Correlation of rate of uterine blood flow and plasma steroid concentrations at parturition in sheep

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Summary. Blood flow transducers were placed around one uterine artery per ewe and observations of the rate of blood flow through this artery and the peripheral plasma concentrations of free, unconjugated oestrone, oestradiol and progestagens were made at intervals of 1–3 days during the last 2–3 weeks of pregnancy and at hourly intervals after spontaneous delivery at term. Rates of uterine blood flow decreased on average by 50% or more within the first hours of parturition and patterns of change in blood flow varied considerably among the animals. Spontaneous daily (*pre partum*) and hourly (*post partum*) changes in uterine blood flow were significantly correlated ($P < 0.01$) with the product of the concentrations of progestagens and of total free oestrogens (oestrone and oestradiol) and with the product of progestagens and oestradiol in peripheral blood.

Introduction

Two lines of evidence suggest that steroid hormones are involved in the vascular changes within the uterus during pregnancy. First, certain patterns of change both in vascular morphology and in rate of blood flow to reproductive tissues occur at specific times during pregnancy and ovarian cycles; the times and patterns of these changes correspond to endocrine changes (Markee, 1940; Phelps, 1946; Huckabee, Crenshaw, Curet & Barron, 1968, 1972; Greiss, 1971). Second, administration of steroid hormones in appropriate amounts and combinations can induce vascular changes which resemble natural vascular phenomena (Williams, 1948; Dickson, Bosc & Locatelli, 1969; Greiss & Anderson, 1969; Caton, Abrams, Clapp & Barron, 1974a; Caton, Abrams, Lackore, James & Barron, 1974b). Both types of data support the idea that steroid hormones may elicit change in the uterine vasculature. However, the data do not establish which of the endogenous hormones affect or to what degree each may be involved in variation of the rate of uterine blood flow among and within normal animals during the course of pregnancy.

Caton, Lackore, Thatcher & Barron (1976) compared changes in rate of uterine blood flow to changes in concentrations of steroid hormones in maternal peripheral plasma of near-term sheep after killing the fetuses *in utero*. Blood flow and hormone concentrations fell rapidly and, although the decrease in flow tended to precede that in hormones, the variables appeared to be related. However, because of the circumstances of the experiment and the limitations of the methods, it was not possible to say whether the variables changed in proportion to each other.

Our present experiment was undertaken to study variation in the rate of uterine blood flow within and amongst normal sheep and to determine whether the variation is associated with changes in concentrations of endogenous hormones in peripheral blood. The studies involved term animals because the changes in uterine blood flow and in hormone concentrations are so great at that time.

Materials and Methods

Animals and measurements

The observations were of 10 sheep of mixed breed who had conceived at known times. The sheep were brought from the breeding barn to the laboratory on Days 115–130 of pregnancy and were housed outdoors in covered 3 × 3 m pens with free access to grain, hay and water. Each day, the sheep were weighed, transferred to a 2 × 0.5 m wheeled cart, brought indoors and left for 1–3 h to become accustomed to the laboratory and activities in it. Once it was established that they were eating and their weight was stable or increasing, they were prepared for surgery.

The sheep were given water but no food for 24 h before surgery. Anaesthesia was induced and maintained by administration, first by mask and then by endotracheal tube, of O₂ and methoxyflurane (Metafane: Pitman-Moore). A 15–20 cm midline abdominal incision was made. The uterine horn selected was that which appeared, by palpation, to contain the most fetal and placental tissue. A 2–3 cm segment of the artery of this horn, just proximal to its first division in the broad ligament, was infiltrated with local anaesthetic to prevent spasm and then was dissected from the peritoneum and adventitia. A flow transducer (C and C Instruments, Inc.) was selected which fitted snugly around the vessel without occluding it (4.5–6 mm diam.). It was sutured in position and the peritoneum was closed over it. A polyvinyl catheter was placed in a femoral artery through a separate incision. The free ends of the catheter and the transducer cable were brought subcutaneously to a pouch fixed to the skin of the flank. Details of the placement and management of catheters in this way have been given elsewhere (Meschia, Wolkoff & Barron, 1959).

