.0	105.0	8.4
	1	19.5	13.0	49.4	1.87	35.8	28.1	106.5	8.7
P9	-8	31.0	17.0	80.0	4.40	41.0	31.0	111.9	11.0
	1	24.7	16.2	64.8	3.12	38.4	22.3	95.7	6.6

Table R.7.7 Neonatal adrenal measurements in individuals (S) from birth to 6 weeks of age. [Age in days, RAT - right adrenal transverse, LAT - left adrenal transverse, RAAP- right adrenal anteroposterior, LAAP - left adrenal anteroposterior, RAC - right adrenal circumference, LAC - left adrenal circumference, RAA - right adrenal area, LAA - left adrenal area, RAL - right adrenal length, LAL - left adrenal length]

S	Age	RAT	RAAP	RAC	RAA	RAL	LAT	LAAP	LAC	LAA	LAL
1	1	21.7	8.9	50.7	1.54	19.3	19.3	10.8	47.5	1.60	16.1
	3	16.6	8.5	40.3	1.10	13.8	19.8	7.3	45.6	1.10	14.3
	5	14.6	8.9	36.6	0.99	9.6	13.8	7.6	35.8	0.88	11.8
	11	11.5	4.9	26.0	0.42	10.1	7.1	4.2	18.3	0.24	8.9
	21	13.1	6.1	29.4	0.58	7.4	10.2	6.7	24.3	0.46	9.3
	41	8.7	6.9	24.6	0.48	6.6					6.9
2	1	16.4	12.7	44.7	1.57	18.5	19.3	12.0	49.6	1.81	18.3
	3	14.2	7.6	37.1	0.92	14.0	17.1	8.3	41.8	1.14	17.6
	5	14.2	6.3	36.0	0.78	13.2	18.4	9.1	42.9	1.25	14.6
	11	13.8	6.1	33.2	0.68	12.3					9.6
	21	10.8	5.4	26.4	0.47	8.1	12.4	5.4	25.6	0.45	8.6
	42	7.2	4.3	19.6	0.27	8.1	8.0	4.7	20.1	0.29	
3	1	15.1	11.0	44.1	1.45	15.3	20.3	9.3	46.7	1.43	15.4
	3	13.6	7.1	32.8	0.75	11.0	11.9	6.7	28.5	0.59	9.7
	5	12.6	6.9	30.5	0.62	8.5	11.9	6.2	30.3	0.61	7.8
	10	8.8	4.8	22.4	0.34	9.1	8.4	4.0	21.1	0.29	7.1
	21	8.5	5.5	23.3	0.39	8.0	7.8	6.7	23.7	0.44	5.9
	45	8.8	5.6	24.2	0.42	7.9	7.9	4.7	21.3	0.32	9.2
4	1	13.0	7.6	32.2	0.75	15.2	17.4	6.9	42.9	1.03	15.3
	3	11.5	5.1	27.6	0.48	10.7	11.8	6.1	30.3	0.60	12.8
	5	9.3	4.9	23.0	0.36	11.0	10.9	4.8	25.7	0.42	7.6
	10	13.3	8.3	35.5	0.91	11.8	12.7	6.9	32.6	0.73	12.5
	21	10.2	6.6	24.0	0.45	7.8	9.3	4.1	19.2	0.25	6.4
	42	10.8	4.8	26.6	0.43	8.4	8.6	4.8	21.5	0.33	6.2
5	1	18.3	6.2	40.5	0.88	18.4	18.7	7.8	48.0	1.30	15.0
	3	9.3	4.2	24.0	0.34	14.9	9.2	5.2	26.2	0.45	8.8
	5	15.7	6.9	36.8	0.85	13.2	13.0	5.9	30.0	0.58	10.3
	21	11.0	5.7	26.0	0.47	10.6	9.1	5.2	22.1	0.35	7.6
	42	10.9	5.7	25.3	0.46	7.7	8.7	5.4	22.2	0.36	8.3
6	1	15.8	8.1	39.9	1.06	18.7	18.1	11.1	45.0	1.51	
	3	15.0	6.7	36.6	0.83	8.6	12.6	6.7	30.2	0.65	
	5	13.9	6.7	33.9	0.75	15.9	10.9	5.1	27.6	0.48	11.7
	10	8.2	5.0	21.4	0.33	7.5	10.5	3.9	23.9	0.32	
	22	12.8	6.0	29.4	0.57	8.6	11.2	6.0	28.6	0.56	7.5
	42	8.0	4.7	21.5	0.32	8.1	7.2	5.2	20.8	0.32	5.9

S	Age	RAT	RAAP	RAC	RAA	RAL	LAT	LAAP	LAC	LAA	LAL
7	1	18.9	8.3	43.0	1.19	14.9	19.1	7.6	47.3	1.25	14.3
	3	13.7	6.3	30.5	0.62	9.5	14.3	7.3	34.3	0.80	8.4
	5	12.4	5.6	29.6	0.55	12.6	13.0	7.1	30.1	0.66	10.0
	10	10.6	5.7	25.3	0.46	7.2	8.0	5.4	21.0	0.33	5.9
	21	8.0	4.8	18.6	0.26	6.5	7.1	4.8	18.6	0.26	6.6
	45	9.1	4.8	21.5	0.32	7.5	8.6	4.4	22.3	0.32	6.2
8	1	22.8	8.4	49.6	1.43	17.3	23.1	11.4	54.4	2.01	20.8
	3	14.5	9.2	39.8	1.13	12.3	15.6	10.2	42.4	1.32	15.1
	5	15.0	5.8	34.9	0.70	6.2	13.1	7.8	32.9	0.78	11.4
	10	14.4	6.9	31.3	0.68	8.1	11.4	6.1	29.0	0.57	9.7
	22	11.4	5.4	27.9	0.50	6.8	11.2	5.9	30.0	0.58	7.9
	43	10.6	5.4	23.6	0.40	7.6	8.7	5.7	24.0	0.42	6.6
9	1	18.9	9.0	46.4	1.39	16.8	16.3	6.6	38.6	0.87	15.2
	3	12.4	5.5	29.6	0.54	9.7	15.1	6.6	34.7	0.76	12.1
	5	10.7	7.6	26.5	0.56	8.9	14.1	3.8	31.6	0.44	10.4
	10	10.0	5.3	26.4	0.46	5.8	6.9	4.7	20.9	0.31	5.5
	21	8.9	5.0	23.9	0.39	8.3	8.6	5.3	24.3	0.41	7.6
	41	10.1	5.9	21.7	0.37	6.6	6.9	5.0	20.5	0.31	8.6
10	1	16.8	12.4	47.3	1.71	19.6	19.7	11.1	49.9	1.75	15.9
	3	18.2	8.4	44.2	1.31	12.1	12.5	6.1	30.4	0.61	11.9
	5	14.5	7.7	35.2	0.86	11.7	15.7	5.8	35.5	0.71	11.2
	11	9.7	3.9	22.0	0.29	7.7	10.8	4.8	24.9	0.40	10.1
	21	11.4	5.7	25.3	0.46	7.5	8.6	5.4	23.6	0.40	6.7
	41	9.7	7.3	26.7	0.55	7.5	12.8	7.6	33.0	0.78	8.0
11	1	19.4	10.2	50.7	1.70	18.9	19.7	11.6	51.1	1.86	15.1
	3	17.1	10.3	43.1	1.36	19.0					
	5	16.2	10.3	41.3	1.27	11.4					
	11	14.4	5.8	31.0	0.61	9.4	10.8	7.5	32.1	0.74	10.5
	21	9.4	5.1	19.1	0.28	9.4	9.1	4.8	22.4	0.34	6.9
	42	12.0	6.9	29.1	0.62	10.0					7.6
12	1	17.6	12.6	44.5	1.55	15.2	24.1	13.7	59.6	2.54	21.8
	3	21.6	11.4	54.4	2.01	17.6	16.6	11.6	46.1	1.60	15.7
	5	15.8	5.1	36.4	0.67	14.3	16.2	8.5	34.2	0.88	14.2
	11	15.5	8.4	40.1	1.09	8.6					10.7
	21	14.1	5.8	30.8	0.60	9.8	10.5	3.7	26.6	0.35	7.4
	42	7.6	5.8	21.5	0.36	6.8	10.1	5.6	25.8	0.46	7.9

Table R.7.8 Neonatal kidney measurements in individuals from birth to 6 weeks of age. [Age in days, RKT - right kidney transverse, LKT - left kidney transverse, RKAP - right kidney anteroposterior, LKAP - left kidney anteroposterior, RKC - right kidney circumference, LKC - left kidney circumference, RKA - left kidney area, LKA - left kidney area, RKL - right kidney length, LKL - left kidney length]

S	Age	RKT	RKAP	RKC	RKA	RKL	LKT	LKAP	LKC	LKA	LKL
1	1	36.9	27.2	106.5	8.57	43.2	40.6	25.5	107.0	8.35	45.7
	3	35.0	23.0	93.9	6.52	41.3	31.6	24.8	92.1	6.55	37.1
	5	35.6	24.7	97.8	7.18	48.3	36.5	26.6	99.3	7.58	44.5
	11	37.0	28.4	103.6	8.32	45.4	35.3	23.5	95.6	6.77	43.1
	21	38.2	22.7	97.4	6.84	49.3	48.6	32.6	128.5	12.33	48.5
	41	41.9	25.3	108.7	8.51	48.0	40.6	27.8	110.6	9.10	46.4
2	1	32.7	28.1	97.9	7.52	43.2	43.4				38.0
	3	35.7	22.2	93.2	6.33	41.8	31.1	20.5	82.5	5.07	45.0
	5	33.1	20.9	84.5	5.30	44.0	39.8	23.7	101.9	7.48	46.8
	11	38.2	24.0	100.8	7.41	52.2					46.9
	21	37.1	20.9	96.6	6.45	45.8	34.1	21.1	89.2	5.78	43.2
	42	40.3	24.5	105.7	8.02	44.5	37.0	26.3	98.9	7.50	42.7
3	1	35.2	30.8	109.0	9.29	41.2	40.6	26.0	105.6	8.27	45.1
	3	33.5	25.1	100.9	7.59	44.9	43.6	26.4	111.0	8.98	44.7
	5	36.1	26.9	103.5	8.14	39.9	29.5	20.6	79.0	4.76	46.9
	10	35.4	24.4	93.7	6.69	42.1	38.9	24.0	102.9	7.63	41.4
	21	44.5	29.2	119.6	10.57	43.0	39.0	23.1	101.7	7.35	42.3
	45	40.0	27.7	107.5	8.75	45.9	37.6	27.9	106.2	8.61	52.0
4	1	32.4	23.6	88.1	5.97	36.8	31.2	24.1	86.1	5.79	41.6
	3	33.2	23.2	92.0	6.36	40.3	34.6	21.6	88.7	5.80	41.0
	5	30.5	24.5	89.1	6.16	38.1	31.5	23.0	86.5	5.74	41.0
	10	35.2	23.9	91.8	6.40	41.1	31.9	25.3	90.6	6.40	43.3
	21	32.9	28.8	100.2	7.89	43.8	34.4	24.0	96.8	6.97	46.8
	42	32.6	25.5	94.1	6.83	42.8	49.4	35.1	132.8	13.48	56.1
5	1	31.5	19.1	80.4	4.71	40.5	33.1	27.3	96.2	7.20	38.9
	3	30.4	22.0	85.1	5.49	38.2	34.7	22.6	91.3	6.19	36.3
	5	33.1	20.7	85.6	5.40	40.8	32.2	24.2	90.3	6.27	39.8
	21	34.1	21.4	91.2	6.02	43.6	40.9	25.9	110.1	8.79	43.0
	42	36.2	19.6	92.2	5.80	44.7	45.7	30.4	123.3	11.27	50.7
6	1	37.2	25.0	99.2	7.38	41.1	35.8	25.5	95.4	6.99	38.2
	3	34.0	23.5	90.6	6.24	43.4	36.5	24.8	99.4	7.38	40.4
	5	41.0	28.5	113.7	9.67	43.7	36.3	27.7	104.4	8.35	41.3
	10	42.4	28.6	111.6	9.41	41.2	42.8	25.6	110.0	8.70	47.9
	22	43.4	26.1	116.0	9.49	49.0	40.3	25.3	102.6	7.81	33.5
	42	51.0	29.4	130.9	12.10	44.3	42.5	30.1	115.9	10.20	47.6

