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Induction of Parturition in the Ovine

Ernest L. Harman

INDUCTION OF PARTURITION IN THE OVINE

BY

ERNEST L. HARMAN

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INDUCTION OF PARTURITION IN THE OVINE

Abstract

ERNEST L. HARMAN

Under the supervision of Associate Professor A. L. Slyter

The effects of flumethasone and prostaglandin $F_{2\alpha}$ (PGF) on the time of parturition were studied in the ewe. Pregnant ewes with known conception dates were allotted into a 3 x 3 x 2 factorial designed experiment (1973 study) and into a completely random designed experiment (1974 study). In the 1973 study treatments consisted of three hormones (flumethasone, PGF and saline control), three levels (low, .5 mg flumethasone, 5 mg PGF, 1 cc saline; medium, 1.0 mg flumethasone, 10 mg PGF, 2 cc saline; high, 1.5 mg flumethasone, 15 mg PGF, 3 cc saline) and two routes of administration (IM and IV) with nine ewes per treatment. Treatments in the 1974 study were 2 mg flumethasone, 15 mg PGF and 4 cc saline. There were 39 ewes per treatment. All treatments in the 1974 study were administered IM in a 4 cc volume. In both studies treatments were administered on day 141 of gestation and a ewe was considered to have been successfully induced if she lambed within 72 hours post-treatment.

In the 1973 study 78% of the ewes treated at the medium level with PGF by the IM route of administration were induced with a mean interval of 32.79 ± 8.22 hours. Flumethasone treatment at the high level by either IM or IV administration also resulted in 78% of ewes lambing within 72 hours of treatment with a mean interval of 42.00 ± 3.12 and 42.93 ± 6.71 hours, respectively.

In the 1974 study 89% of the ewes given flumethasone were induced with an average response interval of 50.9 ± 2.3 hours. Thirty-three percent of the PGF-treated ewes were induced with a mean response interval of 41.7 ± 5.9 hours. Only 7.7% of the saline control ewes lambed within the 72-hour period following injection. The mean interval from treatment to parturition (all ewes included) was significantly reduced by both flumethasone ($P<.01$) and PGF ($P<.05$) treatments when compared to that for control ewes.

Initial total progestins (day 141 of gestation) of control ewes averaged 7.86 ng/ml, while initial mean progestin level for flumethasone-treated ewes was 7.08 ng/ml. PGF-treated ewes initial progestin levels averaged 8.63 ng/ml. Final (within 24 hours of parturition) mean progestin levels of control, flumethasone- and PGF-treated ewes were 2.97, 3.11 and 4.16 ng/ml, respectively. Least squares analysis of variance indicated no significant differences between treatment groups in initial or final progestin levels.

Initial total estrogen levels of control ewes averaged 145.01 pg/ml. Initial mean level of estrogen for flumethasone-treated ewes was 167.83 pg/ml. PGF-treated ewes averaged 137.83 pg/ml. Final estrogen levels were 250.34, 279.22 and 244.49 pg/ml for control, flumethasone- and PGF-treated ewes, respectively. There was no statistical difference indicated by least squares analysis of variance between treatment groups in either initial or final estrogen levels.

INDUCTION OF PARTURITION IN THE OVINE

This thesis is approved as a creditable and independent inv stigation by a candidate for the degree, Doctor of Philosophy, and is acceptable for meeting the thesis requirement for this d gree. Acceptance of this thesis does not imply that the conclusions r ached by the candidate are necessarily the conclusions of the major department.

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LIST OF ABBREVIATIONS AND SYMBOLS

c	Curie(s)
cpm	counts per minute
dpm	disintegrations per minute
IM	intramuscular
IV	intravenous
ng	nanogram(s), gram x 10^{-9}
PBS	Phosphate buffered saline
pg	picogram(s), gram x 10^{-12}
PGF	Prostaglandin $F_{2\alpha}$
RIA	radioimmunoassay
µl	microliter(s)

INTRODUCTION

In recent years management has become a more important factor in the success of a livestock enterprise than in past years. Rapidly increasing costs of production without parallel increasing market prices for livestock are narrowing the producer's profit margin. If producers are to continue to raise livestock economically, research must result in new management techniques which will enable producers to make a fair return on their labor and capital input.

