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Total and Free Plasma Concentrations of Progesterone, Cortisol and Oestradiol-17β during Pregnancy, Parturition and Early Lactation in Sows

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Abstract

The total (bound plus free) concentrations of progesterone, 20α-dihydroprogesterone, oestradiol-17β and cortisol were determined in the plasma of sows at three stages during pregnancy and more intensively from 5 days pre-partum to 5 days post-partum. The free fractions of progesterone, oestradiol-17β and cortisol were measured in the same samples by a rate dialysis method. Up to day 110 of gestation, the amounts of free hormone in plasma did not fluctuate independently of their total concentrations. During farrowing, the total and free concentrations of progesterone and cortisol varied independently of each other, whereas total and free oestradiol-17β declined simultaneously. The initiation of parturition was associated with a decrease in circulating total progesterone, and was accentuated by a decrease in the free fraction ($P<0.005$) so that its active free concentration was only 20% of its day 1 pre-partum value. Total and free cortisol concentrations rose rapidly during labour so that at 12–18 h after birth of the first piglet 30% of that cortisol in maternal plasma was free hormone.

Introduction

It has been suggested recently that bound steroid is taken up directly by target cells (Pardridge 1981; Siiteri *et al.* 1982). However, the biologically active component of a steroid hormone is generally considered to be the small fraction of it which circulates free or unbound to plasma proteins (Westphal and Forbes 1963; Hoffman *et al.* 1969; Ekins 1981, 1982). The role of steroid hormones in the pig during pregnancy, parturition and lactogenesis has been studied previously in terms of the total concentrations of circulating hormone (Robertson and King 1974; Ash and Heap 1975; Baldwin and Stabenfeldt 1975; Taverne *et al.* 1982; Willcox *et al.* 1983*a*). Collectively, these studies have shown that during late pregnancy plasma progesterone concentration declines progressively from about 47·7 to 25·4 nmol l^{-1} by 2 days pre-partum. During the next 48 h plasma progesterone then declines rapidly to less than 9·5 nmol l^{-1} during farrowing so that the initiation of parturition in sows is associated with a low plasma concentration of progesterone. In late pregnancy cortisol concentrations vary between 27·6 and 110·4 nmol l^{-1} in individual sows and are maximal during farrowing. Oestradiol-17β in plasma gradually increases during the last week of gestation to 2·1–3·3 nmol l^{-1} and remains elevated during farrowing until after delivery of the placentae.

The possibility that free hormone concentrations change independently of total hormone concentrations has not been examined in the pig. We have recently developed a rate dialysis technique for measuring the free steroid fraction in undiluted plasma at 37°C (Willcox *et al.* 1983*b*). We now report a study of the relationship between total and free concentrations of plasma progesterone, cortisol, and oestradiol-17β, and the total concentration of 20α-dihydroprogesterone during pregnancy, parturition and early lactation of sows.

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Materials and Methods

Chemicals

All chemicals were analytical grade. Crystalline steroids and activated charcoal were purchased from Sigma Chemical Co. (Sydney). [1,2,6,7-^{3}H]progesterone (3·7–4·8 TBq mmol^{-1}), [1,2,6,7-^{3}H]cortisol (3·0–3·9 TBq mmol^{-1}), [2,4,6,7-^{3}H]oestradiol-17β (3·1–4·1 TBq mmol^{-1}) and 20α-hydroxy[1,2,6,7-^{3}H]pregn-4-en-3-one (1·5–2·6 TBq mmol^{-1}) were purchased from Amersham International (Amersham, U.K.). Lutalyse (prostaglandin $F_{2\alpha}$) was donated by The Upjohn Co. (Sydney).

Animals

Sows (Landrace, Large White and Landrace × Large White) were housed intensively in a commercial piggery (Baconfield Piggery, Bullsbrook, W.A.). The means ± s.e.m. gestational length of sows (n = 3682) in this piggery is 114·0 ± 0·5 days and the parities and the litter sizes of the animals recruited into this study varied between 1–11 and 8–13, respectively. Sows were kept individually in crates from 1 week pre-partum to weaning at 3–4 weeks post-partum. They were tethered by a neck collar during pregnancy, parturition and lactation but were otherwise unrestrained. All animals were given free access to water.