S	Age	RKT	RKAP	RKC	RKA	RKL	LKT	LKAP	LKC	LKA	LKL
7	1	29.0	21.1	80.9	5.06	37.7	36.4	21.4	95.7	6.45	42.6
	3	30.5	25.8	90.1	6.39	39.4	44.4	27.2	115.0	9.77	44.0
	5	33.5	26.9	96.9	7.13	47.4	42.8	29.9	114.5	9.98	44.2
	10	33.7	21.8	88.3	5.79	48.4	38.0	27.7	104.6	8.38	42.8
	21	37.5	24.1	98.2	7.13	40.7	41.6	26.8	106.7	8.52	42.2
	45	43.4	25.6	109.7	8.68	50.6	46.1	33.1	127.1	12.27	47.9
8	1	40.4	26.5	105.1	8.28	41.9	39.8	27.2	106.0	8.50	41.2
	3	27.4	21.5	75.9	4.51	38.9	35.8	29.9	98.1	7.36	37.5
	5	33.5	23.4	91.2	6.28	43.8	35.9	28.6	102.9	8.20	40.6
	10	33.2	25.7	94.4	6.90	40.6	39.5	25.7	104.0	8.03	40.1
	22	44.4	30.8	118.4	10.64	41.4	46.0	27.7	115.4	9.74	44.2
	43	44.9	27.7	116.8	9.92	46.2	33.5	31.7	105.4	8.81	45.5
9	1	38.5	26.1	103.3	8.01	47.7	38.1	28.0	104.8	8.44	47.4
	3	39.4	26.9	104.4	8.26	41.6	33.9	21.1	87.0	5.57	37.9
	5	40.1	26.7	105.9	8.41	47.8	39.3	24.5	99.9	7.39	47.0
	10	35.0	23.6	93.5	6.56	41.8	38.1	23.1	101.7	7.35	41.8
	21	38.2	26.6	102.8	8.02	48.1	40.9	25.7	105.8	8.60	46.2
	41	53.8	26.5	129.1	11.14	51.8	43.3	24.2	109.4	8.36	51.9
10	1	38.5	32.3	111.1	9.74	49.2	34.7	27.2	99.3	7.65	38.9
	3	47.5	36.6	127.3	12.30	40.9	35.6	26.4	101.4	7.80	36.4
	5	33.5	29.7	101.0	8.07	44.9	46.0	33.1	125.7	12.06	46.0
	11	40.8	28.7	111.8	9.44	48.0	40.6	26.9	109.3	8.85	47.2
	21	35.2	27.3	101.4	7.93	49.8					48.4
	41	45.9	30.2	119.8	10.77	45.0	51.2	37.3	141.8	15.35	50.3
11	1	37.5	22.0	96.7	6.66	37.5	32.7	24.1	88.7	6.10	40.2
	3	38.5	29.5	101.1	7.62	40.4	37.6	27.8	102.2	8.08	42.0
	5	31.5	20.1	81.9	4.97	39.1	36.4	24.0	96.3	6.92	44.0
	11	41.7	23.7	104.9	7.80	43.5	44.1	27.0	113.1	9.33	47.9
	21	36.6	23.4	97.1	6.93	43.4	36.2	26.3	97.7	7.35	40.1
	42	43.3	22.9	107.1	7.86	48.3	42.2	29.3	114.9	9.94	47.7
12	1	35.9	24.9	94.5	6.82	42.0	33.5	27.4	95.4	7.14	46.6
	3	34.9	27.4	98.1	7.51	42.0	36.0	22.8	92.9	6.37	43.8
	5	35.4	25.9	95.9	7.10	44.6	41.0	29.5	114.4	9.90	49.7
	11	36.7	28.6	102.0	8.13	42.6	35.5	27.1	98.5	7.53	51.6
	21	40.0	25.9	105.1	8.18	45.0	37.1	29.0	107.8	8.90	42.9
	42	41.8	23.2	106.8	8.31	44.0	46.0	30.6	123.6	11.34	49.6

Table R.7.9 Consecutive adrenal and kidney measurements taken for calculation of coefficients of variation for ultrasound measurement of these organs in 1-3 day old neonates. [AL & KL- adrenal & kidney length in longitudinal section, otherwise abbreviations as for Table R.7.4]

M	AT	AAP	AC	AA	AL	KT	KAP	KC	KA	KL
1	20.8	11.5	49.8	1.78	16.2	30.2	22.3	84.9	5.50	38.9
2	22.0	11.4	53.8	1.97	18.5	35.0	23.8	93.8	6.62	38.5
3	19.4	13.1	52.0	2.03	18.9	33.1	21.7	88.2	5.76	40.1
4	18.5	11.9	50.3	1.84	18.2	36.4	22.0	95.3	6.79	41.1
5	19.7	10.8	48.8	1.67	18.9	33.0	23.7	88.2	5.94	41.9
1	18.0	10.4	46.9	1.54	18.9	28.7	20.4	78.2	4.66	42.0
2	19.9	11.6	51.1	1.86	17.0	27.5	16.0	71.0	3.56	41.4
3	19.1	10.5	47.1	1.53	17.8	31.1	19.0	77.7	4.46	41.7
4	17.5	9.6	43.0	1.38	15.6	31.1	20.5	82.3	5.05	39.8
5	18.7	10.6	44.3	1.44	16.2	29.6	19.3	79.4	4.65	41.3
1	19.6	11.6	48.6	1.72	16.5	32.0	23.5	86.6	5.79	38.9
2	16.9	10.4	44.2	1.41	14.8	31.6	24.3	87.8	5.99	39.9
3	15.2	7.7	43.5	1.14	17.0	32.2	23.8	89.7	6.17	41.3
4	17.2	7.7	43.5	1.14	18.7	28.9	24.7	85.9	5.80	37.3
5	19.9	10.0	46.5	1.49	19.4	28.2	24.2	83.8	5.53	42.1
1	20.0	12.4	49.6	1.84	18.6	37.1	26.1	105.1	8.22	42.5
2	18.9	12.0	49.1	1.78	16.5	36.3	23.7	100.5	7.32	46.2
3	20.7	10.5	51.2	1.75	16.5	28.9	27.2	89.7	6.39	44.7
4	21.6	10.8	58.7	2.14	16.5	35.9	27.7	100.8	7.89	44.5
5	18.2	9.4	47.1	1.45	15.3	35.4	23.8	100.5	7.35	46.7
1	16.9	8.2	38.4	1.00	15.1	29.7	20.1	78.8	4.69	41.4
2	15.6	8.7	37.6	1.02	16.3	30.7	26.5	92.5	6.79	48.2
3	17.9	9.0	44.2	1.29	15.8	30.1	22.9	87.3	5.81	43.5
4	16.3	8.8	43.9	1.27	17.3	29.8	21.9	82.9	5.26	42.3
5	15.6	8.0	42.7	1.15	18.3	31.2	20.1	80.4	4.83	41.3
1	14.3	9.2	37.9	1.06	17.3	29.2	23.1	81.6	5.21	38.9
2	15.1	7.4	36.7	0.89	16.9	30.8	23.5	85.3	5.65	37.7
3	16.9	8.1	40.3	1.06	16.5	30.7	22.5	82.9	5.30	37.1
4	15.3	7.3	39.7	0.98	18.1	32.4	23.7	89.5	6.14	37.9
5	15.7	7.8	40.5	1.05	15.9	32.7	24.2	91.3	6.39	36.3

M	AT	AAP	AC	AA	AL	KT	KAP	KC	KA	KL
1	20.1	10.5	52.7	1.81	17.2	37.7	27.3	103.4	8.26	45.1
2	20.1	12.0	54.6	2.07	18.3	34.6	26.2	101.1	7.76	43.8
3	18.9	10.4	48.4	1.61	15.6	37.8	26.5	104.3	8.18	43.7
4	19.0	9.3	48.1	1.49	16.9	39.4	26.9	108.9	8.18	42.3
5	21.6	11.3	52.2	1.88	17.1	38.0	28.4	104.0	8.41	45.0
1	19.8	7.0	43.3	1.06	18.0	33.9	22.6	91.3	6.19	36.8
2	17.5	6.9	41.7	0.99	18.1	35.9	21.1	90.4	5.90	36.6
3	16.1	8.5	40.3	1.10	18.5	33.0	25.9	95.9	7.10	36.5
4	15.3	9.6	41.7	1.24	16.4	36.0	21.9	92.8	6.24	35.8
5	18.3	8.5	44.5	1.26	17.4	33.5	23.0	88.2	5.92	37.5
1	18.4	8.2	44.2	1.22	19.1	37.0	23.6	100.3	7.29	49.7
2	14.9	9.9	39.3	1.16	17.1	31.0	23.0	87.5	5.86	44.9
3	18.2	10.9	44.4	1.34	20.1	39.6	26.8	94.4	6.99	45.8
4	17.7	11.0	47.7	1.63	18.7	30.3	23.4	86.5	5.77	44.8
5	18.6	9.8	48.8	1.57	17.2	34.0	23.5	94.8	6.68	46.7
1	19.8	7.4	43.2	1.10	18.5	36.7	23.6	96.3	6.86	41.5
2	16.7	10.0	46.5	1.49	17.7	40.6	26.6	109.0	9.02	41.8
3	18.4	9.5	45.9	1.41	18.1	37.8	26.4	102.0	7.99	44.1
4	18.8	8.6	44.7	1.28	18.9	38.2	30.6	107.5	9.06	41.7
5	17.5	10.1	46.5	1.50	20.6	39.2	26.6	105.7	8.38	41.8
1	15.4	7.8	39.4	1.01	14.3	30.3	22.7	82.9	5.33	39.1
2	15.0	7.5	37.5	0.92	12.4	30.0	21.6	82.7	5.21	36.8
3	16.5	9.6	41.7	1.24	12.6	34.2	22.2	90.3	6.06	37.8
4	13.4	8.1	35.6	0.90	13.1	34.4	20.0	90.0	5.69	37.1
5	15.7	9.6	41.7	1.24	13.5	33.1	22.3	89.0	5.92	35.6
1	20.2	8.7	50.4	1.50	18.7	26.6	23.3	80.8	5.14	39.4
2	17.5	6.6	48.2	0.92	18.7	29.2	23.5	85.3	5.65	41.8
3	17.2	7.2	43.1	1.07	19.2	30.8	21.8	82.8	5.24	42.3
4	17.2	7.5	42.2	1.08	19.8	31.2	22.6	85.0	5.54	42.5
5	16.9	6.3	39.9	0.88	18.9	31.0	23.6	85.4	5.67	41.8