The ability to time parturition is a management technique by which producers might be able to cut production costs and/or reduce livestock losses. Purebred breeders and small commercial breeders who are able to obtain accurate breeding dates for their livestock could make more efficient use of available facilities and labor if they were able to accurately control the time of parturition. Large commercial breeders could use induced parturition as an effective "clean-up" technique. Animals that have not given birth by a stipulated time could all be treated at one time and observed closely during the expected lambing period. This could reduce losses in lambs born late in the lambing period since losses at this period are usually higher than the herd average. The increased losses in neonates born at the end of the lambing period may be due to increased birth weights of fetuses carried past normal term and/or lack of adequate supervision during the parturition process.

Cattle that carry their fetus well past term have less time for involution of the uterus in preparation for the subsequent breeding

season. By inducing those animals which have not calved by the last week of the "drop period," a producer should enhance the chance of a cow rebreeding during the subsequent breeding season. At the present time involution time is not a problem in the ewe because ewes are not normally rebred for 5 to 6 months post-lambing. However, if hormonal manipulation enables breeding during the normal anestrous season, involution time will also become important to the sheep producer.

The objectives of this study were (1) to determine if prostaglandin $F_{2\alpha}$ (PGF) and flumethasone are effective for the induction of parturition and (2) to determine the endogenous circulating levels of estrogens and progestins at normal and induced parturition.

REVIEW OF LITERATURE

Numerous studies have been conducted concerning the induction of parturition in domestic animals. However, the mechanisms which initiate parturition have not been definitely established. Many investigators at present hold differing views as to the major physiological factors initiating parturition in domestic animals.

Factors Initiating Parturition

It is generally accepted that progesterone plays a role in the maintenance of pregnancy in mammals (Cole and Cupps, 1969). Many investigators consider the "progesterone block" to be one of the major factors in the maintenance of pregnancy and its removal the major factor in the initiation of parturition (Csapo and Wiest, 1969; Schofield, 1968). It has been shown experimentally that plasma levels of progesterone usually fall just before parturition (Fylling, 1971; Liggins, 1969; Donaldson et al., 1970). However, Bassett et al. (1969) found that plasma concentrations of progesterone in term ewes vary greatly. They found that concentrations fell gradually over the last 2 weeks of gestation in some cases, while in other cases a fall in concentration was very slight or absent. In addition, Bengtsson and Schofield (1963) found that in cases of prolonged pregnancy due to exogenous administration of progesterone the fetal membranes often ruptured at term, but the maternal cervix failed to dilate. Liggins et al. (1972) stated that a fall in plasma levels of progesterone was essential for normal delivery but was not an essential prerequisite for

labor. These findings indicate that, while the level of plasma progesterone plays a role in maintenance of pregnancy, it is probably not the triggering mechanism for parturition. Thorburn et al. (1972) suggested that progesterone's role in pregnancy might be that of suppressing uterine activity and prostaglandin synthesis.

A steady rise in plasma levels of estrogen just before parturition has been shown experimentally by Bedford and co-workers (1972), Challis (1971), Liggins et al. (1972) and Comline and Silver (1972). The rise in maternal estrogens just before parturition may have several effects on the birth process. A high estrogen level may reduce the effects of the "progesterone block" (Bedford et al., 1972), it may cause the release of oxytocin (Bengtsson and Schofield, 1960), or according to Liggins et al. (1972) cause the synthesis of prostaglandins. It has also been shown that estrogen may increase the permeability of some membranes (Heap et al., 1971). This increase in permeability of membranes may play an important role in the ability of maternally administered corticoids to induce premature parturition if given near term. However, Liggins and his co-workers (1972) have shown experimentally that a rise in circulating estradiol-17β does not always precede induced parturition. Normal parturition could be different as at term many other hormonal factors could be present and interacting. Both Liggins et al. (1972) and Bedford et al. (1972) discounted estrogen's role as being the triggering mechanism for parturition.

It has been demonstrated that oxytocin release can be inhibited by progesterone or enhanced by estrogen (Roberts and Share, 1969; Hindson et al., 1969; Hindson and Ward, 1973). Fuchs (1971) has also demonstrated that genital stimulation can cause the release of oxytocin. Chard (1972) has shown that there was little change in oxytocin levels during the first part of parturition, but that there was a sudden rise at the moment of birth. This evidence tends to show that oxytocin probably enhances parturition but does not initiate the process.

