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Feto-placental steroids and parturition in the ewe

by

Frederick Nimrod Thompson, Jr.

A Dissertation Submitted to the

Graduate Faculty in Partial Fulfillment of

The Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Department: Veterinary Physiology and Pharmacology
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For the Graduate College

Iowa State University
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1973

TABLE OF CONTENTS

Page

I. INTRODUCTION 1

II. REVIEW OF THE LITERATURE 3

A. The Adrenal Gland and Parturition 3

1. Naturally occurring variations in gestation length 3
2. Experimental means of altering gestation length 5
3. Corticosteroids in pregnancy 8
4. Effects of corticosteroids on the pregnant myometrium 18

B. Progesterone 19

1. Effects of progesterone on the myometrium 19
2. Interaction of glucocorticoids and progesterone 23
3. Plasma progesterone levels, kinetics and metabolism 25

C. Estrogens 31

1. Estrogen synthesis in the pregnant female 31
2. Estrogen levels and metabolism in the late pregnant female 34
3. The action of estrogens on the uterus 38

D. Other Substances Affecting Myometrial Function 41

1. Prostaglandins 41
2. Oxytocin, vasopressin and myometrial activity 43

III. MATERIAL AND METHODS 49

A. General 49

B. Surgery 49

1. Adrenalectomy 49

2. Maternal cannulations and ovariectomy 52
3. Fetal cannulations 54
4. Acute cannulations 57

C. Hormone Assay Procedures 58

1. General 58
2. Corticoid assay 60
3. Progesterone assay 62
4. Estrogen assays 64

D. Statistical Analysis 67

IV. RESULTS 68

A. General 68

B. Corticoids 71

C. Progesterone 81

D. Estrone 95

E. Estradiol 106

F. Acute Cannulations 109

G. Fetal Cannulations 114

V. DISCUSSION 117

A. Corticoids and Parturition 117

B. Progesterone and Parturition 126

C. Estrogens and Parturition 132

D. Interrelationships of Corticoids, Progesterone and Estrogens at Parturition 139

VI. SUMMARY 143

VII. LITERATURE CITED 146

VIII. ACKNOWLEDGMENTS 168

IX. APPENDIX 169

LIST OF TABLES

		Page
Table 1.	Plasma cortisol levels in sheep and late pregnant women	10
Table 2.	Mean gestation length and number of lambs born	68
Table 3.	Mean steroid levels for the period day -10 through day +2 in the utero-ovarian (UO) and jugular (J) plasma	71
Table 4.	Plasma corticoids (ng/ml) in intact and adrenalectomized (AD) ewes	75
Table 5.	Plasma corticoids (ng/ml) in ovariectomized (OV) and adrenalectomized-ovariectomized (AD-OV) ewes	79
Table 6.	The effects of treatments, days and treatment X day interaction on utero-ovarian (I) and jugular (II) plasma corticoids	80
Table 7.	Jugular plasma progesterone (ng/ml) in ewes	82
Table 8.	Utero-ovarian plasma progesterone (ng/ml) in ewes	83
Table 9.	The effects of treatments, days and treatment X day interaction on utero-ovarian (I) and jugular (II) plasma progesterone	87
Table 10.	Plasma progesterone (ng/ml) in ewes bearing single and multiple lambs	88
Table 11.	Jugular plasma progesterone (ng/ml) in intact and adrenalectomized (AD) ewes	92
Table 12.	Plasma estrone (pg/ml) in intact and adrenalectomized (AD) ewes	96
Table 13.	Correlation coefficients of utero-ovarian (UO) and jugular (J) plasma estrone with plasma corticoids and estradiol	100

Table 14.	The effects of treatments, days and treatment X day interaction on utero-ovarian (I) and jugular (II) plasma estrone	103
Table 15.	Plasma estrone (pg/ml) in ovariectomized (OV) and adrenalectomized-ovariectomized (AD-OV) ewes	104
Table 16.	The mean utero-ovarian progesterone/total estrogen ratio in intact ewes	105
Table 17.	Plasma estradiol (pg/ml) in intact and adrenalectomized (AD) ewes	107
Table 18.	Plasma estradiol (pg/ml) in ovariectomized (OV) and adrenalectomized-ovariectomized (AD-OV) ewes	108
Table 19.	The effects of treatments, days and treatment X day interaction on utero-ovarian (I) and jugular (II) plasma estradiol	109
Table 20.	Plasma corticoids in the utero-ovarian vein (V) and uterine artery (A) following removal of the fetal lambs	110
Table 21.	Mean plasma progesterone levels in the utero-ovarian vein (V) and uterine artery (A) following removal of the fetal lambs	112
Table 22.	Plasma progesterone and corticoids (ng/ml)in umbilical arterial (A) and venous (V) plasma	113
Table 23.	Fetal plasma corticoids and maternal jugular plasma progesterone (ng/ml) in a ewe	115
Table 24.	Fetal and maternal plasma corticoid concentrations (ng/ml)	116

Table A1.	Preparation of protein binding solutions	169
Table A2.	Preparation of scintillation fluids	170
Table A3.	Preparation of celite column	170
Table A4.	Preparation of estrogen assay reagents	171
Table A5.	Preparation of uterine cytosol	171
Table A6.	Gestation length and number of lambs born to ewes in the four treatment groups	172
Table A7.	Mean steroid levels in intact control ewes as used in the analysis of variance	174
Table A8.	Mean steroid levels in adrenalectomized ewes as used in the analysis of variance	175
Table A9.	Mean steroid levels in ovariectomized ewes as used in the analysis of variance	176
Table A10.	Mean steroid levels in adrenalectomized-ovariectomized ewes as used in the analysis of variance	177
Table A11.	Progesterone levels in the utero-ovarian vein (V) and the uterine artery (A) following removal of the fetal lambs	178

LIST OF FIGURES

		Page
Figure 1.	Standard curves in steroid assays	73
	1A. Cortisol (ng/ml) standard curve 1B. Progesterone (ng/ml) standard curve 1C. Estrone (pg/ml) standard curve 1D. Estradiol (pg/ml) standard curve	
Figure 2.	Total plasma corticoids (ng/ml) in the utero-ovarian (UO) and jugular (J) veins of control and adrenalectomized (AD) sheep before and after parturition	77
Figure 3.	Plasma progesterone (ng/ml) in the utero-ovarian (UO) and jugular (J) veins of control sheep before and after parturition	85
Figure 4.	Plasma progesterone (ng/ml), before and after parturition, in the utero-ovarian (UO) and jugular (J) veins of sheep carrying single or multiple fetal lambs	91
Figure 5.	Plasma progesterone (ng/ml) in the jugular vein of control and adrenalectomized (AD) sheep before and after parturition	94
Figure 6.	Plasma estrone (pg/ml) in the utero-ovarian (UO) and jugular (J) veins of control sheep before and after parturition	98
Figure 7.	Plasma estrone (pg/ml) in the utero-ovarian vein of control and adrenalectomized (AD) sheep before and after parturition	102

I. INTRODUCTION

This investigation was undertaken to better define the plasma steroidal changes that occur in the parturient ewe. Although parturition can now be induced in several species with a massive dose of glucocorticoids, the mechanism of action is not understood and retained placenta is an associated problem.

It is felt that the livestock wastage associated with parturition can be reduced and the labor involved in closely watching livestock can be more intensely utilized if the parturition process is scheduled. Consequently, if the physiological events at parturition are elucidated, the basis for a more rational means of parturition induction, regardless of species, would perhaps be possible.

Parturition is a complicated process involving the interrelationship of several factors. It is usually regarded that the progesterone block of the myometrium is withdrawn at or before parturition permitting the released oxytocin to stimulate myometrial activity. The fetal adrenal cortex also appears to be eminently related to the parturition process. The significance and timing of the increased prepartum glucocorticosteroid concentrations in conjunction with the other main steroids, in both the maternal and fetal circulation, is not completely known. Among the parameters to be considered are the significance of the rise in fetal glucocorticoids

to the maternal organism and whether the rise of the gluco-corticoids in the maternal organism is integral to parturition. Identification of those steroid hormone changes that have a causal relationship to parturition is the objective of this study.

II. REVIEW OF THE LITERATURE

A. The Adrenal Gland and Parturition

1. Naturally occurring variations in gestation length

Prolonged gestation due to genetic causes has occurred in the Guernsey and Holstein breeds (Holm, 1967). Postterm Holstein fetuses continued to grow *in utero* but failed to deliver at term. Following delivery by Caesarean section these calves were weak and died in a hypoglycemic crisis (Holm, 1958). Hypoplasia of the pituitary and the adrenal has been reported to be prominent in these calves and treatment with glucocorticoids has been used with some efficacy (Holm *et al*., 1961). In contrast, postterm Guernsey fetuses did not grow *in utero* beyond the size of a seven month fetus and again gestation was prolonged until the fetus died (Kennedy *et al*., 1957). These fetuses appeared to lack a pituitary in all instances. Plasma progesterone levels were maintained in both breeds during the postterm period (Holm and Short, 1962). Uterine insensitivity to oxytocin was also manifested by these animals.

Binns *et al*. (1959) reported a prolonged gestation disease of sheep accompanied by an absence or displacement of the fetal pituitary resulting from the teratogenic effects of *Veratrum californicum* (Binns *et al*., 1963). Fetal adrenals from such postmature lambs converted *in vitro* very little ^{14}C-progesterone into cortisol as compared to the maternal

adrenals (Van Kampen and Ellis, 1972). Ewes fed the South African shrub Salsola tuberculata during the last 50 days of gestation had abnormally long gestations (Basson et al., 1969). Hypophysial and adrenal atrophy were among the most significant features exhibited by these postmature lambs.

Prolonged gestation usually accompanied anencephalic human pregnancies in the absence of hydramnios (Comerford, 1965; Milic and Adamsons, 1969; Turnbull and Anderson, 1969a). Anencephalic infants had small adrenals with a marked reduction of the fetal zone. The more hypoplastic the fetal adrenals appeared the longer was the length of pregnancy (Turnbull and Anderson, 1969b). Termination of anencephalic pregnancy proved difficult as these patients did not readily respond to oxytocin. Likewise, pregnant Addisonian patients maintained on replacement therapy had a mean gestation length of 41 weeks compared to 39 weeks for control women (Osler, 1962). Of the six patients who had to have their pregnancies terminated by induced labor, four had passed term by 8, 18, 21 and 24 days.

Conversely, hyperplasia of the fetal adrenal has been associated with prematurity. Anderson et al. (1971) reported that infants delivered as a result of premature labor had greatly increased adrenal weights as compared to those infants of similar gestational age delivered because of pregnancy complications or therapeutic abortions. Oppenheimer (1969) related cyst formation in the outer adrenal cortex of

the fetus to *in utero* stress and prematurity. Similarly, fetal adrenal hyperplasia with thymic atrophy was a consistent finding in fetuses from habitually aborting Angora goats (Van Rensburg, 1965; Van Rensburg, 1971). These natural diseases have focused attention on the fact that the fetus makes a critical contribution to its delivery.

2. Experimental means of altering gestation length

Electrocoagulation of the pituitary of the fetal lamb resulted in a failure of the initiation of parturition (Liggins *et al*., 1966). Infusion of adrenocorticotropic hormone (ACTH) into one of twin lambs, both with pituitary lesions and beyond term, resulted in parturition on the sixth day of infusion. If more than 70% of the fetal pituitary was destroyed before day 134 of pregnancy, parturition failed to occur (Liggins *et al*., 1967). However, spontaneous delivery followed if the pituitary lesion involved less than 60% of the gland or if the surgery was performed after day 134 of gestation. Pregnancy was prolonged in one ewe with an intact fetal pituitary but there was extensive hypothalamic damage in this case. Destruction of the pituitary of one fetus in the case of multiple pregnancies did not delay the expected time of parturition. Adrenocortical hypoplasia was most prominent among the many signs of impaired trophic influences in the hypophysectomized fetal lamb (Liggins and Kennedy, 1968). Comline *et al*. (1970a) referred to a reduced epinephrine:

norepinephrine ratio found in the adrenal medulla from hypophysectomized fetal lambs. However, Liggins (1969a) reported that in an attempt to ablate the fetal adrenals by formalin injections only the medulla was destroyed and the young were born spontaneously at term.

Complete removal of the adrenal glands from sheep fetuses has resulted in the failure of the ewes to undergo parturition (Drost and Holm, 1968). Pregnancy lasted 157 days or more in eight ewes in which this operation was successfully performed. In two multiple pregnancies where the intact fetus died and the adrenalectomized (AD) fetus remained alive, pregnancy was prolonged. Adrenal blockage by metyrapone administration also prolonged gestation in rats (Parvez _et al._, 1972). ACTH infusions had no effect on a single postmature lamb fetus that had been both hypophysectomized and AD (Liggins _et al._, 1966). Premature parturition occurred when ACTH was infused into single lambs for sufficient duration and in sufficient dosage to cause fetal adrenal weight to reach or exceed that of term lambs (Liggins, 1968). However, neither estradiol-17β nor the other adenohypophyseal hormones alone had this effect. The other adenohypophyseal hormones given in conjunction with ACTH did not shorten the interval from infusion to parturition.

A dose of 25 to 50 mg of cortisol given to the fetal lamb late in gestation was followed by lambing several days later (Van Rensburg, 1967; Halliday and Buttle, 1968; Liggins, 1968). Likewise, infusions of dexamethasone (9-alpha-fluoro-

16-alpha methylprednisolone, DXMS), a potent glucocorticoid, at a dose of 0.05 to 0.1 mg/24 h were sufficient to cause parturition when given to the fetus (Liggins, 1969b). The interval from infusion until parturition was found to be dose dependent, but a minimum of 48 h was necessary to cause parturition even at higher dose levels. The administered DXMS was equivalent to 1.25 to 2.5 mg of cortisol as DXMS has a potency of approximately 25 times that of cortisol (Travis and Sayers, 1965). Since the fetal lamb three days prepartum had a production rate (PR = metabolic clearance rate X plasma concentration) of 2.6 mg cortisol/day (Nathanielsz _et al._, 1972), the administered DXMS was within the physiological range. Infusions of deoxycorticosterone into the fetus were ineffective (Liggins, 1969b). Therefore, the ability of cortisol to cause premature parturition depends upon the glucocorticoid rather than the mineralocorticoid activity.

Although there have been several recent reports of the effects of glucocorticoids on pregnancy, Roboson and Sharaf (1952) reported that either ACTH or cortisone given to pregnant rabbits or mice would interrupt pregnancy. Their findings included some abortions, but their treatment mainly produced death _in utero_. A single injection of 20 mg of DXMS to cows that were 260 days in gestation regularly caused premature parturition 22 to 56 h later (Adams, 1969). Likewise, parturition was induced within 48 h in 10 of 23 ewes by a single injection of 10 to 20 mg of DXMS (Adams and Wagner,

1970). The mean age of gestation in these ewes was 141 days. Fylling et al. (1973) were able to abort 2 of 4 ewes at midpregnancy by giving 2.5 mg of DXMS four times daily for five days starting at day 88 of pregnancy. Thus a substantial difference in dose is necessary if administered to the dam rather than the fetus. Kendall and Liggins (1972) reported that DXMS administration hastened birth in rabbits. This effect has also been achieved in the horse (Alm et al., 1972) and the sow (North et al., 1972). However, pregnant women did not abort following 6 mg DXMS/day for 10 days during their third trimester of pregnancy (Warren and Cheatum, 1967).

Bosc (1971) reported that DXMS did not cause premature birth when injected into four ewes bearing hypophysectomized fetuses, but was effective in five ewes bearing sham-operated fetuses. However, somewhat in contrast, Liggins et al. (1966) reported that ACTH infusion into a hypophysectomized post-mature lamb fetus was followed by parturition. Bosc (1971) further reported that hypophysectomy of ewes did not cause any alterations in the timing of lambing.

3. Corticosteroids in pregnancy

Quantitatively cortisol was found to be the principal glucocorticoid secreted by the adrenal gland of the adult sheep (Bush and Ferguson, 1953; Lindner, 1959). The cortisol: corticosterone ratio in sheep adrenal vein plasma was 15 to 20:1. Plasma cortisol binding capacity in sheep did not in-

crease during pregnancy (Lindner, 1964; Paterson and Hills, 1967) and estrogen administration had no effect on sheep plasma cortisol levels (Lindner, 1964). The concentration of transcortin in ovine plasma expressed as cortisol binding capacity had a mean value of 24 μg cortisol/L plasma (Paterson and Hills, 1967). At physiological cortisol levels 39±5% of the cortisol in sheep plasma was protein bound compared to 86% to 95% in humans (Lindner, 1964). Therefore, the half life of cortisol in sheep was less than 30 min (Paterson and Harrison, 1968) compared to 1.5 to 2.0 h in the human (Peterson *et al.*, 1955). However, cortisol PR was less in pregnant women (Migeon *et al.*, 1968) than in pregnant ewes (Beitins *et al.*, 1970; Dixon *et al.*, 1970). Plasma cortisol concentration is a function of these parameters and sheep had much lower plasma cortisol concentration than women (Table 1).

Changes found in the plasma cortisol concentration of pregnant ewes approaching parturition depend on whether serial plasma samples were examined through parturition. Saba (1965) reported that plasma cortisol levels in pregnant sheep decreased from 2 μg/100 ml plasma at 50 days prepartum to 0.5 μg/100 ml plasma 10 to 1 days before parturition. Paterson and Harrison (1967) made use of isotope dilution techniques to study dynamics of cortisol metabolism in pregnant ewes with autotransplanted left adrenal glands. The metabolic clearance rate (MCR) rose from 0.60 to 0.66 L plasma/min to 0.86 L/min several days before lambing while the cortisol secretion rate

Table 1. Plasma cortisol levels in sheep and late pregnant women

	Condition species	Mean plasma cortisol concentration (μg/100 ml plasma)	Assay method	Other or environmental relating factors	Source
1.	non-pregnant ewes	2.3	fluorometric		Paterson and Harrison (1968)
2.	non-pregnant ewes	1.4	competitive protein binding		Dunn *et al.* (1969)
3.	pregnant ewes	0.6	fluorometric	trained	Lindner (1964)
4.	sheep	1.1	fluorometric	yarded	Lindner (1964)
5.	pregnant ewes (134-140 days)	1.37	competitive protein binding	post surgical	Dixon *et al.* (1970)
6.	pregnant ewes (120 days)	5.26	competitive protein binding	post surgical & venipuncture	Beitins *et al.* (1970)
7.	pregnant women (3rd trimester)	26.9	fluorometric		Rosenthal *et al.* (1969)
8.	pregnant women (3rd trimester)	25.8	fluorometric		Adadevoh and Akinla (1971)

remained relatively constant. Thus, near term a decrease was found in the plasma cortisol concentration. This fall in the plasma cortisol concentration was attributed to an expansion of the plasma volume (Paterson and Harrison, 1968). However, they did not examine any ewes closer than three days prepartum. Basson _et al_. (1969) examined the plasma cortisol concentration in ewes throughout gestation. Cortisol values decreased at about 120 days of gestation with the lowest values since conception found at approximately 138 days. Then plasma cortisol levels rose quickly from these low values to relatively high values as parturition approached. Likewise, Obst and Seamark (1972a) stated they found an increase in plasma corticoids in ewes during the 48 h before parturition.

Brush (1958) reported a non-significant rise in plasma corticosteroids in cows approaching parturition. However, Adams and Wagner (1970) found a significant rise in this specie during the last four days prior to parturition. Pellet _et al_. (1970) found a significant rise in plasma corticoids in the hamster during the latter part of gestation, but no correlation was found to exist between plasma corticoids and number of fetuses. Plasma corticosterone levels in rats were significantly elevated the last three days of pregnancy (Milkovic _et al_., 1973).

Maternal plasma levels of transcortin in women were found to rise progressively from about 3 mg/100 ml early in pregnancy to 7 mg/100 ml by the sixth month (Doe _et al_., 1964).

These increased transcortin levels are considered to be a secondary effect to the rise in plasma estrogens (Sandberg and Slaunwhite, 1959). Likewise, total plasma cortisol rose steadily from a normal value of 7.8 μg/100 ml in non-pregnant women to 26.9 μg/100 ml in the third trimester of pregnancy (Rosenthal et al., 1969). However, cortisol PR was not changed by pregnancy but increased only at labor (Migeon et al., 1968). Since pregnant women do not manifest signs and symptoms of hypercorticoidism (Cushing's syndrome), the rise in plasma corticoids in pregnant women (Friedman and Beard, 1966) may be of little biological significance. A marked rise in plasma cortisol was found in women during labor and delivery (Adadevoh and Akinla, 1971).

The increase in the fetal lamb adrenal weight was an early clue of fetal adrenal function in gestation (Comline and Silver, 1961) since, during the last seven to ten days of pregnancy, fetal adrenal weight was found to approximately double. Recently a marked increase in the fetal lamb's plasma cortisol levels close to parturition has been reported (Bassett and Thorburn, 1969; Comline et al., 1970b; Nathanielsz et al., 1972). However, the fetal lamb's adrenal is capable of responding to stress much earlier in gestation. Hemorrhage of the fetal lamb as early as day 90 of gestation stimulated ACTH release (Alexander et al., 1971a) and infusion of Clostridium welchii Type D toxin into the peritoneal cavity of the fetal lamb on day 107 of gestation resulted in an

increased fetal adrenal growth rate (Liggins, 1969a). ACTH was consistently present in the fetal lamb's plasma after day 130 of gestation (Alexander *et al.*, 1971b).

The 11β-hydroxylase enzyme activity appeared to increase very rapidly the final few days of gestation in the fetal lamb (Anderson *et al.*, 1972). In addition to stimulating the conversion of cholesterol into pregnenolone (Stone and Hechter, 1954), ACTH specifically stimulated 11β-hydroxylase activity in adult rat adrenal tissue (Griffiths and Glick, 1966). A much greater *in vitro* conversion of isotopically labelled pregnenolone into 11-deoxycortisol and cortisol was found in adrenal tissue from a lamb born at day 116 after ACTH infusion than in adrenal tissue from an untreated 122 day fetal lamb (Anderson *et al.*, 1972). Only at labor did conversion of pregnenolone to cortisol predominate over the conversion of pregnenolone to 11-deoxycortisol, but more cortisol was produced with increasing gestational age. Infusions of 11-deoxycortisol had no effect on pregnancy termination. Progesterone was transformed almost as efficiently into cortisol as was pregnenolone. Thus the pathway of pregnenolone through 17α OH-pregnenolone was not quantitatively as significant to cortisol synthesis in the adrenal of the ovine fetus as in the adult. This is in contrast to the findings of Vinson and Whitehouse (1967) who found little difference in the corticosteroid biosynthetic pathways of fetal and adult sheep adrenals.

The high adrenal activity of the neonate in other species is similar to that of the lamb where the plasma cortisol level on day +1 was found to be higher than any other postnatal day (Paisey and Nathanielsz, 1971). This suggests that the fetal pituitary-adrenal function of most species would be similar. The newborn calf was reported to have a rapid fall in plasma cortisol levels during the first two weeks of life (Eberhart and Patt, 1971; Nathanielsz *et al.*, 1972). Plasma and adrenal concentrations of corticosterone were high in the newborn rat as well as the adrenal responsiveness to ACTH (Levine and Treiman, 1969). Humans do not seem to exemplify this same phenomenon as the plasma cortisol in cord blood from newborn humans was found to be considerably lower than maternal blood (Adadevoh and Akinla, 1971; Jarvinen *et al.*, 1971).

The regulation of hypothalamic function in the fetal lamb as parturition approaches is not well defined. Sheep fetal plasma pH values were remarkably stable from day 125 of gestation until the parturition process, and occasionally they remained unchanged through delivery (Comline and Silver, 1970; Meschia *et al.*, 1970). Likewise, fetal blood gas tensions (PO_2 and PCO_2) during the same period did not change markedly. During the last 7 to 10 days of gestation, sheep fetal thyroxine concentrations decreased to maternal values (Hopkins and Thorburn, 1971). The fact that the fetus was found to be hyperthyroid relative to the mother is incongruous with the

finding that the fetal lamb's brain temperature was 0.4 to 0.8C higher than the maternal aortic blood (Abrams et al., 1969). Thorburn et al. (1972) speculated that with maturation of the hypothalamus the fetus becomes aware of its hot environment and the thermal stress stimulates the release of corticotrophin-releasing factor from the hypothalamus.

Corticosteroid values from blood collected acutely from adrenal veins of fetal lambs revealed that adrenal glands of sheep fetuses 1 to 3 weeks before term secrete substantial quantities of cortisol and corticosterone (Jones et al., 1964). Also in acute experiments sheep fetal adrenals were found to secrete increasing quantities of both cortisol and corticosterone with increasing gestational age (Alexander et al., 1968). The largest increase occurred very late in gestation.

Corticosteroid concentrations in unanesthetized, catheterized sheep fetuses were found to have little relation to maternal concentrations (Bassett and Thorburn, 1969). The fetal plasma corticosteroid concentrations remained generally below 2 µg/100 ml until several days before parturition. At this time a pronounced increase in fetal plasma corticoids began. Peak values were found on the day of parturition (Bassett and Thorburn, 1969; Comline et al., 1970b; Nathanielsz et al., 1972). These peak concentrations of corticoids in the circulation of the fetal lamb were considerably above those in the maternal circulation. Nathanielsz et al. (1972) found the mean fetal plasma cortisol values immediately pre-

ceding birth to be from 25 to 30 μg/100 ml plasma. Mean values calculated from Bassett and Thorburn (1969) and Comline et al. (1970b) would be lower. The 50 h preceding normal vaginal delivery were associated with an approximate eightfold increase in cortisol turnover (Comline _et al._, 1970b). Cortisol turnover rate increased from a mean of 10.94 μg/h earlier in gestation to 88.17 μg/h close to parturition. This more rapid turnover of cortisol immediately before parturition was comparable with the rate of infusion of DXMS necessary to induce parturition. In conjunction with these increases, the fetal cortisol PR rose from 0.9 mg/day at four or more days prepartum to 2.6 at three days prepartum and 9.2 at two and one days prepartum (Nathanielsz _et al._, 1972).

Plasma corticoids were higher in the umbilical vein than in the umbilical artery in human infants delivered at term (Smith and Shearman, 1972). A distinct surge of corticoid levels was noted in both the artery and the vein in infants delivered at 35 to 37 weeks of gestation.