Table R.7.10 Consecutive adrenal measurements for calculation of coefficients of variation for ultrasound measurement of this organ in 40-45 day old neonates. [AL & KL- adrenal and kidney length in longitudinal section, otherwise abbreviations as for Table R.7.4]

M	AT	AAP	AC	AA	AL
1	10.5	5.1	24.0	0.42	9.4
2	10.5	6.4	25.1	0.66	9.6
3	9.6	6.5	25.8	0.49	9.8
4	9.6	5.6	31.6	0.46	9.7
5	10.6	5.4	25.4	0.41	9.4
1	7.9	4.3	19.6	0.27	8.1
2	7.9	5.4	21.0	0.33	8.3
3	9.4	5.4	23.0	0.37	8.4
4	8.6	4.7	21.3	0.32	7.6
5	8.6	5.5	23.8	0.41	7.3
1	10.5	5.6	25.8	0.46	7.9
2	9.6	5.5	24.0	0.42	9.4
3	10.5	5.6	25.8	0.46	8.0
4	11.4	5.4	27.3	0.48	8.8
5	10.8	4.8	26.6	0.43	8.4
1	7.9	5.7	21.4	0.35	8.9
2	8.6	4.8	21.5	0.32	8.3
3	7.9	4.7	20.1	0.29	6.8
4	7.9	5.4	21.0	0.33	7.9
5	10.6	5.4	23.6	0.40	7.6
1	10.2	5.7	25.3	0.46	7.6
2	8.6	6.1	23.2	0.41	7.7
3	7.1	5.6	19.9	0.31	8.8
4	7.9	6.0	21.9	0.37	8.3
5	9.4	5.8	24.1	0.43	7.7
1	8.7	7.0	24.6	0.48	9.0
2	7.9	6.2	22.2	0.38	9.1
3	7.9	5.6	21.3	0.35	8.5
4	8.7	6.0	23.2	0.41	8.6
5	7.9	5.6	21.3	0.35	6.6

M	AT	AAP	AC	AA	AL
1	7.2	7.0	22.2	0.40	8.4
2	5.7	5.2	17.1	0.23	8.8
3	7.9	6.0	21.9	0.37	7.3
4	7.9	6.5	22.6	0.40	7.8
5	9.6	7.3	26.7	0.55	7.5
1	8.7	4.0	20.6	0.27	7.2
2	8.7	4.4	21.1	0.30	8.2
3	7.9	5.6	21.3	0.35	8.0
4	8.7	6.4	23.8	0.44	6.8
5	8.6	4.8	21.5	0.33	6.2
1	10.2	6.6	26.6	0.53	7.6
2	9.4	5.8	24.1	0.43	10.0
3	7.9	6.8	23.1	0.42	8.4
4	8.6	5.6	22.5	0.38	10.9
5	8.7	5.7	24.0	0.42	9.8
1	8.6	5.4	22.2	0.36	8.3
2	9.4	4.5	22.3	0.33	11.4
3	9.4	5.0	23.0	0.37	8.4
4	11.8	4.3	26.6	0.40	8.3
5	9.4	6.1	24.5	0.45	8.0
1	8.6	4.0	19.2	0.25	7.5
2	7.7	4.8	21.5	0.32	7.1
3	7.4	5.0	21.7	0.34	6.7
4	7.2	5.4	23.6	0.40	8.3
5	7.9	4.7	21.3	0.32	7.6
1	13.1	7.6	33.0	0.78	8.0
2	9.6	7.7	27.2	0.58	7.5
3	11.4	6.8	29.1	0.61	8.5
4	8.7	6.4	23.8	0.44	8.2
5	9.6	5.6	24.2	0.42	7.7

Table R.8.1 Saliva (S) and plasma (P) progesterone levels in 6 normal women in early pregnancy (8-12 weeks gestation) one hour prior to and then following the administration of a 400mg progesterone pessary (Cyclogest) vaginally.

Time (hr of day)	S1	P1	S2	P2	S3	P3	S4	P4	S5	P5	Time	S6	P6
09.00	0.46	55	0.37	52	0.53	52	0.50	40	0.64	60	11.00	0.81	101
09.30	0.57	52	0.40	44	0.58	46	0.51	40	0.52	51	11.30	0.93	89
10.00	0.40	51	0.30	57	0.55	46	0.41	48	0.50	46	12.00	0.89	112
10.30	0.34	47	0.34	62	0.65	49	0.35	52	0.35	41	12.30	3.51	115
11.00	1.19	53	0.39	87	2.10	60	0.46	59	0.61	49	13.00	1.32	107
11.30	0.42	57	0.38	68	4.90	60	0.64	86	0.60	53	13.30	1.44	110
12.00	0.36	68	0.43	62	53.60	74	0.55	91	0.80	51	14.00	0.91	124
12.30	0.34	66	0.90	65	25.30	84	0.45	78	0.62	54	14.30	1.00	131
13.00	0.32	60	0.97	65	4.59	87	0.35	69	0.40	45	15.00	0.90	130
14.00	0.46	70	1.57	80	19.50	71	0.75	78	0.60	66	16.00	1.12	135
15.00	0.39	71	13.00	101	5.79	91	1.30	96	0.65	55	17.00	1.18	136
16.00	2.43	70	16.00	103	6.20	82	21.39	112	0.50	50	18.00	1.04	136
17.00	6.70	69	23.00	111	3.70	99	14.90	124	1.15	56	19.00	0.94	117
18.00	11.19	81	29.00	154	4.40	101	12.69	115	1.68	52	20.00	1.10	114
19.00	6.65	73	37.00	90	3.29	102	2.20	79	1.26	53	21.00	0.88	128
20.00	0.69	92	63.00	102	3.10	86	32.29	71	2.60	57	22.00	1.06	120
21.00	4.09	72	106.00	115	3.70	102	103.50	58	1.75	66	23.00	0.89	124
22.00	3.60	72	177.00	124	3.70	84	226.00	67	1.91	67	24.00	0.92	132
07.00							36.50	111					
08.00			28.00	114	1.60	79	16.50	90	1.38	73	10.00	1.35	119
09.00	12.50	87	10.30	93	2.10	60			1.30	63	11.00	1.12	119
10.00	13.10	93	10.50	78	2.29	58			1.25	67	12.00	1.29	127

Table R.8.2 Saliva (S) and plasma (P) progesterone levels in 6 normal women in early pregnancy (7-12 weeks gestation) one hour prior to and then following the administration of 400mg micronised progesterone (Utrogestan) orally.

Time (hr of day)	S1	P1	S3	P3	S4	P4	S5	P5	S6	P6	Time	S2	P2
09.00	0.64	72	0.85	98	0.38	37	0.48	52	1.28	114	10.00	0.53	49
09.30	0.65	59	0.79	99	0.31	36	0.32	47	1.19	111	10.30	0.47	50
10.00	0.58	66	0.68	96	0.45	38	0.41	49	1.16	115	11.00	0.30	48
10.30	0.64	65	0.69	100	0.45	37	0.42	44	1.14	112	11.30	0.32	49
11.00	0.72	75	0.83	96	1.21	125	0.49	55	14.68	1537	12.00	0.35	47
11.30	0.74	66	0.84	83	2.54	237	0.39	48	9.91	841	12.30	0.69	44
12.00	0.63	75	7.67	464	1.69	161	0.56	78	8.84	1081	13.00	0.87	58
12.30	0.68	73	20.20	1263	1.64	145	0.76	85	7.89	744	13.30	0.76	47
13.00	5.26	733	15.50	614	2.08	200	3.90	405	6.28	461	14.00	1.05	63
13.30	4.18	479	8.66	428	2.02	167	2.35	266	4.83	319	14.30	2.52	128
14.00	2.76	263	7.26	383	1.29	89	2.25	215	3.95	266	15.00	2.95	153
15.00	1.65	155	3.27	298	1.02	96	1.38	133	2.76	225	16.00	2.23	142
16.00	1.43	138	2.05	113	0.75	92	0.91	102	2.21	212	17.00	1.95	122
17.00	1.24	145	1.83	166	0.92	84	0.59	69	1.83	154	18.00	1.22	110
18.00	1.04	121	1.12	163	0.73	75	0.44	66	1.91	152	19.00	1.19	108
19.00	0.92	136	0.84	159	0.39	43	0.43	49	0.87	133	20.00	1.60	134
20.00	0.83	103	0.96	128	0.32	48	0.47	49	1.13	105	21.00	10.32	649
21.00	0.74	107	0.93	139	0.27	36	0.36	47	1.08	107	22.00	5.86	336
22.00	0.71	103	0.83	130	0.31	44	0.40	43	1.18	103	23.00	4.32	293
08.00	0.59	58	0.92	96	0.32	38	0.29	36	0.93	56	08.00	1.76	156
09.00	0.65	62	0.79	84	0.40	47	0.28	37	0.97	73	09.00	1.88	144
10.00	0.60	61	0.71	79	0.35	44	0.25	35	0.75	71	10.00	1.89	126
11.00											11.00	2.20	110

Table R.8.3 Saliva (S) and plasma (P) progesterone levels in 6 normal pregnant women (26-33 weeks gestation) one hour prior to and then following the administration of a 400mg progesterone pessary (Cyclogest) vaginally.