The role of the fetus in the initiation of parturition has been speculated since 1898 (Liggins et al., 1966). Liggins et al. (1966), in experiments in which the fetal pituitary was destroyed, found that, if 70% or more of the pituitary was destroyed, pregnancy was prolonged until interrupted by caesarean section 10 to 34 days beyond term.

In another study by Liggins and Kennedy (1968) it was found that if the pituitary was left intact but the fetus was hypophysectomized parturition was extended until exogenously initiated. They further found that the ablation of the pituitary was followed by adrenal hypoplasia. Studies by Drost and Holm (1968) have shown the importance of the fetal adrenal in parturition. In an experiment with 36 ewes they found that if fetal lambs were adrenalectomized the lambs were carried an average of 15 days past term. The range of gestational period was 154 to 180 days. Liggins (1968) found that parturition began in ewes after the fetal adrenals weighed 680 ± 158 milligrams. Liggins et al. (1966) destroyed the pituitary of twin fetal lambs. Ten days beyond term ACTH was infused into one lamb. Six days later

delivery occurred. In the infused lamb the adrenals weighed 740 mg, while the noninfused lamb's adrenals weighed only 293 milligrams. Liggins (1968) also initiated parturition in pre-term lambs by infusing dexamethasone intrauterine and found that parturition began in ewes after the fetal adrenals weighed at least 532 milligrams. These studies all lend support to the hypothesis that the fetus exercises great control over its own time of parturition through the fetal hypothalmus-pituitary-adrenal axis.

It has been shown that stimulation of the fetal adrenal glands with glucocorticoids will initiate parturition (Van Rensburg, 1967; Liggins, 1968, 1969). However, there are conflicting reports as to whether glucocorticoids will initiate parturition when given to the dam rather than directly to the fetus.

Studies by Liggins (1969) indicated that parturition in sheep could be initiated by infusion of dexamethasone at .05 to .25 mg per 24 hours for 4 to 10 days, depending on gestational age at the beginning of infusion. Higher dosages and later gestational age at the start of infusion resulted in shorter duration of infusion necessary to initiate parturition. In addition, Liggins (1969) found that the infusion site was of upmost importance. In cases of administration to the ewe rather than to the fetus, parturition was not induced. Only infusion directly into the fetus resulted in parturition prior to term. However, Bosc (1971, 1972) found that dexamethasone infused into ewes at the start of the lambing period would initiate parturition in 45 ± 5.7 hours.

Adams (1969), Adams and Wagner (1970), Evans et al. (1971) and Wagner et al. (1971) have studied the effectiveness of dexamethasone for induction of parturition in cattle. The above investigators administered 20 to 25 mg intramuscular (IM) injections of dexamethasone to beef cattle and in most cases were able to initiate parturition within 72 hours after injection.

Several investigators (Osinga et al., 1971; Skinner et al., 1970; Jochle, 1971) have successfully induced parturition with flumethasone, another synthetic corticoid, but the percent successfully induced has been variable. The differences appear to be related to the dosage given, although different investigators reported both good and poor results with the same levels. According to Jochle (1971) flumethasone has been injected into the dam at the same levels and the same gestational age of fetus with varying results. Levels recommended for induction of parturition in sheep range from .5 to 1.5 milligrams.

The answer to the conflicting reports as to the site of corticoid administration and effectiveness of induction of parturition seems to be the gestational age of the fetus at the time of corticoid administration. In studies in which dexamethasone was given to dams without induction of parturition, the gestational age of the fetus was less than 119 days (sheep). However, in studies in which maternal administration of dexamethasone resulted in successful initiation of parturition, animals were generally at or very near term. In studies in which Adams (1969) induced parturition in cattle by maternal administration of dexamethasone, the gestational range of the treated

animals was 262 to 280 days. However, in cattle of the gestational range of 235 to 257 days, Adams (1969) was able to induce parturition in only one of four animals by maternal corticoid administration. The stage of pregnancy and the site of administration, therefore, appear to influence the effectiveness of induction of parturition by exogenous glucocorticoids. The reason for gestational age affecting effectiveness of administration site might be the permeability of the fetal membranes at the time of administration.