Experimental Design and Sampling

Samples of blood were obtained from two different groups of sows as follows:

(1) Pregnant sows, sampled once and subdivided according to their gestational age: nine sows between days 3–43 (stage I of pregnancy), seven sows between days 51–63 (stage II) and seven sows between 97–107 days (stage III).

(2) Parturient sows, sampled from 5 days pre-partum to 5 days post-partum.

Eight sows were sampled at intervals of 12 h from day 5 to day 1 pre-partum, 6 h from day 1 pre-partum to day 1 post-partum, 12 h on day 2 post-partum and daily thereafter. One sow (895E) was induced to farrow by intramuscular injection of 12·5 mg prostaglandin $F_{2\alpha}$ (Lutalyse) on day 114 of gestation; farrowing commenced 11 h later. The seven other sows sampled all farrowed spontaneously.

Blood was obtained from an ear vein of unrestrained sows by venipuncture with a 24-gauge needle which was connected to a 2·5-ml syringe via clear vinyl tubing (Dural Plastics, Sydney). This enabled blood to be obtained with minimal interference to the animal; most samples were taken while the sow was lying down and/or asleep. The blood was collected into heparinized tubes (Disposal Products, Adelaide), stored on ice and centrifuged within 30 min to separate the plasma fraction. Plasma was stored at −15°C in six aliquots until analysed.

Radioimmunoassays

Progesterone, cortisol, oestradiol-17β and 20α-dihydroprogesterone were measured by specific radioimmunoassays. Antisera to progesterone, 20α-dihydroprogesterone and oestradiol-17β were raised in rabbits against conjugates of 4-pregnen-3,20-dione–3-carboxymethyloxime–bovine serum albumin, 20α-dihydroprogesterone–3-carboxymethyloxime–bovine serum albumin and 1,3,5(10)-oestratrien-3,17β-dione-6-one–6-carboxymethyloxime–bovine serum albumin, respectively. Steroids showing more than 1% cross-reactivity were pregnenolone (10%) and 5α-pregnanedione (45%) for the progesterone antiserum (42-B5), corticosterone (30%) for the cortisol antiserum. None of the 21 steroids tested with the oestradiol-17β antiserum (44-B5) showed more than 1% cross-reactivity. Progesterone cross-reacted slightly (1%) with the 20α-dihydroprogesterone antiserum (6-B5); all of the 19 other steroids tested showed less than 1% cross-reactivity with this antiserum. The assay procedures have been described previously (Henderson *et al.* 1983). Progesterone and 20α-dihydroprogesterone were extracted from plasma before assay with efficiencies of 67·6 ± 2·9% (mean ± s.e.m., n = 14 assays) and 54·1 ± 3·0% (n = 4 assays), respectively, using light petroleum (60–80°C b.p.). Oestradiol-17β and cortisol were extracted from plasma before assay with efficiencies of 81·8 ± 1·6% (n = 5 assays) and 83·5 ± 2·5% (n = 13 assays), respectively using diethyl ether. Isolation of progesterone and cortisol by chromatography indicated that purification of the organic extracts of plasma before assay was unnecessary. Regression analysis of samples assayed for progesterone and cortisol directly or after thin-layer chromatography on silica gel yielded correlation coefficients of 0·95 (n = 28 samples) and 0·98 (n = 11 samples), respectively. The efficacy of direct extraction of oestradiol-17β and 20α-dihydroprogesterone from sow plasma was checked by addition of known amounts of steroid to the plasma prior to assay. Recovery of 1·8, 3·7 and 5·5 pmol of steroid to plasma samples (n = 10) was

100–112%. Solvent blanks were ≤18 fmol per tube for the progesterone, 20α-dihydroprogesterone and oestradiol-17β assays and ≤40 fmol per tube for the cortisol assay. The limits of the sensitivity of the assays (per tube) were 37 fmol for oestradiol-17β, 64 fmol for progesterone and 20α-dihydroprogesterone and 138 fmol for cortisol. The intra- and interassay coefficients of variation of the four steroid assays were all <7 and 14%, respectively.

