FETAL–MATERNAL CORTICOSTEROID RELATIONSHIPS IN SHEEP DURING LATE PREGNANCY

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Summary. Plasma corticosteroids were measured by competitive protein-binding techniques in the jugular and uterine venous plasma of parturient sheep. Plasma corticosteroids began to rise 48 to 72 hr *ante partum* in control ewes and were significantly elevated at parturition in both the control and ovariectomized ewes. Corticosteroid levels in adrenalectomized and adrenalectomized–ovariectomized ewes were markedly lower than in control ewes. Daily corticosteroid levels were frequently higher in the uterine plasma compared to the jugular plasma in all treatment groups. In sheep, the corticosteroid levels in immediate samples, however, were usually slightly greater in the uterine artery before the lambs were removed compared to those in the uterine vein. This concentration difference existed even when the plasma corticosteroid levels were significantly elevated in the umbilical circulation late in gestation when levels in the umbilical artery were slightly greater than those in the umbilical vein. It was concluded that fetal plasma corticosteroids are not transferred significantly to the maternal plasma in sheep during late pregnancy.

INTRODUCTION

Much recent work indicates that the fetal lamb's adrenal is related to the timing of parturition (Liggins, Grieves, Kendall & Knox, 1972). Corticosteroid levels in blood samples from indwelling cannulae rose markedly several days *ante partum* in fetal lambs with little relationship between fetal and maternal values (Bassett & Thorburn, 1969). Maternal corticosteroid levels in ewes began to rise approximately 2 days before parturition, but were considerably less than those in the fetuses (Drost, Kumagai & Guzman, 1973).

Paterson & Harrison (1967) did not find evidence that fetal cortisol was quantitatively transferred to the ewe while using isotope dilution techniques to investigate cortisol secretion and metabolism in pregnant ewes. Other investigations concerning transplacental passage of cortisol in sheep, however, have reported results to the contrary. In ewes 134- to 140-days pregnant, a selective resistance to placental passage of cortisol was found, with fetal cortisol

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more readily transferred to the mother than the reverse (Dixon & co-authors, 1970). Recently, the plasma cortisol concentration was reported to rise at parturition in an adrenalectomized ewe (Harrison, Heap & Paterson, 1972). Although the increased fetal corticosteroids may act to promote uterine prostaglandin synthesis (Liggins & Grieves, 1971), the question of placental corticosteroid transfer is of considerable importance and is further examined in this report.

MATERIALS AND METHODS

Animals

Western crossbred ewes with known breeding dates were randomly assigned to one of four treatment groups. There were seven ewes in the intact control group, six in the adrenalectomy group (AD) and four ewes each in the ovariectomy (OV) and adrenalectomy-ovariectomy (AD-OV) groups. Other ewes were used for immediate and long-term fetal blood sampling.

Surgery

Adrenalectomies were performed under sodium pentobarbital anaesthesia on approximately Day 90 of gestation. Before operation, the animals were given 2.0 mg dexamethasone. Both adrenals were removed in a two-step procedure during the same operation which involved bilateral lumbar incisions. The approach to the adrenals was retroperitoneal. Following surgery, the animals were given 5.0 mg deoxycorticosterone acetate and 25.0 mg cortisone acetate. Later, during blood sampling, this dosage was reduced to 2.5 mg deoxycorticosterone acetate and 12.0 mg cortisone acetate daily.

The completeness of the adrenalectomy was ensured by discontinuing replacement therapy several days *post partum* which resulted in severe weakness. In addition, a necropsy was conducted and suspicious tissue was fixed for histopathological examination.

On approximately Day 132 of gestation, the designated ewes were ovariectomized and uterine and jugular venous cannulae were inserted. A uterine vessel was cannulated according to the method described by Meschia, Makowski & Battaglia (1970). An 18-cm portion of Silastic (Dow Corning, Midland, Mich.) tubing (i.d. 1.016 mm, o.d. 2.032 mm) was positioned in the vein. After a cannula was secured in place, it was filled with heparinized saline (1000 U/ml), closed, and fixed to the skin under tape. The ewes were housed in a heated barn and fed a standard hay and grain ration.

Blood samples were collected daily between 08.00 and 09.30 hours. The blood was chilled and centrifuged and the plasma was harvested and stored at -20°C until assay.

Fetal sampling

Blood samples were obtained with syringe and needle from the umbilical artery and vein of fetal lambs in six ewes. In addition, the uterine artery and vein were cannulated in these ewes. Blood samples were obtained from the uterine vessels while the fetuses were *in utero* and at 1, 4, 8 and 24 hr after

removing the lambs. Vinyl tubing (i.d. 0.762 mm, o.d. 1.168 mm) was used to cannulate the uterine artery and the uterine vein was cannulated as previously described.