Bassett and Thorburn (1969) measured the plasma corticosteriod levels of normal sheep fetuses in utero. They found little relation between maternal and fetal circulating corticosteroid levels. However, there is disagreement on this point as Dixon and his co-workers (1970) found that during the latter fifth of gestation the ewe made a contribution to fetal plasma cortisol levels. These findings agree with those of Anderson et al. (1973) who found substantial cross-placental transfer of corticoids. This might explain part of the marked increase of fetal corticosteroid levels beginning several days before parturition with a peak at birth which Bassett and Thorburn (1969) found in their studies. Beitins et al. (1970) stated that there was very little permeability of the fetal placenta to corticosteroids until the fetus was at or very near term. Therefore, one could assume that the permeability of the fetal placenta has a great deal of effect on the effectiveness of administration site and timing when attempting to induce parturition with glucocorticoids.

The mechanism by which exogenous glucocorticoids initiate parturition is not fully understood. However, it has been shown that elevated fetal corticosteroid levels stimulate the synthesis and release of PGF (Liggins and Grieves, 1971; Liggins et al., 1972). The leutolytic effects of PGF have been demonstrated in the experiments of Barrett et al. (1971), Thorburn and Nicol (1971) and McCracken et al. (1972). The experiments by Liggins et al. (1972) indicated, however, that fetal corticoid levels do not directly affect the dam's PGF concentrations, as no appreciable increase in fetal PGF was found in fetal cotyledons during parturition. Another indication of an indirect action of glucocorticoids on maternal PGF synthesis was indicated by the findings of Liggins et al. (1972) that maternal administration of dexamethasone did not raise maternal PGF concentrations until the fetal placenta was permeable to corticosteroids.

A possibility as to the mechanism of PGF stimulation by increased levels of fetal corticosteroids might be the rise in maternal estrogens which Thorburn et al. (1972) reported after fetal infusion of glucocorticoids. Liggins and his co-workers (1972) found that estrogen may stimulate the release of PGF and that PGF in turn may stimulate the release of estrogen, thus causing the explosive maternal rise of both PGF and estrogen at parturition.

Inskeep (1973) stated that PGF may prove to be a useful agent for the induction of parturition. Lauderdale (1972) has shown that 45 to 150 mg PGF will cause abortion in cows from 40 to 120 days of gestation. Deihl and his co-workers (1974) induced parturition in gilts

with an average gestational age of 109 days by the administration of 5 mg of PGF. Parturition occurred, on the average, 85 hours after injection. Buckle and Nathanielsz (1975) have demonstrated that injections of PGF will also cause premature parturition in laboratory rats. Liggins et al. (1972) stated that in sheep PGF may be a smooth muscle stimulant or may change the sensitivity of the uterus to oxytocin.

The preceding studies, although indicating a definite role in parturition for glucocorticoids, estrogen, progesterone, oxytocin, PGF and the hypothalmo-pituitary-adrenal axis, shed little light on the actual triggering mechanism of parturition. One possibility that may be the trigger for parturition is the maturation of the hypothalymic thermoreceptors. Thorburn and his co-workers (1972) demonstrated that the temperature of the fetal brain was .4 to .8 C higher than maternal blood. As the fetal thermoreceptors mature, it could be postulated that the fetus may become aware of its hot, wet environment with a subsequent release of steroids by the fetal adrenals due to stress. This adrenal steroid release could increase estrogen and PGF levels, reduce progesterone levels and finally result in parturition. Some support for this hypothesis is given by the studies of Liggins (1968) who found that adrenal growth could be stimulated and corticosteroid secretion initiated during the last third of gestation by the infusion of ACTH into the fetal lamb.

Hormonal Induction of Parturition

Regardless of the mechanism responsible for triggering parturition, it has been shown that fetal infusion of certain glucocorticoids will initiate parturition during early pregnancy and that maternal infusion will induce parturition at or near term. However, interval from injection to parturition and dosage levels are variable. PGF also might be effective for the induction of parturition, although little data concerning its effectiveness are available.

Several investigators have used different glucocorticoids, alone or in combination with other hormones, to induce parturition. Dr. Robert Welch (unpublished data) has used the corticosteroid opticortenol (dexamethasone trimethylacetate) to induce parturition. Cows thus treated have shown a decrease in the incidence of dystocia, retained placentas or milk fever. Most other investigators, however, have not obtained the same results regarding adverse side effects.