Free Steroid Fraction

The free fractions of progesterone, cortisol, and oestradiol-17β were measured in duplicate by rate dialysis. Briefly, a dialysis cell of two identical compartments of volume V separated by a semipermeable membrane of area A was filled with the same sample (in this case undiluted plasma). Tracer hormone was added to one compartment initially so that after dialysis for time t, the isotope distribution H was $(h_1-h_2)/(h_1+h_2)$ where h_1 and h_2 were the amounts (or radioactivities) of hormone in compartments 1 and 2. The free hormone fraction f was calculated from

$$-\ln(h_1-h_2)/(h_1+h_2) = 2ADft/V\,,$$

where D is the 'membrane diffusion coefficient', and comprised the diffusion coefficient of the hormone in plasma, membrane thickness and a factor relating the diffusion area of the membrane to its total area. In practice the 'cell permeability constant', $2AD/V$, was determined for a particular membrane and size of cell from preliminary experiments using hormone solutions of known free fraction. Thereafter, the free fraction of unknown solutions of hormone were obtained from their isotope distribution after dialysis for a known time. The method has been validated for sow plasma and yields values comparable to those obtained by centrifugal ultrafiltration (Willcox *et al.* 1983*b*). The intra- and interassay coefficients of variation were 4–6% for cortisol and progesterone, and were 7·9 and 10·3%, respectively, for oestradiol-17β. The free concentration of each steroid was obtained by multiplication of its free fraction and total plasma concentration, determined separately by radioimmunoassay.

Statistical Analysis

Results are expressed as mean ± s.e.m., and subjected to Student's paired t-test (Snedecor and Cochran 1980) between days. Transformation of the data to logarithms or arcsin did not affect the levels of significance.

Table 1. Concentration of total and free steroids in plasma during pregnancy in the sow
Values are expressed as the mean ± s.e.m. of 7–9 observations; n.d., not determined

Steroid	Stage of pregnancy		
	I (days 3–43)	II (days 51–63)	III (days 97–100)
Progesterone			
Total (nmol/l)	57·6 ± 3·5	45·2 ± 2·9	34·0 ± 1·9
Free (%)	7·3 ± 0·1	7·2 ± 0·3	7·6 ± 0·2
Free (nmol/l)	4·1 ± 0·3	3·2 ± 0·3	2·5 ± 0·3
20α-Dihydroprogesterone			
Total (nmol/l)	3·8 ± 0·3	2·5 ± 0·3	2·8 ± 0·3
Cortisol			
Total (nmol/l)	39·5 ± 9·7	41·9 ± 8·8	27·3 ± 5·5
Free (%)	10·9 ± 2·2	10·6 ± 2·0	13·8 ± 1·0
Free (nmol/l)	5·5 ± 2·5	5·0 ± 1·7	4·1 ± 1·1
Oestradiol-17β			
Total (nmol/l)	0·18	0·316 ± 0·084	2·31 ± 0·26
Free (%)	n.d.	4·5 ± 0·2	4·8 ± 0·1
Free (nmol/l)	n.d.	0·015 ± 0·004	0·106 ± 0·015

Results

Pregnancy

The total and free concentrations of hormonal steroids in the maternal plasma of sows were determined at three intervals during pregnancy (Table 1). Although mean total

progesterone declined as pregnancy progressed, the amount of circulating progesterone was variable between sows. In contrast, the free progesterone fraction was constant at 7·2–7·6% of its total concentration throughout pregnancy in all sows, so that the free progesterone concentration varied directly with its total plasma concentration. The concentration of 20α-dihydroprogesterone was low throughout all stages of pregnancy and ranged from 5·2 nmol l^{-1} in one sow (stage I) to 1·7 nmol l^{-1} in another sow (stage II). Total and free plasma concentrations of cortisol declined overall as pregnancy progressed. In different sows, cortisol ranged from 87·5 nmol l^{-1} (stage I) to 11·6 nmol l^{-1} (stage III) whereas the free cortisol fraction varied between 26·2% (stage I) and 5·0% (stage II). However, high free concentrations of cortisol were not correlated with high total concentrations of hormone. Oestradiol-17β was undetectable (<0·18 nmol l^{-1}) in all sows for the first 43 days of pregnancy and was measurable in only four of the seven sows sampled during the middle third of pregnancy. However, by late pregnancy the total concentration of oestradiol-17β had risen to 1·4–3·3 nmol l^{-1} in the seven sows sampled, 5% of which circulated as free hormone.