A significant transfer of cortisol was found to occur between the maternal and fetal circulations in three sheep studied by Beitins _et al._ (1970) and in three sheep studied by Dixon _et al._ (1970). These studies employed isotope dilution techniques with ^{3}H-cortisol infused into the fetal lamb and ^{14}C-cortisol into the ewe. The six pregnant ewes were from 120 to 140 days pregnant. A marked transplacental passage of fetal cortisol to the maternal side occurred in every

case. However, the fetus contributed very little to the total cortisol concentration of the ewe, even though a large percentage of the cortisol originating in the fetus crossed into the maternal circulation. Although only a small percentage of the maternal hormone was transferred to the fetus, the fetal plasma corticoid concentration was found to be composed of a significant amount of cortisol originating from the mother. Even though definite breeding dates were not known, Beitins et al. (1970) stated that perhaps maternal to fetal transfer increased with gestational age since the lightest lamb had a negligible maternal fetal transfer. In support of this, placental permeability to urea increased as gestation progressed in sheep (Meschia et al., 1965). Apparently the sheep's placenta is impermeable to hormones such as glucagon (Alexander et al., 1971c), ACTH (Alexander et al., 1971b) and LH (Foster et al., 1972), but not to a peptide such as oxytocin (Noddle, 1964).

Placental permeability to corticoids has been investigated in other species. Adrenalectomized pregnant rats given saline had plasma corticosterone levels on day 21 of pregnancy equal to those of normal pregnant rats of the same gestational age (Milkovic et al., 1973). In humans infusion of ^{3}H-cortisol into the maternal circulation resulted in substantial quantities of the free hormone appearing in the fetal plasma and amniotic fluid (Abramovich and Wade, 1969). Cortisol PR was determined in an AD pregnant woman while taking only

DXMS as replacement therapy (Charles *et al.*, 1970). These rates were 1.5 and 3.8 mg/24 h in the second and third trimesters, respectively. It was felt that the cortisol calculated was produced by the fetus. Cortisol also appeared to readily pass from mother to fetus in the rhesus monkey (Bashore *et al.*, 1970).

Adrenalectomized mothers of various species have given birth to viable young. Adrenalectomized rats provided with 0.9% saline became pregnant and delivered viable young as readily as intact animals (Thoman *et al.*, 1970). They gave birth to relatively large litters and lactated sufficiently to maintain the young through 21 days. Newborn pups from AD mothers had significantly higher circulating corticosterone than pups from normal mothers. Adrenalectomized women properly managed with gluco- and mineralocorticoid replacement therapy have had normal pregnancies (Charles *et al.*, 1970; Rosenburg, 1970).

4. Effects of corticosteroids on the pregnant myometrium

In vivo and *in vitro* effects of corticosteroids on the pregnant myometrium have been reported. Water soluble corticosteroids significantly increased the amplitude of contraction of myometrial strips from near term women and mice (Mossman and Conrad, 1969). The sensitivity of the mouse uterus to hydrocortisone was greatest at term and methylprednisolone application to a mouse uterine preparation prior to oxytocin

resulted in a lowered tonus and an increased amplitude of contraction. Cortisol pretreatment of an *in vitro* rat uterine preparation caused an increased response to oxytocin in both tonus and amplitude (Zasztowt, 1971). The administration of 50 mg hydrocortisone with oxytocin to women in labor was said to have resulted in an increased amplitude of contraction of the uterus over that seen with oxytocin alone (Zasztowt, 1971).

B. Progesterone

1. Effects of progesterone on the myometrium

Corner and Allen (1929) prepared a crude extract from corpora lutea that maintained pregnancy in castrated does (Allen and Corner, 1929). The extract became known as progesterone. Later an inhibitory effect of progesterone was demonstrated *in vivo* to the oxytocic activity of pituitrin on the uterine muscle of rabbits (Allen and Reynolds, 1935).

Csapo (1961), based on the observation in which twins were born two months apart from a bicornuate uterus, proposed a "local block" effect of progesterone on the myometrium. This "block" was proposed to interfere with excitation, conduction of impulses in, and hence, contraction of the uterus. This "block" supposedly resulted from the diffusion of progesterone from the placenta to the myometrium. A concentration gradient of progesterone from myometrial placental attachment sites where it was highest to lower concentrations

found in the anti-placental regions and plasma supports this concept (Kumar and Barnes, 1965; Wiest, 1967). While many of the electrophysiological characteristics of this "block" such as raising the resting membrane potential and the negative staircase phenomenon are refuted by Kao (1967), progesterone is undoubtedly responsible for the maintenance of pregnancy. Evidence for this "block" was presented in such experiments as the prevention of abortion by progesterone treatments of OV placenta-dislocated rats (Csapo and Wiest, 1969). Removal of ovaries and placenta from rabbits late in pregnancy resulted in a pronounced increase in electrical and mechanical myometrial activity 24 h post surgery. However, exogenous progesterone injections blocked the evolution of this activity (Csapo and Jung, 1963). Myometrial activity produced by prostaglandin (PG)$F_{2\alpha}$ in the rabbit was also significantly reduced by progesterone (Porter and Behrman, 1971).

Miller (1967) proposed that uteri from various species contained both alpha (excitatory) and beta (inhibitory) adrenergic receptors. The effect of adrenergic nerve stimulation depends on the relative dominance of one of these two adrenergic receptors. In the myometrium the relative dominance of these two receptors appears to be under hormonal control (Miller and Marshall, 1965). Pretreatment of immature rabbits with estrogen caused the isolated uterus to contract upon hypogastric nerve stimulation. This effect was

blocked by an alpha-adrenergic blocking agent, phentolamine. However, pretreatment with estrogen followed by progesterone blocked the response to nerve stimulation. This inhibition was prevented by the beta-adrenergic blocking agent, propranolol.

There is some evidence that the sex steroids may modify the oxytocin releasing reflex. An increased release of oxytocin resulted in response to vaginal distension in non-pregnant sheep treated with estrogens, whereas progesterone treatment resulted in a decreased release (Roberts and Share, 1969).

In the rabbit, a specie where progesterone production is extrauterine, parturition was delayed by the systemic administration of progesterone (Csapo, 1956). Progesterone was capable of inhibiting the spontaneous contractility of human term pregnant or non-pregnant myometrial strips _in vitro_ and abolished oxytocin stimulated contractions if adequate concentrations were used (Kumar _et al._, 1962). The same results were not achieved _in vivo_. High doses of progesterone (6 to 7 mg/min for a total dose of 700 to 1000 mg) were infused into 10 women who were either in early spontaneous or oxytocin-induced labor (Kumar _et al._, 1963). Uterine activity was monitored via an intra-amniotic catheter. No effect on uterine activity was seen in 5 out of 10 cases. In the five who did show a diminution in uterine activity the effect was minimal (one case) to moderate (four cases). The activity

was never completely abolished in any patient.

The time and course of parturition was unaffected by giving 40 mg progesterone daily to ewes starting seven days before expected parturition (Bengtsson and Schofield, 1963). However, when the dose was increased to 80 mg or 150 mg progesterone/day, a high proportion of the pregnancies was affected. Some sheep lambed at the expected time, but most of the others were delivered by Caesarean section. Ewes given a single injection of 80 mg progesterone during the early stages of parturition showed a decline of intrauterine pressure waves and lambs were born up to seven days late (Hindson et al., 1968).

To further elucidate the "local block" mechanism, Liggins et al. (1972) have studied plasma and myometrial progesterone changes in ewes following DXMS (1 mg/day to the fetus) induced parturition. Jugular plasma progesterone normally fell from a mean value of 17 ng/ml before DXMS to 1.8 ng/ml on the day of parturition. Myometrial progesterone concentrations were 18.0 ng/g in control animals not in labor and 8.0 ng/g during labor in the DXMS-treated ewes. In sheep receiving 100 mg progesterone/day the mean plasma progesterone values before DXMS and on the day of parturition were not different. Progesterone concentrations in the myometrium at parturition in these sheep did not differ from concentrations in normal sheep 7 to 28 days from term. Thus, although DXMS can significantly reduce myometrial proges-

terone concentration in induced ewes compared to ewes lambing at normal term, this response does not appear to be a prerequisite for initiation of parturition. A dosage of 200 mg progesterone/day completely blocked parturition.

Uterine activity started after the usual latent period of 50 h in two ewes receiving 150 mg progesterone/day while DXMS was being infused into the fetuses (Liggins et al., 1972). However, in spite of normal uterine activity, the cervix failed to dilate and fetal death ultimately resulted from amniotic infection.

Progesterone has also been reported to prevent premature corticoid-induced parturition in cattle (Jochle et al., 1972). Progesterone (100 mg/day) given three days before and five days after administration of 10 mg DXMS on day 270 of pregnancy prevented premature parturition.

2. Interaction of glucocorticoids and progesterone

Corticoid-induced parturition in cattle was preceded by a drop in peripheral plasma progesterone levels (Edqvist et al., 1972; Evans and Wagner, 1971; Schams et al., 1972). Associated with this change was a marked rise in the estrone concentrations (Edqvist et al., 1972), but luteinizing hormone levels appeared unaffected (Schams et al., 1972). In sheep a subthreshold dose of DXMS (4 mg/day) caused no change in progesterone concentration whereas effective doses (10 mg/day for 4 to 5 days starting at day 133 of gestation) were

associated with a marked decrease in peripheral plasma progesterone levels (Fylling, 1971). Likewise, the administration of ACTH to the fetal lamb was associated with a fall in the maternal progesterone concentration before parturition (Liggins et al., 1972).

Further evidence of ovarian-uterine-adrenal interactions came from a study on the effect of various steroids on the decidual reaction in mice (Velardo, 1957). Following unilateral uterine traumatization, the OV pseudopregnant mouse will respond to progesterone administration with an increased weight of the traumatized cornu compared to the contralateral cornu. A 1:1 ratio of ACTH to progesterone inhibited the response of the traumatized cornu from 440% down to 100%. Administration of cortisone:progesterone and cortisol:progesterone at ratios of 1:1 and 2:1, respectively, gave results similar to the ACTH studies.

In humans transcortin had a greater affinity for cortisol but the affinity for progesterone was not insignificant (Rosenthal et al., 1969). The concentration of plasma progesterone was so low under non-pregnant conditions that it did not affect the binding of cortisol. However, during pregnancy the elevated progesterone level displaced cortisol from transcortin. Early in pregnancy the ratio of transcortin-bound progesterone to transcortin-bound cortisol was .15 and rose to .29 in the third trimester. Concurrently the unbound levels of cortisol rose.

A tissue specific progesterone binding protein in rat uterine cytosol was physicochemically indistinguishable from transcortin (Milgrom and Baulieu, 1970a). Cortisol was better able to displace ^{3}H-progesterone from this protein than was progesterone (Davies and Ryan, 1972; Milgrom and Baulieu, 1970a). However, either incubation of intact uterine horns with labelled progesterone or cortisol or the *in vivo* injection of these labelled hormones resulted in labelling the progesterone binding protein with progesterone but not with cortisol (Milgrom and Baulieu, 1970b)

3. Plasma progesterone levels, kinetics and metabolism

Peripheral plasma progesterone concentrations in the pregnant ewe have been measured recently by a number of investigators using gas-liquid chromatography (Basson *et al.*, 1969; Stabenfeldt *et al.*, 1972) and competitive protein binding techniques (Basset *et al.*, 1969; Fylling, 1970; Moore *et al.*, 1972; Thorburn and Mattner, 1971). Plasma progesterone levels during the first 50 days of pregnancy were low (2 to 3 ng/ml). These levels were not different from peak concentrations found during the luteal phase in cycling non-pregnant ewes (Thorburn and Mattner, 1971). Following this period plasma progesterone concentrations were reported by most investigators to rise steadily to 10 to 20 ng/ml at day 120 to 140. The sex of the fetus did not influence maternal progesterone levels (Moore *et al.*, 1972). Ewes carrying twin

fetuses had significantly higher peripheral plasma progesterone levels compared to ewes carrying single lambs according to Basset et al. (1969). Usually a decline in plasma progesterone levels occurred during the last week of gestation. The initial decrease in plasma progesterone preceded any rise in peripheral plasma estrogens (Thorburn et al., 1972).

The PR of progesterone increased throughout gestation (Bedford et al., 1972a). Ewes that delivered lambs >4 kg had a higher progesterone PR (48 to 104 mg/day) than ewes that had lambs <4 kg (10 to 27 mg/day). The mean MCR of progesterone, 3.48 L/min, was relatively stable during pregnancy. In agreement with this high MCR the half life of progesterone in the non-pregnant ewe was found to be only 4 min (Short, 1961).

The secretion of progesterone by the maternal adrenal in sheep was small compared to the total PR as the adrenal progesterone secretion rate varied from 0.7 to 4.5 ng/min (100 to 600 μg/day) in three pregnant ewes (Linzell and Heap, 1968). However, the maternal adrenal appeared to be related to progesterone production, since there was a reduction in urinary excretion values for pregnanetriol and pregnanediol in an AD pregnant woman (Charles et al., 1970).

Maternal cholesterol was regarded as the principal precursor of the large amount of steroids elaborated by the human placenta at mid pregnancy (Diczfalusy, 1969). Evidence that progesterone production in the human was chiefly a

maternal-placental function came from the observation that fetal demise did not significantly reduce the concentration of plasma progesterone, whereas delivery of the placenta resulted in a sharp reduction in progesterone levels (Lurie *et al.*, 1966). Progesterone synthesis proceeded from pregnenolone in sheep placental preparations (Ainsworth and Ryan, 1967), but the contribution of maternal cholesterol to placental progesterone production in sheep was not known (Ryan, 1969).

In ewes the maintenance of gestation was independent of corpora lutea after the first trimester (Casida and Warwick, 1945; Denamur and Martinet, 1955). The ovarian contribution to the maternal plasma progesterone levels in ewes was significant as the ovary secreted 3 to 4 mg progesterone/day until about 20 days before parturition, and then declined (Moore *et al.*, 1972). According to Edgar and Ronaldson (1958) ovine corpora lutea stopped secreting progesterone about day 120 of pregnancy. However, Fylling (1970) did not observe the normal increase in plasma progesterone found in late pregnancy in OV ewes.

Progesterone levels were found to be much higher in uterine venous blood than in peripheral blood of an OV pregnant ewe (Bedford *et al.*, 1972b). Essentially identical results were obtained from intact ewes (Moore *et al.*, 1972; Thorburn and Mattner, 1971). Thus maternal progesterone seems to have been chiefly elaborated from the placenta in

the ewe. Utero-ovarian (UO) plasma progesterone levels in sheep rose from 6.3 ng/ml at day 61 of gestation to a peak of 99 ng/ml at day 120 and remained at a fairly high level until day 144 when the level started to decrease and reached less than 1.0 ng/ml 8 h postpartum (Moore *et al.*, 1972). This prepartum decrease in progesterone concentration was not the result of dilution by increased uterine blood flow since uterine blood flow was decreasing between 95 days and term in ewes (Huckabee *et al.*, 1972). Only on the day of parturition was there a steep increase in uterine blood flow (Bedford *et al.*, 1972b).

In the pregnant rat the concentration of uterine progesterone exceeded the plasma concentration until late pregnancy (Wiest, 1970). Labelled progesterone injected into the rat at mid pregnancy was taken up specifically by the myometrium at a level that exceeded that of the plasma (Davies and Ryan, 1972). This uptake was not evident near term. Measurement of the progesterone receptor activity by sucrose density gradient analysis revealed that the activity which was demonstrable at mid pregnancy was markedly reduced near term. These changes were later found to be positively correlated with changes in the progesterone receptor site concentration (Davies and Ryan, 1973).

Progesterone formed *in vitro* by sheep placental preparations did not accumulate but was largely metabolized to derivatives of progesterone (Ainsworth and Ryan, 1967). In

spite of metabolic activity by the placenta, sheep fetal umbilical vein progesterone levels generally rose as pregnancy progressed (Moore *et al.*, 1972). The sex of the fetal lamb had no effect upon umbilical venous progesterone levels. Umbilical levels reached a maximum of 20 to 24 ng/ml in the latter days of gestation, but never reached uterine vein levels. Most of the plasma progesterone found in the sheep fetus had been transferred from the mother as only a minimal amount of that produced by the placenta went directly to the fetus (Gurpide *et al.*, 1972).

Progesterone levels in umbilical vein plasma from human fetuses at birth were approximately twice as high as levels in the umbilical artery (Gandy, 1968, cited by Solomon and Fuchs, 1971; Zander, 1961). In contrast to sheep, sex had an influence on fetal umbilical progesterone levels in rhesus monkeys as female fetuses had higher levels than did male fetuses (Hagemenas and Kittinger, 1972). The concentrations of 20α and 17α OH-progesterone were higher in the umbilical artery than in the umbilical vein of the human fetus (Zander, 1961). This information implies a secretion of progesterone toward and metabolism by the fetus.

Sheep fetal blood was found to have a remarkable capacity to metabolize progesterone to 20α OH-progesterone *in vitro* (Nancarrow and Seamark, 1968). Enzymatic activity in fetal lamb blood was calculated to metabolize in excess of 13 mg progesterone/h. Since 20α OH-progesterone was a weaker

gestagen, this was regarded as a protective measure regulating the amount of gestagen going to the fetus. In fact, incubation of progesterone with whole blood from sheep resulted in the metabolism of progesterone to 20α OH-progesterone (Van der Molen and Groen, 1968). Besides placental and whole blood metabolism of progesterone, the fetal liver was prominent in progesterone metabolism (Gurpide et al., 1972). Approximately 70% of the progesterone present in the fetal hepatic circulation was extracted and metabolized primarily to pregnanediol.

Plasma progesterone concentrations were found to be rising toward term in women (Csapo et al., 1971; Llauro et al., 1968; Yannone et al., 1969). Llauro et al. (1968) concluded that labor began without any relationship to progesterone levels though a fall in these levels invariably occurred with cervical dilation. Yannone et al. (1969) studied progesterone levels serially in two women one week before and during spontaneous labor. Labor ensued in these women with no decrease in plasma progesterone. In a study of a larger population the protein bound progesterone remained constant throughout pregnancy and labor. However, since progesterone levels were highest at term, the biologically active progesterone was also maximal at the onset of labor (Yannone et al., 1969). However, Csapo et al. (1971) reported that plasma progesterone decreased before the onset of parturition.

The duration of labor was significantly prolonged in women with the highest levels compared to those with low progesterone levels (Csapo *et al.*, 1971). In keeping with this association, Johansson (1968) found a relationship between plasma progesterone levels and the ease with which oxytocin could induce labor. The group that responded rapidly to oxytocin had significantly lower plasma progesterone than the slow response group.

The plasma progesterone levels in an oophorectomy intolerant animal such as the goat (Meites *et al.*, 1951) decreased rapidly several days before parturition (Blom and Lyngset, 1970; Thorburn and Schneider, 1972). Those goats carrying twin fetuses tended to have higher peripheral plasma progesterone levels during the second trimester, but later the values in these two groups were similar (Thorburn and Schneider, 1972). Blood from the ovarian vein revealed peak plasma progesterone levels on day 90 which had decreased significantly by day 140 (Blom and Lyngset, 1970). Stabenfeldt *et al.* (1970) found that peripheral plasma progesterone levels in the cow reached a maximum at day 250 of pregnancy, and a marked decline occurred before parturition.

C. Estrogens

1. Estrogen synthesis in the pregnant female

The concept of the fetus and the placenta as a functional metabolic unit for steroid biosynthetic reactions was intro-

duced by Diczfalusy (1964) and later reviewed by Diczfalusy (1969). According to this concept, both the fetus and the placenta are necessary for the bulk of estrogen production during pregnancy. Maternal cholesterol is extensively converted to progesterone and pregnenolone by the placenta. Pregnenolone is rapidly sulphurylated in the three position by a variety of fetal tissues and hydroxylated at the 17α position in the fetal adrenal. Oxidation of 17α OH-pregnenolone sulfate in the fetal adrenal yields dehydroepiandrosterone sulfate (DHAS). This steroid then may be hydroxylated in the fetal liver to form 16α OH-DHAS which is a part of the sequence of events in estriol formation. In the placenta the androgen sulfates are hydrolyzed followed by aromatization. Thus DHAS metabolism in the placenta results in estrone and estradiol formation via dehydroepiandrosterone (DHA) and androstenedione. Estrone and estradiol are metabolized into a number of metabolites including estriol which is quantitatively the most important estrogen found in late human pregnancy urine (Beling, 1971).

Using *in vivo* isotope dilution techniques, DHAS secreted by the maternal adrenals has been calculated to contribute about 40% of the precursors for estradiol production by the human placenta (Siiteri and MacDonald, 1966). Estrone production was calculated to result equally from precursors found in both the maternal and fetal circulations. Estriol production in human pregnancy is probably largely from pre-

cursors found in the fetal circulation, since Frandsen and Stakeman (1964) found that the urinary excretion of estriol in 16 of 17 anencephalic pregnancies was about 1/10 of the normal pregnancy level while the estrone and estradiol excretion was about 1/4 of the normal pregnancy level. The fetal adrenals were of normal size in the one case that presented a normal excretion of estriol.

Pregnant AD women were found to have low urinary estrogen excretion values (Charles *et al.*, 1970; Gurpide and Vande Wiele, 1971). Likewise the excretion of the estrogen precursor, DHA, was markedly reduced in an AD woman (Charles *et al.*, 1970). Administration of 60 mg DHAS to an AD patient at term increased her rate of estrone plus estradiol production from 5 mg/day to 16 mg/day (Gurpide and Vande Wiele, 1971).

Stimulation of the maternal adrenal by ACTH in pregnant women increased the production rates of DHAS and estradiol (MacDonald *et al.*, 1962; MacDonald and Siiteri, 1965). Conversely adrenal suppression by DXMS or hydrocortisone depressed urinary estrogen excretion (Simmer *et al.*, 1966; Warren and Cheatum, 1967). Administration of 6 mg DXMS/day to four women in their third trimester of pregnancy resulted in a 50% to 90% decrease in urinary excretion of the three estrogens (Warren and Cheatum, 1967). Since all three estrogen levels were depressed, these authors stated that the fetal adrenals must have also been depressed.

The *in vitro* conversion of labelled DHA and androstene-

dione to estrogen by placental tissues of the sheep, cow, horse and sow was demonstrated by Ainsworth and Ryan (1966). Placentas from rabbits and guinea pigs were found to lack aromatizing enzymes. Infusions of labelled pregnenolone and progesterone to the maintained isolated sheep fetus resulted in the formation of androstenedione and DHA (Pierrepoint _et al._, 1971). The fetal adrenal was probably the source of these C_{19} steroids since incubations of pregnenolone and progesterone with fetal lamb adrenal tissue produced several C_{19} steroids (Anderson _et al._, 1972). A tenfold increase in the conversion of pregnenolone to androstenedione was found at 143 days and at labor compared to that at 122 days gestation.

A functional feto-placental unit also exists in sheep. Estrone production resulted from incubations with labelled pregnenolone only when fetal adrenal tissue and placental tissue from sheep were incubated simultaneously (Davies _et al._, 1970). Similar results were obtained from experiments with maternal adrenal and placental tissue. Estrone was the major estrogen produced from incubations of sheep placenta with C_{19} precursors (Pierrepoint _et al._, 1970). Lesser amounts of estradiol-17α and estradiol-17β were produced but estriol was not formed.

2. Estrogen levels and metabolism in the late pregnant female

Estriol excretion patterns in the human are characterized by a sharp increase the last 3 to 4 weeks of pregnancy

(Beling, 1971). However, plasma levels of estradiol-17β in five subjects were stable 2 to 3 weeks prior to and through delivery (Rado et al., 1970). Significant fluctuations were found in plasma estrone and estriol levels during this period but no characteristic pattern occurred. The levels found in early labor were not significantly different from those during the final week. One subject with twins had a significantly greater plasma estrogen concentration than those with a single fetus.

Estrone was quantitatively the most important estrogen in bovine plasma (Edqvist et al., 1973; Smith et al., 1973). Both estradiol and estrone increased linearly from 26 days to 5 days before parturition and then more sharply until day 2 prepartum. One or two days prepartum plasma estrogens fell dramatically reaching low levels postpartum. At variance with this Henricks et al. (1972) reported that the highest values for total estrogens in the cow were found at parturition. The plasma progesterone:estrogen ratio has been reported to decrease several days prior to parturition in the cow (Edqvist et al., 1973). Plasma estrogens rose sharply following DXMS administration for parturition induction in cattle (Edqvist et al., 1972) as did the urinary estrogen excretion (Osinga, 1970). During parturition induction plasma estrone levels rose before plasma progesterone levels began to decline (Edqvist et al., 1972).

Goats also manifested a consistent pattern of an increas-

ing plasma estrogen concentration with advancing gestation (Challis and Linzell, 1971). The highest values were found on the day of parturition. There was considerable variation in estrogen levels found in goats although individuals tended to have either consistently high or low levels. Goats with two fetuses had higher estrogen concentrations than those with single fetuses but this difference was not statistically significant.

Estrone was quantitatively the most important estrogen in the plasma of the pregnant ewe with lesser amounts of estradiol-17β (Bedford _et al._, 1972b; Challis _et al._, 1971; Thorburn _et al._, 1972). Plasma estrogen levels were stable until immediately prepartum when they rose sharply. There was a rapid fall in plasma estrogens postpartum, although highest values were reported shortly after parturition. Estrogen PR was positively correlated with plasma levels (Bedford _et al._, 1972b). The duration of this plasma estrogen peak ranged from 4 to 48 h.

Obst and Seamark (1972b) reported the mean plasma estrogen level in 10 ewes in estradiol-17β equivalents using a competitive protein binding assay method. The mean was 19 pg/ml 24 to 32 h before parturition, increasing to 37 pg/ml at 16 to 24 h prepartum and to 98 pg/ml 8 to 16 h before parturition. Challis (1971) reported that the total unconjugated estrogens in five sheep varied from 75 pg/ml to 411 pg/ml on the day of parturition with little change until 2 to 3 days

prepartum. Values of the total unconjugated estrogens as high as 2586 pg/ml plasma were reported in uterine venous blood of one OV ewe (Bedford _et al._, 1972b). Thorburn _et al._ (1972) reported that estradiol-17β values rose as high as 880 pg/ml within a few hours of birth. Their estrone values were not given but an estrone:estradiol-17β ratio of 1.9:1 was stated.