The fetal tarsal vein was cannulated in one fetus that went to term. A 20-cm portion of Silastic tubing (i.d. 0.762 mm, o.d. 1.65 mm) was inserted into the vein to the level of the posterior vena cava and then brought to the surface. Blood samples were obtained daily.

Assay method

Plasma samples were assayed for total corticosteroid content by the competitive binding techniques of Murphy (1967) as modified by Whipp & Lyon (1970). The procedure was altered to include hexane pre-extraction to remove progesterone and use of florisil (40 mg) as the adsorbent for separation of bound and free hormone. The endpoint of the assay was the time taken to reach a pre-set total count.

Although the exogenously administered corticosteroids may be measurable in the assay system, we were unable to demonstrate an elevated corticosteroid level in one non-pregnant adrenalectomized-ovariectomized ewe 12 hr after injection. In addition, since blood samples were taken at a specified interval after treatment and cortisol-binding capacity did not increase in ewes during late pregnancy (Paterson & Hills, 1967), the corticosteroid values recorded were comparable during this interval.

Progesterone and oestrogen concentrations in the ewes in the four treatment groups have been reported (Thompson & Wagner, 1974).

Analysis of data

Mean differences were tested for significance according to Student's *t* test as applied to the analysis of variance (Snedecor & Cochran, 1967). Day 0 was the day of parturition. Day-0 samples were defined as the last which were collected before parturition and Day +1 was the first day on which samples were collected after parturition.

RESULTS

Mean uterine plasma corticosteroid levels in intact control ewes varied from a low of 11.3 ng/ml on Day -3 to peak levels of 32.5 and 35.5 ng/ml on Day 0 and Day +1, respectively (Table 1). Jugular plasma corticosteroid values in control ewes varied from 8.7 ng/ml on Day -3 to 35.1 and 28.2 ng/ml on Day 0 and Day +1, respectively. A comparison of mean corticosteroid concentrations for Day -8 to Day -3 and Day +2 versus Day 0 revealed a significant rise in both the uterine ($P < 0.005$) and jugular ($P < 0.01$) plasma. The location of the greater mean corticosteroid level varied daily between the jugular and uterine veins. This difference was greatest in the control ewes on Day -2 with mean concentrations of 27.4 and 17.5 ng/ml in the uterine and jugular veins, respectively.

Plasma corticosteroid levels in AD ewes were markedly lower than those in control ewes (Table 1). Uterine plasma corticosteroid values were generally

slightly greater than jugular values in AD ewes and corticosteroid levels in AD ewes rose slightly in both sites at parturition.

Plasma corticosteroid levels were also markedly lower in AD-OV ewes than those of the controls. In contrast to those in AD ewes, uterine plasma corticosteroid values in AD-OV ewes were slightly greater than jugular levels on only 3 days (-6, -5 and -2) *ante partum*. On Day 0, plasma corticosteroid values in AD-OV ewes increased slightly in the jugular plasma only.

The mean corticosteroid levels (for the entire period) in OV and control ewes were not different. Corticosteroid values in OV ewes were significantly ($P < 0.005$) elevated on Day 0 compared to Day -3. On Day -2, a smaller difference was noted between the mean corticosteroid concentrations (ng/ml $\pm$ S.E.) in the uterine and jugular veins of OV ewes compared to those in control ewes: uterine, 15.2 ± 2.4 (4); jugular 12.7 ± 3.0 (4) (number of observations).

Table 1. Plasma corticosteroids in control and adrenalectomized ewes

Days*	Intact		Adrenalectomized	
	Uterine vein	Jugular vein	Uterine vein	Jugular vein
-8	15.1 ± 2.0 (6)†	15.5 ± 5.8 (6)	3.1 ± 1.0 (6)	2.7 ± 0.8 (6)
-6	13.3 ± 2.3 (6)	11.0 ± 1.8 (6)	4.1 ± 1.0 (6)	3.1 ± 0.8 (5)
-4	18.9 ± 3.6 (6)	17.5 ± 5.2 (7)	4.7 ± 1.0 (6)	3.3 ± 0.7 (6)
-3	11.3 ± 3.6 (5)	8.7 ± 1.9 (7)	4.5 ± 1.1 (6)	3.6 ± 1.0 (6)
-2	27.4 ± 7.2 (6)	17.5 ± 4.3 (7)	3.7 ± 1.0 (6)	2.8 ± 1.1 (6)
-1	25.6 ± 6.7 (7)	27.3 ± 7.2 (7)	4.4 ± 1.5 (5)	2.4 ± 0.9 (6)
0	32.5 ± 9.0 (6)	35.1 ± 6.9 (7)	6.1 ± 1.3 (5)	4.5 ± 0.9 (6)
+1	35.5 ± 10.9 (3)	28.2 ± 8.4 (6)	4.0 ± 2.2 (4)	3.8 ± 1.7 (6)
+2	11.5 ± 0.6 (2)	8.4 ± 2.6 (5)	2.7 ± 1.4 (4)	3.4 ± 0.9 (6)

* Days before (-) and after (+) parturition.