Time (hr of day)	S1	P1	S2	P2	S3	P3	S4	P4	S5	P5	S6	P6
09.00	1.89	172	2.58	288	0.76	126	1.65	275	1.41	181	1.58	153
09.30	2.10	168	2.67	274	0.73	149	1.95	278	1.46	203	1.24	155
10.00	1.82	179	2.62	465	0.95	141	2.00	311	1.36	182	1.29	176
10.30	1.70	206	2.75	407	0.88	146	1.71	305	1.60	160	1.35	194
11.00	1.51	176	2.74	334	1.02	138	1.73	358	1.51	206	1.16	176
11.30	4.22	183	2.33	393	0.85	132	1.62	324	32.48	216	1.10	198
12.00	5.23	179	2.41	442	0.87	131	1.41	241	13.51	226	2.48	212
12.30	2.61	184	2.53	383	1.93	118	1.53	252	36.15	186	1.17	203
13.00	4.42	182	2.22	337	1.31	142	1.31	271	37.33	203	1.30	232
14.00	3.95	204	2.41	396	1.56	176	1.37	238	72.16	193	1.74	221
15.00	13.82	190	5.62	247	3.61	184	1.50	268	25.34	214	1.29	216
16.00	9.30	226	4.80	335	1.96	188	1.95	302	138.97	236	1.27	226
17.00	6.42	222	5.41	261	2.05	201	1.80	293	37.61	215	1.66	231
18.00	7.15	204	5.22	269	1.51	181	1.85	252	24.02	217	2.47	227
19.00	4.30	168	4.23	280	1.27	188	1.57	291	35.33	160	1.79	225
20.00	6.52	216	21.32	258	1.72	197	1.66	296	21.52	190	1.61	274
21.00	6.61	215	35.22	264	1.43	203	1.92		8.14	259	1.58	251
22.00	4.46	242	11.45	313	1.36	201	3.64		9.89	208	1.60	303
23.00	5.93		8.12		1.39	151	3.22		9.74		1.44	
24.00			8.90		1.41	179	12.90				8.51	
01.00					1.10							
02.00			5.61									
04.00			60.30									
07.00	2.40		36.41						39.62		8.21	
08.00	1.65	167	13.00	311					8.42	173	5.10	170
09.00	1.81		17.91	334	0.95	149	4.95		12.93	169	7.15	155
10.00	1.43	185	20.00	356.00	1.00	168	4.92	347	11.92	174	5.73	207

Table R.8.4 Saliva cortisol levels in 5 normal women in early pregnancy (8-12 weeks gestation) one hour prior to and following the administration of a 400mg progesterone (Cyclogest) pessary vaginally.

Time (hr of day)	1	2	3	4	5
09.00	8.8	16.9	21.4	34.7	14.8
09.30	3.4	11.1	17.8	19.9	11.7
10.00	3.3	8.3	16.0	14.4	8.6
10.30	4.9	6.4	9.8	12.5	8.5
11.00	5.1	4.5	11.2	8.4	7.9
11.30	7.8	3.4	8.1	7.3	6.6
12.00	7.5	3.3	12.2	13.1	6.7
12.30	4.7	11.5	6.1	4.7	8.8
13.00	1.9	11.2	4.3	5.3	13.5
14.00	1.7	6.3	8.8	5.2	6.5
15.00	2.5	3.2	9.8	7.4	4.7
16.00	3.2	4.9	6.4	5.7	2.9
17.00	2.1	2.1	4.5	6.0	5.1
18.00	1.9	3.2	4.6	4.7	5.0
19.00	2.4	5.3	7.9	5.5	6.7
20.00	0.8	5.1	4.1	3.6	3.5
21.00	1.0	3.2	6.1	3.5	1.9
22.00	0.7	4.7	6.2	3.8	2.1
07.00				23.2	
08.00		16.3		14.5	14.6
09.00	7.3	7.6	23.1		22.6
10.00	4.4	3.8	18.6		11.4

Table R.8.5 Saliva (S) and plasma (P) progesterone levels in a women in threatened preterm labour at 21+ weeks gestation one hour prior to and then following the administration of 100mg progesterone (Gestone) intra-muscularly.

Time (hr of day)	S1	P1
16.00	0.83	141
16.30	1.05	116
17.00		142
17.30	1.31	185
18.00	1.41	217
18.30	1.40	197
19.00	1.44	242
20.00	1.83	286
21.00	2.56	319
22.00	2.43	383
23.00	2.21	350
08.00	1.13	
09.00	0.82	149
10.00	0.98	
12.00	0.61	
13.00	0.46	
14.00	0.53	
15.00	0.51	157
16.00	0.42	138
17.00	0.52	149

Table R.9.1 Individual subjects (S), hormone levels throughout gestation, (G in days). [S E1, S E2, S E3, S P - saliva oestrone, oestradiol, oestriol and progesterone respectively; Pl E1, Pl E2, Pl E3, Pl P - plasma oestrone, oestradiol, oestriol and progesterone respectively; SHBG - sex hormone binding globulin, , DHEAS - dehydroepiandrosterone sulphate (µmol/L), ß-hCG - ß-human chorionic gonadotrophin (IU/ml), hPL - human placental lactogen (µIU/ml), PRL - prolactin (mIU/L)] All values are in nmol/L unless otherwise stated.

S	G	S E1	S E2	S E3	S P	Pl E1	Pl E2	Pl E3	Pl P	SHBG	DHEAS	ß-hCG	hPL	PRL
1	135	0.337	0.163	1.08	0.99	22.7	32.0	10.9	121	272	2.58	60.69	4.58	4140
1	159	0.315	0.119	0.84	0.79	31.6	37.5	10.4	114	360	2.69	45.02	6.19	7429
1	173	0.368	0.092	0.61	0.82	34.3	42.3	10.6	113	364	2.12	54.03	7.30	6785
1	187	0.381	0.142	1.05	0.83	37.9	52.3	11.8	118	428	2.35	47.86	8.39	8326
1	201	0.384	0.195	1.14	1.58	35.2	45.8	12.2	152	392	2.21	56.66	9.21	7613
1	215	0.618	0.253	1.75	1.59	58.3	60.8	16.7	186	360	1.82	56.89	12.34	9453
1	229	0.756	0.371	2.91	1.41	63.6	92.7	28.3	222	403	1.84	54.76	12.79	9913
1	243	0.838	0.365	3.49	2.65	70.3	99.3	38.2	255	488	1.74	64.69	11.08	9361
1	256	0.863	0.395	4.83	1.94	70.5	112.5	47.5	277	470	1.52	51.25	13.03	9407
1	269	0.938	0.529	6.39	3.51	69.0	117.5	58.5	365	454	1.49	64.61	10.91	11937
2	140	0.348	0.111	0.83	0.95	24.4	26.5	11.0	133	296	1.91	21.68	3.07	2576
2	153	0.291	0.120	1.06	0.96	20.5	21.3	11.4	175	292	1.76	15.91	3.92	3220
2	167	0.326	0.170	1.15	1.32	29.0	34.0	15.9	131	312	2.06	12.62	5.15	3404
2	181	0.385	0.196	1.26	1.67	31.5	38.6	16.2	232	316	2.71	11.92	5.11	3105
2	196	0.481	0.194	1.39	1.38	34.3	36.9	14.6	204	312	2.32	11.77	6.42	3427
2	210	0.514	0.207	1.22	2.02	30.9	46.5	19.0	242	316	1.50	10.57	7.13	3496
2	224	0.626	0.257	1.64	2.62	31.1	54.0	19.8	326	360	1.63	10.84	8.73	3519
2	238	0.545	0.268	1.49	2.36	35.2	62.5	22.8	310	408	1.73	9.56	8.06	2438
2	245	0.479	0.233	1.46	2.06	33.5	66.0	26.4	387	404	1.95	9.92	9.86	3266
2	252	0.649	0.339	2.44	3.28	35.6	61.1	25.5	321	360	1.82	11.09	9.47	4232
2	266	0.955	0.361	3.26	2.67	45.3	77.1	35.9	343	424	1.96	10.73	8.69	4094
3	175	0.327	0.091	1.95	1.19	17.8	36.4	17.1	130	372	1.81	14.23	5.45	3105
3	189	0.386	0.130	1.72	1.55	14.7	42.7	21.2	220	360	2.16	15.37	7.37	3450
3	203	0.391	0.150	2.87	1.83	16.2	47.1	28.1	239	392	1.60	14.00	8.39	3657
3	217	0.296	0.164	3.22	2.43	13.6	48.3	24.7	247	430	1.53	13.76	8.69	4531
3	231	0.295	0.161	3.70	2.60	15.6	58.4	25.5	326	480	1.68	20.09	10.75	3519
3	245	0.289	0.235	4.51	3.31	18.1	58.2	33.0	411	406	1.25	17.61	10.52	3864
3	259	0.410	0.229	6.55	3.91	21.9	84.2	53.9	438	508	1.26	20.49	11.10	4255

S	G	S E1	S E2	S E3	S P	Pl E1	Pl E2	Pl E3	Pl P	SHBG	DHEAS	ßHCG	HPL	PRL
4	145	0.347	0.127	0.93	0.90	24.4	28.2	8.5	84	368	2.98	13.38	1.82	1794
4	162	0.406	0.147	0.96	0.75	27.2	37.2	10.3	97	464	3.06	7.54	2.29	1978
4	176	0.461	0.198	1.39	0.87	30.9	51.9	17.5	136	512	2.74	11.10	3.31	2507
4	187	0.460	0.214	1.53	1.26	29.4	58.3	18.6	177	444	2.69	12.81	4.02	2208
4	201	0.605	0.231	2.11	1.23	33.7	53.7	16.4	150	360	2.64	5.01	4.55	2208
4	211	0.749	0.146	2.15	1.37	47.7	54.5	17.4	149	472	2.51	10.20	5.45	3059
4	225	0.570	0.184	1.85	1.32	34.4	62.3	23.2	218	536	2.31	10.23	5.86	2990
4	239	0.431	0.188	2.51	1.61	31.6	59.9	28.9	251	500	2.08	13.92	7.31	2369
4	253	0.584	0.241	3.39	1.74	40.7	76.8	40.9	288	532	2.40	7.62	6.77	3634
4	259	0.547	0.247	3.28	1.58	35.5	66.7	34.9	225	436	2.32	6.04	5.69	3749
4	268	0.831	0.306	4.05	1.58	55.6	80.2	40.5	259	504	1.85	4.47	4.81	4163
4	273	0.946	0.401	5.23	1.73	42.2	94.7	48.5	291	536	2.13	4.69	5.75	4301
5	135	0.350	0.091	0.62	1.17	14.6	24.3	7.9	150	248	3.72	17.49	2.21	2438
5	143	0.347	0.086	0.64	1.36	15.6	23.5	7.6	155	272	3.44	15.32	3.53	3105
5	156	0.391	0.150	1.05	1.72	18.2	33.2	12.7	175	300	3.25	12.37	4.46	3243
5	162	0.384	0.221	1.16	2.05	20.3	38.2	13.8	251	296	3.43	13.45	4.06	3450
5	176	0.371	0.208	1.21	2.03	18.8	34.3	12.1	169	301	2.96	16.08	4.98	4209
5	187	0.438	0.234	1.51	2.06	23.2	38.4	15.6	254	360	3.06	18.54	6.58	4163
5	201	0.536	0.237	1.70	1.95	28.0	45.1	15.9	216	332	3.72	15.84	6.95	4301
5	215	0.576	0.193	1.79	2.21	26.4	44.2	17.6	276	348	3.09	18.15	8.30	4416
5	227	0.603	0.205	2.72	3.10	21.9	48.2	25.1	328	380	3.34	24.06	8.90	4623
5	241	0.634	0.328	4.07	4.09	28.1	60.0	39.2	390	352	2.74	25.49	10.66	4393
5	255	0.677	0.385	5.93	4.26	28.5	71.7	56.2	426	368	2.06	17.03	8.87	5198
5	263	0.551	0.250	4.27	4.09	32.3	53.0	39.4	428	404	2.76	14.44	8.95	5221
5	269	0.840	0.563	7.00	5.16	33.2	102.6	87.3	534	380	2.54	12.60	8.52	4531
5	275	0.455	0.405	3.54	2.96	37.2	81.8	32.3	303	442	3.84	12.45	7.14	4324