Plasma concentration of total estrogens in pregnant ewes was higher in the UO vein than in the J vein (Bedford _et al._, 1972b) indicating a uterine site of estrogen biosynthesis. This difference was marked in one ewe but negligible in two others.

In contrast to the rise in plasma estrogens only at parturition in the pregnant ewe, the urinary excretion of total estrogens was generally found to increase gradually from about the tenth day of pregnancy (Fevre _et al._, 1965). Total urinary estrogens were much greater in ewes bearing twin fetuses compared to a ewe with a single fetus (Fevre and Rombauts, 1966). The main urinary estrogen excreted in the pregnant ewe was estradiol-17β along with considerable amounts of estrone. Urinary estrone excretion was found to be lower in OV pregnant ewes than in normal ewes (Fevre, 1967).

Estrogens found in the plasma of the sheep fetus 125 days or less of gestational age were mainly sulpho-conjugates with an insignificant amount of unconjugated estrogens (Findlay and Cox, 1970). The concentration of estrogens in the

fetuses increased with gestational age. Plasma estrogens in one lamb given ACTH were higher than in normal term fetuses (Thorburn *et al.*, 1972).

3. The action of estrogens on the uterus

It was first demonstrated in 1925 that estrogens had myometrial stimulating properties (Frank *et al.*, 1925). Excised uteri from OV estrogen treated rats exhibited rhythmic contractions whereas uteri from untreated rats were quiescent. Reynolds (1935) postulated that estrogen was the hormone responsible for the onset of parturition on the basis that estrogens increase greatly at the end of gestation and due to the known action of this hormone. If estrogens are responsible for the onset of parturition, it would be expected then that estrogen administration would have this effect. However, such experiments have proved inconclusive since it depended upon when estrogens were given and the species (Deanesly, 1966). Estrogen treatment prolonged gestation in the rabbit presumably due to luteotrophic properties of estrogens in this specie. Estrogens have also prolonged gestation in rats and cats, although the increased uterine contractions have caused the fetuses to die.

Rats ovariectomized 48 h prior to term did not deliver or delivered only a fraction of their litters (Csapo, 1969). Oxytocin was ineffective under these conditions. The administration of 10 μg estradiol propionate 24 h before term pre-

vented the arrested labor and normal litters were delivered at term. Reduction of the dose of estrogen to 5 µg affected therapeutic efficacy only slightly but a reduction to 2.5 µg did so markedly. Although uteri from OV rats responded normally to electrical stimulation, this potential was not revealed in response to oxytocin. Estrogen therapy restored reactivity to oxytocin to the extent of the intact controls.

A direct action of estradiol-17β was found upon the myometrium of women at term (Pinto *et al.*, 1964). Uterine contractility was measured by recording intra-amniotic pressure and the mammary effect via an *in situ* cannula. The intravenous administration of 100 mg estradiol-17β produced a significant increase in uterine activity without an effect on mammary contractility. The uterine contractions elicited by estradiol had different characteristics compared to those following 2 to 4 mU oxytocin and oxytocin following estradiol produced a significant increase in uterine activity compared to oxytocin alone. Following estradiol administration cervical ripeness invariably was observed within a period of 3 to 6 h.

The first intrauterine pressure waves occurred 24 h after stilbestrol administration to late pregnant ewes (Hindson *et al.*, 1967). In 3 out of 7 treated ewes unaided delivery of viable lambs followed labor of a normal duration (12 to 13 h). Labor may not have been normal as two ewes had a protracted labor and delivered only with assistance.

In two other ewes the intrauterine pressure waves increased for 36 h, but they did not deliver in response to these contractions. Following the injection of stilbestrol in mid pregnancy to seven ewes, two aborted a few days later and the five others went to term. At term 4 of these 5 ewes required assistance to effect delivery as cervical dystocia was prominent.

Estrogens administered to cyclic ewes on days 11 and 12 of the estrous cycle resulted in a significant regression of corpora lutea by day 13 (Stormshak et al., 1969). In hysterectomized ewes estrogens failed to cause regression of corpora lutea. On this basis it can be postulated that estrogens stimulate the release of a luteolytic factor from the uterus. This may be related to the report that estrogens stimulated the release of $PGF_{2\alpha}$ from the uterus of non-pregnant guinea pigs (Blatchley et al., 1971). Estradiol-17β administration following progesterone injections to OV non-pregnant ewes resulted in an increase of PGF levels in the peripheral plasma similar to that observed in intact ewes on day 14 of the estrous cycle (Caldwell et al., 1972). This treatment was ineffective in ewes that had been both OV and hysterectomized or in OV ewes receiving only progesterone. This sequence of events was reversed in a pregnant ewe as estradiol-17β rose markedly following intra-aortic infusions of $PGF_{2\alpha}$ (Liggins et al., 1972).

Estrogens stabilized the uterine muscle fiber membranes

in OV rats by raising the resting membrane potential (Marshall, 1959). Thus many local impulses were not propagated, but rhythmic contractions resulted from impulses exceeding threshold. Areas of the estrogen dominated uterus showed pacemaker characteristics with a series of action potentials preceding and accompanying each contraction. In contrast, uterine pacemaker areas were not evident in the progesterone dominated uterus and action potentials did not consistently precede or accompany contractions.

D. Other Substances Affecting Myometrial Function

1. Prostaglandins

Among the effects PG have on reproduction, several workers have demonstrated that PG can cause regression of the corpus luteum. Infusions of $PGF_{2\alpha}$ into either the ovarian artery or uterine vein of ewes were followed by a drop in peripheral plasma progesterone and hastened onset of estrous behavior (Thorburn and Nicol, 1971). In the peripheral plasma of cyclic ewes PGF rose slightly by day 13 of the estrous cycle and was significantly elevated on day 14 when luteal regression had begun (Caldwell *et al.*, 1972). Similar effects have been observed in the hampster (Lukaszewska *et al.*, 1972) and the cow (Louis *et al.*, 1972).

Prostaglandins of the E and F series stimulated the human pregnant myometrium *in vitro* (Bygdeman, 1965). Karim *et al.* (1968) were able to induce labor in 10 women at or near

term by the intravenous infusion of $PGF_{2\alpha}$. Since this initial report many investigators have reported the successful use of PG either to induce labor at term or as an abortifacient (Karim, 1971). Uterine contractions following PGE_2 or $PGF_{2\alpha}$ administration were similar to those seen during spontaneous labor. Prostaglandins have also been used successfully to abort early pregnant cows (Lauderdale, 1972). Conversely indomethacin, a drug that blocks PG synthesis, prolonged gestation in rats (Chester *et al.*, 1972).

A rise in PGF levels in the uterine venous plasma of sheep and goats has been reported to occur during the 24 h before parturition (Thorburn *et al.*, 1972). In another report $PGF_{2\alpha}$ uterine venous plasma levels only rose to detectable levels when ewes were in labor (Liggins and Grieves, 1971). In a study of the temporal relationships of estrogens to $PGF_{2\alpha}$ levels in the uterine venous plasma of an OV ewe at parturition, total unconjugated estrogen levels were found to rise during the last 30 h of pregnancy whereas $PGF_{2\alpha}$ levels remained low until 8 h before lambing when their concentration increased sharply (Challis *et al.*, 1972). Uterine plasma levels of progesterone were declining before the rise in either estrogen or PG levels .

Prostaglandin $F_{2\alpha}$ concentrations in the maternal cotyledons of sheep rose markedly following infusion of DXMS into the fetal lamb (Liggins and Grieves, 1971). Subsequently concentrations in the myometrium rose during labor, but levels

in the fetal cotyledons did not change significantly during parturition induction. Concentrations of $PGF_{2\alpha}$ were also high in the maternal cotyledons and myometrium when uterine activity was suppressed by progesterone. Endometrial tissue from women in labor contained PG of the E and F series 10 to 30 times higher than amniotic fluid which also contained appreciable quantities of these PG (Karim, 1972).

In addition to a direct effect on the myometrium PGE_2 or $F_{2\alpha}$ have been shown to promote the release of oxytocin from the posterior pituitary (Gillespie *et al.*, 1972). Oxytocin levels rose from undetectable levels in two men before PG infusion to levels similar to women undergoing PG-induced labor. Prostaglandin-induced uterine contractions were suppressed by ethanol (Karim and Sharma, 1971) as was the release of oxytocin (Fuchs and Wagner, 1963). However, ethanol did not suppress uterine contractions induced by exogenous oxytocin (Karim and Sharma, 1971).

2. Oxytocin, vasopressin and myometrial activity

Stimulation of the cervix and vagina of the cat induced an increase in uterine activity (Ferguson, 1941). In a cross-circulation study, Peeters *et al.* (1965) were able to confirm that genital stimulation resulted in oxytocin release. Genital stimulation of one ewe usually resulted in the elicitation of the sensitive and specific milk ejection response in the other cross-circulated ewe. Oxytocin has been postulated to

be involved in the parturition process on the basis of two sets of facts: 1) the cervix and vagina are stretched during parturition; 2) the similarity between the pattern of uterine contractions during spontaneous labor and those elicited by the intravenous injection of oxytocin (Caldeyro-Barcia *et al.*, 1971).

Oxytocin lowered the membrane potential and thus was thought to lower the electrical threshold of the uterine muscle fiber (Jung, 1961) with the end result being an increase in the force and frequency of contraction. The estrogen dominated myometrium was more sensitive to oxytocin than was the progesterone dominated myometrium (Marshall, 1968).

The importance of an intact uterine-hypothalamic nervous pathway for normal parturition is controversial. Spinal cord transection in the thorax of pregnant rabbits resulted in a large number of dystocias despite the fact that parturition was initiated within the normal time (Beyer and Mena, 1970). Likewise, discrete lesions in the hypothalamic paraventricular nucleus of the cat resulted in prolonged pregnancy and dystocia (Nibbelink, 1961). Spinal anesthesia also interrupted labor in the cow (Fitzpatrick and Walmsley, 1962) However, there are several reports indicating that interruption of spinal pathways did not affect normal delivery in several species such as the rat (Gale and McCann, 1961) and the sow and the dog (Marshall and Chassar Moir, 1952).

The results of current investigations suggest that

detectable oxytocin levels in the blood occurred sporadically in labor and that spontaneous uterine activity did not depend primarily on increased concentrations of oxytocin. Serial plasma samples taken from 33 women during labor revealed the presence of oxytocin only in 83 of 264 samples (Gibbens _et al._, 1972). Although no relation was found between the appearance of oxytocin and uterine contractions, the incidence of positive results increased with full dilatation of the cervix. Cobo (1968) made continuous recordings of mammary pressures and amniotic fluid pressure during spontaneous labor in 20 subjects. The response of the mammary gland was used as a criterion of oxytocin release. Uterine activity increased exponentially at the end of the first stage of labor, but this was not accompanied by significant milk ejecting activity. The threshold sensitivity of the mammary gland to oxytocin remained unchanged throughout labor.

Umbilical arterial plasma oxytocin levels were significantly higher than were the umbilical venous plasma levels in the human fetus at labor (Chard _et al._, 1971). Fetal plasma oxytocin levels were higher than maternal levels. Fetal samples taken during labor had higher oxytocin levels than those taken during Caesarean section. Vasopressin in umbilical plasma was present in an even higher concentration than was oxytocin. As with oxytocin there was a significant umbilical arterio-venous difference in vasopressin levels. However, vasopressin appears not to be instrumental in partu-

rition as vasopressin was approximately 30 times less active than oxytocin on the human term uterus and vasopressin-induced contractions were unphysiological in nature (Cibils *et al.*, 1961). Both fetal posterior pituitary hormones were absent in the cord plasma of an anencephalic fetus at normal delivery (Chard *et al.*, 1971). Thus the importance of the fetal posterior pituitary in parturition is open to dispute.

Oxytocin plasma levels in animals in labor have been higher than corresponding human samples (Chard *et al.*, 1970). This difference is probably explainable on the basis of a difference in sampling locations. Animal samples were usually taken from the external J vein which was in direct line with pituitary drainage, whereas human samples were taken from the antecubital vein. In goats plasma oxytocin was low (Chard *et al.*, 1970) or not detectable (Folley and Knaggs, 1965) until the second stage of labor when it became markedly elevated. Only during the actual delivery process were high concentrations of oxytocin found in ewes (Fitzpatrick and Walmsley, 1965).

Spontaneous uterine activity in pregnant women was slight until the last 4 to 8 weeks of pregnancy when it increased faster as labor approached (Caldeyro-Barcia and Sereno, 1961; Csapo and Sauvage, 1968). The human uterus also responded to oxytocin throughout pregnancy but the response became greater in the latter part of gestation. Uterine contractions in cows began approximately two days

prepartum (Gillette and Holm, 1963). In ewes uterine activity remained low until about 30 h before delivery (Ward, 1968, cited in Bedford *et al.*, 1972b). A gradual increase in uterine activity was found after this time which became most pronounced the last several hours of gestation. However, Hindson *et al.* (1965) did not record uterine pressure waves in ewes until the last 12 h of pregnancy. These increased progressively with the approach of delivery. A general increase in uterine sensitivity to oxytocin was found in sheep as parturition approached (Bengtsson and Schofield, 1960; Hindson *et al.*, 1969). The onset of parturient uterine pressure waves was even more abrupt in rabbits as they only occurred at the time of delivery (Fuchs, 1964; Schofield, 1968).

Csapo and Sauvage (1968) proposed a theory to explain the evolution of uterine activity and the initiation of labor in humans based upon the ratio of uterine volume to progesterone. According to this theory uterine volume and progesterone affect uterine activity in opposite directions. When these two factors are in balance or the ratio increases gradually, pregnancy continues. However, pregnancy terminates when the balance is upset. In the last few weeks of pregnancy the ratio of uterine volume to placental progesterone was increasing at a faster rate than earlier in pregnancy. It was postulated that the mechanism of increased uterine activity following rupture of the fetal membranes was by a mechanism

other than by decreased uterine volume as the loss of 500 to 1000 ml of amniotic fluid from women at term resulted in either a lowered or unaltered uterine pressure. They speculated that loss of amniotic fluid allowed the fetal membranes to withdraw from the uterine wall and this interfered with progesterone distribution.

III. MATERIAL AND METHODS

A. General

Breeding dates were recorded while observing a Dorset ram twice daily when allowed to mix with Western cross bred ewes in a small enclosure. The ewes were randomly assigned to one of four treatment groups; either intact controls, OV (ovariectomized), AD (adrenalectomized) or AD-OV (adrenalectomized-ovariectomized). Additional ewes were bred for a fetal cannulation study and for a study of progesterone production following removal of the fetal lambs.

The ewes were sheared before surgery. Following surgery they were housed in pairs in small stalls in a heated barn and fed hay *ad libitum* and 0.5 pounds of a 14% grain ration daily. Blood samples were collected daily (0800 to 0930) following implantation of the cannulas. Replacement therapy for AD ewes was given following blood collection. The first site to be sampled daily was randomized so that either the J vein or the UO was sampled first on alternate days. Immediately after collection the blood was chilled, centrifuged, the plasma harvested and stored at -20C until assayed.

B. Surgery

1. Adrenalectomy

Adrenalectomies were performed on approximately day 90 of gestation. Preoperatively the ewes were given 40.0 mg chlor-

promazine intravenously, 2.0 mg atropine subcutaneously, and 2.0 mg DXMS intravenously. Anesthesia was induced and maintained with Na pentobarbital intravenously. Following anesthesia induction a cannula was placed in the J vein through which further increments of anesthetic agent and Plasmadex®[2] with electrolytes were administered. Both adrenals were removed during the same operation in a two step procedure similar to that described by Berg and Tilton (1968). Two incisions, one on each side of the ewe, were required. Removal of both adrenals through a single incision (Buck and Bond, 1966) was not felt to allow adequate visualization of the contralateral adrenal gland. A horizontal incision was begun at the posterior edge of the last rib and 2 cm ventral to the transverse processes of the lumbar vertebrae. The incision was continued posteriorly for about 12 cm through the skin, fascia and lateral abdominal muscles. The approach to the adrenal glands was retroperitoneal. Blood vessels entering and leaving the adrenal gland were ligated in an effort to achieve hemostasis. The adrenal gland was carefully freed of its attachments down to the region of its hilar vein. Since the right adrenal was more closely attached to the vena cava than the left, removal of the right adrenal was more hazardous. A

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[1]Thorazine®, Smith, Kline and French, Philadelphia, Pa.

[2]Diamond Laboratories, Des Moines, Ia.

Kapp Beck blood vessel clamp[1] was placed onto the vena cava and the adrenal gland. The adrenal gland was then bluntly dissected free from its final attachments. The vena cava tissue held by the jaws of the vascular clamp on the right side was closed by an interlocking suture pattern before the clamp was removed. Either 3-0 or 4-0 non-absorbable suture material with an attached needle was used. Finally the abdominal musculature was closed with a simple continuous pattern using number 2 catgut and the skin was closed with Vetafil®[2].

Immediately after adrenalectomy the ewe was given 5.0 mg deoxycorticosterone acetate (DOCA) and 25.0 mg cortisone acetate (CA) intramuscularly. This dosage was given daily but after cannulation was reduced to 2.5 mg DOCA and 12.0 mg CA daily. This latter dosage was found to maintain the plasma Na and K concentrations at levels reported for normal sheep (English _et al._, 1969). Procaine penicillin G (1,400,000 U) and streptomycin (1.75 g) were given intramuscularly daily for several days following surgery and a nitrofurazone[3] dressing was applied to the surgical wounds. Dexamethasone (1.0 mg) was occasionally administered to counteract anorexia.

The completeness of the adrenalectomy was checked by

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[1]Weck Surgical Instruments, Philadelphia, Pa.

[2]Bengen and Company, Hanover, Western Germany.

[3]Furacin®, Eaton Laboratories, Norwich, N. Y.

withholding replacement therapy starting several days postpartum. Severe weakness ensued 2 to 3 days after replacement therapy was stopped. As a further check on the completeness of the procedure, a post mortem examination of the adrenal sites was carried out and suspicious tissue saved for histopathologic examination.

2. Maternal cannulations and ovariectomy

Ovariectomy was performed during the same procedure for cannulation of the UO vein. Preoperatively the same drugs were administered as stated for the adrenalectomy procedure with the exception that ewes that were not AD did not receive DXMS. Following induction of Na pentobarbital anesthesia, with the animal in lateral recumbency, an indwelling vinyl[1] cannula (ID .058" X OD .080") was positioned in the J vein via a thin-walled 12 gauge needle. The external end of the cannula was fitted to an adapter[2] and sutured to the animal's neck.

Ovariectomy was accomplished with the ewe in dorsal recumbency through a midline abdominal incision that began close to the mammary gland and extended anteriorly for approximately 20 cm. A Carmalt forcep was placed proximal to the ovary. The ovarian vessels were ligated and the stump trans-

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[1]Becton-Dickinson and Co., Rutherford, N. J.

[2]Plastic tubing adapter, Size C, Clay-Adams, Parsippany, N. J.

fixed using Vetafil®. Finally the ovary was excised distal to the forceps.

If the animal had been OV, then the cannulation of the UO vein was accomplished through this incision, but otherwise a low flank incision was made on the side of the ewe containing the pregnant horn. Silastic®[1] tubing (ID .040" X OD .080") was used for the cannulation of the UO vein. First a small incision was made in the perimetrium over the uterine venous arcade along the lesser curvature of the pregnant horn as described by Meschia et al. (1970). The vein selected was dissected free of surrounding fascia and two lengths of suture material (2-0 silk) were passed around the vein. This exposed segment of the vein was incised with iris scissors between the ligatures and a 18 cm portion of the cannula was inserted into the vein. The results of several post mortem examinations revealed that the tip of the cannula had not advanced into the vena cava. If the cannula either met resistance near its final location or blood was not readily obtainable, the position of the cannula was changed. The ligatures around the vein were then used to secure the cannula in place. Another ligature was placed several cm from the incision to tie the cannula to the serosal surface of the uterus. The perimetrium was closed over the cannula and the cannula was exteriorized through the incision in the abdominal wall. Sufficient length of cannula was

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[1]Dow Corning, Midland, Mich.

left in the abdominal cavity to allow for uterine movement. The abdominal musculature was closed with number 3 chromic catgut using a simple interrupted pattern and the skin was closed with Vetafil®. A blunt 18 gauge needle was placed in the end of the cannula. The needle and cannula were held in apposition by a nylon strap[1] that was applied by using a tie gun[1]. Finally the cannula was filled with sterile heparinized saline (1000 U/ml), closed and the end sutured to the dorsum of the ewe. All cannulas were covered with 3" Elastikon®[2] tape.

3. Fetal cannulation

Either the fetal carotid artery or the fetal tarsal vein was cannulated between days 130 and 135 of pregnancy. The venous cannula was Silastic® tubing (ID .030" X OD. 065") with approximately 20 cm of one end marked to enter the vein. The arterial cannula was Silastic® tubing (ID .030" X OD .065") with a 12 cm end designed to enter the artery. A drop of Medical Silastic 382 Elastomer[3] was placed behind this segment. Two pieces of non-absorbable suture material with attached needles were placed in this material. A blunted 20 gauge needle was placed in the exteriorized end of both cannulas and nylon straps as previously described were used to

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[1]Extracorporeal Medical Specialties, Church Rd., Mt. Laurel Twp., N. J.

[2]Johnson and Johnson, New Brunswick, N. J.

[3]Dow Corning, Midland, Mich.

secure the needle to the cannula. Before placement the cannulas were filled with heparinized saline and closed.

A low flank incision was made on the side of the ewe where the fetal lamb could most easily be palpated. A cannula was first placed in the UO vein as previously described. For the fetal venous cannulation a purse string suture pattern was placed in a relatively avascular portion of the myometrium near the hind limbs of the fetal lamb and an incision was made in the myometrium within the placed sutures. Allis tissue forceps were used to compress the fetal membranes against the myometrium in order to prevent loss of fetal fluid. A hind limb was pulled through the incision and the purse string suture temporarily closed around the limb. A longitudinal incision was made over the lateral surface of the hock. The tarsal vein was stripped of fascia and two non-absorbable ligatures were placed around the vein. After the vein was opened with iris scissors, the cannula was inserted. Then the ligatures were tied around the vein and cannula. The skin was closed and a stay suture for the cannula was fixed to the skin immediately distal to the skin incision. The purse string suture was removed, the limb returned to the uterus, and after leaving sufficient cannula material in the uterus to allow for movement, the fetal membranes were closed with 1-0 catgut. The uterus was closed using an infolding pattern. The cannula was brought through the abdominal incision which was then closed in layers. Finally the exteriorized end of the cannula

was fixed to the dorsum of the ewe and covered with tape as previously described.

A low flank incision was likewise used in the procedure to cannulate the fetal carotid artery. The portion of the uterus containing the fetal lamb's head and neck was exteriorized. The ventral surface of the fetal lamb's head and neck was compressed against the uterine wall to prevent loss of fetal fluids and an incision approximately 6 cm in length was made in the myometrium over the anterior ventral surface of the fetal lamb's neck. The incision was continued through the fetal membranes and skin. Several Allis tissue forceps were placed around the incision to compress together the fetal skin, placental membranes and the uterine wall. A short segment of the fetal carotid artery was exposed and the artery was tied off anteriorly. A piece of suture material and a bulldog clamp were placed around the proximal end of the artery. The carotid artery was opened between the anterior ligature and the clamp. The cannula was advanced to the level of the clamp. Then subsequent removal of the clamp allowed the cannula to be inserted. Hemostasis was maintained during cannula insertion by applying tension to the positioned ligature which was tied after cannula placement. The ligatures attached to the cannula were used to stabilize the cannula by incorporating muscle and fascia before being tied. The fetal skin was closed with a simple interrupted suture pattern. After leaving some excess cannulation material in the

uterus, the fetal membranes, uterus and abdominal musculature were closed as previously described. Finally the fetal cannula was exteriorized and attached to the ewe as was described for the UO cannula.

4. Acute cannulations

The tip of the pregnant horn of the uterus was exteriorized through a low flank incision. The UO vein was first cannulated as previously described. A branch of the uterine artery was also cannulated using vinyl tubing (ID .030" X OD .046") with 18 cm of the cannula inserted into the artery. The uterus was opened and the umbilical cord delivered through the incision. Blood samples were then obtained simultaneously from the umbilical vein and artery by a syringe and needle. At approximately the same time blood samples were also obtained from the uterine artery and vein. These samples were immediately centrifuged, the plasma harvested and frozen at -20C. The fetal lamb or lambs were removed after ligating the umbilical vessels. The uterus was then closed as usual, the cannulas brought through the abdominal incision and this incision closed. The cannulas were fixed to the dorsum of the ewe under tape as previously described. Blood samples were collected from the uterine artery and vein at 1, 4, 8, and 24 h post initial sampling. Several 48 h samples from the vein were also collected. The passage of the fetal membranes was noted.

C. Hormone Assay Procedures

1. General

Competitive protein binding techniques based on the principles described by Murphy (1964, 1967) were used for the steroid hormone assays. Sensitive, precise and specific micro-methods for the quantitation of steroid hormones in blood have been developed utilizing these principles. These methods are based upon the competition between the steroid extracted from the blood and the radioactive labelled hormone for binding sites on the corticosteroid binding globulin of dog plasma in the corticoid and progesterone assay. A similar competition is utilized in the estrogen assay for binding sites in rabbit uterine cytosol. The reactions are quantitated by counting the label remaining bound to the binding sites. The larger the amount of unlabelled steroid in the system, the smaller is the amount of labelled steroid bound to the protein.

Solvents used for the extraction of plasma and chromatography were of the highest purity available. These solvents were either Nanograde from Mallinckrodt Chemical Works, Pesticide grade from Fisher Scientific Co. or Spectrograde from Eastman Kodak Co. Conical centrifuge tubes used were subjected to a sulfuric:nitric acid bath (3:1) followed by ten rinses each of distilled water and water from a Millipore Super Q[1]

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[1]Millipore Corp., Bedford, Mass.

complete system (distilled-filtered-deionized). Glass pipettes were washed first with a detergent, then water from the Super Q and finally rinsed with ethyl alcohol. Much use was made of disposable pipettes and tubes. The corticoid binding globulin was contained in dog plasma collected 36 to 48 h after adrenalectomy and used without further refinement.

