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THE CONCENTRATION OF UNCONJUGATED OESTRONE, OESTRADIOL-17α AND OESTRADIOL-17β IN THE MATERNAL PLASMA OF THE PREGNANT EWE IN RELATION TO THE INITIATION OF PARTURITION AND LACTATION*

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Summary. The concentration of unconjugated oestrone, oestradiol-17α and oestradiol-17β has been measured in the maternal jugular venous plasma of pregnant sheep every 6 hr over the period 108 hr before to 72 hr after parturition. Measurable levels of each oestrogen were present 108 hr before parturition and by about 40 hr, the concentration began to increase markedly. Levels of 200 to 350 pg/ml for oestrone and 100 to 150 pg/ml for oestradiol-17α and for oestradiol-17β were found just before parturition. At 12 hr after parturition, the concentration of oestrone and oestradiol-17β had fallen to < 15 pg/ml. Levels of oestradiol-17α remained elevated for a longer period after parturition and did not fall to <35 pg/ml until 48 to 60 hr after parturition.

INTRODUCTION

The work of Liggins, Kennedy & Holm (1967) and Liggins & Kennedy (1968) has aroused considerable interest in the rôle of the steroid hormones of fetal and/or placental origin in the initiation of parturition. In the pregnant sheep, Findlay & Cox (1970) found high levels of oestrogen sulphates in the fetal plasma while Challis (1971) and Challis, Harrison & Heap (1971) found that, in this species, a sharp rise in the concentration of total unconjugated oestrogens occurred just before parturition. In the maternal plasma of the goat during the last few days of pregnancy, Challis & Linzell (1971) found a rise in the concentration of the total unconjugated oestrogens while Thorburn, Nicol, Bassett, Shutt & Cox (1972) found a rise in unconjugated oestrone and oestradiol-17α which was paralleled by a rise in the secretion of oestrogens from the conceptus.

The rise in plasma oestrogens in the pregnant sheep before parturition has been investigated in detail, using a method which has been developed to measure the individual unconjugated oestrogens in plasma (Robertson, Smeaton &

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Durnford, 1972). The report documents the changes in the maternal jugular venous plasma concentration of unconjugated oestrone, oestradiol-17α and oestradiol-17β in eight sheep over the period 108 hr before to 72 hr after normal parturition and the initiation of lactation.

MATERIALS AND METHODS

Animals

Eight Western ewes were mated by a ram fitted with a marking harness and the time of mating was recorded. Throughout gestation, the ewes were housed in individual pens. Over the period beginning 6 days before the estimated time of parturition, the ewes were watched continuously, with the exception of the period from 02.00 to 08.00 hours, and blood sampling was commenced. Two of the eight ewes lambed within this 6-hr period.

Blood sampling

Blood samples (10 ml) were collected by needle puncture into heparinized tubes every 6 hr at 02.00, 08.00, 14.00 and 20.00 hours and the sampling was continued until 72 hr *post partum*. Immediately after collection, the tubes were chilled in iced water, centrifuged and 2·5-ml aliquots of plasma were transferred into glass vials which were sealed and kept frozen at $-20°$ C until used for assay.

The time of collection of all samples was subsequently standardized, taking the time of parturition as 0 hr. As it was impracticable to carry out estimations of oestrone, oestradiol-17α and oestradiol-17β on all plasma samples collected from each animal, the ewes were divided into two groups depending upon whether they had a single lamb (four ewes) or twins (four ewes) and pooled samples were made for each 6-hr period within the two groups. Thus, each pooled sample contained aliquots from four ewes. In order to obtain an estimate of the between-animal variation and to determine the effect, if any, of the sex of the fetus, individual estimates were carried out on the plasma samples taken from each animal during the sampling period 12 to 7 hr before parturition.

Oestrogen determination

Unconjugated oestrone, oestradiol-17α and oestradiol-17β were measured by the method of Robertson *et al.* (1972) using 1 ml plasma.

Oestrone and oestradiol-17β were estimated from a standard curve for oestradiol-17β while oestradiol-17α was estimated from one for oestradiol-17α.

The sensitivity of the method has been estimated to be 11 pg for oestrone, 13 pg for oestradiol-17β and 34 pg for oestradiol-17α. All assays were carried out in duplicate and the values obtained were corrected for procedural losses.