Mean and pulsatile rates of uterine blood flow were measured with a NARCO RT 500 electromagnetic flow meter and were recorded continuously with electronic apparatus (NARCO Physiograph DPM 4-55). During measurements, animals were awake and standing or lying unrestrained in the wheeled cart. Flow transducers were calibrated *in vitro* before and after use in each ewe. In 3 ewes, flow transducers broke or became detached from the blood vessel before parturition.

Several factors not directly related to the endocrine milieu were associated with transitory changes in rate of uterine blood flow. Their effects were minimized by management of animals and collection of data as follows. (1) Rates of uterine blood flow were uniformly low each day when animals were first brought into the laboratory, but rose during the next 30 min. Only after flow had risen and stabilized was it considered representative. (2) Rate of flow changed abruptly with any loud noise or unusual activity in the laboratory. The times of such disturbances were recorded and values taken then were not used to calculate rates for that day. (3) In most ewes, rate of flow tended to be higher in the late afternoon or evening than in the morning, presumably a diurnal effect (Lanz, Caton, Schlereth & Barron, 1977; Walker *et al.*, 1977). For consistency, only data obtained between 08:00 and 11:00 h were used to calculate flow for that day. (4) Minor fluctuations in blood flow appeared to be related to uterine contractions and to changes in body position (Lanz *et al.*, 1977). To minimize these effects, 20 consecutive values measured at 1-min intervals were averaged to obtain a figure representative for that day and time period.

Animals were allowed at least 4 days to recover from surgery before measurements of uterine blood flow and withdrawal of blood samples were taken at 1- to 3-day intervals. Arterial blood samples were withdrawn daily for 20 min at a constant rate while blood flow was monitored. Samples were centrifuged as soon as possible thereafter; plasma was separated and stored at –20°C until steroid analysis.

Imminent parturition was identified by enlargement of the udder which became pink and warm to touch, distended teats, oedematous vulva which changed from a pale pink to a violet-like colour, a mucoid discharge from the vagina and the more sedentary behaviour of the animals. In some ewes, there was a change in the rate and pattern of variation of uterine blood flow (see below). When one or more of these signs appeared, the ewes were watched more

closely and flow was monitored for longer than the customary 3 h. An observer was present with 8 of the ewes to note the exact time of delivery of lambs and placentas. Birth had probably occurred within 1 h of arrival of the observer for 2 ewes because their lambs were not yet standing and had not been cleaned of amniotic fluid, there was a palpable pulse in the stump of the umbilical cord and the placenta had not been passed.

Radioimmunoassay of steroids

Oestrone and oestradiol. Plasma (3–4 ml) was extracted twice with 2 volumes of anhydrous diethyl ether. Oestrone and oestradiol were isolated from the dried ether by column chromatography (Sephadex LH-20).

Antiserum to oestrone-6-carboxymethyloxime conjugated to bovine serum albumin was prepared in rabbits. After correction for endogenous plasma levels, mean $\pm$ s.d. recovery of 20, 50, and 100 pg oestrone added to ewe plasma was 24.1 ± 1.9 , 51.6 ± 3.6 and 99.9 ± 5.9 pg, respectively (8 samples each). The sensitivity was 5 pg oestrone and the inter- and intra-assay coefficients of variation were 14.4 and 4.7% respectively.

Antiserum to 6-keto-oestradiol conjugated to bovine serum albumin was obtained from Dr G. D. Niswender, Colorado State University. When 20, 50 and 100 pg oestradiol were added to ewe plasma, recovery after correction for endogenous plasma levels was 18 ± 2.3 , 53.5 ± 3.5 and 100.6 ± 10.4 pg/ml, respectively (4 samples each). The sensitivity of the assay was 10 pg and the inter- and intra-assay coefficients of variation were 15.5 and 6.2%, respectively.