S	G	S E1	S E2	S E3	S P	PI E1	PI E2	PI E3	PI P	SHBG	DHEAS	ßHCG	HPL	PRL
6	141	0.121	0.088	0.50	0.58	11.7	20.2	4.3	71	252	2.71	46.41	2.31	2921
6	155	0.136	0.063	0.60	0.53	10.8	22.5	4.6	79	316	2.98	35.64	3.58	3013
6	170	0.163	0.103	0.79	0.85	15.0	25.3	8.3	88	356	3.81	28.97	4.38	3450
6	197	0.224	0.141	0.95	0.74	16.5	38.0	7.2	92	384	3.32	38.13	5.41	5589
6	210	0.176	0.115	0.95	0.94	14.8	28.9	8.9	104	432	2.84	39.24	6.34	4922
6	238	0.174	0.142	1.36	0.81	16.0	47.5	10.5	136	420	2.70	45.44	8.23	5336
6	253	0.215	0.142	1.66	1.50	18.8	46.2	11.6	149	396	2.76	48.59	8.45	5796
6	267	0.284	0.180	3.27	1.42	18.5	54.5	21.5	147	420	2.59	44.73	8.88	5635
6	282	0.186	0.144	2.17	1.74	18.0	56.0	21.9	242	444	3.04	35.16	8.08	5520
6	287	0.218	0.181	2.37	2.22	19.2	47.5	18.5	248	428	2.01	35.38	8.45	5704
7	137		0.104	0.54	1.50	17.4	20.2	9.5	75	360	2.40	19.25	2.85	1472
7	146	0.302	0.131	0.73	0.96	28.2	23.9	10.1	81	381	2.38	16.77	2.98	2806
7	161	0.283	0.133	0.98	1.94	27.3	30.0	12.6	109	407	2.06	15.53	4.03	2231
7	175	0.334	0.142	1.43	1.17	24.6	28.2	14.7	104	380	1.99	11.94	3.52	2162
7	182	0.366	0.141	1.49	1.35	26.6	28.4	13.5	115	429	3.06	12.53	4.03	2392
7	196	0.372	0.145	1.56	1.16	30.1	31.4	14.7	126	439	2.51	11.49	4.53	3174
7	210	0.370	0.186	1.65	1.47	27.5	36.2	17.4	162	440	2.33	14.87	5.27	3197
7	224	0.480	0.276	1.99	1.65	33.5	44.5	22.6	234	444	2.25	13.15	5.69	4508
7	237	0.493	0.271	2.11	1.86	34.1	39.6	23.3	241	405	1.79	11.80	6.95	3289
7	252	0.400	0.237	1.83	2.10	38.3	41.2	19.6	225	424	1.81	15.02	6.06	3795
7	266	0.447	0.268	3.37	2.30	40.7	44.0	28.5	269	420	1.38	15.56	7.07	3749
7	273	0.692	0.402	4.19	2.57	56.7	65.1	45.6	282	452	2.17	16.47	8.22	4025
7	280	0.866	0.427	4.74	2.75	54.3	64.3	42.3	334	460	1.82	15.03	12.56	4071

S	G	S E1	S E2	S E3	S P	Pl E1	Pl E2	Pl E3	Pl P	SHBG	DHEAS	βHCG	HPL	PRL
8	133	0.308	0.093	0.56	0.86	15.6	20.2	6.5	75	312	4.90	22.40	2.87	1679
8	150	0.319	0.108	0.62	1.23	18.8	23.1	8.1	119	280	4.30	20.51	3.56	1403
8	164	0.358	0.124	0.76	1.01	18.7	24.5	9.4	125	320	4.80	14.41	4.42	1702
8	178	0.389	0.150	0.94	1.21	25.9	32.5	11.0	138	332	4.40	17.45	5.41	2139
8	192	0.497	0.171	0.98	1.24	26.3	36.8	11.3	150	336	3.90	22.15	6.55	2185
8	206	0.403	0.178	1.21	1.57	28.4	47.0	15.9	212	340	4.10	17.66	7.50	2162
8	219	0.645	0.281	1.12	1.34	38.1	57.3	13.2	196	340	4.30	16.92	7.86	2852
8	234	0.625	0.248	1.57	1.46	34.6	49.7	17.9	201	360	3.70	16.03	6.96	3657
8	248	0.841	0.336	1.97	1.92	46.9	82.5	24.3	333	362	3.60	19.58	8.52	3381
8	262	0.754	0.278	2.99	1.71	49.1	86.7	36.3	321	412	3.20	14.88	8.56	2944
8	268	0.926	0.416	3.67	1.87	52.8	102.9	43.9	391	452	3.40	12.79	7.61	2898
9	158	0.253	0.132	1.74	1.13	12.2	33.6	20.5	146	336	2.13	12.13	4.85	2806
9	177	0.231	0.151	1.79	1.21	9.9	36.2	19.7	148	342	1.87	10.64	5.53	3197
9	187	0.371	0.126	1.98	1.38	10.4	38.9	21.5	158	344	1.42	13.19	7.03	4186
9	198	0.368	0.166	1.73	1.15	9.9	41.6	21.1	168	380	1.41	15.35	8.04	3358
9	211	0.464	0.218	2.28	1.73	11.3	53.2	29.5	180	374	1.56	17.14	9.27	4048
9	225	0.450	0.239	2.40	1.90	14.7	61.8	27.9	205	436	1.58	22.70	11.34	4968
9	239	0.413	0.223	2.97	3.52	17.2	68.8	32.3	293	428	1.51	21.16	12.18	5635
9	253	0.429	0.245	4.82	2.38	14.6	68.3	54.5	351	416	0.85	21.05	12.79	3864
9	267	0.585	0.543	8.42	3.79	30.3	194.3	95.7	489	580	0.62	24.29	13.53	6279
10	143	0.560	0.220	1.50	1.36	19.8	61.4	10.0	101	380	5.23	11.73	3.37	2093
10	157	0.533	0.374	2.02	1.45	16.8	79.6	14.1	115	372	4.52	11.41	3.83	2185
10	170	0.525	0.327	2.26	1.47	19.4	85.5	16.5	118	344	3.98	12.87	4.31	2438
10	184	0.722	0.302	2.10	1.60	31.0	106.8	20.0	162	440	4.41	16.40	6.58	2300
10	198	0.779	0.341	2.84	2.01	23.8	103.4	19.7	177	424	3.74	16.98	6.33	2714
10	212	0.820	0.338	2.48	1.82	23.5	97.7	20.1	157	456	3.38	18.10	7.28	2760
10	228	0.754	0.328	3.12	1.77	23.2	97.8	31.3	231	500	3.29	22.90	9.10	2484
10	242	0.770	0.445	5.80	2.31	28.7	183.6	63.4	337	552	3.38	22.20	11.00	3910
10	255	0.743	0.343	7.10	2.04	26.7	179.6	61.2	290	540	3.64	20.50	10.61	3128

S	G	S E1	S E2	S E3	S P	PI E1	PI E2	PI E3	PI P	SHBG	DHEAS	βHCG	HPL	PRL
11	195	0.612	0.209	1.47	1.34	42.2	61.4	9.6	123	428	3.10	13.42	8.05	3151
11	209	0.642	0.226	1.54	1.50	48.8	66.7	12.8	167	452	2.69	14.89	10.09	3956
11	223	0.633	0.255	1.89	1.36	50.4	80.9	15.8	275	472	2.48	18.25	5.10	4002
11	237	0.630	0.277	2.21	2.52	52.7	86.6	19.3	268	464	2.16	20.58	6.37	3749
11	251	0.764	0.311	2.36	2.71	55.2	99.8	22.2	278	468	1.65	19.59	11.69	4025
11	266	0.986	0.486	4.85	3.32	83.5	116.3	38.7	334	484	2.18	19.15	15.00	4485
11	279	1.036	0.484	4.68	2.90	83.2	108.4	40.3	336	424	1.57	19.70	16.32	5060
11	288	0.849	0.553	4.02	2.25	77.9	131.5	25.8	320	476	1.79	19.94	18.62	5428
12	148	0.458	0.123	0.95	1.02	44.2	32.9	9.5	128	508	2.71	17.44	4.05	920
12	163	0.611	0.242	1.19	1.31	53.5	39.5	13.1	156	552	2.70	17.96	4.36	1219
12	177	0.541	0.229	1.51	1.39	51.6	44.1	16.9	214	592	2.54	17.11	5.49	1725
12	189	0.732	0.309	1.44	1.82	62.8	56.5	19.7	186	650	3.40	22.43	7.21	1587
12	205	0.691	0.251	1.96	2.04	51.5	60.6	24.5	246	604	2.73	20.55	8.08	2806
12	217	0.647	0.259	1.86	1.90	46.9	66.4	23.5	245	628	2.29	31.85	9.22	1840
12	232	0.656	0.321	2.75	3.06	48.6	73.9	28.3	410	596	2.25	31.45	10.74	3381
12	245	0.832	0.344	3.33	3.84	60.0	82.6	38.4	455	701	2.44	37.68	11.50	2921
12	259	1.020	0.465	5.68	4.18	57.5	99.4	57.3	510	692	2.48	37.45	10.32	3496
13	142	0.201	0.054	0.67	0.61	13.5	21.4	8.3	81	328	0.94	5.81	2.27	1012
13	157	0.233	0.062	0.70	0.65	20.7	21.9	10.0	79	340	1.13	5.02	3.02	1288
13	176	0.258	0.103	1.15	0.74	20.3	31.2	12.3	123	408	1.06	3.81	4.02	1748
13	189	0.224	0.097	0.95	0.92	22.5	32.4	13.2	95	381	0.91	4.06	4.87	2070
13	206	0.228	0.093	1.12	1.36	26.6	38.9	15.9	152	404	0.75	5.29	5.74	2967
13	219	0.230	0.133	1.15	1.52	22.7	49.5	21.3	197	428	0.75	5.45	7.78	2070
13	233	0.285	0.102	1.42	1.77	24.3	47.9	20.3	210	456	0.72	6.21	8.41	2300
13	248	0.286	0.130	1.81	2.03	27.4	60.0	26.5	250	500	0.66	7.43	8.64	2921
13	259	0.279	0.158	2.42	2.12	29.0	67.3	34.2	252	482	0.67	9.00	8.93	6624
13	267	0.311	0.200	3.15	3.49	24.7	77.3	39.2	344	532	0.68	8.06	8.26	5635