Plasma samples collected from one ewe were assayed in their entirety in one assay. All plasma samples were assayed in duplicate and a set of standards accompanied each rack of unknowns which consisted of 12 duplicate samples.

Grade A standards of hydrocortisone, progesterone, estrone and estradiol-17β were purchased from Calbiochem[1]. Microgram amounts of the standards were weighed on a Cahn Electrostatic Balance[2] prior to dilution for use in the assays. All isotope labelled steroids were purchased from New England Nuclear Corp. and generally used without further purification. Occasionally labelled steroids were chromatographed after received in the laboratory according to Quesenberry and Ungar (1964). Solubilizers used were either Biosolv #3[3] or Triton X-100[4].

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[1]Calbiochem, La Jolla, Calif.

[2]Cahn Instrument Co., Paramount, Calif.

[3]Beckman Instruments, Fullerton, Calif.

[4]Sigma Chemical Co., St. Louis, Mo.

2. Corticoid assay

Plasma samples were assayed for total corticoid content by the competitive binding technique (Murphy, 1967). Plasma samples and cortisol standards (0.2 ml) were pipetted into 16 X 125 mm screw cap disposable tubes. Standards used were 0, 3, 6, 12, 24, 36, 48 and 60 ng/ml concentration prepared in distilled water. Assay samples and standards were first extracted with 5 ml of hexane to remove progesterone. The tubes were then centrifuged to separate the hexane and plasma. The plasma was frozen at the bottom of the tubes in a dry ice-alcohol bath and the hexane discarded. After the plasma thawed, 4.5 ml of methylene chloride were added and the tubes were shaken and centrifuged as previously described. The plasma or water phase on top of the solvent was removed by aspiration. One ml of the methylene chloride extract was transferred to each of two 12 X 75 mm disposable glass tubes (unknowns) or each of three tubes (standards). The methylene chloride was then evaporated in a 45C water bath. One ml of the corticoid-binding globulin (CBG) 1,2-^{3}H hydrocortisone solution (Table A1) was added to each tube (224 pg hydrocortisone). The tubes were incubated for five min in a 45C water bath, 15 min in a 10C water bath and 40 mg of washed florisil[1] (60-100 mesh) were added sequentially to each tube. The tube was mixed slowly on a Vortex mixer for 30 s and returned to

[1]Fisher Scientific Co., Pittsburgh, Pa.

the 10C water bath for five min. Following this an 0.5 ml aliquot of the supernatant was transferred using an Eppendorf 500 pipette[1] to a scintillation vial. Ten ml of scintillation fluid A (Table A2) were added, mixed and the radioactivity determined in a Beckman LS100 Scintillation Counter.

The slope of the regression line was calculated from the data of the standards using time to a preset total count as the end point. The calculated slope was used in the following equation to calculate the mean concentration of corticoids in the unknown samples.

$$Y_i - \bar{y} = b(X_i - \bar{x})$$

The equation was solved for X_i (concentration of corticoid in unknown sample) as follows:

$$X_i = \frac{Y_i - \bar{y} + b\bar{x}}{b}$$

The terms of the equation were defined as follows:

Y_i = Average time required to count the unknown duplicates

$\bar{y}$ = Mean time required to count standards

b = Slope of the standard curve

X_i = Corticoids (ng/ml) in unknown

$\bar{x}$ = Mean corticoids (ng/ml) for all standards

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[1]Brinkman Instruments, Westbury, N. Y.

3. Progesterone assay

Plasma samples were assayed for progesterone by the competitive protein binding technique of Neil *et al.* (1967). Two ml of J plasma or 0.3 to 0.5 ml of UO plasma were pipetted into 16 X 125 mm screw cap tubes. Background value for the assay was determined by including three tubes each containing 2.0 ml of distilled water. The accuracy of the assay was determined by assaying plasma of known progesterone concentration (0.5 ng/ml and 3.0 ng/ml). Tracer amounts of $4\text{-}^{14}C$ progesterone, equivalent to 170 counts per min (cpm) were added to each tube and directly to two scintillation vials for recovery data. The samples were then extracted three times with 10 ml petroleum ether. During each extraction the tubes were shaken manually for 30 s and centrifuged. The plasma was frozen in the bottom of the tubes in a dry ice-alcohol bath and the ether was decanted into conical centrifuge tubes. The ether was then evaporated by placing the tubes in a 45C water bath. After the ether extract had evaporated, the sides of the tubes were rinsed with a small aliquot of petroleum ether which was also evaporated. The samples were spotted on 20 X 20 cm thin-layer silica gel chromatogram sheets, MN-Polygram® (N-HR/UV254, Machery-Nagel, Duren, W. Germany)[1] with 0.2, 0.1 and 0.1 ml of benzene:methylene chloride (1:1). Six lanes were marked off

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[1]Purchased from Brinkman Instruments.

by scoring with a needle. Concentrated progesterone was spotted on the outside lanes of each sheet and a sample on each of the four inner lanes. The sheets were submitted to ascending chromatography in diethyl ether:benzene (2:1). The progesterone control spots were located by their absorption under ultraviolet light and the area in each sample lane corresponding to these spots was delineated. The sample spots were scraped into tightly curled bunches and placed in disposable Pasteur pipettes previously packed with a small amount of glass wool. The glass wool was washed with sulfuric:nitric acid (2:1) and distilled water and the prepared pipettes were rinsed with absolute methanol before use. The progesterone was eluted in 3.0 ml absolute methanol. For each sample 0.9 ml of the eluate was placed in a scintillation vial. The eluate was allowed to dry in the vials and 10 ml scintillation fluid B (Table A2) were added. This was counted for ^{14}C so that recovery values could be calculated. Two other 0.9 ml aliquots of the eluate were placed in 12 X 75 mm tubes and evaporated in a 45C water bath under a stream of filtered air.

Three sets of progesterone standards prepared in absolute methanol corresponding to 0.0, .25, .5, 1.0, 3.0, 5.0 and 10.0 ng of progesterone were placed in 12 X 75 mm tubes and evaporated. One ml of CBG 1,2-^{3}H corticosterone solution (Table A1) was added to each sample and standard tube (35 pg corticosterone). The tubes were then placed in a 45C bath

for five min after which they were removed and placed in a 10C water bath for 10 min. At the end of this period each tube sequentially received 75 mg florisil. The tube was then shaken slowly on a vortex mixer for 30 s, returned to the 10C water bath for five min and 0.5 ml of the supernatant was transferred to a scintillation counting vial using an Eppendorf 500 pipette. Ten ml of scintillation fluid A (Table A2) were added, mixed and the radioactivity was determined.

A standard curve was drawn on semi-log paper using cpm from the progesterone standards. A value for each sample was determined from this curve and corrected according to the following equations to give a value in ng progesterone/ml for each sample.

$$\frac{(^{14}\text{C cpm} - {}^{14}\text{C bkg})\ (^{14}\text{C aliquot correction factor})}{^{14}\text{C total cpm} - {}^{14}\text{C bkg}} = \%\ \text{recovery}$$

$$\frac{1}{\%\ \text{recovery}} \times \underset{\text{read from graph}}{\text{ng progesterone}} = \underset{\text{corrected for loss}}{\text{ng progesterone}}$$

$$\underset{\text{corrected for loss}}{\text{ng progesterone}} \times \frac{\text{assay aliquot correction factor}}{\text{plasma extracted (ml)}} - \underset{\text{background}}{\text{assay}} =$$

progesterone ng/ml plasma

4. Estrogen assays

Plasma samples were assayed for estrone and estradiol according to the radio-ligand assay technique described by

Korenman (1970). Plasma samples were extracted twice with 10 ml anhydrous diethyl ether[1]. A new can of ether was opened for each assay. The amount of plasma assayed varied from 0.5 to 2.0 ml of UO plasma and 1.0 to 4.0 ml of J plasma. The amount of plasma assayed depended on the relationship to the time of lambing and, therefore, the expected estrogen concentration. In each assay two distilled water samples (1.0 ml) were included as blanks. Following addition of ether, the tubes were shaken manually for 30 s, centrifuged, the plasma frozen in a dry ice-alcohol bath and the ether phase decanted into conical centrifuge tubes. The ether extracts were dried in a 45C water bath. The sides of the tubes were then washed down with a small aliquot of ether and redried.

The dried extracts were next transferred to a prepared celite column (Table A3) with three X 0.3 ml of iso-octane. Solvents were forced through the columns under 2 psi air pressure. The columns were first washed with 8.0 ml iso-octane and the eluate discarded. Next estrone (E_1) was eluted from the columns with 4.0 ml 15% ethyl acetate-iso-octane. Finally estradiol (E_2) was eluted from the columns with 5.0 ml 30% ethyl acetate-iso-octane. The E_1 and E_2 fractions were evaporated in a 45C water bath. The sides of the tubes were washed with a small aliquot of ethyl acetate

[1]Spectrograde, Eastman Kodak Co., Rochester, N. Y.

and the tubes redried.

The E_1 and E_2 extracts were reconstituted in 0.6 ml of tris-HCl-buffer (Table A4) using a Vortex mixer. Aliquots (0.2 ml) of this solution were transferred to each of two 12 X 75 mm polypropylene tubes containing 0.25 ml of tris-HCl-ethylene glycol buffer (Table A4) for the E_1 fractions or to tubes containing 0.24 ml of the buffer for the E_2 fractions. Twenty µl of ^3H estrogen (6,7-^3H estradiol-17β or 6,7-^3H estrone) containing approximately either 380 pg E_1 or 268 pg E_2 were added to each tube. Finally rabbit uterine cytosol solution (Table A5) was added to each tube (0.03 ml for E_1 and 0.04 ml for E_2). Thus all tubes contained 0.5 ml.

A duplicate set of standards for E_1 (0, 10, 25, 50, 100, 200, 300, 400, 500, 600 pg) and E_2 (0, 5, 10, 20, 40, 100, 200 pg) were included in each assay. Standard solutions were prepared such that 0.2 ml would yield the desired amount of estrogen in the standards. This amount (0.2 ml) was added to each standard tube and the other reagents added as for the unknowns. The standard and unknown tubes were mixed by gentle rocking and incubated 18 to 24 h at 4C.

Following incubation, 0.5 ml of charcoal-dextran-tris suspension (Table A4) was added to each tube. The tubes were briefly shaken, incubated in a 15C water bath for 15 min and centrifuged. The supernatant was then poured into a counting vial and 10 ml of scintillation fluid C (Table A2) were added. The percentage of tritiated estrogen bound to cytosol for each

standard was calculated and used to plot a standard curve. Blanks were used to determine the 100% bound. Values in pg/ml for the plasma samples were determined from the standard curve. The concentration of estrogen (E_c) in pg/ml plasma was calculated according to the following formula:

$$E_c = \frac{T_t}{T_a}(E)\frac{1}{V}$$

T_t = Total amount of tris-HCl used for each sample (0.6 ml)

T_a = Amount of tris-HCl used per assay tube

E = Pg of estrogen read from standard curve

V = Volume (in ml) of plasma extracted

D. Statistical Analysis

The effect of treatments, days and treatment X day interaction on plasma corticoids, progesterone, estrone and estradiol levels in both UO and J plasma was examined by analyses of variance (Steel and Torrie, 1960). Using the data in the analyses of variance, correlation coefficients were calculated between the various steroid levels. Mean differences were calculated according to Student's t test.

IV. Results

A. General

Twenty-one ewes were in the four treatment groups as shown in Table 2. The mean gestation lengths and number of lambs born to each group are also given. The individual gestation length and number of lambs born to each ewe are shown in Table A6.

Table 2. Mean gestation length and number of lambs born

	Control	AD[a]	OV[b]	AD-OV[c]
No. ewes	7	6	4	4
Mean gestation length in days	145.8	146.2	143.7	141.8
Live births				
Single	3	3	2	1
Sets of multiple	3	3	1	2
No. dead lambs	2	0	1	1

[a]Adrenalectomized.

[b]Ovariectomized.

[c]Adrenalectomized-ovariectomized.

Days before (-) and after (+) parturition are given in the appropriate tables in this section. Day 0 was defined as the last day a blood sample was collected before parturition and day +1 was defined as the day the first samples were

collected following parturition.

Adrenalectomy procedures and further surgery for cannulations and ovariectomy were attempted on a considerably greater number of ewes than those that eventually carried their young to an apparently normal parturition. Losses from the experiment came from deaths of the ewes, abortions and incomplete adrenalectomies. Surgery was initially performed with halothane inhalation anesthesia. However, following the report of Comline and Silver (1970) of a large number of ewes aborting after halothane anesthesia, Na pentobarbital became the anesthetic agent. A total of nine AD ewes either died or aborted following ovariectomy and cannulation procedures. Since none of the intact control ewes died or aborted following placement of UO cannulas, the AD ewes were regarded as great surgical risks. Four AD ewes were judged not to have been completely adrenalectomized. Adrenal tissue was found at necropsy in one ewe while the other three did not exhibit profound weakness following replacement therapy removal.

Utero-ovarian cannulas remained patent for as long as 39, 35, 32, 27 and 26 consecutive days. Seventy per cent of the UO cannulas remained open for 8 or more days after initial insertion. It was not uncommon to be unable to secure a blood sample from a UO cannula one day and to be successful the next day. Thirty per cent of the UO cannulas were not patent postpartum.

In order to perform the analyses of variance, data from days that did not have values for all eight possible determinations were excluded. Therefore, data from 72 of 273 ewe days were excluded from the analyses of variance. Overall mean values for the entire sampling period based on these 201 ewe days are given by treatment groups in Table 3. The mean values for this statistical treatment of the data are presented in Tables A7 through A10. These mean values are slightly different from those determined using all the data which are presented in tables in this results section. The means used in the analyses of variance and the appropriate mean squares were used for the "Studénts" t tests except where specified.

B. Corticoids

A typical cortisol standard curve is shown in Figure 1A.

Precision for the procedure was calculated by the method of Snedecor (1952) from the results of duplicate measurements on sheep plasma using the formula

$$S = \sqrt{\frac{\Sigma d^2}{2N}}$$

Table 3. Mean steroid levels for the period day -10 through day +2 in the utero-ovarian (UO) and jugular (J) plasma

		1	2	3	4
Treatments		Intact control	AD	OV	AD-OV
No. observations		61	66	38	36
Corticoids (ng/ml)	UO	20.8	4.0	19.1	4.0
	J	20.6	3.2	18.2	4.0
Progesterone (ng/ml)	UO	61.8	55.7	46.9	52.4
	J	15.5	7.5	10.3	9.9
Estrone (pg/ml)	UO	167.1	59.7	91.1	54.0
	J	128.6	51.5	74.1	51.7
Estradiol (pg/ml)	UO	31.8	22.9	34.2	22.4
	J	33.7	16.8	29.4	18.3

Corticoids UO 1 vs 2, 1 vs 4 $P<.001$.

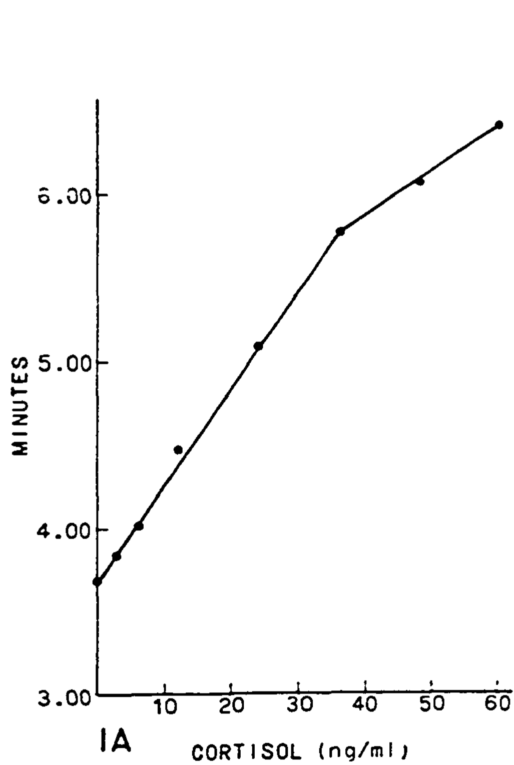
Corticoids J 1 vs 2 $P<.001$, 1 vs 4 $P<.005$.

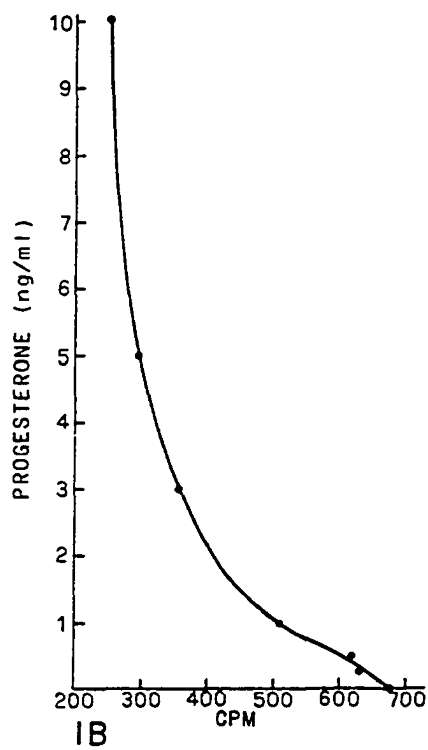
Progesterone J 1 vs 2 $P<.005$, 1 vs 3 $P<.05$, 1 vs 4 $P<.025$.

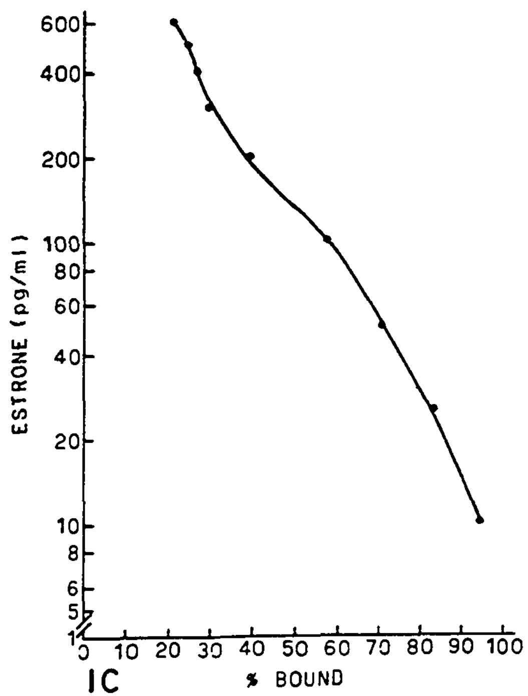
All other comparisons between groups were non-significant.

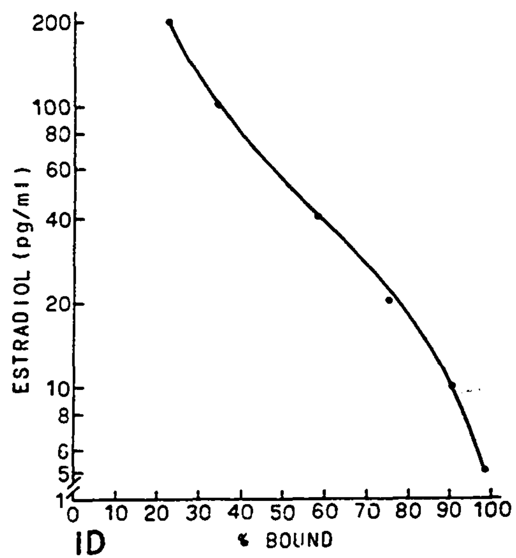
Figure 1. Standard curves in steroid assays

1A. Cortisol (ng/ml) standard curve
1B. Progesterone (ng/ml) standard curve
1C. Estrone (pg/ml) standard curve
1D. Estradiol (pg/ml) standard curve


MINUTES
6.00
5.00
4.00
3.00
10 20 30 40 50 60
IA
CORTISOL (ng/ml)


PROGESTERONE (ng/ml)
10
9
8
7
6
5
4
3
2
1
200 300 400 500 600 700
CPM
IB


ESTRONE (pg/ml)
600
400
200
100
80
60
40
20
10
8
6
5
1
0 10 20 30 40 50 60 70 80 90 100
IC
% BOUND


ESTRADIOL (pg/ml)
200
100
80
60
40
20
10
8
6
5
1
0 10 20 30 40 50 60 70 80 90 100
ID
% BOUND

where S is the coefficient of variation between duplicate observations, d is the difference between the two results of a duplicate measurement and N is the number of duplicate measurements. These S values were determined from differences in time to a preset count. The S values were as follows: 3.79 (0-5 ng/ml, n=40); 5.71 (5-10 ng/ml, n=42); 9.76 (10-20 ng/ml, n=39); 8.98 (>20 ng/ml, n=42).

Mean UO plasma corticoid levels in intact control ewes varied from a low of 11.3 ng/ml at day -3 to peak levels of 32.5 and 35.5 ng/ml at day 0 and day +1, respectively (Table 4). Jugular plasma corticoid values in control ewes varied from 8.7 ng/ml at day -3 to 35.1 and 28.2 ng/ml at day 0 and day +1, respectively. A comparison of mean corticoid concentrations on days -8 to -3 and +2 vs day 0 revealed a significant rise in both the UO ($P<.005$) and J ($P<.01$) plasma.

The location of the greater corticoid levels varied between the J and UO veins (Figure 2) except at day -2 when the UO value was greater in all six control ewes. The differences (21.0, 7.8, 10.4, 12.1, 1.3 and 2.0 ng/ml) were significant ($P<.05$) when tested according to a paired observation analysis (Steel and Torrie, 1960).

Plasma corticoid levels in AD ewes were invariably markedly lower compared to intact controls (Table 4, Figure 2). The difference in the overall corticoid concentration (Table 3) between control and AD ewes was highly significant ($P<.001$). Utero-ovarian plasma corticoid values were generally

Table 4. Plasma corticoids (ng/ml) in intact and adrenalectomized (AD) ewes[a]

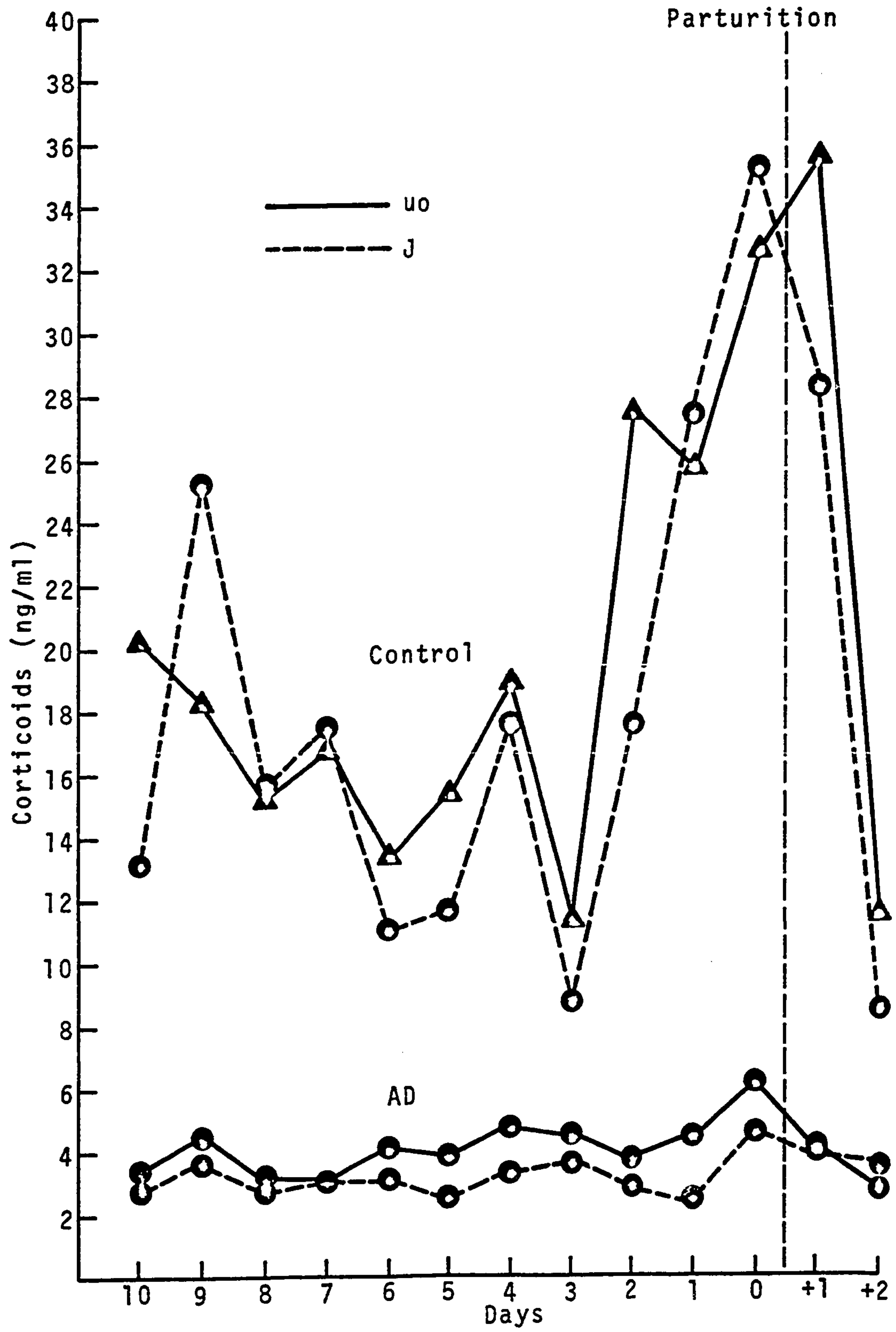
Days[b]	Intact		AD	
	UO	J	UO	J
- 10	20.1± 6.3 (6)[c]	13.1±3.1 (5)	3.3±0.8 (3)	2.7±1.0 (5)
- 9	18.3± 2.4 (6)	25.2±6.6 (6)	4.5±1.7 (4)	3.6±1.1 (5)
- 8	15.1± 2.0 (6)	15.5±5.8 (6)	3.1±1.0 (6)	2.7±0.8 (6)
- 7	16.7± 1.9 (6)	17.5±6.1 (6)	3.0±0.9 (6)	3.0±1.0 (6)
- 6	13.3± 2.3 (6)	11.0±1.8 (6)	4.1±1.0 (6)	3.1±0.8 (5)
- 5	15.3± 1.2 (7)	11.6±2.3 (7)	3.8±0.8 (6)	2.5±0.7 (6)
- 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)	4.6±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)

[a]Plasma levels in utero-ovarian (UO) and jugular (J) veins.