Progestagens. Progestagens were measured after extraction of 0.2–0.5 ml plasma with 2,2,4-trimethylpentane (iso-octane) without further chromatographic purification. Antiserum to 11 α -hydroxyprogesterone hemisuccinate conjugated to bovine serum albumin was a gift of Dr J. L. Fleeger, Texas A & M University. This antiserum cross-reacts with other progestagens, principally 5 α -pregnane 3,20-dione (approximately 20%) and 20 α - and 20 β -dihydroprogesterone (5%). Addition of 100, 300 and 500 pg progesterone to ewe plasma resulted in recovery of 103.6 ± 6.2 , 308.5 ± 16.7 and 531 ± 31.0 pg, respectively (6 samples each) after correction for endogenous levels. Sensitivity of the assay was 20 pg and inter- and intra-assay coefficients of variation were 12.6 and 4.8%, respectively.

Results

All animals delivered spontaneously within 2–3 days of their expected dates of parturition. Nine of the 10 ewes produced live lambs whose birthweights are given in Table 1. All looked healthy; they nursed and gained weight during the ensuing 10 days that they were observed.

Table 1. Summary of observations and birthweight of animals studied

Ewe	No. of days observed	Observations		Lamb birthweight (g)
		<i>Pre partum</i>	<i>Post partum</i>	
788	18	12	12	3390
35	30	10	8	2792, 2076 (twins)
28	4	3	9	Not recorded
E19	3	2	10	3110
27	11	10	10	2962
160	25	21	11	3208
742	11	11	4	2810
81	9	8	6	2778 (stillborn)
707	16	10	0	4435
189	18	12	0	3223

As variation among and within individual ewes was great, data for each ewe are given in Text-fig. 1. More than 150 sets of observations of the 10 ewes were made for periods as long as 30 days. Most data, however, were obtained during the last 2 weeks of pregnancy. Statistical analyses were made only on the 100 sets of observations which were complete (Table 1), i.e., those sets for which there were valid measurements for the rate of uterine blood flow and for peripheral concentrations of each of the 3 steroid hormones at the same time.

Blood flow

There appeared to be 3 patterns of change in rate of uterine blood flow during the last 2 weeks of pregnancy (see Text-figs 1a, 1b and 1c). In the first pattern (Text-fig. 1a), there was a steady increase throughout the period, often with a secondary, abrupt rise during the last few days before parturition. In one ewe, the highest rate was measured during the early post-partum period. In the second pattern (Text-fig. 1b), the rate of flow showed little change until after parturition. Ewe E19 was included here although she may have been observed for too short a time to detect a pre-partum rise in uterine blood flow. The rate of flow in Ewe 788 was very high from the start of observations and was as high as the rates observed just before parturition in ewes depicted in Text-fig. 1(a). In the third pattern (Text-fig. 1c), rate of flow tended to fall. Ewe 742 delivered a live, healthy lamb but did not allow it to nurse (it was raised by bottle and thrived). The lamb of Ewe 81 was of normal size (2800 g) but stillborn. The conditions of its tissues suggested it had died within 24 h of delivery.

In all the ewes, the rate of blood flow fell rapidly after parturition and reached, on average, a value of one-third the pre-partum rate within 2 h (Text-fig. 1). After that, changes were less rapid and by 24–72 h, depending on the animal, rates of flow were no longer measurable, presumably because the change in diameter of the uterine artery distorted its relationship to the lumen of the flow transducer. In 3 ewes, there was a secondary, transient increase in uterine blood flow between the 2nd and 4th h *post partum*. The increase occurred after spontaneous delivery of the placenta and was not associated with suckling or with any discernible change in the animals' condition.

Hormone concentrations

Data for individual ewes are shown in Text-fig. 1. In 3 sheep, there was a transient rise of oestrone, of oestradiol or of both hormones between the 10th and 15th days *pre partum*. Concentrations of progestagens tended to fall gradually during the last days of pregnancy. Concentrations of oestradiol and oestrone increased within hours of parturition, oestradiol rising before and oestrone after the birth. Oestrogen levels fell rapidly *post partum*, although in 3 ewes (Text-fig. 1), there was a secondary, transient rise of one or more hormones between the 2nd and 4th h. This rise, at least 1 h after spontaneous delivery of the placenta, was not associated with any obvious external change but appeared to coincide with the secondary increase in rate of uterine blood flow described above.