S	G	S E1	S E2	S E3	S P	Pl E1	Pl E2	Pl E3	Pl P	SHBG	DHEAS	βHCG	HPL	PRL
14	145	0.133	0.044	0.57		7.0	24.3	10.2	127	324	0.95	3.88	3.27	1909
14	167	0.144	0.053			7.4	21.6	11.1	134	320	1.31	2.46	4.03	1978
14	181	0.117	0.047			6.2	19.0	12.6	173	304	1.03	2.23	4.97	2461
14	194	0.208	0.057			6.9	24.2	13.4	187	328	0.83	2.16	5.73	6118
14	208	0.248	0.061			8.5	26.0	20.6	325	320	0.86	3.05	7.12	5750
14	222	0.417	0.059			7.3	25.4	23.5	359	304	0.79	3.34	7.83	4715
14	236	0.372	0.073			8.9	39.3	35.8	461	320	0.66	4.64	8.11	5589
14	250	0.208	0.095			9.9	34.5	44.6	563	312	0.61	4.29	9.09	7245
14	257	0.351	0.126	3.98		11.2	49.2	52.0	502	324	0.40	4.41	9.36	6486
14	271	0.363	0.119	3.46		11.7	43.1	47.9	544	328	0.76	3.75	9.01	7038
15	142	0.191	0.107	1.47	1.06	12.4	27.4	9.8	128	380	1.18	27.65	2.71	1334
15	156	0.182	0.107	1.54	1.00	12.7	27.2	9.0	118	344	1.15	19.54	2.86	1495
15	170	0.361	0.124	1.30	0.96	18.5	26.9	8.5	82	432	1.17	22.51	3.50	2185
15	184	0.230	0.112	1.87	1.16	16.9	30.8	13.7	131	450	1.05	21.33	4.91	1771
15	191	0.316	0.176	2.04	2.77	19.1	26.6	12.6	145	456	0.63	20.99	5.47	2392
15	198	0.212	0.145	2.09	2.46	16.4	30.2	16.3	141	368	0.72	18.28	4.91	2024
15	205	0.301	0.206	1.72	1.47	18.9	27.4	14.9	143	436	0.71	25.53	5.32	2576
15	209	0.337	0.208	2.31	1.24	22.9	37.5	17.8	147	498	1.04	27.59	5.56	2668
15	219	0.412	0.103	2.10	1.76	19.2	40.5	21.8	155	524	1.04	31.04	6.95	2737
15	226	0.375	0.098	2.80	2.11	19.3	30.6	20.8	138	492	0.89	32.25	6.75	3335
15	233	0.413	0.153	3.42	2.91	20.2	55.3	36.7	281	580	1.14	35.97	7.52	2783
15	240	0.396	0.177	3.63	2.76	21.5	43.8	28.3	252	512	0.82	31.16	6.95	3289

S	G	S E1	S E2	S E3	S P	Pl E1	Pl E2	Pl E3	Pl P	SHBG	DHEAS	βHCG	HPL	PRL
16	139	0.293	0.128	0.89	0.99	13.7	28.7	9.3	144	424	2.07	8.53	2.86	2185
16	153	0.297	0.143	0.94	1.36	14.1	31.2	10.6	202	495	2.00	6.90	4.00	2507
16	167	0.270	0.168	1.10	1.41	17.0	36.7	11.7	186	452	1.86	4.66	4.76	2392
16	181	0.285	0.167	1.23	1.50	16.8	42.1	13.0	223	458	2.03	4.43	5.36	2415
16	195	0.269	0.190	1.51	2.08	14.7	40.5	13.6	271	561	1.66	5.13	9.66	2461
16	209	0.407	0.221	1.63	2.26	16.7	49.3	14.8	324	564	1.57	6.03	8.48	2530
16	223	0.412	0.299	1.68	2.69	19.0	54.2	16.6	376	632	1.65	5.41	7.38	2116
16	237	0.543	0.351	2.17	2.76	22.5	87.1	23.7	449	680	2.04	6.63	8.62	2737
16	250	0.351	0.351	2.54	3.50	18.6	71.2	27.1	436	588	1.40	10.20	4.58	2576
16	258	0.455	0.397	4.28	4.05	24.8	82.9	38.3	477	592	0.83	13.99	6.23	2944
17	142	0.420	0.128	0.96	1.52	34.9	55.6	9.0	199	440	3.36	37.09	3.80	2829
17	156	0.391	0.109	1.50	1.53	37.8	64.2	16.0	264	488	2.65	35.36	6.04	3565
17	170	0.407	0.148	1.72	2.93	35.7	68.0	15.4	258	508	2.36	38.63	6.34	3450
17	185	0.363	0.210	1.90	2.65	32.2	75.9	19.4	336	512	2.64	45.24	7.38	3588
17	198	0.352	0.149	2.52	3.28	34.6	96.1	22.9	437	487	2.32	47.71	9.04	3680
17	226	0.394	0.192	3.81	5.07	29.4	96.4	37.7	543	516	1.75	59.74	12.13	4577
17	240	0.436	0.344	11.06	8.99	31.2	135.3	79.0	785	664	1.12	75.63	18.29	6026
18	153	0.117	0.048	0.57	0.64	11.0	22.7	8.2	83	544	1.80	5.78	2.89	2093
18	168	0.131	0.083	0.63	0.84	17.4	30.1	10.6	90	572	1.79	3.74	3.12	2116
18	182	0.112	0.093	1.38	0.94	14.7	42.3	19.2	96	548	1.54	3.93	4.10	2714
18	195	0.135	0.073	1.48	1.49	16.7	37.6	14.6	157	580	1.56	3.29	5.26	3289
18	210	0.212	0.114	1.38	1.31	16.6	45.8	16.6	184	592	1.52	4.22	6.30	3335
18	227	0.302	0.155	2.11	1.88	16.9	58.4	19.5	243	604	1.55	7.91	9.58	5244
18	237	0.200	0.131	1.49	1.98	17.4	60.2	18.5	261	648	1.48	9.06	9.24	4439
18	251	0.261	0.137	2.56	2.09	17.0	49.5	22.3	309	640	1.35	13.83	11.62	4623
18	265	0.308	0.165	2.56	2.75	19.0	56.3	22.0	344	589	1.49	20.20	13.92	4531
18	279	0.337	0.332	4.08	2.80	24.1	84.1	28.6	346	604	1.84	19.20	14.96	7751
18	286	0.354	0.203	2.48	3.41	31.6	69.9	20.2	367	542	1.44	17.83	12.63	5106
18	290	0.628	0.377	6.99	6.27	27.3	83.1	40.2	353	524	1.51	19.35	11.92	5313

S	G	S E1	S E2	S E3	S P	PI E1	PI E2	PI E3	PI P	SHBG	DHEAS	ßHCG	HPL	PRL
19	141	0.219	0.098	0.71	1.29	20.7	25.2	6.8	136	332	1.58	26.31	2.14	4186
19	157	0.255	0.096	1.09	1.22	20.2	28.2	10.3	165	360	1.43	18.77	2.64	4140
19	171	0.298	0.135	1.53	1.85	25.4	41.6	15.4	216	404	2.17	16.59	3.23	5037
19	185	0.350	0.157	1.35	1.91	29.9	49.0	16.7	246	400	1.76	15.55	4.08	5980
19	199	0.336	0.166	1.84	1.93	31.0	57.2	18.8	257	450	1.44	19.74	4.73	5221
19	213	0.499	0.194	1.97	2.11	38.1	67.4	21.0	298	470	1.38	21.28	5.01	5842
19	227	0.476	0.200	2.18	2.21	37.7	69.8	22.7	325	526	1.24	21.12	5.59	6624
19	241	0.581	0.236	2.20	1.85	41.2	73.7	23.2	320	448	1.59	21.57	5.61	5290
19	255	0.601	0.238	2.88	2.78	39.7	76.9	28.9	339	496	1.56	23.64	5.92	6141
19	263	0.606	0.194	2.03	2.03	40.0	66.2	17.9	300	548	1.43	21.71	6.69	6233
19	269	0.946	0.378	3.68	2.43	58.8	110.5	37.6	416	540	1.46	21.73	5.81	6394
20	140	0.421	0.146	0.82	0.75	27.2	24.8	9.4	89	430	1.13	23.67	3.93	1127
20	154	0.415	0.183	0.89	0.92	27.5	28.8	12.4	108	492	0.98	22.94	4.43	2668
20	168	0.494	0.146	1.08	1.11	32.5	34.3	10.8	110	528	0.93	24.57	6.30	3795
20	183	0.584	0.244	1.12	1.15	47.8	42.1	13.5	138	528	0.77	14.75	6.76	3910
20	209	0.390	0.110	0.89	1.75	33.6	36.7	17.5	237	530	0.72	16.85	7.49	8671
20	225	0.547	0.157	1.41	1.54	48.7	52.8	19.5	201	576	1.09	12.63	8.85	3887
20	239	0.642	0.209	1.38	1.71	52.0	45.1	13.6	212	552	0.87	14.41	9.99	3634
20	259	0.507	0.185	1.98	2.17	43.9	52.0	19.6	248	554	0.78	13.65	11.82	3979
20	277	0.588	0.141	0.84	2.23	38.0	41.7	8.1	258	560	1.34	11.73	8.72	3864

Table R.10.1 Hourly saliva F levels (nmol/L) in individual women from various groups. [N - normal women with regular cycles; OC - taking a combined oral contraceptive pill; S - following superovulation with HMG]