Adams (1969) was able to initiate parturition in 19 of 22 head of Holstein-Friesian cross cows with 20 mg dexamethasone in an IM injection or with 20 mg dexamethasone in combination with 5 mg of dienestrol. The day of pregnancy for these cows ranged from 262 to 280 days. Parturition began from 22 to 56 hours after treatment. However, five calves died within 6 days of delivery and all cows had retained placentas with the exception of one that was 280 days pregnant. There was also a high incidence of dystocia and metritis associated with calving.

In another study by Adams and Wagner (1970) parturition was induced in 46 of 54 cows and in 11 of 23 sheep. Twenty mg of dexamethasone were administered to cattle and 10 mg to sheep. The mean interval to parturition for cattle was 49 hours and for sheep the interval was 43 hours. In cattle several fetuses had to be repositioned, 27 cows had retained placentas and 24 subsequently showed signs of metritis. There were no retained placentas, increased lambing difficulty or obvious metritis in the induced sheep. A group of cattle which Adams and Wagner (1970) administered an oral progestin (chloro-17-acetoxy progesterone) during the induction period had less placental retention and postpartum metritis problems. Although only a small number were treated in this manner, they felt that the treatment might prove beneficial as all five cows given the oral progestin rebred within 90 days.

Wagner et al. (1971) were able to induce parturition in 311 cows using either 20 mg dexamethasone or 7.5 mg flumethasone 7 to 14 days before term. Fifty-three percent of the cattle retained fetal membranes, with the incidence of retained membranes in direct proportion to early gestational age. Cattle with retained placentas were treated within 24 hours postpartum with 4 million units of penicillin and 5 g of streptomycin. Subsequent fertility did not appear to be affected by retained placentas thus treated.

Gaverick and his co-workers (1972) found that, if 6 mg of estradiol benzoate were injected in cattle along with 20 mg dexamethasone, the incidence of retained placentas was comparable to that of a

control group. However, in a group injected with 20 mg dexamethasone without estradiol benzoate there was a 50% incidence of placental retention.

Osinga et al. (1971) were able to initiate parturition within 48 hours in 24 of 27 dairy cows of the gestational range of 273 days using 2.5, 5.0 or 10.0 mg flumethasone in IM injections. The three animals which did not respond to treatment were treated at the 2.5 mg level, indicating that 2.5 mg flumethasone was not an effective dosage for induction of parturition. However, 5.0 mg was effective. These animals were treated with intrauterine antibiotic and no attempt was made to manually remove retained placentas. Placental discharge was spontaneous within 4 to 7 days, followed by apparent normal uterine involution and postpartum fertility.

Parturition was successfully induced within 72 hours in 60% of 60 ewes which were of the gestational range of 141 to 143 days by Skinner et al. (1970). Treatments used were IM injections of .5, 1.0 and 1.5 mg flumethasone. In addition, three ewes were treated with .25 mg flumethasone but did not respond to treatment, indicating that .25 mg was not effective for induction of parturition in the ewe. In this experiment no adverse postpartum side effects were encountered. This agrees with the findings of Adams and Wagner (1970) who encountered no postpartum problems in sheep in which parturition was induced with dexamethasone.

The preceding studies indicate that the elective induction of parturition in cattle and sheep by the use of corticosteroids is

possible, although timing of treatment, levels and response interval have not been adequately established. In cattle adverse side effects such as dystocia and retention of fetal membranes are a problem. The problem of retained placentas, however, can be overcome with proper postpartum management. There appear to be no adverse side effects in sheep due to corticosteroid induced lambing.

Estrogen and Progesterone Levels in Pregnant Ewes

Low physiological levels of estrogen and progesterone made accurate determination of physiological levels almost impossible until the development of sensitive protein-binding assays by Murphy (1964) and improved upon in 1967 by Murphy. The highly sensitive radioimmunoassays developed by Thorburn et al. (1969) have also greatly improved accuracy in determination of levels of estrogen and progesterone. Due to the relatively short period of time assay systems have been available for accurate measurement of physiological levels of both estrogen and progesterone, reliable data concerning their levels in the pregnant ewe are limited. In addition, levels reported have been determined on a limited number of animals.