Correlations

Analysis of variance revealed statistically significant differences among ewes for uterine blood flow, and for concentrations of all three hormones as shown in Table 2. Significant trends with time ($P < 0.01$) were detected for all responses with the exception of the sum of concentrations of oestrone and oestradiol ($P < 0.20$).

Text-fig. 1. Data from individual ewes: (a) those ewes in which uterine blood flow increased; (b) those in which uterine blood flow tended to be stable; and (c) those in which uterine blood flow tended to decrease during the last days of gestation. P = parturition.

Table 2. Correlations between uterine blood flow and steroid hormones alone and in combinations

Hormones	Correlations†			
	Gross	Partial	<i>Pre partum</i>	<i>Post partum</i>
Oestrone	0.291*	0.145	0.202	0.060
Oestradiol	0.085	0.044	0.246	-0.073
Oestrone + oestradiol	0.200	0.113	0.308	-0.011
Progestagen	0.444**	0.212	0.346	0.185
Oestrone/progestagen	-0.215	-0.166	-0.170	-0.083
Oestradiol/progestagen	-0.285*	-0.185	-0.156	-0.149
Oestradiol + oestrone/progestagen	-0.258*	-0.204	-0.172	-0.105
Oestrone × progestagen	0.600**	-0.204	-0.172	-0.105
Oestradiol × progestagen	0.275*	0.440**	0.464**	0.725**
(Oestradiol + oestrone) progestagen	0.542**	0.339**	0.595**	0.460*
Log ₁₀ (oestradiol + oestrone) progestagen	0.622**	0.509**	0.621**	0.590**
Oestradiol + oestrone + progestagen	0.272*	0.202	0.152	0.051

* $P < 0.05$; ** $P < 0.01$.

† See text for explanation.

Discussion

These data appear to be the most extensive to date of rate of uterine blood flow during the days before and hours after spontaneous parturition. In spite of efforts to avoid collection and incorporation of stress-related data, there was considerable variation amongst ewes ($P < 0.01$). The amount of variation confirmed previous observations of pregnant sheep in which uterine blood flow was estimated by a diffusion equilibrium method that expressed rate in ml/min per kg tissue nourished (Caton, Crenshaw, Wilcox & Barron, 1979). In the present study, rate of blood flow varied by day of pregnancy ($P < 0.01$) and showed a general tendency to increase, a finding which was anticipated since the total mass of tissue contained within the uterus also tends to increase. But this pattern, too, was subject to variation. In almost every animal, there were one or more days of pregnancy during which the blood flow was lower than on days either before or after. During the last days of pregnancy, another variation in patterns occurred—a large increase in uterine blood flow in 3 ewes, no change in 2 and decreased flow in the other 2 ewes.

The fall in rate of uterine blood flow *post partum* was almost as rapid as that observed when fetuses of near-term sheep were killed *in utero* (Caton *et al.*, 1976) and was more rapid than that observed in a single post-partum bitch (Assali, Dasgupta, Kolin & Holms, 1958). The transient rise of oestrogen concentrations between the 10th and 15th days *pre partum* usually lasted only 1 day, but the concentrations at that time sometimes equalled or exceeded concentrations measured at parturition. The transient rise in concentration in 3 ewes of one or more steroids between the 2nd and 4th h *post partum* occurred after expulsion of the placenta and the source of the hormone at that time is therefore problematic. We also have no explanation for the difference in timing of the oestrogen peaks, oestradiol before and oestrone after parturition.