[b]Days before (-) and after (+) parturition.

[c]Mean±S.E., (number of observations).

Figure 2. Total plasma corticoids (ng/ml) in the utero-ovarian (UO) and jugular (J) veins of control and adrenalectomized (AD) sheep before and after parturition


Parturition
uo
J
Control
AD
Corticoids (ng/ml)
Days
40
38
36
34
32
30
28
26
24
22
20
18
16
14
12
10
8
6
4
2
10
9
8
7
6
5
4
3
2
1
0
+1
+2

slightly greater than J values in AD ewes. The exception was day -7 when the values from the two locations were the same. Plasma corticoid levels in AD ewes rose on day 0 and then decreased.

In OV ewes mean plasma corticoid levels decreased on day -1 in both the UO and J plasma (Table 5). Compared to the intact controls plasma corticoid concentrations from OV ewes were significantly lower in both sampling locations only on day -1 (UO, P<.001 and J, P<.005). The overall corticoid means (Table 3) were not significantly different for these two treatment groups. On day 0 there was a significant elevation in the plasma corticoid levels in OV ewes compared to day -3 (UO, P<.001 and J, P<.005). In contrast to intact control ewes, there was no difference in plasma corticoid levels in the UO vein compared to the J vein in OV ewes on day -2.

Plasma corticoid levels in AD-OV ewes (Table 5) were markedly lower than those levels in intact controls (Table 4). The overall corticoid means (Table 3) were significantly different (P<.005) between these two treatment groups. Utero-ovarian plasma corticoid levels were slightly greater than J levels on only three days (days -6, -5 and -2) prepartum. Plasma corticoid values in AD-OV ewes generally rose in the UO vein in a period from days -8 through -5 and then continuously declined through day +2. The same general pattern existed for plasma corticoid levels in the J vein.

Treatments significantly (P<.01) affected the plasma

Table 5. Plasma corticoids (ng/ml) in ovariectomized (OV) and adrenalectomized-ovariectomized (AD-OV) ewes[a]

Days[b]	OV		AD-OV	
	UO	J	UO	J
-10	17.2± 7.4 (2)[c]	14.1± 5.2 (2)		
- 9	30.6±13.2 (2)	30.5±10.0 (2)	2.3±1.0 (3)	2.9±1.5 (3)
- 8	20.7± 9.0 (4)	23.5±11.5 (4)	3.2±1.5 (3)	3.9±1.8 (3)
- 7	12.6± 1.5 (3)	22.8± 7.9 (4)	3.1±0.7 (4)	3.4±0.9 (4)
- 6	15.9±11.2 (2)	28.1± 9.3 (4)	4.3±1.6 (4)	3.5±1.1 (4)
- 5	12.5± 4.4 (4)	15.7± 6.0 (4)	5.8±2.2 (4)	3.2±0.8 (4)
- 4	18.6± 5.7 (4)	15.7± 7.7 (4)	5.4±1.6 (4)	5.5±1.1 (4)
- 3	16.1± 2.0 (4)	13.0± 2.7 (4)	5.3±1.6 (4)	6.0±1.3 (4)
- 2	15.2± 2.4 (4)	12.7± 3.0 (4)	5.2±2.3 (3)	4.0±0.7 (4)
- 1	8.8± 1.1 (4)	11.4± 3.6 (4)	3.2±1.9 (3)	5.5±1.7 (4)
0	41.1±10.9 (4)	36.0±12.1 (4)	2.9±0.1 (2)	5.7±1.2 (4)
+ 1	16.2± 1.2 (2)	20.8±14.3 (4)	2.6±0.7 (2)	3.5±0.9 (3)
+ 2	14.6± 2.3 (3)	13.2± 4.1 (4)	1.7±1.7 (2)	2.0±2.0 (3)

[a]Plasma levels in utero-ovarian (UO) and jugular (J) veins.

[b]Days before (-) and after (+) parturition.

[c]Mean±S.E., (number of observations).

corticoid levels in both locations (Table 6). Also the corticoid levels were found to significantly (P<.01) vary with days and there was a significant (P<.01) treatment X days interaction.

Table 6. The effects of treatments, days and treatment X day interaction on utero-ovarian (I) and jugular (II) plasma corticoids[a]

Source of variation	Degrees of freedom	Mean square	F
I			
Treatment	3	3163	11.18**
Animals (treatment)	17	283	
Day	12	197	4.19**
Treatment X day	35	119	2.53**
Residual	133	47	
II			
Treatment	3	3677	12.86**
Animals (treatment)	17	286	
Day	12	227	3.20**
Treatment X day	35	140	1.97**
Residual	133	71	

[a]Based on data in Tables A7 to A10.

**P<.01.

C. Progesterone

A typical progesterone standard curve is shown in Figure 1B. Blank values corrected for recovery of added tracer had a mean value of 0.88 ng/ml (n, 55). Recovery of ^{14}C-progesterone added to the plasma samples for estimation of losses due to procedures including chromatography were 59%, 61%, 58% and 64% in four representative assays. Two progesterone standards prepared in blank sheep plasma were included in each assay. The results were 0.39 ng±0.11 (mean±S.E., n=12) for the 0.5 ng standard and 3.80 ng±0.64 (mean±S.E., n=13) for the 3.0 ng standard. The coefficient of variation determined from cpm was 31.20 (0.0 - 5.0 ng, n=55), 18.11 (6.0 - 10.0 ng, n=44), 20.63 (11.0 - 20.0 ng, n=33) and 16.21 (>20.0 ng, n=129).

Utero-ovarian plasma progesterone levels were markedly higher than J levels in all treatment groups (Tables 7, 8). Figure 3 illustrates the relationship of UO plasma progesterone levels to peripheral levels in control ewes. In each treatment group the overall mean UO plasma progesterone level was significantly (P<.001) higher than the comparable J level (Table 3). A significant correlation (r=0.415, P<.001) existed between UO and J plasma progesterone levels.

Peripheral progesterone concentrations remained relatively constant from day -10 to day -2, with some slight decline toward the end of this period. These concentrations began to decrease sharply on day -1 and were <1.0 ng in all groups by day +1.

Table 7. Jugular plasma progesterone (ng/ml) in ewes

Days[a]	Intact	OV[b]	AD[c]	AD-OV[d]
-10	17.0±4.1 (5)[e]	10.9±3.7 (2)	10.7±2.6 (5)	
- 9	18.8±6.4 (6)	14.4±7.1 (2)	10.4±2.3 (5)	13.6±7.7 (3)
- 8	20.6±5.7 (6)	15.7±8.0 (4)	9.7±1.4 (6)	14.8±9.5 (3)
- 7	19.5±3.9 (6)	20.2±8.5 (4)	7.1±1.1 (6)	13.1±4.6 (4)
- 6	16.6±4.6 (7)	13.9±7.4 (4)	11.1±3.9 (5)	11.0±3.8 (4)
- 5	17.3±4.8 (7)	10.5±4.6 (4)	10.0±2.7 (6)	10.8±2.9 (4)
- 4	16.1±3.7 (7)	11.5±4.2 (4)	7.3±1.8 (6)	9.8±5.1 (4)
- 3	15.0±4.1 (7)	13.1±6.4 (4)	9.8±2.6 (6)	9.2±3.6 (4)
- 2	16.2±4.8 (7)	10.9±5.3 (4)	5.8±1.4 (6)	9.6±5.1 (4)
- 1	11.7±3.0 (7)	5.8±3.6 (4)	5.3±1.6 (6)	9.0±4.0 (4)
0	8.7±2.6 (7)	7.3±5.7 (4)	5.6±2.2 (6)	5.8±4.3 (4)
+ 1	0.8±0.4 (6)	0.3±0.3 (4)	0.6±0.2 (6)	0.9±0.5 (3)
+ 2	0.9±0.3 (7)	0.2±0.2 (4)	0.5±0.2 (6)	0.5±0.5 (3)

[a]Days before (-) and after (+) parturition.

[b]Ovariectomized.

[c]Adrenalectomized.

[d]Adrenalectomized-Ovariectomized.

[e]Mean±S.E., (number of observations).

Table 8. Utero-ovarian plasma progesterone (ng/ml) in ewes

Days[a]	Intact	OV[b]	AD[c]	AD-OV[d]
-10	55.9± 9.9 (5)[e]	62.0±15.6 (2)	57.4± 8.1 (4)	
- 9	87.4±21.2 (6)	54.5±15.7 (2)	62.1±18.6 (4)	41.2± 8.9 (3)
- 8	70.7± 4.6 (6)	62.3±21.3 (4)	55.5±10.8 (6)	62.5±19.3 (3)
- 7	95.9±35.0 (4)	53.8±25.4 (3)	50.4± 7.5 (6)	53.0±22.3 (4)
- 6	70.6±16.6 (6)	47.8±27.6 (2)	82.8±14.5 (6)	83.1±11.6 (4)
- 5	74.2±13.8 (7)	65.5±17.1 (4)	71.8±11.0 (6)	72.4±19.1 (4)
- 4	63.2±11.0 (6)	67.6±19.3 (4)	136.2±91.2 (6)	63.2±20.0 (4)
- 3	58.9± 9.7 (5)	63.6±16.9 (4)	63.1±12.9 (6)	48.4±17.7 (4)
- 2	47.9±11.9 (6)	51.6±14.3 (4)	39.1± 5.3 (6)	41.6±25.9 (3)
- 1	41.1±10.0 (7)	30.8±13.5 (4)	50.1±17.0 (5)	49.8±34.3 (3)
0	30.3± 9.7 (5)	21.9± 9.8 (4)	24.0± 9.1 (5)	9.4± 3.7 (2)
+ 1	9.0± 8.8 (3)	0.9± 0.9 (2)	2.1± 0.5 (4)	0.2 (1)
+ 2	1.6± 1.6 (2)	1.6± 0.9 (3)	1.9± 0.9 (4)	0.4 (1)

[a]Days before (-) and after (+) parturition.

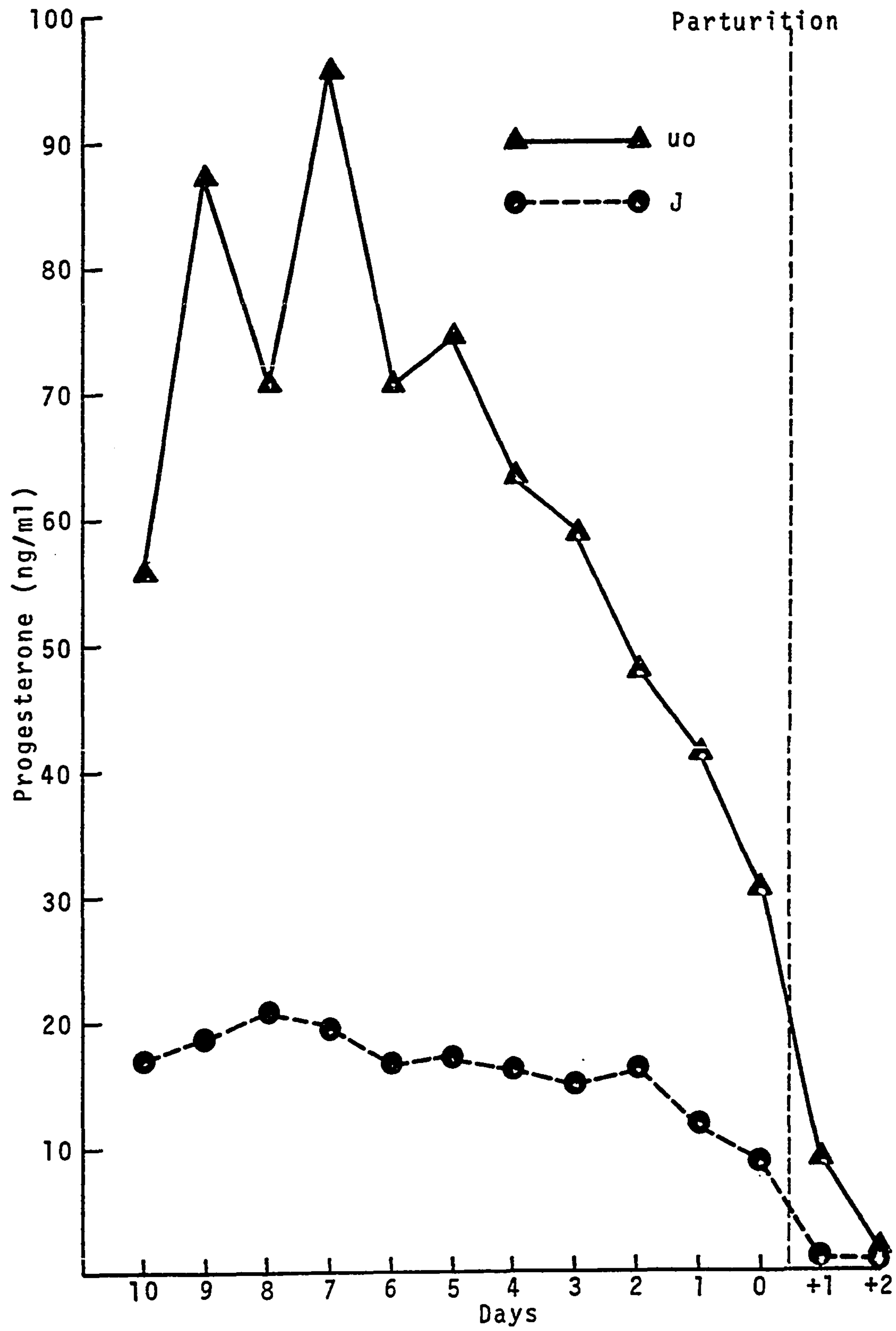
[b]Ovariectomized.

[c]Adrenalectomized.

[d]Adrenalectomized-ovariectomized.

[e]Mean±S.E., (number of observations).

Figure 3. Plasma progesterone (ng/ml) in the utero-ovarian (UO) and jugular (J) veins of control sheep before and after parturition



Progesterone (ng/ml)
100
90
80
70
60
50
40
30
20
10
Parturition
uo
J
10
9
8
7
6
5
4
3
2
1
0
+1
+2
Days

Progesterone levels in the UO plasma declined slightly in all groups from the period days -10 through -4 compared to the period days -3 through -1 (Table 8). A prepartum decline in progesterone levels was evident earlier in the UO plasma (day -4) than in the J plasma (day -1) in control ewes (Figure 3).

By day +2 both UO and J progesterone levels had declined to 1.9 ng/ml or less in all groups. At this time UO and J concentrations were not significantly different. This decline in progesterone values before and continuing postpartum was undoubtedly responsible for the significant (P<.01) day effect on progesterone levels (Table 9).

Progesterone determinations were performed on plasma samples from two control and two AD ewes from days -16 through -10. Mean UO progesterone value for these control ewes for days -14 through -16 was 63.5 ng/ml compared to 52.5 ng/ml for days -10 through -8. Mean UO progesterone values from AD ewes for the same periods, respectively were 49.5 ng/ml and 44.3 ng/ml. These differences were not significant in either group.

Intact control ewes carrying more than one fetal lamb invariably had higher plasma progesterone values than ewes with one fetal lamb (Table 10, Figure 4). Analysis of the data in Table 10 revealed that ewes bearing multiple lambs had significantly (P<.05) higher J progesterone levels than ewes with single lambs during the entire prepartum period

Table 9. The effects of treatments, days and treatment X day interaction on utero-ovarian (I) and jugular (II) plasma progesterone[a]

Source of variation	Degrees of freedom	Mean square	F
I			
Treatments	3	1626	0.22
Animals (treatment)	17	7498	
Day	12	4622	2.83**
Treatment X day	35	988	0.60
Residual	133	1634	
II			
Treatments	3	399	0.70
Animals (treatment)	17	571	
Day	12	183	7.63**
Treatment X day	35	25	1.04
Residual	133	24	

[a]Based on data in Tables A7 to A10.

**P<.01.

while UO progesterone values were significantly different only on days -10, -1 and 0 (P<.025). After UO progesterone values were plotted from control ewes according to numbers of fetal lambs (Figure 4), it was evident that UO plasma progesterone levels were steadily declining 4 to 7 days prepartum

Table 10. Plasma progesterone (ng/ml) in ewes bearing single and multiple lambs

Days[a]	Jugular		Utero-ovarian	
	Single	Multiple[b]	Single	Multiple[b]
-10	7.0±2.7 (2)[c]	23.7±0.6 (3)	40.7± 5.1 (3)	78.8± 5.4 (2)
- 9	7.1±1.5 (3)	30.7±8.2 (3)	59.1±11.1 (3)	115.7±36.5 (3)
- 8	10.0±1.8 (3)	31.2±7.1 (3)	66.3± 4.6 (3)	75.0± 8.2 (3)
- 7	7.4±3.3 (2)	25.6±0.6 (4)	53.1±19.0 (2)	138.7±57.7 (2)
- 6	7.2±1.2 (3)	23.4±6.0 (4)	40.4±14.8 (3)	101.5±27.7 (2)
- 5	4.7±1.0 (3)	26.8±3.3 (4)	51.6±10.2 (3)	91.2±19.6 (4)
- 4	7.9±1.5 (3)	22.2±4.0 (4)	46.7±10.5 (3)	79.8±14.9 (3)
- 3	5.1±1.6 (3)	22.6±3.7 (4)	42.9± 6.4 (2)	69.5±12.7 (3)
- 2	4.9±0.3 (3)	24.7±5.1 (4)	28.7± 3.6 (3)	67.2±18.2 (3)
- 1	5.2±1.3 (3)	16.6±3.7 (4)	20.3± 2.1 (3)	62.0±10.0 (4)
0	3.8±1.7 (3)	12.5±3.6 (4)	8.5± 3.4 (2)	44.8± 7.1 (3)
+ 1	0.3±0.1 (2)	1.1±0.5 (4)	0.2± 0.1 (2)	26.8 (1)
+ 2	0.5±0.3 (3)	1.3±0.4 (4)	0.0± (1)	3.3 (1)

[a]Days before (-) and after (+) parturition.

[b]Three ewes delivered twin lambs and one ewe delivered triplets.

[c]Mean±S.E., (number of observations).

The number of ewes carrying multiple/single lambs in the control group was 4/3, 1/3 in the OV group, 3/3 in the AD group and 2/2 in the AD-OV group. Since the number of fetal lambs significantly affected peripheral plasma progesterone levels, comparisons between groups should account for this variable.

There was no significant effect of ovariectomy on either J (Table 7) or UO (Table 8) progesterone levels when the daily means were compared. This was in agreement with the fact that treatments did not significantly affect plasma progesterone levels (Table 9). However, based on overall means (Table 3), peripheral plasma progesterone levels were significantly ($P<.05$) reduced in OV ewes compared to control ewes. This effect may be attributable to a greater ratio of multiple:single lambs in the control groups. From days -10 to -4, OV ewes with single lambs had a mean J progesterone value of 10.2 ng/ml compared to 7.3 ng/ml for similar intact ewes during this period. Utero-ovarian plasma progesterone levels in control and OV ewes with single lambs were 51.1 and 56.9 ng/ml, respectively, for this time period.

Jugular plasma progesterone levels were compared in intact control ewes to AD ewes after each group was normalized to three ewes carrying single lambs and three carrying twin lambs. Intact control animals had consistently higher J plasma progesterone levels compared to AD ewes (Table 11, Figure 5). This difference in J plasma progesterone (based

Figure 4. Plasma progesterone (ng/ml), before and after parturition, in the utero-ovarian (UO) and jugular (J) veins of sheep carrying single or multiple fetal lambs

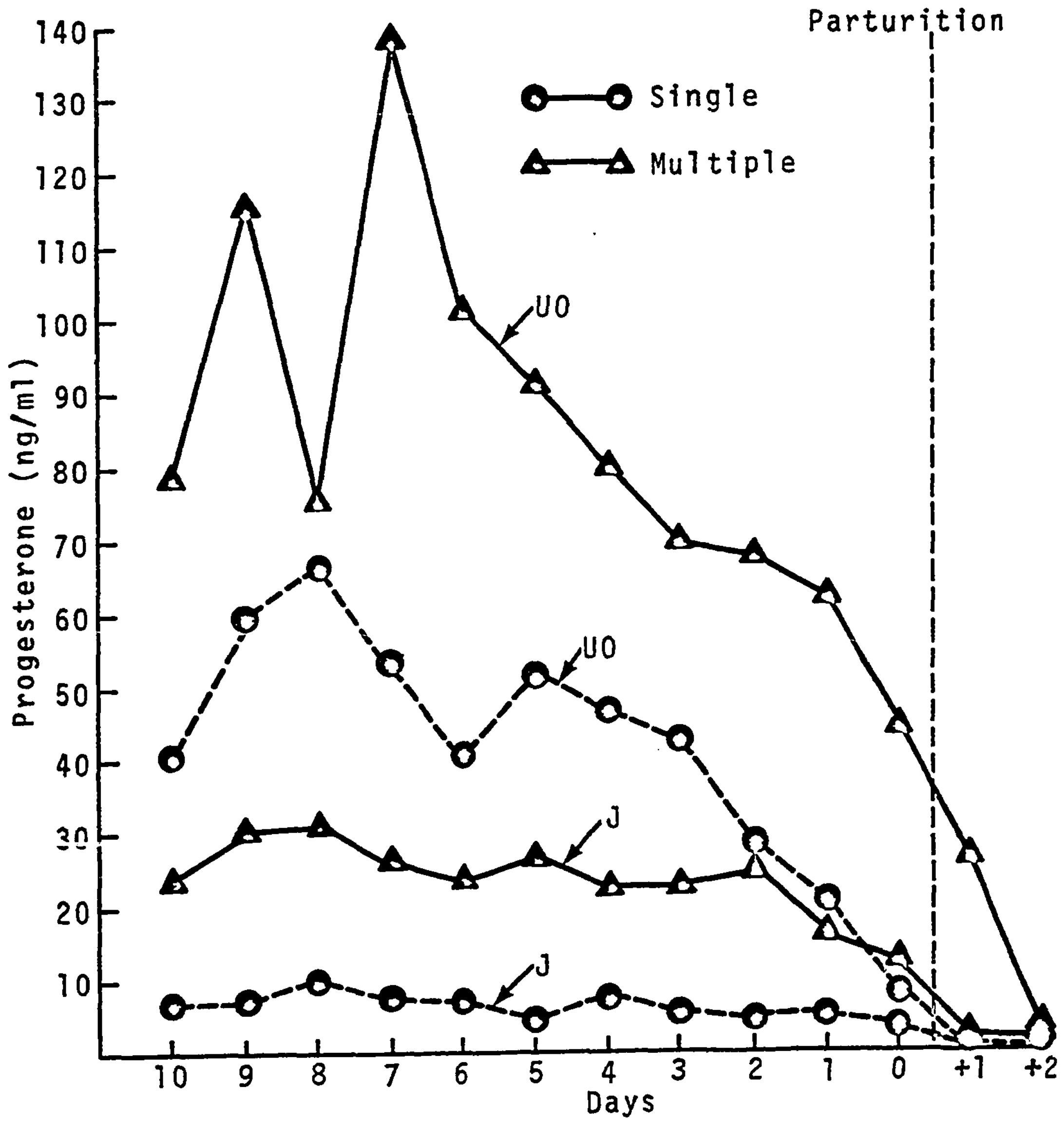

Parturition
Single
Multiple
Progesterone (ng/ml)
Days
UO
UO
J
J
140
130
120
110
100
90
80
70
60
50
40
30
20
10
10
9
8
7
6
5
4
3
2
1
0
+1
+2

Table 11. Jugular plasma progesterone (ng/ml) in intact and adrenalectomized (AD) ewes[a]

Days[b]	9	7	5	3	1
Intact	18.9±6.4 (6)[c]	18.6±4.7 (5)*	16.5±5.6 (6)	13.4±4.4 (6)	7.5±2.8 (6)
AD	10.4±2.3 (5)	7.1±1.1 (6)	10.0±2.7 (6)	9.8±2.6 (6)	5.6±2.2 (6)

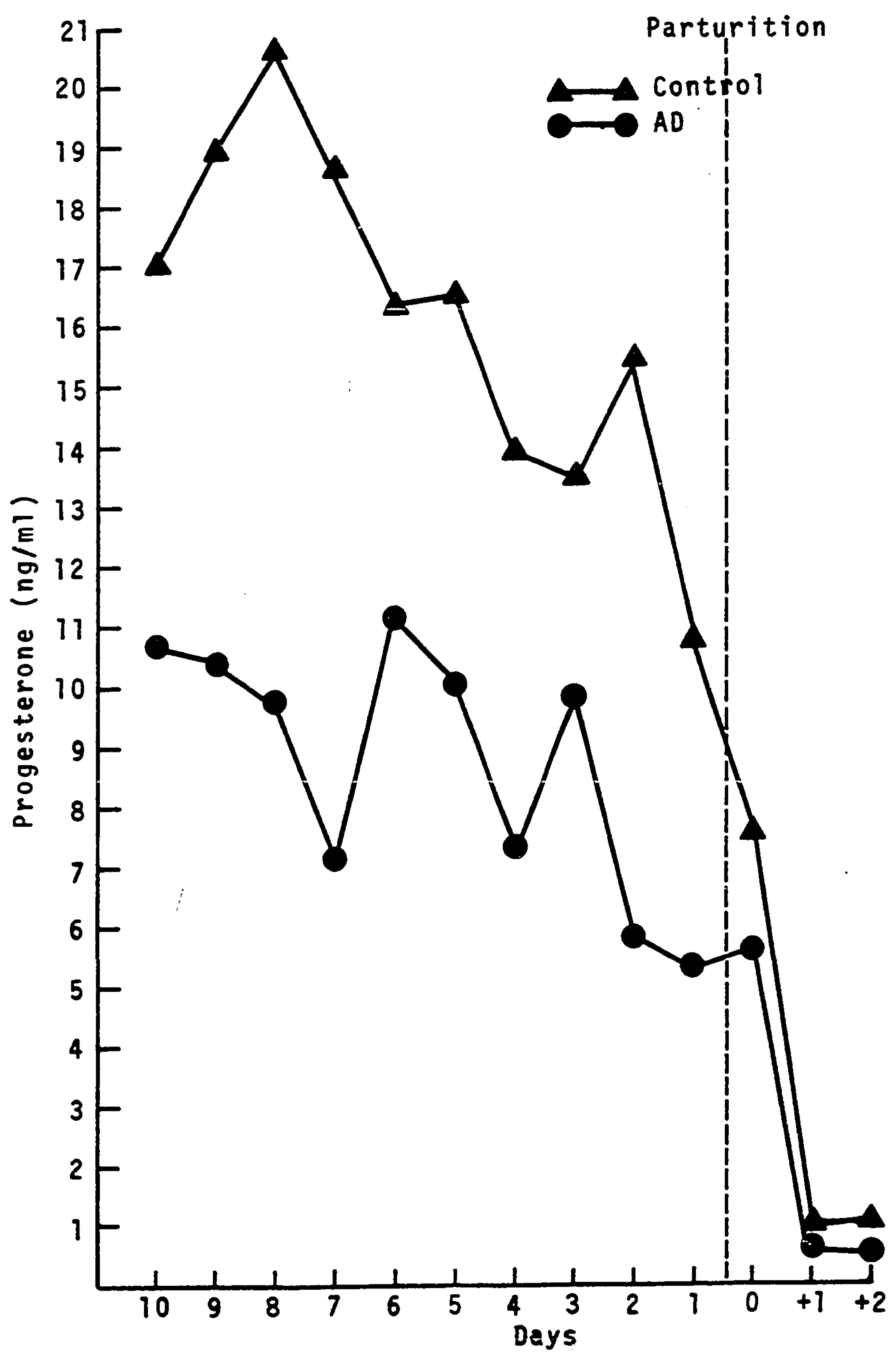
[a]Each group had three ewes bearing single fetal lambs and three ewes bearing twin fetal lambs.