According to Bassett and Thorburn (1973), following fertilization, peripheral plasma concentrations of progesterone increase at about the same rate as in the luteal phase of the estrous cycle. They further stated that the normal decline at 13 to 16 days seen in the progesterone levels of the cycling ewe does not occur in the pregnant ewe. Rather, the ewe maintains the peak level of the estrous cycle of about 2 to 3 ng/ml until approximately 50 days into gestation. From day 50 there is

a steady increase in circulating levels until a peak of 12 to 20 ng/ml is attained at day 130 to 141 of gestation. Levels decline the last 4 to 5 days, such that at the time of parturition levels are approximately 2 ng/ml (Fylling, 1971). In a pregnant ewe which had been given dexamethasone to induce parturition at day 140 of gestation, Fylling (1971) found that a similar pattern of progesterone was evident; namely, a rapid decline in progesterone levels just prior to parturition. Similar declines have been shown in ewes in which premature parturition had been induced with ACTH or Synacthen, a synthetic ACTH (Thorburn et al., 1972; Liggins et al., 1972; Bassett and Thorburn, 1973).

Studies conducted by Matter and Thorburn (1970), Thorburn and Matter (1971) and Thompson (1973) have shown that in ewes bearing singles placental progesterone output was approximately 4 mg per day at day 100 of gestation, while in ewes bearing twins daily output was approximately 8 mg per day. Five days prior to parturition a single bearing ewe's placental progesterone output was approximately 36 mg per day. Ewes bearing twins averaged approximately 56 mg per day during the same time period.

Total estrogen concentration in the pregnant ewe generally ranges below 50 pg/ml until a sharp rise occurs around 48 hours pre-parturition (Challis et al., 1971; Thorburn et al., 1972). However, Rawlings and Ward (1973) have reported levels of estrogen of approximately 20 to 100 pg/ml until 48 hours prepartum and approximately 200 to 900 pg/ml within 24 hours of lambing. It is generally agreed that estrogen concentration in

the peripheral plasma drops to near undetectable levels within 2 days postpartum.

Liggins et al. (1972) have shown a normal pattern of estrogen concentration in the peripheral plasma of the ewe in which the fetus was infused with dexamethasone. The levels remained below 100 pg/ml until 48 hours prepartum and then rose dramatically to around 300 pg/ml at parturition.

In most studies reported there was considerable variation in levels of estrogens. This was probably due to the effect of sex of lambs, weight of lambs and/or nutrition of the ewe (Bassett and Thorburn, 1973). In addition, variation between animals appeared to be substantial and the numbers involved in individual studies were generally limited.

MATERIALS AND METHODS

1973 Study

Breeding dates were obtained for purebred and crossbred ewes during August through October of 1972 by the use of marker rams with dye-painted briskets. Ewes were checked twice a day for "rattle" marks and their date of marking recorded. Dye colors used were changed every 17 days and the last marking date recorded for a particular ewe was considered to be her conception date.

Ewes were then randomly allotted within day of breeding into a 3 x 3 x 2 factorial experiment with 9 head per cell. Random allotment was accomplished as outlined by Steel and Torrie (1960). Treatments employed are shown in table 1.

TABLE 1. EXPERIMENTAL DESIGN - 1973 STUDY

	Treatment					
	PGF mg		Flumethasone mg		Saline cc	
Level	IM[a]	IV[b]	IM	IV	IM	IV
Low	5	5	.5	.5	1	1
Medium	10	10	1.0	1.0	2	2
High	15	15	1.5	1.5	3	3

[a] Intramuscular.
[b] Intravenous.

On day 141 of gestation, as determined from apparent conception dates, ewes were examined and those determined to be pregnant were weighed and given their respective treatment. From the time of

treatment until lambing ewes were observed at least hourly to determine time of parturition.

Data recorded were pre-lambing ewe weight, total weight of lambs born, lambing difficulty score (1 = normal, ..., 4 = extremely difficult), evidence of retained placenta, time of lambing and type of birth. Hours from treatment to parturition were then calculated.

All ewes used in this study were maintained at the University Sheep Unit, Brookings, South Dakota, and were involved in production studies.

1974 Study

Conception dates were obtained for purebred and crossbred ewes during August through October of 1973 in the same manner as for the 1973 study. Some of the 1974 study ewes had been used in the 1973 study. Ewes were then randomly allotted within day of breeding into three treatments. Treatments used for the 1974 study were 2 mg flumethasone, 15 mg PGF and a physiological saline control. All treatments were given in an equal volume (4 cc) of physiological saline.