Parturition

Among those sows which farrowed spontaneously, total progesterone concentrations varied between 29·6 and 73·5 nmol l^{-1} for the 3 days preceding parturition but declined slightly overall. At day 1 pre-partum total progesterone was 41·0 ± 2·5 nmol l^{-1} but it declined to 11·5 ± 1·0 nmol l^{-1} (P<0·001) on the day of delivery (Fig. 1). The decline in plasma progesterone concentration was followed by parturition in all sows: individual progesterone concentrations were all less than 14·6 nmol l^{-1} on the day of farrowing and declined to between 1·1 and 3·8 nmol l^{-1} by day 2 post-partum (Fig. 1). The free progesterone fraction remained unchanged from day 4 pre-partum to day 1 pre-partum at 7·6 ± 0·4% and 7·2 ± 0·4%, respectively. It then declined significantly (P<0·005) to 5·1 ± 0·1% at farrowing but by day 2 post-partum it had returned to pre-partum levels of 7·1 ± 0·5%. The concentration of free progesterone was variable among sows ranging from 2·2 to 5·1 nmol l^{-1} with a mean value of 3·2 ± 0·3 nmol l^{-1} at day 3 pre-partum. The free concentration declined slightly to 2·9 ± 0·3 nmol l^{-1} at day 1 pre-partum and then declined significantly (P<0·001) to 0·57 ± 0·06 nmol l^{-1} by the beginning of farrowing. After farrowing, the free concentration decreased further to 0·16 nmol l^{-1} by day 2 post-partum. However, for individual sows the timing of the fall in free progesterone concentrations ranged from day 4 pre-partum to day 1 pre-partum (Fig. 2). The total concentration of 20α-dihydroprogesterone varied independently of both total and free progesterone concentrations in maternal plasma. Total circulating concentrations of 20α-dihydroprogesterone did not exceed 4·7 nmol l^{-1} pre-partum and declined to less than 1·6 nmol l^{-1} post-partum. In individual sows, this decline commenced at 9–22 h before delivery.

The free fraction of oestradiol-17β was 5·3 ± 0·7% over the perinatal period so that changes in the total concentration of oestradiol-17β accurately reflected fluctuations in the free hormone concentration. However, total and free oestradiol-17β varied by as much as threefold among different sows pre-partum. In all four sows analysed, total and free concentrations of oestradiol-17β were higher just before parturition (sows 852F, 723H, 836F) whether or not farrowing was initiated by prostaglandin (sow 895E). The plasma levels of bound and free oestradiol-17β began to decline (P<0·05) after delivery of the first piglet and were undetectable by 36 h post-partum (Figs 1*b* and 2).

Total and free concentrations of cortisol in maternal plasma were variable before farrowing, ranging from 21·2 to 169·7 nmol l^{-1} and 1·7–34·5 nmol l^{-1}, respectively (Figs 1*c* and 2). However, in six of the seven sows parturition was associated with a rapid rise in plasma cortisol just prior to delivery, which reached a maximum about 12–18 h after the start of farrowing (P<0·05 for all seven sows), and declined about 12 h later to