† Means $\pm$ S.E. in ng/ml (number of observations).

A greater corticosteroid concentration was frequently found in the uterine than in the jugular plasma (Table 1), but no concentration difference in any treatment group was noted between these sites after calculation of the mean levels for the entire period.

Uterine arterial and venous plasma corticosteroid levels were measured in six ewes 139- to 146-days pregnant before and after their fetuses were removed (Table 2). A greater but non-significant corticosteroid concentration was present in the artery in five of the six ewes before the lambs were removed. The ewe that was the exception was one of two ewes which was 139-days pregnant. The mean $\pm$ S.E. were 101.2 ± 14.6 and 104.3 ± 14.0 ng corticosteroid/ml plasma in the uterine vein and artery, respectively. After the lambs were removed, an increased frequency of a greater corticosteroid concentration in the uterine vein than in the uterine artery (six of sixteen comparisons) was noted. Again, this difference was not significant. At 24 hr after lamb removal, the mean levels had decreased to 54.1 ng/ml in the artery and 52.2 ng/ml in the vein.

The mean plasma corticosteroid levels in the umbilical circulation were significantly ($P < 0.001$) elevated at Days 142 to 146 of gestation compared to

Day 139 (Table 3). In the four comparisons at Days 142 to 146 of gestation, a greater concentration was present in the umbilical artery than in the umbilical vein, but this difference was not significant. Earlier in gestation, no consistent pattern was noted in the corticosteroid concentrations present in the artery and vein.

Fetal plasma corticosteroid levels were measured in one long-term cannulated fetal lamb for a period of 13 days before birth. Beginning at Day -7, fetal plasma corticosteroids increased to 18.5 ng/ml from levels ranging from 0.2 to 6.4 ng/ml during the period from Day -12 to Day -8. From Day -6 to

Table 2. Plasma corticosteroids in the uterine vein and uterine artery following removal of the fetal lambs

Hr after removal of lambs	Day 139*		Day 142		Day 144		Day 145		Day 146	
	V	A	V	A	V	A	V	A	V	A
0	110.6 126.7	132.1 96.0	59.4	59.8	66.0	85.2	149.0	156.0	95.2	96.7
1	139.0 125.2	165.2	39.7	31.3	92.8	109.8	163.7	166.0	50.6	42.1
4	52.1 78.3	47.5	64.2	50.8	119.8	162.8	66.0	78.3	42.1	20.6
8	90.6 119.8		83.8	87.1	45.2	67.8			58.3	63.7
24	72.2 39.9		42.1	30.4	54.6	70.8			53.0	6.11

Values are expressed as ng corticosteroid/ml plasma. V, uterine vein; A, uterine artery.

* Gestational age (two ewes on Day 139).

Table 3. Plasma corticosteroids in the umbilical artery and vein of ewes

	Gestational age (days)							
	139		142		144		145	
Vein	7.5 ^a	25.2 ^b	23.7 ^a	38.2 ^b	97.5	164.2	159.0	148.3
Artery	14.4 ^a	19.9 ^b	22.9 ^a	43.9 ^b	135.8	166.2	174.4	152.1

Values expressed in ng/ml plasma.

^{a, b} Twin fetuses.

Day -2, levels ranged from 22.9 to 47.9 ng/ml. A blood sample collected within 3 hr of parturition had a plasma corticosteroid level of 157.6 ng/ml. Thus the level of 91.6 ng/ml found on Day -1 probably was representative of an increase that began as early as 48 hr *ante partum*.

Maternal adrenalectomy did not affect gestation length (145.8 days for control and 146.2 days for AD ewes) and all AD ewes gave birth to live lambs.