On day 141 of gestation, as determined from apparent conception dates, ewes were examined and those determined to be pregnant were weighed, a blood sample taken by jugular vein puncture and given their respective treatment by an IM injection. Subsequent blood samples were taken at 1 p daily until lambing. Ewes not lambing by day 16 post-treatment were re reated with their respective treatment as it was assumed that they conceived to the cycle following the last observed breeding date. Parameters recorded were the same for the 1974 study as for the 1973 study. In addition sex of lambs born was recorded and a

sex ratio calculated (1 = all female, 2 = 33% male, 3 = 50% male, 4 = 66% male, 5 = all male).

Blood samples were collected in heprinized tubes and placed in an ice bucket until all collections were made for a particular day. They were then centrifuged for 10 minutes at 1,800 X G and plasma placed in a 5 ml plastic culture tube and frozen at -10 C until assayed. Samples assayed for estrogen and progesterone were the first four collected and/or the last two collected prior to lambing.

All ewes used in the 1974 study were, as in the 1973 study, maintained at the University Sheep Unit, Brookings, South Dakota, and were involved in production studies.

All blood samples assayed in this study were assayed by radioimmunoassay. The basic principles of this radioimmunoassay (RIA) have been outlined by Parker (1974). The assay essentially involves an antigen-antibody binding reaction. An antibody specific for the hormone to be measured is placed in constant amounts with an unknown quantity of that hormone. A constant amount of radioactively labeled hormone is then placed into the assay system. Due to direct competition between labeled and nonlabeled hormones for antibody binding sites, the more labeled hormone present the less unlabeled hormone present in the unknown sample. This reaction allows a standard curve to be developed for known quantities of hormone. Unknown amounts of hormone can then be determined from the standard curve.

Estrogen Assay

Total estrogens were measured by the technique described by Parker (1974) with slight modifications.

All glassware used in this assay was washed by soaking in Micro washing solution (International Products Corp.) for at least 2 hours at 95 C. It was then rinsed twice with tap water and four times with distilled water and dried in an oven at 100 C.

The antibody used in this study for estrogen was supplied by Dr. R. D. Randel while he was at the U. S. Range Livestock Experiment Station, Miles City, Montana. The antibody was characterized and validated by Stellflug (1972). It was used at a dilution of 1:60,000 in phosphate buffered saline (PBS, .1 M phosphate; pH, 7.0).

The scintillation cocktail consisted of 7.0 g 2,5-diphenyloxazole (PPO), .3 g 1,4-bis(-(5-phenyloxazolyl))-benzene (POPOP) and 100 g naphthalene dissolved in 1 liter of dioxane.

Extraction fluid consisted of freshly opened anesthesia grade anhydrous ether.

A suspension of .25% charcoal (Norit A) and .25% dextran T-70 in PBS was used to remove unbound estrogen from the assay system.

Standard solutions of estradiol-17β (Sigma Scientific) were made by serial dilution in twice distilled ethanol such that 50 µl contained 0, 12.5, 25.0, 50.0, 100.0 and 200.0 picograms. All standard and PBS solutions were stored at 5 C when not in use.

Extraction and RIA of Estrogen. The extraction procedure was identical to the procedure described by Parker (1972) and will not be

detailed in this thesis. Percent recovery from 10 assays averaged 88 ±2.1% and was not significantly different.

The RIA procedure was essentially the same as that outlined by Parker (1974) except that each assay consisted of 50 sample tubes (25 duplicate pairs of each sample to be assayed), two water blank tubes, two total counts and two recoveries. In addition, duplicate standards were included in each assay.

An outline of both the extraction procedure and the RIA procedure is listed in appendix A. Two-tenths ml plasma was extracted for each assayed collection date. The assayed value was then corrected for procedural losses and converted to a per ml basis. The assay is considered to be for total estrogens.

Validation of Estrogen Assay. Plasma from a pregnant ewe was stripped free of steroids by heating for 5 minutes at 45 C. One-half g of florsil was then added to the plasma which was then mixed at moderate speed on a vortex mixer for 5 minutes. The tube was allowed to settle for 5 minutes and the supernatant extracted with a pasteur pipette. This procedure was repeated four times. The extracted plasma was then allowed to cool in an ice bath for 10 minutes. Nonlabeled estradiol from solutions used for standard curves was then added to .2 ml plasma and incubated at 45 C for 5 minutes. The tubes were then allowed to cool for at least 10 minutes in an ice bath. The resulting samples, in addition to a sample of stripped plasma with no estradiol added, were extracted and a RIA ran on them. The resulting means and standard errors are presented in table 2.