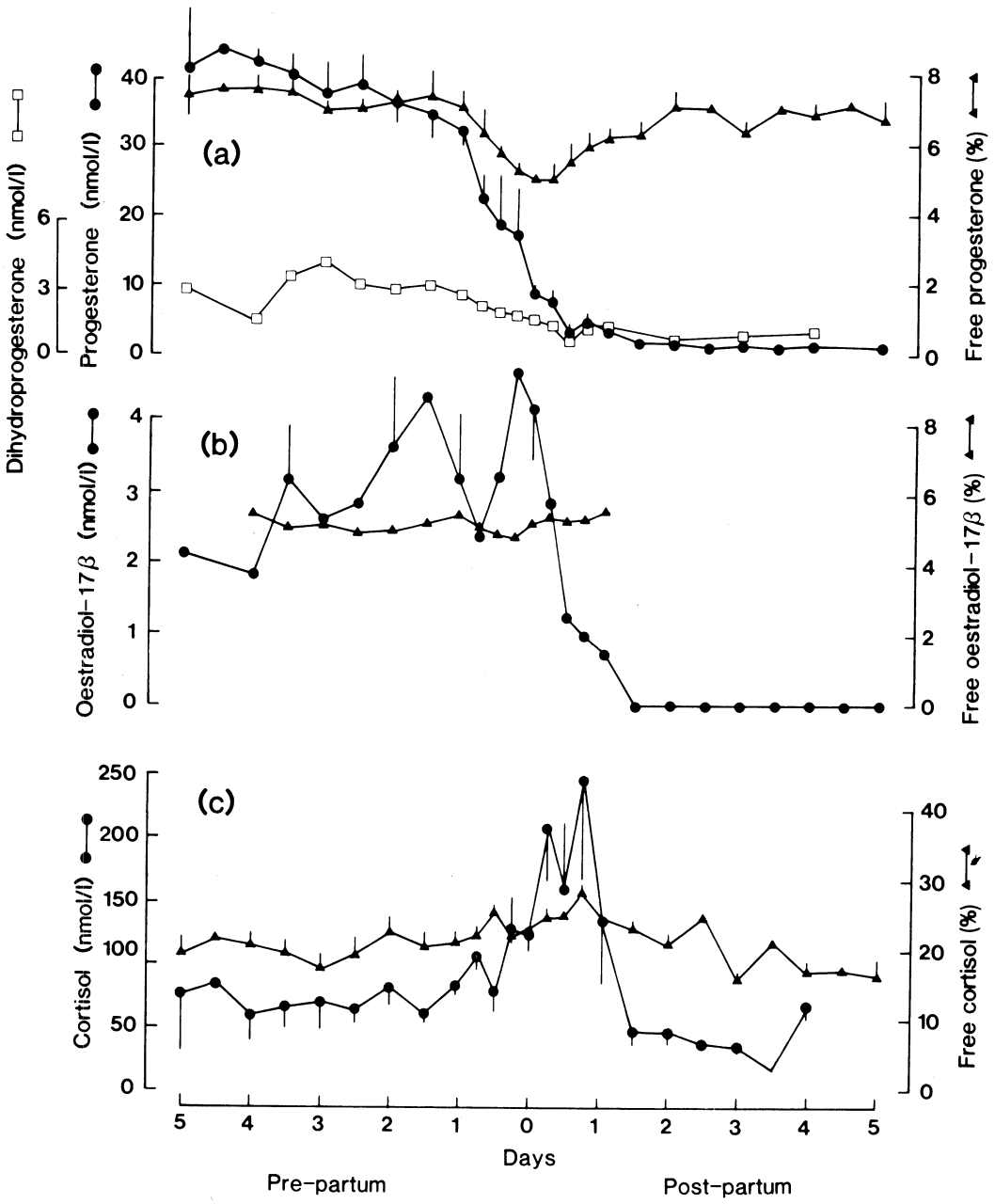

Fig. 1. Changes in the total concentration and free fraction of steroid hormones in the plasma of sows from 5 days pre-partum to 5 days post-partum. Results are expressed as the mean ± s.e.m. for seven sows (progesterone and cortisol), three sows (20α-dihydroprogesterone) and three sows (oestradiol-17β). The beginning of day 0 is denoted by the birth of the first piglet. (*a*) Total progesterone, total 20α-dihydroprogesterone and percentage free progesterone; (*b*) total and percentage free oestradiol-17β; (*c*) total and percentage free cortisol.

reach pre-partum values 36 h after birth (Fig. 1*c*). The free cortisol fractions in the pre-partum period varied between 11·5 and 33·3% in different animals. The free cortisol fraction increased progressively from 17·5 ± 2·1% at day 3 pre-partum to 23·1 ± 1·1%