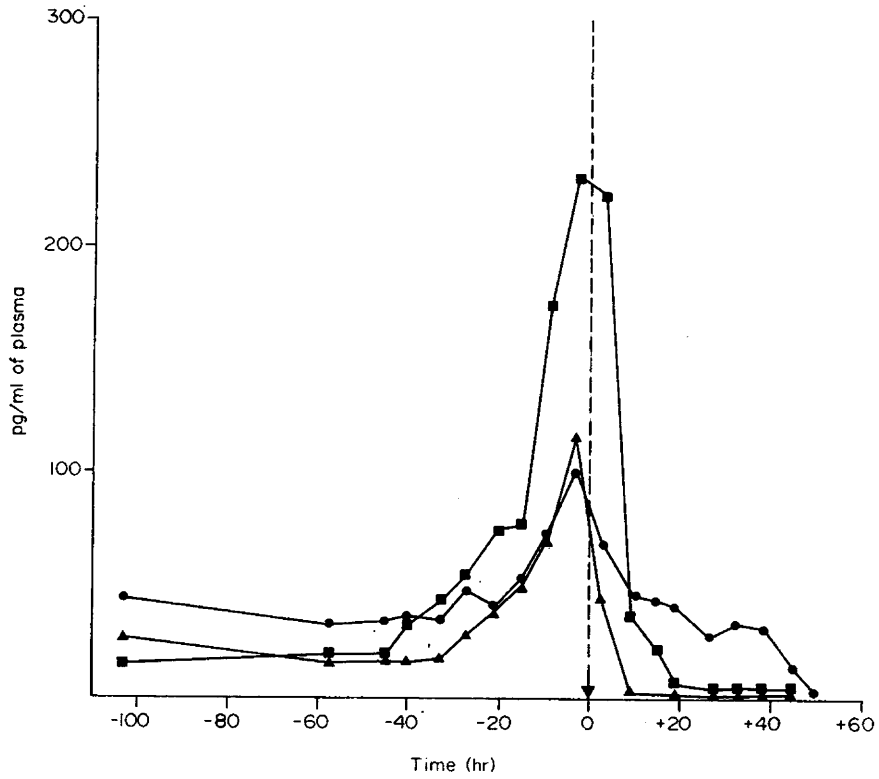
RESULTS

Table 1 shows the time of onset of parturition, the sex of the lamb(s) and the time interval in hours between the last *pre-partum* sample and parturition for each of the ewes within the two groups.

Table 1. Gestation length, sex and weight of lambs born to 'single lamb' and 'twin lamb' groups of ewes

Ewes	*Sex of lamb(s)*	*Weight of lamb(s) (kg)*	*Time of lambing (hours)*	*Time interval (hr) between* pre-partum *sample and parturition*	*Gestation length (days)*
'Single lamb' group					
B21	M	3·3	02.00 to 08.00	0 to 6·00	144
R35	M	5·1	13.45	5·75	148
Y39	F	5·8	15.30	1·50	148
Y40	M	5·9	02.00 to 08.00	0 to 6·00	148
'Twin lamb' group					
B34	F,F	3·3,2·9	15.30	1·50	149
G34	M,M	3·0,3·3	19.30	5·50	145
G34	M,M	2·8,2·3	13.00	5·00	144
R36	M,F	2·5,2·3	10.45	2·75	145

There could be a difference of up to 6 hr between the time of the last sample taken and the time of parturition because blood samples were taken only every 6 hr at fixed times of the day.