Time	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10
7.00	18.6	23.2	2.4	12.3	21.9	23.0	14.1	13.6	13.5	4.0
8.00	16.3	8.2	7.7	9.2	14.6	30.0	10.0	15.2	20.6	6.2
9.00	8.4	6.1	11.1	9.1	8.3	12.6	2.7	12.8	8.5	9.9
10.00	7.1	3.9	16.4	3.5	6.6	7.4	5.3	8.7	5.3	8.6
11.00	5.4	3.4	8.5	5.4	5.5	4.7	4.6	7.4	4.6	4.5
12.00	5.3	2.2	5.6	5.2	5.4	4.7	6.1	6.1	4.7	2.5
13.00	9.2	5.5	4.4	3.2	13.4	7.3	5.2	3.7	5.6	5.8
14.00	6.2	4.1	4.2	2.5	11.7	4.4	3.6	4.5	4.7	5.9
15.00	4.1	3.5	5.1	5.4	9.7	3.8	3.5	4.3	3.0	2.9
16.00	4.5	2.9	2.9	3.2	6.2	4.9	0.6	4.1	4.3	1.4
17.00	7.2	4.3	0.6	1.1	5.6	5.0	2.2	3.3	3.5	2.8
18.00	9.3	1.7	1.1	3.3	3.2	10.7	1.5	3.1	2.7	2.6
19.00	4.6	3.4	0.9	3.6	2.8	5.7	0.5	2.5	2.3	3.2
20.00	3.7	2.1	1.0	1.6	2.7	4.0	0.6	3.6	1.7	0.8
21.00	1.6	0.5	0.7	1.2	2.6	1.7	0.3	2.1	0.8	1.5
22.00	1.4	1.0	1.0	1.2	1.3	1.1	0.2	1.9	0.9	1.9
23.00	0.9	1.1	0.5	0.4	1.6	1.8	0.8	0.7	1.1	1.8
	OC1	OC2	OC3	OC4	OC5	OC6	OC7	OC8		
7.00	18.1	21.3	24.7	10.2	23.0	8.0	10.1	8.8		
8.00	21.3	11.6	25.1	18.3	19.5	17.2	13.1	18.2		
9.00	15.0	8.6	16.6	14.0	12.6	5.3	11.6	24.6		
10.00	11.3	6.3	11.2	8.5	8.1	7.3	12.0	19.1		
11.00	8.9	4.7	7.0	7.6	6.6	4.5	10.7	8.8		
12.00	6.1	4.2	5.1	7.1	5.8	1.6	7.2	5.4		
13.00	5.3	3.4	4.8	4.4	6.2	2.3	4.3	6.9		
14.00	8.1	2.5	2.8	6.1	5.9	6.3	4.7	5.2		
15.00	6.4	3.2	1.5	5.2	6.3	3.9	4.4	5.6		
16.00	4.9	3.5	1.5	4.1	4.7	8.1	2.8	8.3		
17.00	2.7	2.8	1.0	2.4	3.7	4.2	5.9	6.4		
18.00	3.1	2.4	0.6	2.3	2.9	3.3	4.4	9.2		
19.00	2.2	1.3	1.0	1.7	4.4	2.9	2.9	3.9		
20.00	2.1	1.5	0.8	0.6	5.8	4.3	3.0	3.8		
21.00	1.1	1.4	1.8	1.1	5.3	3.1	3.1	2.2		
22.00	0.6	1.2	0.2	1.2	2.8	2.9	2.5	0.9		
23.00	0.9	0.7	3.0	1.6	2.1	1.0	1.3	1.5		
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
7.00	6.3	10.2	11.6	24.1	17.9	20.4	12.3	14.2	29.5	17.0
8.00	19.0	11.3	21.5	16.7	18.8	15.6	15.6	15.5	21.0	21.0
9.00	19.5	11.7	11.9	11.1	20.3	8.2	10.9	8.9	8.6	15.1
10.00	10.2	8.1	9.8	9.5	9.1	5.5	6.8	6.8	5.8	8.4
11.00	8.2	6.1	5.6	7.6	3.6	4.7	5.5	3.7	6.6	5.3
12.00	5.3	6.6	4.7	5.1	6.0	4.6	7.9	8.6	4.5	6.8
13.00	4.4	10.7	5.5	4.5	7.7	5.8	5.4	4.5	3.6	7.4
14.00	6.0	7.7	4.3	5.7	8.8	4.7	5.1	3.4	3.5	8.3
15.00	5.9	4.3	8.2	4.4	4.3	3.0	9.3	4.2	3.4	6.9
16.00	3.4	4.2	5.1	4.2	2.6	3.9	8.0	5.0	8.6	5.5
17.00	5.2	2.4	5.3	4.1	2.4	3.6	4.6	3.9	11.2	3.4
18.00	4.3	2.9	4.8	3.1	1.5	2.7	3.7	2.6	4.1	4.9
19.00	2.2	1.4	5.1	2.9	8.7	2.4	9.6	1.4	3.6	5.2
20.00	1.7	1.3	5.3	3.6	3.8	1.7	2.5	1.7	1.6	3.2
21.00	2.0	0.9	2.9	4.0	2.6	0.8	1.8	2.0	1.9	2.6
22.00	1.5	2.0	3.0	2.5	2.9	0.8	2.4	2.4	1.7	2.5
23.00	1.7	1.9	1.9	1.9	1.7	1.1	2.3	1.6	1.3	3.3

Table R.10.2 Hourly saliva F levels (nmol/L) in individual women in early (EP) and late (LP) pregnancy; and hourly saliva P levels (nmol/L) in individual women in late pregnancy.

Time	EP1	EP2	EP3	EP4	EP5	EP6	EP7	EP8	EP9
7.00	32.0	7.8	6.7	4.4	11.0	24.0	19.6	12.3	15.5
8.00	16.3	21.2	20.5	15.8	15.3	26.0	25.6	19.2	26.2
9.00	8.4	15.4	14.7	10.4	12.2	15.4	12.7	28.5	15.9
10.00	6.9	17.3	8.9	4.9	10.6	9.8	11.0	11.4	13.1
11.00	5.5	11.2	7.9	7.9	6.6	10.1	9.9	14.3	13.5
12.00	8.2	9.6	5.7	5.3	7.4	8.6	9.8	5.6	9.5
13.00	9.6	6.5	4.9	4.1	5.6	5.5	15.4	5.4	7.1
14.00	6.4	3.9	4.2	6.2	8.2	5.2	10.3	9.3	7.4
15.00	4.2	4.9	5.6	6.8	5.3	6.3	6.3	6.8	7.9
16.00	5.4	5.2	8.2	8.2	4.5	4.9	6.1	3.6	7.0
17.00	4.1	3.3	5.0	2.9	3.6	4.1	5.8	3.8	8.3
18.00	1.9	2.6	4.3	5.0	3.6	2.6	6.2	1.5	6.7
19.00	2.4	2.2	3.5	2.6	3.1	1.7	3.7	2.4	4.9
20.00	2.9	1.0	3.2	2.8	2.6	2.1	2.8	1.5	3.8
21.00	1.4	1.7	2.1	2.8	3.1	1.7	2.6	2.1	3.3
22.00	2.7	1.8	2.0	2.7	1.9	1.3	2.4	1.8	1.9
23.00	2.1	1.6	1.5	3.2	1.8	2.1	1.6	1.4	6.3
	LP1 F	LP2 F	LP3 F	LP4 F	LP5 F	LP6 F	LP7 F	LP8 F	LP9 F
8.00	25.7	14.0	27.0	14.0	26.3	31.5	25.8	13.5	16.1
9.00	14.7	19.3	29.1	32.0	23.2	32.6	18.9	15.2	20.6
10.00	21.6	14.2	19.7	20.2	18.9	23.8	15.3	19.5	13.4
11.00	13.2	11.5	15.8	19.0	13.2	23.9	12.4	18.2	13.0
12.00	11.0	10.9	15.3	15.7	14.3	23.8	10.5	14.0	18.5
13.00	11.6	9.9	16.9	15.3	10.5	19.4	10.4	11.0	12.3
14.00	9.6	9.9	15.7	12.0	9.6	19.0	9.3	11.7	8.2
15.00	9.2	11.0	12.5	12.3	9.4	21.0	8.7	13.6	11.8
16.00	10.4	9.5	14.9	11.0	6.2	18.6	12.1	10.9	9.6
17.00	10.2	9.0	18.6	11.8	6.6	16.9	9.0	10.4	10.8
18.00	7.9	8.0	13.2	10.9	8.7	13.3	8.8	9.8	96.0
19.00	6.8	7.9	9.9	10.0	5.3	12.8	10.1	7.4	8.4
20.00	6.8	4.5	10.0	6.8	8.0	10.3	9.0	7.2	7.6
21.00	5.9	4.8	8.6	7.3	5.5	14.5	8.6	6.7	8.1
22.00	5.5	4.6	8.1	5.0	6.1	14.1	9.1	6.5	7.0
	LP1 P	LP2 P	LP3 P	LP4 P	LP5 P	LP6 P	LP7 P	LP8 P	LP9 P
8.00	3.0	1.4	3.5	2.2	1.2	3.6	4.6	3.9	6.8
9.00	2.4	1.4	2.4	2.4	1.4	3.8	4.0	4.1	3.9
10.00	2.2	1.4	2.8	2.7	1.3	4.2	5.2	3.0	3.4
11.00	2.2	1.2	3.0	2.4	1.5	3.3	4.4	3.0	4.5
12.00	2.3	1.1	4.5	2.9	1.6	3.9	3.9	4.5	4.8
13.00	2.0	1.4	4.7	3.0	1.5	4.2	4.8	3.4	3.6
14.00	2.3	1.2	5.2	3.2	1.5	3.0	4.6	4.2	4.8
15.00	2.2	1.4	4.8	2.6	1.4	3.7	3.1	2.7	4.4
16.00	1.6	1.2	4.6	2.6	1.5	3.7	5.4	3.2	3.6
17.00	1.8	1.1	5.0	2.6	1.4	3.3	5.3	3.3	4.5
18.00	2.1	1.0	5.1	2.4	1.1	3.2	5.0	3.9	4.2
19.00	2.7	1.1	4.7	2.5	1.3	3.0	5.1	3.6	4.0
20.00	2.0	1.2	4.8	2.5	1.5	2.8	4.9	3.1	3.7
21.00	2.0	0.9	3.2	2.4	1.3	3.7	5.1	3.3	3.1
22.00	2.1	1.4	4.6	2.1	1.9	3.9	5.0	3.0	3.6

Table R.10.3 Hourly saliva F levels (nmol/L) in individual women on postpartum days 1-3.

Time	PP1 1		PP1 3	PP1 4		PP1 6
8.00	20.1		15.6	18.7		26.3
9.00	18.7		16.4	8.3		37.8
10.00	12.0		13.0	7.2		23.5
11.00	14.2		10.4	35.0		15.6
12.00	11.0		8.4	18.0		30.6
13.00	6.5		7.4	12.0		34.5
14.00	11.5		6.5	6.2		31.0
15.00	9.5		5.6	6.6		22.9
16.00	6.6		4.3	27.0		12.6
17.00	11.5		4.0	11.6		10.3
18.00	9.5		4.4	11.9		6.9
19.00	7.0		5.0	8.8		21.0
20.00	7.0		9.7	5.6		14.2
21.00	4.0		7.3	3.9		9.7
22.00	3.4		4.9	4.4		7.6
	PP2 1	PP2 2	PP2 3	PP2 4	PP2 5	PP2 6
8.00	19.0	11.5	11.1	23.5	18.0	22.6
9.00	21.5	17.5	22.3	11.4	16.5	28.4
10.00	16.0	9.5	10.6	10.0	11.6	16.9
11.00	11.5	6.7	7.1	9.4	4.5	13.1
12.00	9.5	5.4	11.9	3.6	1.8	10.7
13.00	4.4	14.1	12.4	5.4	7.0	22.8
14.00	6.5	6.7	23.2	5.1	4.5	37.5
15.00	4.8	6.6	12.7	4.4	9.2	12.4
16.00	6.2	5.8	7.9	6.0	4.8	8.7
17.00	6.2	1.6	6.4	6.9	4.0	5.4
18.00	4.9	2.0	5.2	7.9	1.3	8.7
19.00	5.8	6.2	4.8	6.2	9.0	5.5
20.00	2.0	3.7	4.2	4.4	5.0	10.6
21.00	3.0	2.2	8.8	4.1	2.5	8.4
22.00	3.0	1.8	6.4	6.8	2.0	6.8
	PP3 1	PP3 2	PP3 3	PP3 4	PP3 5	PP3 6
8.00	25.8	17.2	23.8	36.5	22.8	13.4
9.00	25.4	14.0	8.4	18.0	15.7	26.0
10.00	15.1	8.1	8.7	11.1	10.3	11.2
11.00	9.4	4.4	6.7	8.2	7.1	8.3
12.00	7.3	3.7	4.2	17.0	5.3	6.6
13.00	171.0	3.0	4.2	2.0	2.1	2.9
14.00	3.7	3.7	3.0	2.8	4.6	5.6
15.00	4.7	2.6	5.7	4.0	3.2	5.4
16.00	6.0	1.0	3.6	6.6	2.9	6.8
17.00	5.7	1.2	5.8	6.0	2.2	10.7
18.00	8.6	2.4	4.1	2.4	2.9	8.6
19.00	5.5	6.3	9.2	4.6	1.7	5.2
20.00	3.5	6.9	6.0	3.5	1.1	3.9
21.00	3.7	2.5	4.8	3.0	1.0	2.8
22.00	2.1	2.7	1.9	2.0	1.5	5.4

Table R.10.4 Hourly saliva F levels (nmol/L) in individual women on postpartum days 4 and 5.