[b]Days before parturition.

[c]Mean±S. E., (number of observations).

*$P < .05$.

Figure 5. Plasma progesterone (ng/ml) in the jugular vein of control and adrenalectomized (AD) sheep before before and after parturition


Parturition
Control
AD
Progesterone (ng/ml)
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
10 9 8 7 6 5 4 3 2 1 0 +1 +2
Days

on Table 11), however, was only significant on day -7 ($P<.05$). Based on overall means (Table 3), peripheral plasma progesterone levels in AD ewes were significantly ($P<.005$) reduced compared to control ewes. A comparison in the daily UO concentrations between these two groups yielded no particular trend or significant difference. Also, the overall UO levels were not significantly different.

Peripheral plasma progesterone values from AD-OV ewes were consistently lower than control values (Table 7). These differences were not significantly different on any particular day, but the overall mean J progesterone levels (Table 3) were significantly reduced ($P<.025$). A similar trend was not evident in UO values from these two groups (Table 8).

D. Estrone

A typical estrone standard curve is shown in Figure 1C. Water blanks were used to determine the 100% bound level in the standard curve and therefore were not quantitated. The coefficient of variation calculated from cpm for the entire estrone range found in the plasma samples was 13.07 (n=90).

During days -10 to -5, UO plasma estrone levels in control ewes ranged from 52.3 pg/ml to 77.2 pg/ml (Table 12, Figure 6). Beginning at day -4 estrone levels averaged over 100 pg/ml and reached peak values at parturition. Maximum estrone concentrations of 2970 pg/ml (UO) and 2329 pg/ml (J) were from an intact ewe sampled within an hour after parturi-

Table 12. Plasma estrone (pg/ml) in intact and adrenalectomized (AD) ewes[a]

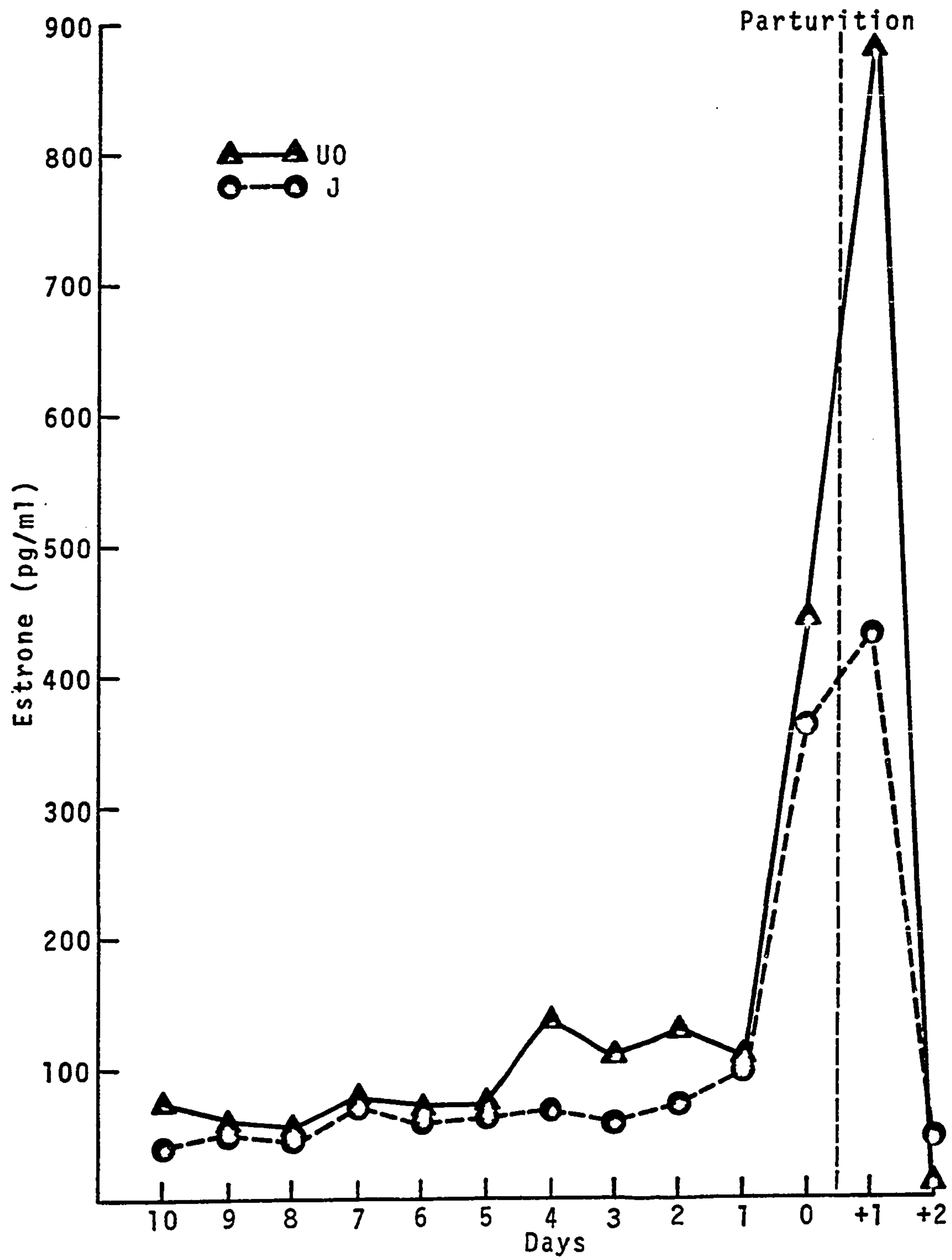
Days[b]	Intact		AD	
	UO	J	UO	J
-10	72.0± 25.0 (3)[c]	38.0± 10.2 (4)	44.3± 8.7 (4)	22.5± 4.8 (4)
- 9	59.4± 9.7 (6)	48.8± 10.3 (6)	30.1± 8.9 (4)	47.4±19.2 (5)
- 8	52.3± 7.7 (6)	44.5± 7.7 (6)	37.1± 5.7 (6)	38.2±10.6 (6)
- 7	77.2± 27.8 (6)	76.2± 17.7 (6)	53.3±17.5 (6)	39.7±11.0 (6)
- 6	68.5± 10.5 (5)	58.7± 12.8 (6)	58.1±17.3 (6)	41.3±11.0 (5)
- 5	74.8± 23.2 (7)	61.1± 8.6 (7)	34.2± 6.5 (6)	28.5± 5.3 (6)
- 4	137.1± 56.6 (6)	67.4± 13.1 (7)	41.1± 8.0 (6)	31.7± 5.4 (6)
- 3	110.2± 29.6 (5)	57.7± 8.3 (6)	51.9±14.5 (6)	49.0± 7.2 (6)
- 2	127.0± 28.6 (6)	72.7± 17.5 (7)	39.5± 8.9 (6)	43.5±16.6 (6)
- 1	108.8± 22.1 (7)	96.7± 17.2 (7)	77.3±25.2 (5)	57.2±17.7 (6)
0	443.5±169.0 (5)	363.1±133.6 (7)	180.0±36.8 (5)	172.8±29.2 (6)
+ 1	877.4±703.2 (4)	432.6±378.0 (7)	111.8±55.2 (4)	59.1±19.8 (6)
+ 2	11.3± 11.3 (2)	48.8± 19.4 (7)	24.4±10.7 (4)	19.9± 4.2 (6)

[a]Plasma levels in utero-ovarian (UO) and jugular (J) veins.

[b]Days before (-) and after (+) parturition.

[c]Mean±S.E., (number of observations).

Figure 6. Plasma estrone (pg/ml) in the utero-ovarian (UO) and jugular (J) veins of control sheep before and after parturition


Parturition
900
800
700
600
500
400
300
200
100
Estrone (pg/ml)
UO
J
10
9
8
7
6
5
4
3
2
1
0
+1
+2
Days

tion. If these values had not been included in the estrone mean for day +1, the means for day +1 would have been 179.8 and 116.5 pg/ml for UO and J plasma, respectively. Only in two intact control ewes was there a marked elevation (>400 pg/ml) in UO estrone values on both days 0 and +1. Therefore, peak estrone levels generally had a duration of 24 h or less.

Daily mean J plasma estrone levels from intact ewes were consistently but not significantly lower than UO levels. Overall mean J estrone levels (Table 3) also were not significantly lower than UO estrone values. During the period days -10 to -2, J estrone concentrations from intact control ewes fluctuated between 38.0 and 76.2 pg/ml (Table 12). Jugular values began a steep prepartum rise on day -1 that continued through parturition.

By postpartum day +2, estrone levels had fallen sharply in both sampling locations. Estrone values from ewes carrying multiple young were not different from ewes carrying a single fetal lamb.

The correlation coefficient between UO and J estrone was 0.973 ($P<.0001$). Correlation coefficients, presented in Table 13, delineate the relationship of estrone to estradiol and corticoid concentrations. One should note that there is a stronger correlation between estrone and J estradiol than estrone and UO estradiol levels.

Treatments had a significant ($P<.01$) effect on both UO and J estrone levels (Table 14). Estrone levels varied

Table 13. Correlation coefficients of utero-ovarian (UO) and jugular (J) plasma estrone with plasma corticoids and estradiol[a]

	Corticoids		Estradiol	
	UO	J	UO	J
UO	0.390	0.378	0.663	0.835
J	0.358	0.369	0.651	0.816

[a]All values P<.001.

significantly (P<.01) with the day and treatment X day produced a significant (P<.01) interaction. A treatment effect attributable to adrenalectomy appeared to be present since daily mean estrone concentrations in both sampling sites were consistently lower in AD ewes compared to values from intact controls (Table 12, Figure 7). The difference between these treatment groups was only significant on days 0 and +1 (P<.025). The difference in the overall means (Table 3) between these two groups was not significant. The maximum UO plasma estrone concentration from an AD ewe was 300 pg/ml. This sample was collected approximately 3 h prepartum. In contrast to this value, three UO values from control ewes on day 0 were 953, 630 and 456 pg/ml. These values were determined from plasma samples collected no closer than 12 h to parturition.

Plasma estrone levels from AD-OV ewes were consistently

Figure 7. Plasma estrone (pg/ml) in the utero-ovarian vein of control and adrenalectomized (AD) sheep before and after parturition

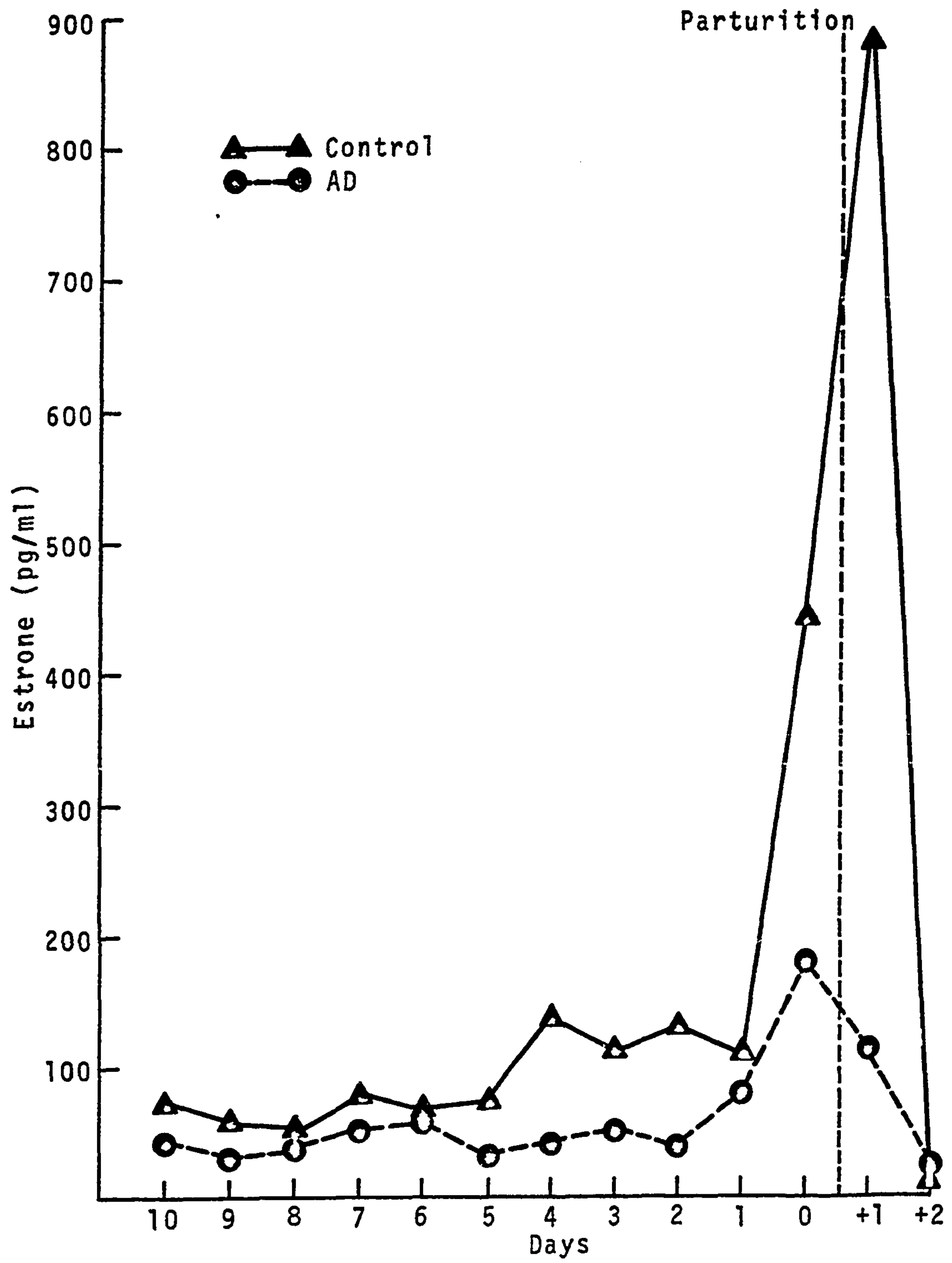

Parturition
Control
AD
Estrone (pg/ml)
900
800
700
600
500
400
300
200
100
10
9
8
7
6
5
4
3
2
1
0
+1
+2
Days

Table 14. The effects of treatments, days and treatment X day interaction on utero-ovarian (I) and jugular (II) plasma estrone[a]

Source of variation	Degrees of freedom	Mean square	F
I			
Treatments	3	300427	6.06**
Animals (treatment)	17	49578	
Day	12	115455	4.53**
Treatment X day	35	100827	3.96**
Residual	133	25472	
II			
Treatments	3	179805	5.87**
Animals (treatment)	17	30640	
Day	12	75990	4.11**
Treatment X day	35	58723	3.18**
Residual	133	18491	

[a]Based on data in Tables A7 to A10.

**$P<.01$.

lower in both the UO and J plasma compared to intact ewes (Tables 12, 15). However, only estrone values from days 0 and +1 were significantly ($P<.025$) greater in the intact ewes. Overall mean estrone values (Table 3) were not different between these two groups.

Table 15. Plasma estrone (pg/ml) in ovariectomized (OV) and adrenalectomized-ovariectomized (AD-OV) ewes[a]

Days[b]	OV		AD-OV	
	UO	J	UO	J
-10	54.5±10.4 (3)[c]	42.3± 8.3 (3)		
- 9	74.5±17.7 (3)	48.0± 9.0 (3)	51.5±13.3 (3)	44.3±15.5 (3)
- 8	66.4±14.6 (3)	31.0±10.5 (4)	48.0± 6.1 (3)	46.0±13.0 (3)
- 7	70.9±18.7 (3)	33.8± 1.9 (4)	55.5± 8.6 (4)	44.8± 7.4 (4)
- 6	137.6±81.6 (3)	48.7± 9.3 (4)	57.8±15.6 (4)	50.4± 8.7 (4)
- 5	52.3±13.1 (3)	51.1± 7.0 (4)	54.4± 7.3 (4)	45.9±11.5 (4)
- 4	58.7± 7.6 (4)	54.0±11.7 (4)	52.5±12.1 (4)	58.3±10.1 (4)
- 3	58.5± 7.2 (4)	77.1±14.2 (3)	56.3±12.9 (4)	57.3±19.4 (4)
- 2	74.7±15.2 (4)	54.6±12.4 (4)	60.5±20.8 (3)	54.1±13.2 (4)
- 1	119.6±50.8 (4)	106.1±28.5 (4)	48.0± 9.0 (3)	77.6±36.3 (4)
0	255.3±80.1 (4)	236.0±92.8 (4)	69.8± 8.3 (2)	169.1±99.6 (4)
+ 1	45.0± 6.0 (2)	132.9±82.2 (4)	29.4±12.6 (2)	43.0± 6.1 (3)
+ 2	25.4± 4.6 (3)	26.8± 8.0 (4)	24.0± 7.5 (2)	24.4±11.9 (3)

[a]Plasma levels in utero-ovarian (UO) and jugular (J) veins.

[b]Days before (-) and after (+) parturition.

[c]Mean±S.E., (number of observations).

Utero-ovarian estrone values from OV ewes did not exhibit the minor prepartum rise at days -4, -3 and -2 as did values from the intact control ewes (Tables 12, 15). These daily differences between OV and control ewes were not significant. On day 0, UO estrone levels were significantly ($P<.05$) greater in control ewes compared to OV ewes. This difference was significant ($P<.01$) in both sites on day +1. Jugular values from OV ewes before day -1 appeared to be generally lower than those from intact controls and similar to J values from AD and AD-OV ewes. However, overall mean estrone values were higher from OV ewes than those from AD and AD-OV ewes (Table 3). Overall J estrone concentrations were not different between control and OV ewes.

As parturition approached the progesterone/total estrogen (estrone plus estradiol) ratio became much smaller (Table 16). The change in this ratio was greatest from days -1 to +1.

Table 16. The mean utero-ovarian progesterone/total estrogen ratio in intact ewes

Days before and after parturition												
-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	0	1	2
1121[a]	1148	861	962	957	779	402	546	295	322	61	7	92

[a]Ratio = $\frac{\text{ng progesterone/ml plasma}}{\text{ng estrone + ng estradiol/ml plasma}}$.

E. Estradiol

A typical estradiol curve is shown in Figure 1D. Prepartum UO estradiol levels in control ewes ranged from 77% to 15% of the UO estrone levels in the same ewes. The disparity seen between UO and J progesterone and estrone levels was not exhibited by estradiol levels (Tables 17, 18). In fact, it was not unusual for J plasma estradiol levels to exceed UO levels. Overall UO and J estradiol means (Table 3) were not significantly different in any treatment group. Utero-ovarian plasma estradiol levels rose only on day 0 and were significantly ($P<.05$) higher than day -1 levels in each treatment group.

Jugular plasma estradiol levels varied significantly ($P<.01$) with treatments, but UO levels did not (Table 19). Estradiol levels also varied significantly ($P<.01$) with the day and there was a significant ($P<.01$) treatment X day interaction. Overall mean estradiol levels (Table 3) were lower in both UO and J plasma in AD and AD-OV ewes compared to intact control ewes.

Table 17. Plasma estradiol (pg/ml) in intact and adrenalectomized (AD) ewes[a]

Days[b]	Intact		AD	
	UO	J	UO	J
-10	13.6± 4.0 (4)[c]	12.2± 1.9 (4)	27.0± 4.9 (4)	7.0±2.5 (4)
- 9	16.7± 3.2 (6)	11.9± 3.5 (6)	33.8±10.6 (4)	10.9±2.1 (5)
- 8	29.8±15.9 (6)	8.7± 3.1 (6)	17.1± 2.6 (6)	9.9±1.5 (6)
- 7	34.4±17.7 (6)	17.6± 5.1 (6)	11.0± 4.1 (6)	11.5±1.6 (6)
- 6	18.1± 5.1 (5)	11.7± 5.3 (5)	15.5± 3.7 (6)	17.8±4.1 (5)
- 5	20.6± 4.5 (7)	11.5± 3.6 (7)	17.7± 1.7 (6)	14.9±2.2 (6)
- 4	20.2± 5.6 (6)	10.0± 4.7 (5)	17.1± 2.4 (6)	11.2±2.2 (6)
- 3	21.4±10.7 (4)	17.4± 3.4 (6)	16.3± 2.6 (5)	16.0±6.5 (6)
- 2	35.7±14.5 (6)	30.2±14.6 (7)	14.7± 4.0 (6)	13.2±2.3 (6)
- 1	28.3± 6.0 (7)	54.6±31.3 (7)	24.7± 8.4 (5)	16.6±3.2 (6)
0	56.7±13.7 (5)	74.6±22.7 (7)	64.1±15.2 (5)	49.4±7.6 (6)
+ 1	93.3±60.2 (4)	113.5±72.2 (6)	26.6±16.3 (4)	28.0±9.7 (6)
+ 2	6.8± 6.8 (2)	17.1± 5.1 (6)	8.3± 2.1 (4)	7.4±3.5 (6)

[a]Plasma levels in utero-ovarian (UO) and jugular (J) veins.

[b]Days before (-) and after (+) parturition.

[c]Mean±S.E., (number of observations).

Table 18. Plasma estradiol (pg/ml) in ovariectomized (OV) and adrenalectomized-ovariectomized (AD-OV) ewes[a]

Days[b]	OV		AD-OV	
	UO	J	UO	J
-10	37.0±16.6 (3)[c]	26.1± 9.3 (3)		
- 9	26.6± 4.3 (3)	32.7±12.8 (3)	19.9± 9.6 (3)	21.9± 7.8 (3)
- 8	42.3±17.3 (3)	31.0± 7.4 (4)	19.7± 7.7 (3)	15.0± 4.2 (3)
- 7	21.9± 3.8 (3)	21.3± 6.8 (4)	16.1± 5.9 (4)	14.3± 2.8 (4)
- 6	35.1±15.4 (3)	26.1± 5.6 (4)	16.5± 6.0 (4)	16.5± 6.0 (4)
- 5	19.9± 6.2 (3)	33.0±13.6 (4)	19.2± 6.4 (4)	15.4± 3.5 (4)
- 4	34.5±11.6 (4)	24.6± 8.5 (4)	16.5± 5.6 (4)	19.0± 4.2 (4)
- 3	33.3± 8.1 (4)	36.5± 7.2 (3)	21.2± 5.0 (4)	16.0± 3.9 (4)
- 2	42.1±15.3 (4)	31.6± 4.1 (3)	26.0± 4.8 (3)	14.8± 7.4 (4)
- 1	33.8± 8.1 (4)	38.6±11.5 (4)	20.1± 8.9 (3)	27.5±11.8 (4)
0	75.6±21.3 (4)	53.5±10.0 (4)	90.0±78.0 (2)	87.4±56.9 (4)
+ 1	18.0±18.0 (2)	26.6± 3.2 (4)	3.0± 3.0 (2)	39.4±29.8 (3)
+ 2	14.3± 3.9 (3)	11.9± 6.1 (4)	18.8±12.8 (2)	9.1± 3.4 (3)

[a]Plasma levels in utero-ovarian (UO) and jugular (J) veins.

[b]Days before (-) and after (+) parturition.

[c]Mean±S.E., (number of observations).

Table 19. The effects of treatments, days and treatment X day interaction on utero-ovarian (I) and jugular (II) plasma estradiol[a]

Source of variation	Degrees of freedom	Mean square	F
I			
Treatments	3	1812	0.94
Animals (treatment)	17	1937	
Day	12	2770	6.05**
Treatment X day	35	995	2.17**
Residual	133	458	
II			
Treatments	3	7133	4.14**
Animals (treatment)	17	1723	
Day	12	4524	4.98**
Treatment X day	35	2715	2.99**
Residual	133	909	

[a]Based on data in Tables A7 to A10.

**P<.01.

F. Acute Cannulations

Uterine arterial and UO venous plasma corticoid and progesterone levels were acutely determined in six ewes before and after their fetal lambs were removed (Table 20). These ewes were 139 to 146 days pregnant. Although the plasma

Table 20. Plasma corticoids in the utero-ovarian vein (V) and uterine artery (A) following removal of the fetal lambs

Hrs. after removal	139[a,b]		142		144		145		146	
	V	A	V	A	V	A	V	A	V	A
0	110.6[c] 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	67.6			58.3	63.7
24	72.2 39.9		42.1	30.4	54.6	70.8			53.0	61.1

[a]Gestational age.