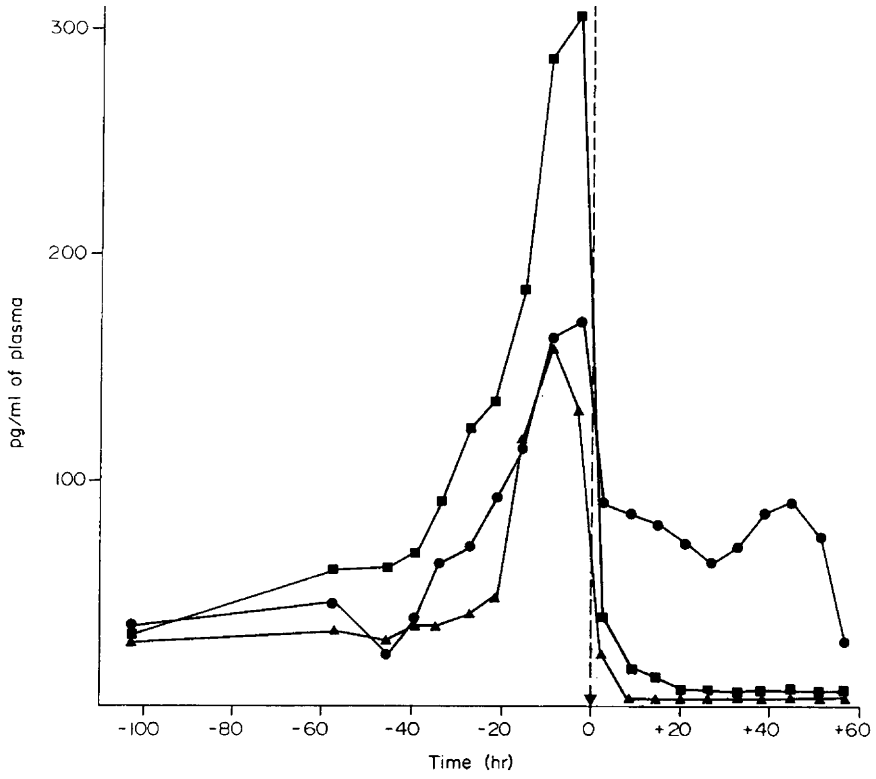


TEXT-FIG. 1. Changes in the maternal jugular venous plasma concentration of unconjugated oestrone (■), oestradiol-17α (●) and oestradiol-17β (▲) in relation to the time of parturition (0 hr), in ewes (n = 4) carrying a single lamb.

It should be noted that the group of ewes which had twin lambs was equally weighted as far as the sex of the lambs was concerned, whereas the group having single lambs was heavily weighted in favour of male lambs.

Plasma oestrogen values

The changes in the concentration of unconjugated oestrone, oestradiol-17α and oestradiol-17β in the pooled plasma samples from each group, over the period 108 hr before, to 60 hr after, parturition are shown in Text-figs 1 and 2.



TEXT-FIG. 2. Changes in the maternal jugular venous plasma concentration of unconjugated oestrone (■), oestradiol-17α (●) and oestradiol-17β (▲) in relation to the time of parturition (0 hr), in ewes (n = 4) carrying twin lambs.

Measurable concentrations of unconjugated oestrone, oestradiol-17α and oestradiol-17β could be detected 108 hr before parturition. In both groups, a rapid increase in the concentration of all the oestrogens was discernible, commencing about 40 hr before parturition and reaching a maximum around the time of parturition. At peak concentration, the mean levels of oestradiol-17α and of oestradiol-17β were very similar and lay within the range of 100 to 150 pg/ml, whereas the concentration of oestrone (225 to 300 pg/ml) was approximately double that of the individual oestradiols. After parturition, there was a rapid fall in the concentration of unconjugated oestrone and oestradiol-17β, to

<15 pg/ml by 10 hr *post partum*. Compared to oestrone and oestradiol-17β, the decline in the concentration of oestradiol-17α was less rapid. Nevertheless, the concentration had dropped to <35 pg/ml by 45 hr *post partum* in the 'single lamb' group and by 60 hr *post partum* in the 'twin lamb' group. This apparently anomalous behaviour of oestradiol-17α will be discussed later.

Table 2 presents the oestrogen concentrations in the plasma samples of individual ewes from each group taken during the period 12 to 7 hr before

Table 2. The concentration of unconjugated oestrogens in individual jugular venous plasma samples of pregnant ewes taken 12 to 7 hr before parturition

Ewes	*Sex of lamb*	*Concentration of oestrogen (pg/ml) (duplicate samples and mean)*		
		Oestrone	*Oestradiol-17α*	*Oestradiol-17β*
'Single lamb' group				
B21	M	275, 258 → 267	98, 110 → 104	81, 89 → 85
R35	M	133, 130 → 132	71, 74 → 72	56, 55 → 56
Y39	F	167, 161 → 164	76, 75 → 76	72, 88 → 78
Y40	M	100, 91 → 96	77, 62 → 70	86, 79 → 83
Group mean ± S.D. Estimate on pooled sample (Text-fig. 1)		165 ± 74 169	80 ± 16 73	76 ± 13 71
'Twin lamb' group				
B34	F,F	313, 338 → 326	180, 173 → 176	110, 90 → 100
G34	M,M	514, 469 → 492	217, 221 → 219	137, 147 → 142
G35	M,F	140, 115 → 128	88, 72 → 80	103, 74 → 87
R36	M,F	230, 265 → 248	183, 183 → 183	214, 171 → 193
Group mean ± S.D. Estimate on pooled sample (Text-fig. 2)		298 ± 154 294	165 ± 59 168	131 ± 48 143

parturition. Considerable variation existed between and within the animals of each group.

If the concentration of individual oestrogens is compared between the groups, the concentration of oestradiol-17β is seen to be marginally significantly higher ($P<0{\cdot}05$) in the 'twin lamb' group. The between-group differences for oestrone and oestradiol-17α are not significant.

The effect of sex, weight and number of the fetuses

It is not possible from the data available in Table 2 to determine whether plasma unconjugated oestrogen levels are influenced by the number of conceptuses or the sex of the fetus(es).