DISCUSSION

Plasma corticosteroid levels have been reported to rise progressively throughout gestation in women (Rosenthal, Slaunwhite & Sandberg, 1969). This increase

has been attributed to an increased protein binding of corticosteroids which itself was secondary to an increased oestrogen level. In sheep, however, the cortisol-binding capacity does not increase during pregnancy (Paterson & Hills, 1967) and is not affected by oestrogens (Lindner, 1964). The cortisol secretion rate in sheep near term has been reported to remain relatively constant while the metabolic clearance rate increases (Paterson & Harrison, 1967). Although the maternal plasma cortisol concentration has been reported to decrease during the last 2 weeks before lambing, the authors did not examine ewes beyond the 3rd day before parturition.

The decrease in maternal corticosteroid values on Day -3 in this report resembles the slight decrease noted in ewes from Day -10 to Day -2 by Drost *et al.* (1973). The significant elevation of the plasma corticosteroid level at parturition contrasts with the lesser increase reported by Drost *et al.* (1973), but the duration of the increase reported here of 48 to 72 hr *ante partum* is similar to the time span they reported. The corticosteroid increase probably occurred before uterine pressure waves began since changes in uterine activity reportedly began only 12 hr before parturition (Hindson, Schofield, Turner & Wolff, 1965). Thus, any stress of impending parturition would not appear to result from changes in uterine activity.

On the basis of the fetal corticosteroid values reported here and by others (Bassett & Thorburn, 1969; Drost *et al.*, 1973), an apparent fetal-maternal corticosteroid concentration difference in favour of the fetus exists in the ewe during late pregnancy. In this study, the corticosteroid level was frequently greater in the uterine plasma than in the jugular plasma. As a further indication of fetal adrenal activity, a slightly greater corticosteroid level was found in the umbilical artery than in the umbilical vein at Days 144 to 146 of gestation. Associated with these increased fetal levels, Comline, Nathanielsz, Paisey & Silver (1970) reported an eight-fold increase in the cortisol turnover rate 50 hr before delivery. Also, the fetal cortisol production rate was found to rise from 0.9 mg/day at 4 or more days before birth to 9.2 mg/day at Days 2 and 1 before birth (Nathanielsz, Comline, Silver & Paisey, 1972).

The rapid passage of fetal cortisol to the mother in ewes less than 140 days pregnant with a resistance to passage in the opposite direction (Dixon *et al.*, 1970) favours the concept that the fetus may augment the maternal corticosteroid level in parturient sheep. In addition, the cortisol concentration in an adrenalectomized ewe maintained only on deoxycorticosterone acetate rose from 1 µg/l on Day 140 of gestation to 4.4 µg/l at parturition (Harrison *et al.*, 1972). The AD ewes in the study reported here were given 12.0 mg cortisone acetate daily in addition to deoxycorticosterone acetate. Assuming that some cortisone-cortisol interconversion occurred, the higher corticosteroid levels before parturition can at least be partly explained. The small increase of approximately 2 ng/ml found at parturition in the plasma of AD ewes in this study, suggests that the fetus could have been a source of corticosteroids for the mother. This was further suggested by the slightly greater concentration found in the uterine than in the jugular plasma of these AD ewes. This difference was not, however, as apparent in the AD-OV ewes.

Although the daily mean corticosteroid level was frequently greater in the

uterine plasma than in the jugular plasma in each treatment group, the difference in the means calculated for the entire period was either non-existent or negligible. Thorburn & Mattner (1971) found a lower progesterone level in jugular venous plasma than in carotid arterial plasma in parturient sheep. The head, therefore, may extract a portion of the corticosteroid level and the jugular uterine difference may simply be a manifestation of metabolic differences.

The fact that a slightly greater corticosteroid concentration was found in jugular plasma on Day -1 and Day 0 of the control ewes, however, is evidence that the fetus was not a significant source of maternal corticosteroids. The same conclusion can be drawn from the greater concentration in the uterine plasma of the control ewes on Day +2.

The marked difference between the corticosteroid levels in the uterine plasma and the jugular plasma of the control ewes at Day -2 was not present in the OV ewes. It seems probable that the ovary did not contribute significantly to this difference since the corticosteroid levels in OV ewes at parturition were equal to those in the control ewes. The concentration difference in the sampling sites in control ewes on Day -2 could perhaps be better explained by an increased secretion between the collection of uterine and jugular samples.

Information about corticosteroid concentrations in the complete uterine circulation was obtained from immediate samples taken from ewes. The fact that corticoid levels were the same or slightly higher in the uterine artery than the uterine vein at a time when the fetal levels were elevated suggests that transplacental passage of fetal corticosteroids in sheep must be quantitatively small even in late gestation. Therefore the increased maternal corticosteroid levels at parturition must be largely the result of an increased maternal adrenal secretion between Day -3 and parturition rather than an augmentation from the fetus.

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