Time	PP4 1	PP4 2	PP4 3	PP4 4	PP4 5	PP4 6
8.00	20.6	32.0	25.2	34.0	27.0	13.8
9.00	13.8	12.1	12.4	20.0	20.9	28.7
10.00	12.3	7.0	8.6	13.8	14.3	15.4
11.00	6.1	6.5	5.2	8.9	8.5	9.2
12.00	6.9	4.9	3.7	10.2	9.5	7.6
13.00	4.2	8.0	4.1	9.1	6.5	4.6
14.00	2.8	3.8	7.6	10.3	5.5	6.2
15.00	2.9	5.2	9.8	11.8	3.7	4.6
16.00	2.9	4.7	11.3	4.7	2.7	6.6
17.00	4.0	4.5	10.2	4.7	2.6	3.2
18.00	4.8	4.8	12.3	4.4	3.4	5.2
19.00	3.8	5.9	7.8	4.1	4.5	8.1
20.00	3.4	3.9	5.4	2.0	1.5	7.0
21.00	3.9	4.0	7.5	1.8	1.4	6.5
22.00	2.3	3.5	3.8	4.2	1.4	8.2
	PP5 1	PP5 2	PP5 3	PP5 4	PP5 5	PP5 6
8.00	9.4	12.1	11.3	12.9	13.4	26.5
9.00	12.6	16.1	8.6	16.0	8.1	16.8
10.00	6.5	7.8	6.4	5.6	5.6	11.4
11.00	4.9	2.5	4.9	4.3	3.4	7.3
12.00	4.1	3.2	4.1	5.1	6.3	4.3
13.00	4.8	4.6	3.1	6.0	7.2	3.4
14.00	8.2	2.5	4.4	10.2	4.6	3.1
15.00	3.7	8.7	6.5	12.4	3.9	5.4
16.00	4.4	7.9	9.2	10.3	2.7	10.5
17.00	3.6	7.1	7.7	3.6	4.1	6.2
18.00	3.4	2.9	6.4	2.5	4.0	6.3
19.00	3.1	1.8	4.7	3.0	1.6	4.6
20.00	4.2	2.7	5.3	11.7	1.2	4.0
21.00	4.9	2.0	4.4	6.9	1.7	3.8
22.00	4.2	1.3	2.9	5.3	2.6	2.3

Table R.10.5 Hourly saliva F levels (nmol/L) in individual women on postpartum days 12-33.

Time	PP12 2	PP12 3	PP12 4	PP12 6	PP19 2	PP19 3	PP19 4	PP19 6
8.00	16.5	14.1	19.8	11.1	11.8	13.2	26.5	22.5
9.00	10.1	7.9	12.0	9.5	10.7	13.7	25.0	23.1
10.00	5.1	4.1	7.3	6.8	11.6	8.8	12.1	12.0
11.00	5.8	3.7	7.8	5.0	7.7	6.1	7.3	8.1
12.00	4.6	2.9	8.0	3.0	7.1	8.1	4.6	4.6
13.00	6.3	3.1	12.1	1.9	6.5	7.4	5.1	10.2
14.00	4.8	2.7	16.5	2.2	5.2	13.9	4.9	7.0
15.00	5.2	7.1	4.4	3.4	4.1	10.3	9.9	3.8
16.00	3.7	5.6	6.3	2.9	4.5	7.1	7.5	2.4
17.00	6.4	2.9	5.3	2.8	3.1	2.3	4.5	3.6
18.00	6.3	1.9	4.2	2.2	3.0	4.4	2.8	2.8
19.00	6.6	3.3	2.6	2.1	4.6	3.6	2.0	2.0
20.00	2.7	2.4	3.4	2.7	5.1	2.6	1.9	1.8
21.00	2.1	2.2	3.1	2.1	1.6	2.3	3.9	2.6
22.00	1.9	2.3	3.4	0.9	1.5	3.5	3.3	2.0
	PP26 2	PP26 3	PP26 4	PP26 6	PP33 2	PP33 3	PP33 4	PP33 6
8.00	16.4	11.5	5.5	11.2	7.3	13.5	5.1	11.3
9.00	10.1	15.9	23.5	30.2	9.8	8.2	10.6	19.3
10.00	8.6	11.6	18.7	18.3	5.5	8.3	22.9	10.8
11.00	5.7	6.3	9.4	9.5	4.2	5.1	14.1	5.6
12.00	3.6	3.5	5.4	6.2	5.3	5.0	8.6	3.7
13.00	2.9	1.4	3.3	3.8	4.4	3.0	3.7	5.1
14.00	2.6	2.6	2.0	4.0	3.8	3.3	4.4	4.0
15.00	7.4	4.8	4.8	2.9	5.5	2.9	7.9	6.3
16.00	3.2	2.5	4.7	8.1	3.7	7.4	8.4	4.2
17.00	4.7	3.4	2.7	5.6	4.3	4.3	6.8	2.7
18.00	5.9	1.8	4.9	3.3	6.2	3.3	4.3	1.9
19.00	6.2	1.3	1.8	2.1	3.6	2.6	3.1	3.3
20.00	1.4	1.6	1.3	6.5	2.3	1.5	2.5	2.6
21.00	5.0	0.9	0.8	12.4	1.8	0.8	1.6	2.4
22.00	1.5	1.9	0.9	3.0	1.5	1.6	1.8	1.9

Table R.10.6 Plasma CBG and total plasma F levels (nmol/L) at 09.00h in individual women from various groups. [N - normal women with regular cycles; OC - taking the oral contraceptive pill; S - superovulated following treatment with HMG; EP - early pregnancy, (12-16weeks gestation); LP - late pregnancy, (37-39weeks gestation); PP1-33 - postpartum days 1 to 33.]

Subject	CBG	F	Subject	CBG	F
N 1	433	301	PP2 1	861	640
2	417	267	2	905	784
3	400	427	3	717	645
4	389	223	4	1307	695
5	662	260	5	745	868
6	455	269	6	1067	784
7	513	281	PP3 1	848	708
8	656	230	2	840	501
9	604	200	3	700	706
10	571	391	4	1280	910
OC 1	1244	296	5	707	549
2	1180	473	6	1059	567
3	1213	296	PP4 1	836	606
4	1246	133	2	825	486
5	1189	233	3	597	468
6	1178	321	4	1174	533
7	1100	331	5	686	596
8	1498	348	6	1081	724
S 1	585	711	PP5 1	835	499
2	731	471	2	757	465
3	535	356	3	573	406
4	538	344	4	1001	575
5	496	446	5	682	532
6	579	478	6	993	436
7	468	286	PP12 1	732	422
8	504	385	2	644	310
9	449	291	3	552	296
10	616	272	4	810	325
EP 1	1194	467	5	599	492
2	949	717	6	733	444
3	538	350	PP19 1	557	304
4	1302	571	2	444	291
5	764	539	3	462	331
6	833	405	4	642	460
7	1042	652	5	548	585
8	896	489	6	579	626
9	845	494	PP26 1	523	338
LP 1	1094	609	2	492	205
2	1063	656	3	460	407
3	1096	670	4	565	510
4	1478	1066	5	502	431
5	971	550	6	524	743
6	1339	695	PP33 1	510	411
PP1 1	1026	720	2	411	243
2	1012	1388	3	482	418
3	803	780	4	584	320
4	1423	860	5	540	332
5	786	971	6	482	609
6	1096	760			

Table R.10.7 Plasma P, E2 and E3 levels (nmol/L) in individual women from various groups. [S - superovulated following treatment with HMG; EP - early pregnancy (12-16 weeks gestation); LP - late pregnancy (37-39 weeks gestation)]

Subject	Plasma P	Plasma E2	Plasma E3
S 1	142	1.53	-
2	210	3.07	-
3	180	3.45	-
4	90	3.26	-
5	169	2.69	-
6	122	1.11	-
7	110	2.08	-
8	116	-	-
9	112	1.14	-
10	167	2.12	-
EP 1	192	10.12	1.56
2	128	10.35	2.49
3	123	3.76	2.33
4	91	16.65	3.1
5	82	8.55	2.66
6	131	15.45	4.85
7	97	10.82	4.14
8	73	8.36	1.95
9	106	8.47	3.15
LP 1	478	48.2	32.7
2	286	47.3	38.2
3	391	53.9	28.3
4	334	40.2	19.7
5	315	35.2	30.2
6	340	82.4	84.7

Appendix 3 Candidate's Publications and Presentations

Publications

Scott EM, Thomas A, McGarrigle HHG, Lachelin GCL.
Serial adrenal ultrasonography in normal neonates.
J Ultrasound Med 9:279-283, 1990

Scott EM, McGarrigle HHG, Lachelin GCL.
The increase in plasma and saliva cortisol levels in pregnancy is not due to the increase in corticosteroid-binding globulin levels.
J Clin Endocrinol Metab 71:639-644, 1990

Presentations

Scott EM, McGarrigle HHG, Lachelin GCL.
Unexpectedly high saliva progesterone levels following vaginal progesterone administration in early pregnancy.
J Endocrinol 119 [suppl.] Abstract 143, 1988

Scott EM, McGarrigle HHG, Lachelin GCL.
Raised free cortisol in pregnancy appears unrelated to progesterone or corticosteroid binding globulin concentrations.
36th Annual Meeting, Society for Gynecological Investigation,
Abstract 164, p163, March 1989

Scott EM, McGarrigle HHG, Lachelin GCL.
Unphysiological saliva progesterone (P) levels following vaginal P administration in early pregnancy.
37th Annual Meeting, Society for Gynecological Investigation,
Abstract 215, p204, March 1990