[b]Two ewes on day 139.

[c]Expressed in ng/ml plasma.

corticoid level was greater in the artery in 5 of the 6 ewes before the lambs were removed, there was no significant difference in the mean plasma corticoid levels from these two sampling locations. The ewe that was an exception was one of two ewes 139 days pregnant. The means ±S.E. were 101.2±14.6 and 104.3±14.0 ng corticoid/ml plasma in the vein and artery, respectively. After removal of the lambs there was an increased frequency of a greater corticoid concentration in the UO vein compared to the uterine artery (6 of 16 comparisons) with no significant difference in the mean concentrations. At 24 h post lamb removal the mean plasma corticoid levels had decreased to 54.1 ng/ml in the artery and 52.2 ng/ml in the vein.

Following removal of the lambs the mean plasma progesterone levels decreased rapidly in both the artery and vein (Table 21). However, this rapid decline in the UO venous plasma progesterone levels was not exhibited by the ewe that was 144 days pregnant (Table A11). None of the ewes had passed their placenta at 24 h post lamb removal. Mean progesterone levels were significantly greater ($P<.01$) in the UO vein compared to the uterine artery both before and 1 h after the lambs were removed (Table 21). This difference was not significantly different in arterial and venous samples collected at 4, 8 and 24 h. The UO venous:uterine arterial progesterone concentration ratio was 5.2 before the lambs were removed. This ratio decreased to 2.4 24 h later. At

48 and 72 h post lamb removal the mean progesterone concentrations continued to decline in the UO vein. Mean values were then 2.0 and 1.8 ng/ml, respectively.

Table 21. Mean plasma progesterone levels in the utero-ovarian vein (V) and uterine artery (A) following removal of the fetal lambs

Hours after removal	V	A	V/A
0	143.0±25.9 (6)[a]	27.4±7.3 (6)**	5.2
1	27.8± 5.4 (6)	9.1±1.6 (5)**	3.1
4	16.7±10.7 (6)	3.1±0.5 (5)	5.4
8	19.9±15.2 (6)	3.1±0.6 (4)	6.4
24	4.0± 1.5 (6)	1.7±0.2 (3)	2.4

[a]Mean±S.E., (number of observations).

**P<.01.

At day 139 of gestation a significantly (P<.025) greater progesterone concentration was found in the umbilical vein compared to the umbilical artery (Table 22). The mean values were 16.5 ng/ml and 6.2 ng/ml in the vein and artery, respectively. After 139 days progesterone levels declined in the umbilical circulation. There was then no apparent arteriovenous concentration difference. In fact, progesterone levels were slightly greater in the artery in 2 of 4 later gestational comparisons.

Conversely, mean plasma corticoid levels in the umbilical circulation were significantly (P<.001) elevated when days 142 to 146 of gestation were compared to day 139 (Table 22). The plasma corticoid levels were higher in the umbilical artery compared to the umbilical vein in the four possible comparisons at days 142 to 146 of gestation. However, the mean difference between these levels was not significantly different. Earlier in gestation at day 139 there was no consistent pattern in the corticoid concentrations found in the artery and vein.

Table 22. Plasma progesterone and corticoids (ng/ml) in umbilical arterial (A) and venous (V) plasma

Gestational age (days)	Sex	Progesterone		Corticoids	
		V	A	V	A
139	M	12.8	3.5	7.5	14.4
	F	26.3	9.4	25.2	19.9
139	__a	6.0	4.0	23.7	22.9
	__a	20.7	7.7	38.2	43.9
142	M	4.6	2.3	97.5	135.8
	M	4.0		70.8	
144	F	1.0	2.8	164.2	166.2
145	F	0.2	1.7	159.0	174.4
146	M	3.4	2.8	148.3	152.1

[a]Sex not recorded.

G. Fetal Cannulations

Fetal plasma corticoids were measured in one chronically cannulated fetal lamb for a period of 13 days prepartum (Table 23). Beginning at day -7 fetal plasma corticoid levels increased sharply to 18.5 ng/ml compared to earlier gestational levels of <1.0 to 4.0 ng/ml. Maternal plasma progesterone had begun a continual prepartum decrease by day -6. A blood sample collected within 3 h of parturition had a plasma corticoid level of 157.6 ng/ml. Thus the ascent to the elevated fetal plasma corticoid level that was found on day -1, 91.6 ng/ml, could have begun as long as 48 h prepartum. Corticoid levels found in plasma samples from this lamb were similar to those found in five other fetuses. Mean values for these fetal lambs were 35.4, 30.9, 45.8, 53.0 and 157.6 ng/ml for the period days -4 through 0, respectively. Plasma corticoid levels in a fetal lamb, in the maternal UO vein and J vein are reported in Table 24. Fetal plasma corticoid levels were higher than levels in the UO vein except at day -2. However, again except at day -2, J plasma corticoid levels were higher than UO levels.

Table 23. Fetal plasma corticoids and maternal jugular plasma progesterone (ng/ml) in a ewe[a]

Days[b]	Fetal corticoids	Maternal progesterone
-12	4.0	18.2
-11	0.2	25.5
-10	6.4	41.7
- 9	0.2	18.3
- 8	1.4	12.3
- 7	18.5	15.0
- 6	15.5	12.3
- 5	32.3	12.1
- 4	57.9	10.9
- 3	22.9	10.1
- 2	37.0	9.1
- 1	91.6	5.0
0	157.6	2.0

[a]Gestation length 146 days.

[b]Days before (-) parturition.

Table 24. Fetal and maternal plasma corticoid concentrations (ng/ml)

Days[a]	Fetus	UO[b]	J[c]
-4	34.0	27.4	44.3
-3	26.3	17.5	20.7
-2	44.9	63.4	46.3
-1	43.1	40.8	62.3
0	166.7	76.0	88.8

[a]Days before (-) parturition.

[b]Utero-ovarian.

[c]Jugular.

V. DISCUSSION

A. Corticoids and Parturition

Maternal plasma corticoid levels have been found to be elevated during the latter part of gestation in several species. Plasma corticoid levels rose progressively throughout gestation in women (Friedman and Beard, 1966; Rosenthal _et al._, 1969). The elevated plasma corticoid levels found in pregnant women have been attributed to increased protein binding which itself was secondary to increased estrogen production (Friedman and Beard, 1966). These increased levels in pregnant women were not due to an increased cortisol secretion since cortisol PR was not altered by pregnancy (Migeon _et al._, 1968). In two other species with much shorter gestation lengths, the rat (Milkovic _et al._, 1973) and the hamster (Pellet _et al._, 1970), plasma corticoid levels were elevated during the latter part of gestation. The portion of gestation with an elevated corticoid level was relatively comparable between these species and humans.

In contrast to the above named species plasma corticoid values from control sheep in this study were only elevated 48 to 72 h prepartum. Basson _et al._ (1969) also found an elevated plasma corticoid level in ewes at parturition. Their data indicated that plasma corticoids had begun to rise several days prepartum. Corticoid values of 8.0 to 15.0 ng/ml

reported by that group for the day of parturition were considerably lower than the mean peripheral value of 32.5 ng/ml found in this study. Plasma corticoids in cows were significantly elevated for a four day period immediately prepartum (Adams and Wagner, 1970). However, in another study plasma corticoids in cows only became elevated 12 h prepartum (Smith et al., 1973). In swine the prepartum corticoid rise had begun at least 48 h before parturition (Molokwu and Wagner, 1973).

Although this elevated plasma corticoid level in sheep could be the result of one or several altered corticoid metabolic parameters, the cortisol secretion rate has been reported to remain relatively constant while the MCR increased several days before lambing (Paterson and Harrison, 1967). Also the corticoid binding capacity was not elevated during pregnancy (Lindner, 1964). Therefore the increase in plasma corticoids appears to be the result of the stress of parturition on the maternal individual. The other possibility is an augmentation of the maternal plasma corticoid level by a transplacental passage of corticoids.

Adrenalectomy significantly lowered plasma corticoid levels. The constant daily replacement therapy resulted in a small daily variation in the plasma corticoid levels in AD ewes (Table 4). Still these levels were highest on day 0 and declined in the postpartum period. Prepartum UO plasma corticoid levels in AD ewes were generally higher than J levels.

These observations together with the significant elevation of UO plasma corticoids compared to J levels in control ewes on day -2 suggest that the uterus or its contents may have been a source of maternal corticoids. Since fetal lamb corticoid levels increased sharply late in gestation (Tables 22, 23, 24) and exceeded levels found during this period in the UO vein (Tables 4, 24), a concentration difference was established between the fetal lamb and mother. The transplacental passage of cortisol from the fetal lamb to the ewe with high resistance to passage in the opposite direction reported by Beitins _et al_. (1970) and Dixon _et al_. (1970) further supports this contention.

Plasma corticosterone levels found in pregnant AD rats presumably originated from the fetal pups (Milkovic _et al_., 1973). Levels found in these AD rats on day 21 of gestation were similar to those found in the intact controls. These findings substantiate the earlier report of Kamoun and Stutinsky (1968) who found a detectable corticosterone level in the maternal plasma of AD rats on day 18.5 of gestation. The corticosterone level in these rats continued to rise until day 20.5 of pregnancy. Cortisol readily passed the placenta from mother to fetus in women (Abramovich and Wade, 1969) and in the rhesus monkey (Bashore _et al_., 1970). An AD pregnant women was found to have a cortisol PR of 1.5 and 3.8 mg/24 h. while taking only DXMS as replacement therapy (Charles _et al_., 1970). This report further supports the passage of corticoids

in the fetal to maternal direction.

The fetal to maternal cortisol transfer reported by Beitins *et al.* (1970) and Dixon *et al.* (1970) was quantitatively small, but their experimental sheep were 140 days or less in gestation. At this time the fetal lamb's adrenal was still relatively inactive compared to the period within three days of birth (Nathanielsz *et al.*, 1972). The fetal cortisol concentration only markedly increased the last few days of gestation in this study (Tables 22, 23) which substantiates results found by Bassett and Thorburn (1969) and Comline *et al.* (1970b). Therefore if a study similar to that of Beitins *et al.* (1970) or Dixon *et al.* (1970) was conducted using ewes within 72 h of lambing, the transplacental passage of fetal cortisol might be greater.

However, even within a day or two of lambing the cortisol PR by the fetal lamb was only 9.2 mg/day (Nathanielsz *et al.*, 1972). Thus if virtually all of the fetal cortisol was transferred to the dam, this would not be sufficient to explain the prepartum maternal plasma corticoid level. This is especially true when it is noted that UO plasma corticoid levels found in this study rose from a mean of 11.3 ng/ml on day -3 to 32.5 ng/ml on day 0 (Table 4). Using the MCR for cortisol in sheep of 1300 L/day (Beitins *et al.*, 1970) or 0.86 L/min (Paterson and Harrison, 1967) the amount of cortisol secretion necessary to elevate the maternal plasma cortisol concentration from 11.3 to 32.5 ng/ml was calculated to

be approximately 28 mg/day. Therefore it would appear that some maternal cortisol secretion or metabolic parameters must change close to parturition in the ewe.

Although differences were found in the timing of the prepartum corticoid rise between control and OV animals, the biological significance of this fact is obscure. There did not appear to be an inability to produce corticoids in OV ewes since plasma corticoid concentrations were nearly identical on day 0 in control and OV ewes. Although the ovary may contribute to the prepartum corticoid rise, the peak levels found in OV animals on day 0 probably result from the adrenal response to the stress of parturition.

Observations in the AD-OV and OV ewes do not support the hypothesis that the uterine contents contributed to the maternal plasma corticoid levels. First, the significant elevation found in the UO plasma corticoid level compared to J levels on day -2 in control ewes was not present in OV ewes. Mean UO plasma corticoid levels in OV ewes were only slightly greater than J levels on this day (Table 5). Second, the consistently greater UO plasma corticoid levels compared to J levels seen in the AD ewes were not present in the AD-OV group. Although plasma corticoids rose in both sampling sites at parturition in the AD ewes (Table 4), UO plasma corticoids fell slightly on this day in the AD-OV group (Table 5).

Information about corticoid concentrations in the complete uterine circulation was obtained from the acutely

cannulated ewes. With the fetal lambs *in utero*, corticoid concentrations were higher in the uterine artery than in the UO vein in 5 of 6 observations (Table 19). Before lambs were removed from three ewes which were 144, 145 and 146 days pregnant a greater plasma corticoid concentration was found in the artery in every instance. This was at a time when fetal corticoids were significantly elevated in the umbilical circulation (Table 22). These results suggest that transplacental passage of fetal plasma cortisol in sheep must be quantitatively small even late in gestation.

The uterine artery may in fact normally have a greater corticoid concentration compared to the UO vein. The stress of surgery undoubtedly promoted adrenal corticoid secretion and this could further mask a uterine arterio-venous difference. This rationale implies that the pregnant uterus metabolizes plasma corticoids. Following removal of the fetal lambs there was an increased incidence of a greater corticoid concentration in the UO vein compared to the uterine artery (6 of 16 comparisons, Table 19). This suggests that the uterus was metabolizing corticoids and that the pregnant uterus does so to a greater extent than the non-pregnant uterus.

Results from this study indicated that the maternal adrenal of pregnant sheep was not necessary for the initiation of normal parturition. Those AD ewes that did not prematurely abort had a mean gestation length almost identical to normal

ewes (Table A6). Since these AD ewes gave birth to live lambs, a low maternal plasma corticoid level was not detrimental to fetal lamb survival. However, many AD ewes aborted following abdominal surgery for placement of UO cannulas, whereas OV and control ewes did not abort after subjection to the same surgery. Therefore, the presence of the maternal adrenal was not necessary for the initiation of parturition, but in fact its absence increased the likelihood of gestational failure in this study. Dickson and Seekins (1963) reported that the stress of starvation caused abortion in two AD ewes. Therefore it is suggested that the low replacement therapy used in this study and the further stress of surgery may have been predisposing factors to abortion. One should be aware that maternal adrenalectomy is not detrimental to fetal survival since AD rats (Thoman _et_ _al_., 1970) and AD women (Charles _et_ _al_., 1970; Rosenburg, 1970) gave birth to apparently normal young.

The literature unquestionably indicates that a functioning fetal pituitary-adrenal axis is integral to the normal timing of parturition. Genetic causes of fetal pituitary hypoplasia and aplasia in calves have been associated with prolonged gestation (Holm, 1967). Prolonged gestation in women has been associated with anencephalic pregnancies (Comerford, 1965). Ingestion of certain teratogenic agents by pregnant ewes that have produced pituitary developmental errors has also been associated with prolonged gestation

(Basson *et al.*, 1969; Binns *et al.*, 1963). In addition either fetal adrenalectomy (Drost and Holm, 1968) or hypophysectomy (Liggins *et al.*, 1966) caused prolonged gestation in sheep. Conversely the administration of either ACTH (Liggins, 1968) or DXMS (Liggins, 1969a) to the fetal lamb hastened the onset of parturition. Results from this study indicate that the mechanism of action of the fetal lamb's adrenal probably involves an increase in plasma corticoid levels a few days before parturition (Tables 22, 23). The glucocorticoids rather than the mineralocorticoids appear to be the adrenal secretion products which are active in parturition induction since infusion of 11-deoxycortisol into a fetal lamb was not effective in parturition induction (Anderson *et al.*, 1972).

There is evidence to indicate that the increasing fetal plasma glucocorticoid levels may have direct and indirect effects on the myometrium. Methylprednisolone in low dosages significantly stimulated the amplitude of contraction of pregnant term human myometrial strips *in vitro* (Mossman and Conrad, 1969). *In vitro* studies with methylprednisolone followed by oxytocin to term mouse uterine strips produced a lowered tonus and an increased amplitude of contraction compared to oxytocin alone. Thus methylprednisolone modulated the uterine response to oxytocin. Cortisol was able to efficiently displace progesterone from its binding protein in rat uterine cytosol (Davies and Ryan, 1972; Milgrom and Baulieu, 1970a), an effect which was negligible in intact rat

uterine tissue (Milgrom and Baulieu, 1970b). However, it can not be concluded from this report that cortisol was not able to displace uterine progesterone in late pregnancy because their investigation was not designed to study the effects of high levels of fetal plasma corticoids on uterine progesterone. Dexamethasone infusions into the fetal lamb were followed by marked increases in the $PGF_{2\alpha}$ content of the maternal cotyledon at 24 h and a rise in the myometrial content at 48 h when the ewes were in labor (Liggins and Grieves, 1971). Thus the increasing fetal cortisol levels near parturition may have an indirect effect on the myometrium by stimulating PG synthesis, and the myometrial stimulating properties of PG are well-known (Karim, 1971).

The administration of DXMS to the pregnant maternal organism of several species has also been reported to induce premature birth (Adams, 1969; Adams and Wagner, 1970; Alm _et al._, 1972; North _et al._, 1972). The dosage necessary to produce this effect was considerably greater when given to the mother rather than the fetus. This suggests that if corticoids interact to initiate the process of parturition by some direct or indirect action on the uterus a fetal source is a favored route. The fact that there was always an interval of at least 48 h from the beginning of DXMS infusion to the fetal lamb before birth occurred suggests that the mechanism of action of corticoids was probably indirect (Liggins, 1969a). Dexamethasone administration to sheep starting as

early as day 88 of gestation produced birth several days later (Fylling *et al.*, 1973). Therefore it can be concluded that the parturition process can be initiated relatively early in gestation.

B. Progesterone and Parturition

Utero-ovarian and J progesterone concentrations were elevated during days -9 to -5 compared to the period closer to parturition (Tables 7, 8). A consistent decline in UO progesterone levels was evident as early as 5 to 7 days prepartum suggesting that the preparation for lambing began a week before parturition. In contrast to the UO decline, a consistent decline in J progesterone values occurred only on days -1 and 0. The decline may have been evident first in the UO plasma simply because the levels were higher there and a similar relative fall in each sampling site only produced a distinct difference in the UO plasma.

Utero-ovarian plasma progesterone values were significantly greater than J values in all treatment groups (Tables 3, 7, 8). Others have reported a similar concentration difference in both ovariectomized (Bedford *et al.*, 1972b) and intact sheep (Moore *et al.*, 1972; Thorburn and Mattner, 1971). Progesterone levels found in the UO vein were also greater than those found in the uterine artery (Table 21). Since there was also no significant effect of ovariectomy on UO plasma progesterone (Table 3), progesterone must be chiefly

elaborated by the placenta in late pregnant sheep.

A decline in peripheral plasma progesterone values several days prepartum was also reported in swine (Molokwu and Wagner, 1973), goats (Thorburn and Schneider, 1972) and cows (Stabenfeldt et al., 1970), species that have a corpus luteum rather than a placental source of progesterone. The fall in peripheral levels in these species and sheep appears indistinguishable. In contrast, peripheral plasma progesterone values rose to term in humans and only during labor did they decrease (Llauro et al., 1968; Yannone et al., 1969).

The decrease in plasma progesterone levels during the last week of gestation in sheep has been associated with an increased progesterone PR and a slight increase in progesterone MCR (Bedford et al., 1972a). The conversion of isotopically labelled progesterone to 20α OH-progesterone was unchanged during this period. In rats during the latter half of gestation enzymatic activity of 20α hydroxysteroid dehydrogenase and 5α steroid dehydrogenase increased greatly in uterine tissue (Flint and Armstrong, 1973). Also in rat uterine tissue both the progesterone receptor activity (Davies and Ryan, 1972) and concentration (Davies and Ryan, 1973) were markedly decreased near term. These reports suggest that at least in the rat, and perhaps in sheep as well, uterine progesterone binding is decreased and progesterone metabolism is accelerated in preparation for parturition.

A concentration gradient of progesterone from the pla-

centa to the myometrium and plasma was found in pregnant women (Wiest, 1967). Myometrial and plasma progesterone were proportional in sheep since a decline in one location was observed in the other (Liggins _et al_., 1972). Therefore, with the decline in plasma progesterone close to parturition, as described in most species, a fall in myometrial progesterone would be expected, thus removing the uterus from a progesterone "block" as described by Csapo (1961) and allowing for uterine activity.

Ewes carrying more than one lamb had consistently higher daily UO progesterone levels and significantly higher J levels than ewes carrying a single lamb (Table 10, Figure 4). Bassett _et al_. (1969) reported a similar effect on J progesterone levels in ewes. Progesterone PR was markedly increased in ewes delivering lambs >4 kg compared to ewes delivering lambs <4 kg (Bedford _et al_., 1972a) indicating that progesterone secretion and plasma values in pregnant sheep are related to both the size and number of fetal lambs.

After normalizing the control group and the OV group to only ewes carrying single lambs, it was evident that ovariectomy did not affect J or UO progesterone. Several investigators have reported that ovarian progesterone secretion was small or nil in late pregnant sheep (Edgar and Ronaldson, 1958; Moore _et al_., 1972). According to calculations made from Moore _et al_. (1972), ovarian progesterone secretion was 0.3 to 1.5 mg/day 4 to 6 days prepartum. These authors

stated that the ovary secreted 3 to 4 mg progesterone daily until about 20 days prepartum and then declined. This calculated amount of ovarian progesterone in the late pregnant ewe is small compared to a total progesterone PR of 14 to 104 mg/day in comparable animals (Bedford et al., 1972a). Fylling (1970) did not observe the normal rise in J progesterone levels late in gestation in ovariectomized ewes. However, in this study OV ewes appeared indistinguishable in this respect from control ewes. Ovariectomized and AD-OV ewes in this study did have a shortened mean gestation length compared to AD and control sheep (Table 2) implying that perhaps ovarian progesterone or some other ovarian factor was important for ewes to carry their lambs to term.

It appears that adrenalectomy affected progesterone metabolism but not progesterone secretion since adrenalectomy reduced J progesterone levels (Table 11) but not UO levels. It could be speculated that the adrenal corticoids normally exert a sparing effect on progesterone metabolism. The loss of potential progesterone precursors, cholesterol and pregnenolone, by adrenalectomy did not appear to affect placental progesterone secretion. Adrenal ablation itself would not appear to have a substantial direct effect on plasma progesterone levels, since adrenal progesterone secretion in pregnant sheep was small (100 to 600 μg/day) in comparison to the mentioned progesterone PR in pregnant ewes (Linzell and Heap, 1968). The same suppression of J, but not UO, progesterone

levels was evident in the AD-OV ewes. Therefore the same phenomenon appears to be present in AD-OV ewes. However, urinary excretion of pregnanediol and pregnanetriol was slightly reduced in an AD pregnant woman (Charles *et al.*, 1970), suggesting that in this patient the maternal adrenal was producing significant amounts of progesterone or was supplying a significant amount of precursors for placental progesterone production.

Following fetal demise in a human pregnancy caused by intra-amniotic hypertonic saline administration, progesterone levels did not significantly decrease until the placenta was delivered three days later (Lurie *et al.*, 1966). Similarly Wiest *et al.* (1966) found that progesterone levels in human pregnancy did not fall rapidly after fetal death but remained elevated as long as three weeks. The excretion of pregnanediol was normal in anencephalic pregnancies (Frandsen and Stakeman, 1964), indicating that placental progesterone production in humans did not involve direct fetal participation. The same conditions may exist in sheep since sheep placental preparations alone converted pregnenolone to progesterone (Ainsworth and Ryan, 1967). However, UO progesterone levels generally fell rapidly following lamb removel (Table 21). Therefore, results from this study appear to differ from these reports in humans. Removal of the fetal lambs may have immediately altered placental function whereas fetal death may not have had an immediate deleterious effect. The possi-

bility strongly suggested by this study is that placental progesterone secretion in the sheep requires fetal participation. Whether this participation is indirect to maintain placental viability or direct in the formation of progesterone was not delineated by this study.

Progesterone concentrations in the umbilical vein of fetal lambs have been reported to generally increase with increasing gestational age (Moore *et al.*, 1972). Results from this study do not completely agree with this report since the greatest progesterone concentrations found in the umbilical vein were at day 139 of gestation (Table 22). Later in gestation these levels fell quite markedly. At day 139 a significantly greater progesterone concentration was found in the umbilical vein compared to the umbilical artery, a difference similar to that found in human fetuses at birth (Gandy, 1968, cited by Solomon and Fuchs, 1971).

Progesterone and pregnenolone were converted equally as well into cortisol by fetal lamb adrenals *in vitro* (Anderson *et al.*, 1972), suggesting that some of the progesterone concentration found in the umbilical vein was potential substrate for cortisol synthesis. This is pertinent because a significant increase in the corticoid concentration (Table 22) was associated with the decrease in the progesterone concentration in the umbilical circulation after day 139. Although progesterone may act as a substrate for fetal adrenal cortisol synthesis, the evidence reported in this study does not sug-

gest that placental progesterone was quantitatively important to corticoid synthesis. A slightly greater progesterone concentration was found in the umbilical artery rather than the vein in 2 of 4 comparisons at days 142 through 146. This would not be expected if placental progesterone served as a significant source of cortisol synthesis. Also the progesterone concentration at this time in the umbilical circulation was very small compared to the corticoid concentration.

Progesterone administration blocked the onset of parturition in sheep (Liggins _et al._, 1972), rabbits (Csapo, 1956) and corticoid-induced cows (Jochle _et al._, 1972). Following daily administration of a progesterone dosage that was slightly less than one that completely blocked the onset of parturition in ewes, the fetal membranes ruptured at the expected time, but the expulsion of the fetus was delayed (Bengtsson and Schofield, 1963; Liggins _et al._, 1972). Therefore, while progesterone did not affect the timing of parturition in these experiments, uterine function did not proceed normally following this progesterone dosage.

C. Estrogens and Parturition

Mean UO estrogen levels in control ewes clearly began to increase 4 to 5 days prepartum with a further dramatic increase at parturition (Table 12). The pattern of the parturient increase in plasma estrogens varied widely between animals in both magnitude and duration. The prepartum in-

crease in UO estrone levels in control ewes several days before parturition is well defined by this study. Other studies have either involved a less frequent sampling routine or insufficient animals were studied to describe this early prepartum increase (Bedford _et al._, 1972b; Challis _et al._, 1972).

Estradiol levels in control ewes were considerably less than corresponding estrone levels. An increase in estradiol levels in both sites occurred only at parturition. Therefore these results substantiate earlier reports that estrone was the main plasma estrogen in the pregnant ewe (Challis _et al._, 1971; Thorburn _et al._, 1972).