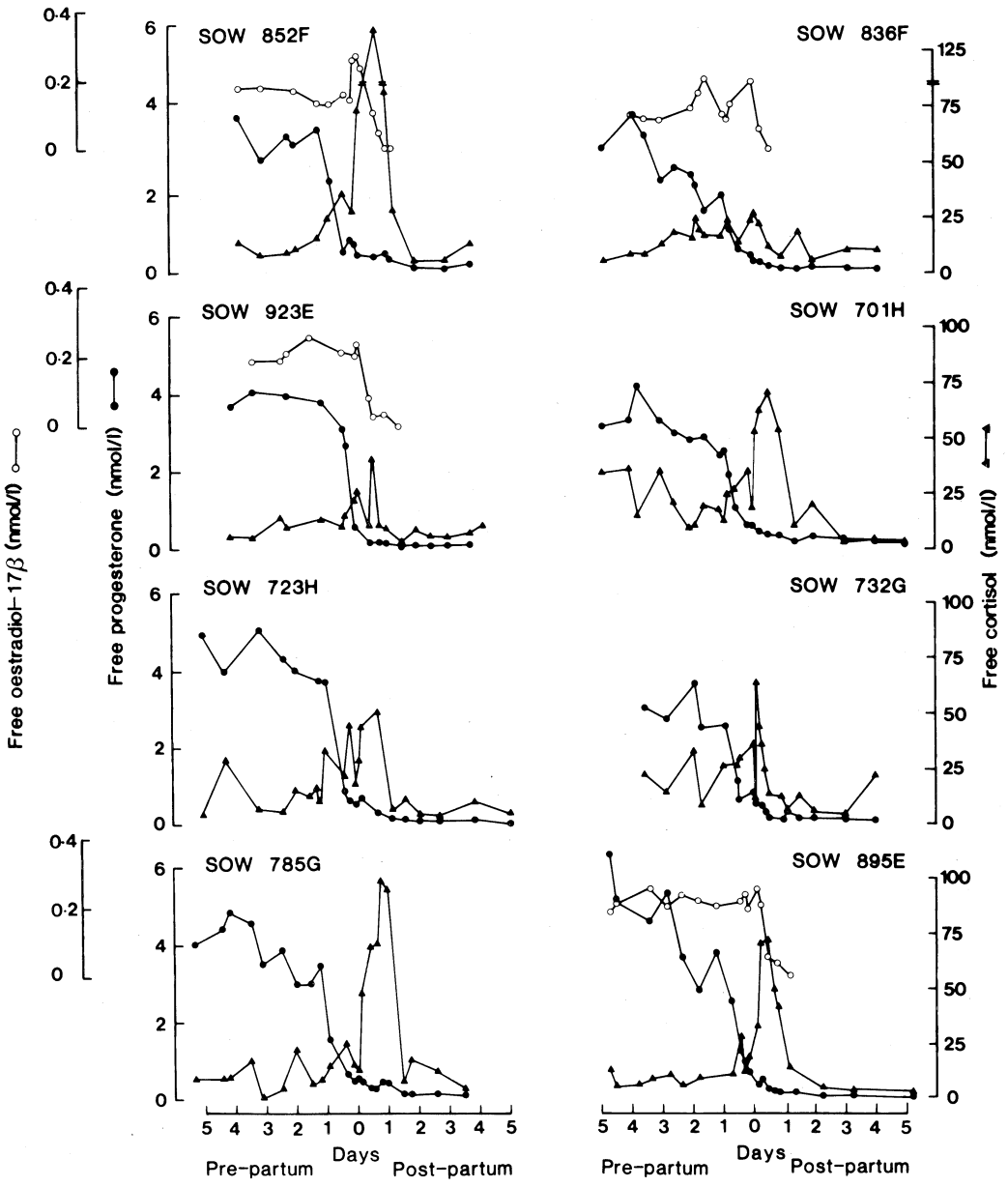

Fig. 2. Changes in the free concentrations of progesterone, oestradiol-17β and cortisol in individual sows from 5 days pre-partum to 5 days post-partum. The initiation of parturition was spontaneous in all sows except sow 895E, whose parturition was induced by an intramuscular injection of prostaglandin $F_{2\alpha}$. The beginning of day 0 is denoted by the birth of the first piglet.

during farrowing ($P<0·05$) and to a maximum of 28·3 ± 1·0% ($P<0·025$) about 18 h after birth of the first piglet. Thereafter it declined to 21·1 ± 1·6% by day 2 post-partum.