DISCUSSION

It is clear from the results that a dramatic increase in the concentration of unconjugated oestrone, oestradiol-17α and oestradiol-17β occurs in the jugular venous plasma of the pregnant ewe beginning at about 40 hr before parturition and reaching a peak at parturition. The concentrations of oestradiol-17α and oestradiol-17β are very similar and are approximately half that of the concentration of oestrone. After parturition, the concentrations of oestrone and oestradiol-17β have dropped to <15 pg/ml by 10 hr *post partum*. The drop in the concentration of oestradiol-17α is not so precipitous, taking approximately 45 hr to reach <35 pg/ml in the case of the 'single lamb' group and 60 hr in the case of the 'twin lamb' group.

The data of Challis (1971) on the change in the level of total unconjugated oestrogens, of Thorburn *et al.* (1972) on unconjugated oestradiol-17β and of Bedford, Challis, Harrison & Heap (1972) on unconjugated oestradiol-17β and oestrone in the blood of the pregnant ewe just before parturition are in agreement with the present findings. Challis, Harrison & Heap (1971) and Bedford *et al.* (1972) have demonstrated that the probable source of those unconjugated oestrogens at this time was the conceptus. In the goat, Challis & Linzell (1971) found higher levels of total unconjugated oestrogens and, as a consequence, were able to demonstrate a gradual rise in plasma oestrogen levels right through pregnancy culminating in a sharper rise over the last week. Thorburn *et al.* (1972) reported an increase in unconjugated oestrone and oestradiol-17β in the maternal jugular venous plasma of goats over the last 2 days of pregnancy and were able to relate this to an increase in the difference between the uterine arterial and venous concentrations. The sensitivity of the method used in the present investigation precludes testing for the presence, or otherwise, of a steady rise in maternal plasma oestrogen level throughout pregnancy in the ewe. In the cow, Robinson, Baker, Anastassiadis & Common (1970) reported a marked rise in the concentration of total oestrone (conjugated and unconjugated) during the last 30 to 14 days before parturition while Robertson (1974) found a rise in the concentration of unconjugated oestrone, oestradiol-17α and oestradiol-17β in the maternal plasma over the last 20 days of pregnancy. In the rat, a sharp rise in the ovarian secretion of oestrogens occurs just before parturition (Yoshinaga, Hawkins & Stocker, 1969) but in the other species studied, i.e. woman (Roy & MacKay, 1962) and the guinea-pig (Challis, Heap & Illingworth, 1971), where a prolonged rise in oestrogen levels during pregnancy was found, it is still uncertain whether this rate of increase accelerates immediately before parturition.

Normally, ewes will ingest the placental membranes at parturition and will imbibe some of the amniotic fluid through licking the lamb. It is possible that the more gradual decline in the level of oestradiol-17α observed after parturition, at a time when the concentrations of oestrone and oestradiol-17β are undetectable, may be due to a steady absorption of this oestrogen or a precursor oestrogen from the ingested membranes. Robertson (1974) did not observe a prolonged elevation in oestradiol-17α in cows where the placental membranes were not ingested. Challis & Linzell (1971) specifically refer to the ingestion of

the shed placental membranes by one of their goats but noted that, 24 hr after parturition, unconjugated oestrogens could not be detected in jugular venous plasma. However, in the method used by these workers for measuring total unconjugated oestrogens, only oestrone or oestradiol-17β was used for the construction of standard curves. It is unlikely that this method would have been sufficiently sensitive to demonstrate a prolonged elevation of oestradiol-17α because oestradiol-17α competes poorly with these two oestrogens for antibody-binding sites (approximately 30% affinity).

The relationship between the maternal plasma levels of cortisol, progesterone, oestrogens and the initiation of parturition has been reviewed by Liggins, Grieves, Kendall & Knox (1972), Thorburn *et al.* (1972) and by Bedford *et al.* (1972).

The relevance of oestrogens to the development of the mammary gland during gestation should be considered. This general area has been reviewed by Cowie (1971) and the general finding from experiments carried out on cows and on goats is that a combination of progesterone and oestrogen would appear to yield the best stimulation in the non-pregnant animal. In the pregnant ewe, it is difficult to postulate a rôle for the oestrogens in mammary growth unless (a) the mammary gland has the ability to concentrate these steroids in the cells, (b) the cells are sensitive to concentrations of oestrogens at levels of <10 pg/ml or (c) the influence of oestrogens is only extended during the last 2 days of pregnancy. The precipitous rise in unconjugated oestrogen concentration in the plasma of the ewe would seem to be more closely linked to the initiation of lactogenesis which occurs within less than 72 hr of parturition.

The events occurring in late gestation in the ewe, i.e. a period of preconditioning to an elevated level of progesterone followed by a decrease in progesterone concentration at the time of the oestrogen surge, mimic the conditions which induce behavioural oestrus during the normal oestrous cycle. They may, therefore, explain the phenomenon of *post-partum* behavioural oestrus which is observed in some ewes at parturition.

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