Jugular estrone levels began to increase on day -2 in control ewes with a marked increase at parturition. This abrupt increase in peripheral plasma estrone levels at parturition substantiates reports by Obst and Seamark (1972b), Challis (1971) and Thorburn _et al_. (1972). The marked elevation of plasma estrogens only at parturition or a few days prepartum does not seem compatible with the gradual increase in urinary estrogen excretion reported to occur throughout pregnancy in the ewe (Fevre _et al._, 1965).

A greater plasma estrone concentration was consistently noted in the UO plasma compared to J levels in control ewes (Table 12). A similar concentration difference was not noted in estradiol levels from day -3 through parturition (Table 17). Also overall estradiol levels did not reflect a concen-

tration difference between these sites. This suggests that the MCR of estrone was greater than estradiol or the interconversion of estrone to estradiol was greater than the reverse. In non-pregnant ewes the MCR for estrone was slightly greater and the partial conversion of estradiol to estrone was greater than that of estrone to estradiol (Kazama and Longcope, 1972). If the same conditions exist in the late pregnant ewe, the lack of an estradiol concentration difference between the UO vein and J vein cannot be explained on this basis.

The duration of the plasma estrone peak was found to be generally less than 24 h. Bedford _et al_. (1972b) reported the duration of this peak to vary from 4 to 48 h, but Thorburn _et al_. (1972) stated that it was confined to 12 h prepartum. The duration of the estradiol peak appears to be similar to that for estrone. It is the opinion of this author that the duration of the estrogen peak was too short to interpret differences in magnitude as being physiologically important. This becomes even more apparent when it is considered that plasma samples were collected once every 24 h. Therefore overall mean estrogen levels in Table 3 should be regarded accordingly.

The highest mean estrone levels were those observed in control ewes on day +1 which was largely the result of the unusually high estrone concentration found in one ewe a few hours postpartum. Even excluding levels found in this ewe

for the determination of the mean estrone values for day +1, there was still a considerable estrogen concentration at this time. Bedford et al. (1972b) found the highest estrogen PR to occur 30 min postpartum in a ewe and Challis et al. (1971) also reported that the highest values they found were 1 h postpartum in a ewe. This suggests that the placenta has a substantial estrogen producing capability even after delivery of the fetuses.

Results from this study did not indicate that plasma estrogen concentrations were elevated in ewes carrying multiple fetuses. Goats with twin fetuses had plasma estrogen levels higher than those carrying one fetus (Challis and Linzell, 1971). Rado et al. (1970) made the same observation in humans and Fevre and Rombauts (1966) reported an increased urinary estrogen excretion in ewes with twin lambs.

The ewe, with a high level of circulating estrogen only at parturition, appears to be different from several species. Plasma estrone, the principal estrogen in cows, gradually increased during the last month of gestation and reached maximum levels 4 to 5 days prepartum (Edqvist et al., 1973; Smith et al., 1973). Estradiol levels exhibited a similar temporal relationship by increasing near parturition but the concentration of estradiol was only about 1/10 that of estrone (Smith et al., 1973). In swine, where estrone was again the principal estrogen, plasma estrone levels were substantial one week prepartum and rose to peak values 2 to

3 days prepartum (Molokwu and Wagner, 1973). In the goat plasma estrogens increased throughout gestation to highest levels immediately prepartum (Challis and Linzell, 1971) which was similar to the urinary estriol excretion pattern in pregnant women (Beling, 1971).

The process of estrogen synthesis in the pregnant woman involves aromatization of preformed androgens by the placenta (Diczfalusy, 1969). The process is commonly regarded as being accomplished by the feto-placental unit. A functional feto-placental unit was found in sheep since estrogen synthesis resulted only when placental tissue was incubated simultaneously with either maternal or fetal adrenal tissue (Davies et al., 1970). Since ovariectomy did not significantly affect plasma estrogen levels and UO estrone levels were greater than J levels, evidence for placental secretion of estrogen was found in this study.

In humans the quantitative importance of the fetal adrenal to estrogen synthesis was provided by the observations of Frandsen and Stakeman (1964) who reported a marked reduction in urinary estrogen excretion in anencephalic pregnancies with hypoplastic fetal adrenals. Dehydroepiandrosterone sulfate secreted by the maternal adrenal was calculated to contribute up to 40% of the precursors for estradiol production in pregnant women (Siiteri and MacDonald, 1966). Similarly ACTH administration to pregnant women stimulated estradiol production (MacDonald and Siiteri, 1965) whereas

DXMS administration to pregnant women depressed urinary estrogen excretion (Warren and Cheatum, 1967). Decreased urinary estrogen excretion values were also found in adrenalectomized pregnant women (Charles *et al.*, 1970; Gurpide and Vande Wiele, 1971). In this study daily plasma estrone levels were consistently reduced in AD ewes and this difference was significant at parturition (Table 12, Figure 4). The comparison of AD-OV ewes to the controls also revealed a general reduction in plasma estrone in the AD-OV group which was significant at parturition. Therefore in the sheep as in women the maternal adrenals appear to be instrumental in the process of placental estrogen synthesis.

A difference was not apparent in estradiol levels in control ewes compared to the other treatment groups (Tables 17, 18). Peak estradiol levels at parturition were not different. Overall estradiol levels were similar in control and OV ewes, but were reduced in the AD and AD-OV ewes (Table 3). These results suggest that more of the estrone precursors compared to the estradiol precursors were being supplied by the maternal adrenal. Since a stronger correlation was found to exist between estrone and J estradiol levels than UO estradiol levels (Table 13), this suggests that estradiol depends more on estrone than other factors.

In contrast to AD and AD-OV ewes, daily UO plasma estrone levels prepartum in OV ewes were not consistently lower than control values (Tables 12, 14). However, daily

J estrone values prepartum were similar to those from AD and AD-OV ewes and thus lower than control values. Therefore while estrone production was not apparently altered in OV ewes there is an indication that estrone metabolism was altered in OV ewes. No explanation is offered for this observation since the only literature that was pertinent indicated that urinary estrone excretion was less in OV ewes (Fevre, 1967). It is concluded from data from this study on the basis of the UO levels that the ovary was not a significant estrogen source for the parturient ewe.

The elevated plasma estrogen level in the parturient ewe appears to be physiologically significant when the effects of estrogens on the myometrium are considered. Estradiol had a direct stimulating effect on uterine contractility in pregnant term women (Pinto _et al_., 1964). Uterine activity was significantly increased by estradiol infusions alone and the response in activity produced by 20 mU of oxytocin following estradiol was significantly greater than the response to oxytocin alone. Thus estradiol potentiated the effects of oxytocin. Cervical ripeness was invariably noticed within a few hours after estradiol administration. Stilbestrol administration to late pregnant ewes resulted in a rise in intrauterine pressure 24 h later (Hindson _et al_., 1967). Delivery of lambs from 3 of 7 ewes followed this rise in intrauterine pressure.

Estrogens had an effect on uterine muscle fibers from

ovariectomized rats (Marshall, 1959). Individual muscle fibers from the untreated rats were quiescent whereas those from estrogen treated rats were rhythmically active. Estrogen treatment raised the resting membrane potential but progesterone treatments raised the resting membrane potential even further. A series of action potentials preceded and accompanied each muscle contraction in the estrogen treated rats but this was not a consistent finding in the progesterone treated rats.

Estrogens may also affect uterine activity by promoting PG synthesis since estradiol administration following prior progesterone injections to ovariectomized non-pregnant ewes resulted in a significant rise in PG levels in the peripheral plasma (Caldwell _et al._, 1972). This was not found in ovariectomized and hysterectomized ewes or in those ovariectomized ewes treated only with progesterone. Estrogens were also reported to stimulate the release of $PGF_{2\alpha}$ from the uteri of non-pregnant guinea pigs (Blatchley _et al._, 1971).

D. Interrelationships of Corticoids, Progesterone and Estrogens at Parturition

In the chronically cannulated fetal lamb fetal plasma corticoids increased markedly at least one day earlier than maternal plasma progesterone began to decline (Table 23). In goats an increase in fetal plasma corticoids also preceded a drop in maternal plasma progesterone prior to parturition (Thorburn _et al._, 1972). Following ACTH infusions into fetal lambs maternal plasma progesterone decreased rap-

idly (Liggins et al., 1972). Peripheral plasma progesterone has also been reported to decrease rapidly in cows following DXMS administration for parturition induction (Edqvist et al., 1972; Evans and Wagner, 1971). These results strongly suggest that corticoids have a negative effect on progesterone and fetal plasma corticoids chronologically increase prepartum before maternal plasma progesterone decreases.

During pregnancy goats have an ovarian source of progesterone whereas sheep have a placental source with a rise in fetal corticoids preceding the prepartum progesterone decrease in both species. Therefore, if the rise in fetal corticoids is instrumental in initiating parturition through a negative effect on progesterone, the mechanism of action is probably indirect. This mechanism may involve PG synthesis, since DXMS infusions into the fetal lamb were followed by increases in the $PGF_{2\alpha}$ content of the maternal cotyledon and myometrium (Liggins and Grieves, 1971). Prostaglandin $F_{2\alpha}$ was luteolytic in cycling ewes (Thorburn and Nicol, 1971) and PGF levels rose in the UO vein 24 h prepartum (Thorburn et al., 1972).

A distinct rise in fetal corticoids in human pregnancy has also been noted close to term (Smith and Shearman, 1972). It could be speculated that since this change is common to many mammalian species it is intimately related to the initiation of parturition. However, pregnant women responded to DXMS dosages with a decreased urinary estrogen excretion (Warren and Cheatum, 1967) whereas DXMS administration to

cows for parturition induction was followed by a marked increase in plasma estrone concentrations (Edqvist *et al.*, 1972). This may represent a species difference, but in women close to term the effect of a massive DXMS dose on plasma estrogen levels is not known.

Dexamethasone infusions into fetal lambs was followed by a rise in maternal plasma estradiol-17β levels if the ewes were more than 130 days in gestation, but not in animals less than 130 days pregnant. Therefore the rise in plasma estrogens normally seen in late pregnant animals was not a prerequisite for lambing (Liggins *et al.*, 1972).

A decrease in plasma progesterone levels was noted prior to parturition (Tables 7, 8). A fall in myometrial progesterone would be expected with this decrease since DXMS infusions into fetal lambs resulted in a decrease in plasma and myometrial progesterone (Liggins *et al.*, 1972). However, parturition occurred with no fall in either plasma or myometrial progesterone levels when 100 mg progesterone was given daily to ewes while their fetuses were infused with DXMS. Therefore, although corticoid-induced parturition was normally associated with a lowering of myometrial and progesterone levels, this response does not appear to be a prerequisite for lambing.

There is evidence that estrogens affect progesterone metabolism since estrogen administration to ovariectomized progesterone treated rats was followed by a decrease in

uterine progesterone and an increased rate of ring A reduction of progesterone (Armstrong and King, 1971). The administration of estrone to rats at midpregnancy also significantly increased the rate of *in vitro* progesterone metabolism by the rat uterus (Howard and Wiest, 1972).

Chronologically in this study maternal plasma progesterone levels began to decrease at least five days prepartum and were preceded by a rise in fetal plasma corticoids. Maternal plasma corticoids were elevated 2 to 3 days prepartum, appearing to result from the stress of impending parturition rather than from the fetus. Finally a sharp rise in plasma estrogens occurred only on the day of parturition with evidence that levels had gradually increased 4 to 5 days earlier.

In a study of the temporal changes at parturition in the uterine venous plasma of one ewe the estrogen concentration rose before the $PGF_{2\alpha}$ concentration (Challis *et al.*, 1972). Therefore prepartum changes in the parturient ewe appear to occur in sequence and there is strong evidence that fetal corticoids precipitate these changes.

VI. SUMMARY

Pregnant ewes were randomly assigned to the following groups: (1) normal controls, (2) adrenalectomized (AD), (3) ovariectomized (OV) and (4) adrenalectomized-ovariectomized (AD-OV). Fetal lambs in other late pregnant ewes were cannulated. The uterine and umbilical circulation was acutely sampled in yet another group of ewes.

Ewes were AD at day 90 of gestation and maintained on replacement therapy. Late in gestation the assigned ewes were ovariectomized. At this time cannulas were placed in the utero-ovarian (UO) and jugular (J) veins. Blood samples were collected daily, the plasma harvested and frozen until assayed. Steroid levels were determined by competitive protein binding techniques. The binding protein was found in adrenalectomized dog plasma for the corticoid and progesterone assays. For the estrone and estradiol assays the binding protein was present in a rabbit uterine cytosol preparation.

Plasma corticoids were significantly ($P<.01$) elevated at parturition in the control and OV group. Corticoid levels were significantly ($P<.001$) lower in AD and AD-OV groups. In either the acutely or chronically sampled fetus, plasma corticoids were markedly elevated late in gestation. No significant difference existed in corticoid concentration between the umbilical artery and vein. Evidence was found to suggest fetal plasma corticoids increased before maternal

progesterone decreased.

A lack of evidence was found in support of a transplacental passage of corticoids from the fetal to the maternal side. A greater corticoid concentration was invariably found in the uterine artery than in the uterine vein in late pregnant ewes. However, quite frequently a greater corticoid concentration was found in the UO vein compared to the J vein in all treatment groups.

Utero-ovarian progesterone concentrations were significantly ($P<.001$) higher than J levels in all treatment groups. Control ewes carrying multiple lambs had significantly ($P<.05$) higher J progesterone levels than ewes with a single fetal lamb. Progesterone levels declined prepartum with a consistent decline in UO levels 5 to 7 days before parturition. Ovariectomy did not affect plasma progesterone levels, whereas J plasma progesterone was consistently lowered in AD and AD-OV ewes.

Progesterone concentrations were significantly ($P<.025$) greater in the umbilical vein than in the umbilical artery at gestational age 139 days. Later in gestation these levels declined and an umbilical venous-arterial difference was not present. Following removal of the fetal lambs uterine progesterone levels decreased.

Estrone concentrations were quantitatively greater than estradiol. Estrone concentrations were clearly greater in the UO than in the J vein, whereas this difference was often not

evident in the estradiol concentrations.

Utero-ovarian estrone concentrations in control ewes increased several days prepartum. A dramatic increase in estrone levels was seen at parturition in both sites. Prepartum estrone levels were consistently reduced in both sites in AD and AD-OV ewes as were J estrone levels in OV ewes.

Adrenalectomy did not affect lamb viability or prolong gestation. Many AD ewes did abort or die following further surgery. Ovariectomy tended to reduce gestation length.

Thus it appears that the following sequence of events presage the occurrence of parturition in sheep.

1. Fetal plasma corticoids begin to increase about a week prepartum and this is accompanied by a decrease in UO vein progesterone levels.
2. Several days prepartum maternal UO estrogen levels begin to increase. Fetal plasma corticoids levels increase sharply at this time while maternal progesterone levels continue to decrease.
3. A further dramatic increase in maternal plasma estrogen levels occurs within 24 h of parturition.
4. On the day of parturition PG levels rise in the UO plasma and myometrial contractions appear approximately 12 h prepartum.
5. Accompanying the expulsion of the fetal lamb plasma oxytocin levels rise sharply.

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IX. APPENDIX

Table A1. Preparation of protein binding solutions

A. Corticoid assay

1. Place 0.28 ml of a stock solution of 1, 2-^{3}H hydrocortisone (0.08 μg/ml) into a round bottom flask.
2. Add 100.0 ml of distilled-deionized water.
3. Add 3.0 ml of adrenalectomized dog plasma.
4. Chill to 4C before use.

B. Progesterone assay

1. Place 0.035 ml of a stock solution of 1, 2-^{3}H corticosterone (100 ng/ml) into a round bottom flask.
2. Add 99.0 ml of distilled-deionized water.
3. Add 1.0 ml of adrenalectomized dog plasma.
4. Chill to 4C before use.

Table A2. Preparation of scintillation fluids

A. Scintillation fluid A

1. Place 5 g of diphenyloxazole into a large container.
2. Add 1 L toluene.
3. Add 100 ml of Biosolv #3 or 250 ml Triton X100.

B. Scintillation fluid B

1. 5 g diphenyloxazole.
2. 1 L toluene.

C. Scintillation fluid C

1. 5 g diphenyloxazole.
2. 1 L toluene.
3. 200 ml Biosolv #3 or 500 ml Triton X100.

Table A3. Preparation of celite column

1. Wash celite (diatomaceous earth Grade 1, Sigma Chemical Co.) in 6 N HCl.
2. Wash with distilled-deionized water until effluent is neutral.
3. Wash with methyl alcohol.
4. Wash with diethyl ether, dry and store in a muffle furnace at 540C.
5. Mix celite with ethylene glycol 2:1 (w/v) and pack the mixture with the aid of iso-octane to a height of 5 cm in 5 ml pipettes. Wash columns with 3.0 ml iso-octane before use.

Table A4. Preparation of estrogen assay reagents

A. Tris-HCl-buffer

Dissolve 1.21 g of THAM® [tris (hydroxymethyl) aminomethane] in 1 L of distilled-deionized water. Adjust pH to 8.0 if necessary.

B. Tris-HCl-ethylene glycol

Mix 80.0 ml tris-HCl-buffer with 20.0 ml ethylene glycol.

C. Tris-charcoal-dextran suspension

Add 1.0 g Norit A (activated charcoal, Sigma Chemical Co.) and 0.1 g dextran to 200.0 ml tris-HCl-buffer.

Table A5. Preparation of uterine cytosol

1. Uteri obtained from 6 day pregnant rabbits, frozen on dry ice and stored at -20C until needed.
2. Homogenize the uteri in tris-HCl-buffer containing 0.25 M sucrose and 0.001 M ethylene-diaminetetraacetate 1:3 (w/v) using a tissue grinder.
3. Centrifuge at 3500 X G for 15 min.
4. Recentrifuge the supernatant at 105,000 X G for 90 min.
5. Place in small ampules and store in liquid N_2.

Table A6. Gestation length and number of lambs born to ewes in the four treatment groups

Control			Adrenalectomized		
Ewe No.	Length[a]	Lambs	Ewe No.	Length[a]	Lambs
419	144	3	313	147	2
421	148	2	409	144	2
422	146	2	411	145	2
308	147	1	413	144	1
293	144	1	417	148	1
289	145	2[b]	423	149	1
298		1			
Mean	145.8			146.2	

[a]Gestation length in days.

[b]Born dead.

Ovariectomized			Adrenalectomized-Ovariectomized		
Ewe No.	Length[a]	Lambs	Ewe No.	Length[a]	Lambs
415	145	1	405	142	1[b]
296		1[b]	414	139	1
301	142	1	503	144	2
424	144	2	502	142	2
Mean	143.7			141.8	

Table A7. Mean steroid levels in intact control ewes as used in the analysis of variance

Day	Corticoids[a] UO[c]	Corticoids[a] J[d]	Progesterone[a] UO[c]	Progesterone[a] J[d]	Estrone[b] UO[c]	Estrone[b] J[d]	Estradiol[b] UO[c]	Estradiol[b] J[d]	N
-10	16.9	13.9	63.0	19.3	42.0	41.7	14.2	12.7	3
- 9	18.3	25.2	87.4	18.9	59.4	48.8	16.7	12.0	6
- 8	15.1	15.5	70.7	20.6	52.3	44.5	29.8	8.7	6
- 7	18.4	21.6	95.9	16.5	56.7	78.1	43.0	17.6	4
- 6	11.5	10.9	82.1	11.8	66.1	59.8	19.7	12.6	4
- 5	15.3	11.6	74.3	17.3	74.8	61.1	20.6	11.5	7
- 4	18.9	19.6	63.3	16.3	137.1	72.3	20.2	12.7	6
- 3	12.5	9.3	61.3	21.8	98.3	55.9	13.9	16.4	4
- 2	27.4	18.3	48.0	17.0	127.0	78.5	35.7	33.3	6
- 1	25.6	26.5	44.1	11.7	108.8	96.7	28.3	54.6	7
0	37.0	40.2	30.3	8.3	443.5	359.0	56.7	80.2	5
+ 1	43.4	45.9	13.4	1.2	1701.0	1284.5	168.0	283.3	2
+ 2	12.1	15.0	3.3	1.7	22.5	12.0	13.5	3.9	1

[a] As expressed in ng/ml plasma.

[b] As expressed in pg/ml plasma.

[c] Utero-ovarian vein.

[d] Jugular vein.

Table A8. Mean steroid levels in adrenalectomized ewes as used in the analysis of variance

Day	Corticoids[a]		Progesterone[a]		Estrone[b]		Estradiol[b]		N
	UO[c]	J[d]	UO[c]	J[d]	UO[c]	J[d]	UO[c]	J[d]	
-10	3.3	2.2	49.5	10.0	43.0	21.0	30.0	5.6	3
- 9	4.5	3.2	62.1	11.6	30.1	52.9	54.8	11.7	4
- 8	3.1	2.7	55.5	9.8	37.1	38.2	17.1	9.9	6
- 7	3.0	3.0	52.1	7.2	53.3	39.7	11.0	11.5	6
- 6	4.3	3.1	76.9	11.1	63.2	41.3	14.3	17.8	5
- 5	3.8	2.5	71.8	10.1	34.2	28.5	17.7	14.9	6
- 4	4.7	3.3	136.3	7.3	41.1	31.7	17.1	11.2	6
- 3	4.5	3.6	63.1	9.8	51.9	49.0	13.6	16.0	6
- 2	3.7	2.8	39.1	5.9	39.5	43.5	14.7	13.2	6
- 1	4.4	2.9	50.1	5.8	77.3	64.6	24.7	17.1	5
0	6.1	4.6	24.0	6.3	180.0	156.9	64.1	51.5	5
+ 1	4.0	4.4	2.1	1.0	111.8	81.4	26.6	31.8	4
+ 2	2.7	3.6	1.9	0.3	24.4	26.2	8.3	7.3	4

[a] As expressed in ng/ml plasma.

[b] As expressed in pg/ml plasma.

[c] Utero-ovarian vein.

[d] Jugular vein.

Table A9. Mean steroid levels in ovariectomized ewes as used in the analysis of variance

Day	Corticoids[a]		Progesterone[a]		Estrone[b]		Estradiol[b]		N
	UO[c]	J[d]	UO[c]	J[d]	UO[c]	J[d]	UO[c]	J[d]	
-10	17.2	14.1	62.0	10.9	45.8	34.5	21.0	25.3	2
- 9	30.6	30.5	54.5	14.4	73.4	40.8	24.0	27.0	2
- 8	25.3	30.0	79.6	20.2	66.4	25.8	42.3	29.1	3
- 7	12.6	16.5	53.8	15.3	70.9	35.3	21.9	23.1	3
- 6	15.9	14.4	47.8	11.1	172.1	57.0	20.1	19.6	2
- 5	12.3	16.0	67.4	12.7	52.3	46.6	19.9	19.8	3
- 4	18.6	15.7	67.6	11.5	58.7	54.0	34.5	24.6	4
- 3	15.5	14.7	68.6	16.1	55.0	77.1	34.6	36.5	3
- 2	17.4	13.3	52.3	9.3	81.6	63.2	53.2	31.6	3
- 1	8.8	11.4	30.8	5.8	119.6	106.1	33.8	38.6	4
0	41.1	36.0	21.9	7.3	255.3	236.0	75.6	53.5	4
+ 1	16.2	10.1	0.9	0.0	45.0	65.0	18.0	28.0	2
+ 2	14.4	9.9	1.6	0.3	25.4	31.0	14.3	10.1	3

[a] As expressed in ng/ml plasma.

[b] As expressed in pg/ml plasma.

[c] Utero-ovarian vein.

[d] Jugular vein.

Table A10. Mean steroid levels in adrenalectomized-ovariectomized ewes as used in the analysis of variance

Day	Corticoids[a]		Progesterone[a]		Estrone[b]		Estradiol[b]		N
	UO[c]	J[d]	UO[c]	J[d]	UO[c]	J[d]	UO[c]	J[d]	
- 9	2.3	2.9	41.3	13.6	51.5	44.3	19.9	21.9	3
- 8	3.2	3.9	62.5	14.8	48.0	46.0	19.7	15.0	3
- 7	3.1	3.4	53.0	13.1	55.5	44.8	16.1	14.3	4
- 6	4.3	3.5	83.1	11.0	57.8	50.4	16.5	16.6	4
- 5	5.8	3.2	72.4	10.8	54.4	45.9	19.2	15.4	4
- 4	5.4	5.5	63.2	9.8	52.5	58.3	16.5	19.0	4
- 3	5.3	6.0	48.4	9.2	56.3	57.3	21.2	16.0	4
- 2	5.2	4.4	41.6	10.8	60.5	61.1	26.0	19.8	3
- 1	3.2	3.9	49.8	6.8	48.0	41.7	20.1	16.0	3
0	2.9	4.1	9.4	1.1	69.8	83.3	90.0	50.3	2
+ 1	1.8	4.8	0.2	0.0	42.0	42.0	6.0	11.7	1
+ 2	0.0	0.0	0.4	0.0	31.5	48.0	6.0	5.4	1

[a] As expressed in ng/ml plasma.

[b] As expressed in pg/ml plasma.

[c] Utero-ovarian vein.

[d] Jugular vein.

Table A11. Progesterone levels in the utero-ovarian vein (V) and the uterine artery (A) following removal of the fetal lambs

	139[a,b] 2[c]		142[a] 2[c]		144[a] 1[c]		145[a] 1[c]		146[a] 1[c]	
Hrs.[d]	V	A	V	A	V	A	V	A	V	A
0	182.8 89.5	61.5 21.8	176.1	29.3	72.1	23.5	233.0	10.0	104.2	18.2
1	13.5 26.0	 12.8	30.9	10.0	50.7	12.0	16.5	5.5	29.0	5.4
4	8.5 6.5	 3.1	8.3	4.6	70.1	3.9	4.1	2.0	2.6	2.1
8	6.3 5.7		5.3	4.3	95.9	3.8	2.8	2.0	3.5	2.1
24	2.4 4.0		1.7	2.0	11.5	1.3	2.1		2.5	1.8
48	2.1				1.9					

[a]Gestational age.

[b]Two ewes on day 139.

[c]Number of lambs.

[d]Hours after removal of the fetal lambs.