We examined the data to ascertain whether spontaneous changes in concentrations of steroid hormones in the peripheral blood could account for normal variation in the rate of blood flow through one major uterine artery. The gross correlations shown in Table 2 represent simple correlations between measurements expressed in several ways; the fact that measurements on a number of ewes were repeated over a period of time was disregarded. The partial correlations were from data adjusted statistically for systematic animal differences and for trends with time. In all but one comparison (uterine blood flow against oestradiol × progestagen concentration), the partial correlation was closer to zero than the gross correlation, and for only one comparison (uterine blood flow against oestrone × progestagen) did the sign of the two coefficients differ.

These relationships can be seen in Text-figs 1(a), 1(b) and 1(c). No concentration of any one hormone was consistently related to uterine blood flow nor were different ratios related to uterine blood flow. The last point was of special interest since the ratios of hormone concentrations appear to affect uterine vascular phenomena in other circumstances (Huckabee *et al.*, 1968; Caton *et al.*, 1974a, b).

The rate of uterine blood flow, however, was closely and consistently related to the product of oestradiol and progestagen concentration, to the product of total free oestrogens (oestrone and oestradiol) and progestagens or to the logarithm of this product (Table 2). Considering the nature of the changes between late pregnancy and the early post-partum period, we thought that the gross and partial correlations obtained might be forced, i.e. were statistically related only because both variables underwent such large and rapid changes at the time of parturition. To evaluate this possibility, data were re-analysed with respect to those obtained up to, but not including, the day of parturition (Text-fig. 2) and those obtained on the day of parturition and afterwards (Text-fig. 3). The results given in Table 2 show that uterine blood flow was still closely related to the product of oestradiol and progestagen, of oestrogens and progestagen and of the logarithm of this product.

Text-fig. 2. Relationship between uterine blood flow and log (oestrone + oestradiol) × progestagen on days up to, but not including, the day of parturition.

The fact that concentrations of oestrogens and of progestagens are expressed in different units (pg and ng, respectively) may give rise to questions concerning the validity of using their product as a variable. There is a precedent for this as these same proportions appear to be important when exogenous hormones are used to affect rate of uterine blood flow (Caton *et al.*, 1974a) or to maintain pregnancy in sheep ovariectomized early in gestation (Lyons, 1943; Foote, Gooch, Pope & Casida, 1957).

The observations suggest each of the 3 hormones studied have some effect on rate of uterine blood flow. This is in agreement with earlier work. Virtually all oestrogens are able to affect either vascular morphology or rate of blood flow, even though there are minor differences in potency among compounds (Caton *et al.*, 1974a, b; Resnick, Killam, Battaglia, Makowski & Meschia, 1974). Also, oestrogens and progestagens appear to work synergistically. Giving progesterone to ovariectomized ewes already receiving an oestrogen (Greiss & Anderson, 1969) induces further increase in blood flow (ml/min). Withdrawal of progestagens or oestrogens from a combined regimen of hormone therapy apparently causes constriction of small blood vessels

Text-fig. 3. Relationships of uterine blood flow and log (oestrone + oestradiol) $\times$ progestagen in 5 ewes on the day of parturition and 2 days thereafter.

and hence decreased blood flow (Markee, 1940). This might explain why the product of concentrations of oestrogen and progestagen was closely related to spontaneous changes in uterine blood flow whereas the product of concentrations of individual hormones was not.

The present data do not detract from the importance of other factors which may affect uterine blood flow. Excitement or stress, as when ewes were first brought into the laboratory each morning, changes in blood gases, pH or systemic arterial pressure may all alter the rate of flow. Similarly, the magnitude of the response to each of these stimuli as well as the response of the uterine vasculature to steroid hormones may vary with age or parity. We did not measure these things and any or all of them may account for some of the variability, depicted in Text-figs 2 and 3, from one ewe to the next and from one day to the next in the same animal.

However, our observations confirm and extend earlier work relating oestrogens and progestagens to vascular phenomena in the reproductive tract. A quantitative relationship between them is shown to exist. Endogenous hormones, as measured and expressed in these experiments, did account for a statistically significant amount of the variation in the rate of flow.

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