Discussion

In most physiological studies the total concentrations of steroid hormones are measured rather than their free concentrations, which may estimate changes in the active fraction of the hormones imprecisely. Available methods for determining free ligand fractions measure either directly or indirectly the amount of free hormone in an equilibrium system *in vitro*, which only approximates the fluctuating steady-state system characteristic of flowing blood. Furthermore, it is assumed that the free fraction of each steroid in plasma remains constant during the measuring process and that added tracer steroid equilibrates with both free and protein-bound steroid. Until now, methods for measuring the free steroid fraction in plasma samples have suffered from various limitations. These include the need for large sample volumes, multiple determinations of radioactivity per sample, correction for variable volume changes which occur when plasma is dialysed against a hypotonic solution, and the difficulty of working at physiological temperatures (Willcox *et al.* 1984). We measured the free fraction of steroids in sow plasma by a rate dialysis method because it enabled the analysis to be carried out at 37°C using undiluted plasma, was suitable for oestradiol-17β and cortisol as well as progesterone, and required only 0·35 ml of sample (Willcox *et al.* 1983*b*). The free concentrations of these hormones in sow plasma have not been reported previously.

This study has confirmed that the total maternal concentrations of circulating progesterone and oestradiol-17β do not change significantly from 97 to 110 days of gestation in pregnant sows. Furthermore, we have shown that a redistribution of circulating progesterone in favour of the bound fraction does occur immediately prior to and during labour in this species. It is unlikely that 20α-dihydroprogesterone synthesis increased relative to progesterone synthesis at this time, because total concentrations of 20α-dihydroprogesterone did not increase during pregnancy and labour (Fig. 1*a*). In all sows the onset of parturition was associated with a sharp decline in the total concentration of progesterone to 22–35% (mean 28%) of its day 1 pre-partum value (Fig. 1*a*). At the same time, the free progesterone fraction decreased significantly ($P<0·005$). Thus at the onset of parturition, the amount of physiologically active free hormone in plasma was only 20% of its day 1 pre-partum value. The importance of progesterone withdrawal in the initiation of parturition in the sow has been emphasized previously (Robertson and King 1974; Ash and Heap 1975; Baldwin and Stabenfeldt 1975; Coggins *et al.* 1977; Martin *et al.* 1978). Withdrawal of this hormone from sow plasma is accentuated by the decrease in the fraction of circulating free progesterone.

The proportion of free oestradiol-17β in plasma was unchanged from mid-pregnancy to parturition so that the amount of free oestradiol-17β in maternal plasma varied as the total concentration of this hormone varied. The total amount of oestradiol-17β in the plasma of pre-partum sows (Fig. 1*b*), measured by specific radioimmunoassay, was similar to that reported by Taverne *et al.* (1978–1979) and Willcox *et al.* (1983*a*). Administration of oestradiol-17β to rats has been shown to increase the number of prolactin receptors and also to promote growth of myoepithelial cells in the mammary gland (Falconer 1980). The lack of significant pre-partum changes in oestradiol-17β in maternal plasma suggest that circulating oestradiol-17β does not participate directly in the initiation of parturition (Willcox *et al.* 1983*a*). In this study oestradiol-17β was first detectable in the peripheral plasma of sows at 53 days of gestation, when lobulo-alveolar development is beginning (Cross *et al.* 1958; Kensinger *et al.* 1982). Thus, in sows oestrogens may be more important to growth and development of the mammary gland than to regulation of the events of parturition.

The concentration of free cortisol was variable among individual sows over the perinatal period but rose sharply in all but one sow 12–18 h after the start of farrowing to comprise approximately 30% of the circulating hormone. The free cortisol concentration at parturition is at least threefold higher than that reported in humans (Rosenthal *et al.* 1969;

Brien 1980). From the limited amount of free steroid data currently available for other species, only the squirrel monkey has as high a concentration of physiologically active free cortisol as that found here in the sow (Siiteri *et al.* 1982). There is a circadian rhythm in plasma cortisol concentrations in the sow (Martin *et al.* 1978) but such a rhythm was difficult to detect at the sampling frequency employed in this study (Fig. 2). However, the increase in plasma cortisol during parturition was greater than fluctuations due to circadian rhythm. Furthermore, our technique of blood sampling interfered minimally with the animals, which were unrestrained except for a loose neck tether. Total corticosteroids increase in maternal and fetal porcine plasma at parturition (Ash and Heap 1975; Silver *et al.* 1979) and decrease rapidly afterwards (Baldwin and Stabenfeldt 1975). A rise in fetal glucocorticoids is implicated in the initiation of parturition in sheep, cows and goats (Thorburn and Challis 1979), but concerted action by at least two fetuses appears necessary for the onset of labour in the sow (Stryker and Dziuk 1975). The concentration of glucocorticoids in fetal plasma is high at parturition (Nara and First 1978) but it is not known if the glucocorticoids cross the placenta. This rise in the levels of circulating glucocorticoid at farrowing is thought to reflect maternal stress (Molokwu and Wagner 1973). However, by sampling at frequent intervals, we showed that the increase in maternal cortisol was maximal 12–18 h after the expulsion of the first piglet (Fig. 1*c*). If the rise in total circulating cortisol was due solely to maternal stress, it would be expected to occur at the beginning of parturition. Thus, the increase in total concentration of cortisol in plasma toward the end of labour (Fig. 1*c*), accompanied by an even greater increase in free cortisol (Fig. 2), may be more important to the triggering of post-partum events such as lactogenesis.

Most of the steroid hormone in mammalian blood is bound with high affinity to corticosteroid binding globulin (CBG), α_1-acid glycoprotein and sex hormone binding globulin and with low affinity to albumin (Grant *et al.* 1967; Westphal 1971). As pregnancy advances in humans, cortisol is displaced from CBG by progesterone due to increased amounts of progesterone in plasma relative to cortisol and a shift in the relative affinity of CBG in favour of progesterone (Rosenthal *et al.* 1969). By the end of the first stage of human labour, the total and free plasma concentrations of cortisol increase so that about 2·5-fold more cortisol circulates unbound (Willcox *et al.* 1984). We measured the total and free concentrations of progesterone (Fig. 1*a*) and cortisol (Fig. 1*c*) in sow plasma because both hormones are bound to CBG, whose concentration remains relatively constant throughout the porcine perinatal period at 15–30 nmol cortisol bound per litre of plasma (Martin *et al.* 1978). In the sows studied here, the total amount of circulating progesterone declined just prior to and during parturition so that increased binding of progesterone by CBG may have displaced cortisol from the protein and increased the proportion of unbound cortisol in plasma. However, the total and free concentrations of cortisol rose during farrowing, when progesterone concentrations were low, suggesting that the capacity of CBG and albumin for cortisol at this time were exceeded.

Possibly, the onset of parturition in sow 895E had started prior to the injection of prostaglandin since this sow delivered its first piglet within 11 h of prostaglandin administration and the perinatal profiles of total and free steroids in the plasma of this sow were similar to the seven sows which farrowed spontaneously. The administration of prostaglandin $F_{2\alpha}$ or its analogues to late-pregnant sows normally induces parturition within 30 h (Diehl *et al.* 1974; Coggins *et al.* 1977; Hartmann *et al.* 1983). This is consistent with the view that prostaglandin $F_{2\alpha}$ causes luteolysis of the corpus luteum to remove the inhibition of parturition by progesterone (Thorburn and Challis 1979). Once parturition begins, changes in maternal concentrations of other steroid hormones occur which facilitate labour.

In conclusion, the total and free fractions of plasma progesterone and cortisol vary independently of each other during farrowing in sows. The initiation of parturition is associated with withdrawal of total progesterone, and is accentuated by a decrease in the

putative active free fraction. Total and free concentrations of oestradiol-17β did not vary independently of each other throughout pregnancy and parturition. They probably mediate mammogenesis rather than parturition since bound and free oestradiol-17β declined rapidly with the onset of labour. The rise in total and free cortisol which occurred late in labour may influence post-partum events rather than be merely a response to the stress of labour itself.

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