TABLE 2. ABILITY OF ASSAY TO MEASURE ESTRADIOL ADDED TO FLORSIL STRIPPED PLASMA

No. samples	Estradiol added pg/ml	Estradiol measured pg/ml	Corrected estradiol pg/ml	SE
5	0	2.43	2.43	.19
5	12.5	14.64	12.21	1.68
5	25.0	29.60	27.17	2.30
5	50.0	50.55	48.12	3.97
5	100.0	101.09	98.66	8.53

Parallelism, a good indicator of the validity of an assay system (Midgey et al., 1969), was also performed to see if the extraction of varying quantities of plasma would yield comparable results when converted to a per ml basis. The test indicated that the assay was measuring the desired compound.

Progesterone Assay

Progesterone 1,2,6,7-^{3}H having a specific activity of 105 c/mMole was obtained from New England Nuclear Corp. and used without further purification. Labeled progesterone was received in a .25 ml quantity and diluted to approximately 20,000 dpm per 100 µl with PBS.

The scintillation cocktail consisted of analytical grade toluene (Mallinckrodt) and scintillation grade Triton X-100 (Research Products International) in a 2:1 mixture. In addition, 7.0 g PPO and .3 g POPOP were added per liter of cocktail.

The extraction fluid consisted of 99 Mole % pure benzene (Fisher) and 99 Mole % pure hexane in a 2:1 mixture.

A suspension of .25% charcoal and .025% dextran T-70 in PBS was used to remove unbound progesterone from the assay system.

The antibody used in this assay was kindly supplied by Dr. G. D. Niswender, Colorado State University, Fort Collins, Colorado.

Standard solutions of progesterone were prepared by serial dilution of progesterone (Sigma Scientific) to the desired concentration in ethanol such that 50 µl contained 0, .025, .05, .1, .25, .5, .75, 1.0 and 1.5 nanograms.

As in the estrogen assay, all standard and PBS solutions were stored in a refrigerated incubator at 5 C except when in use.

Extraction Procedure for Progesterone. To extract progesterone from plasma samples, .1 ml aliquots of each sample were placed in 15 x 85 mm test tubes. Then, taking five at a time, 2 ml of benzene:hexane were added to each tube and all five were mixed for 30 seconds on a vortex mixer, stoppered with a number 4 cork stopper and placed in a rack. After all samples to be extracted for a particular day had been thus treated, the rack was placed in a freezer at -20 C for approximately 12 hours in order to freeze the plasma. The tubes were then removed from the freezer six at a time and the nonfrozen fraction poured into a 12 x 75 mm test tube. The benzene:hexane was then dried down under a stream of nitrogen gas.

Recovery was determined as detailed by Parker (1974) for estrogen extraction, except that progesterone 1,2-^{3}H was utilized rather

than tridiated estrogen. Percent recovery for 10 assays averaged 92 ± 3.2%. Assays did not differ significantly in percent recovery.

RIA Procedure for Progesterone. The RIA procedure for progesterone was identical to the procedure used for estrogens except for the addition of .2 ml of PBS to each tube prior to addition of charcoal-dextran solution and allowing all tubes to incubate for 15 minutes in an ice bath prior to centrifugation. An outline of the procedure is contained in appendix B.

Validation of Progesterone Assay. Various steroids (Sigma Scientific) were dissolved in twice distilled ethanol such that 50 µl contained the desired quantity of steroid. Fifty µl of the various solutions containing from 25 to 2,000 pg were added to 12 x 75 mm assay tubes and carried through the identical assay used for the plasma being assayed for progesterone. Table 3 indicates the various steroids and quantities measured in addition to progesterone. It is evident from the table that the antibody is somewhat specific for progesterone, although there was some cross-reactivity with 17$_\alpha$OH progesterone and pregnenolone. Also, there was cross-reactivity with corticosterone. This was ignored due to less than 5% of corticosterone 1,2-^{3}H being extracted with the extraction procedure utilized in this study. This is in agreement with results reported by Murphy (1967) who found that less than 2.0% of corticosterone and less than 1.0% cortisol was extracted by petroleum ether of which hexane is a refined product. The

TABLE 3. CROSS-REACTIVITY OF PROGESTERONE ANTIBODY WITH VARIOUS STEROIDS

Steroid assayed	Concentration, pg								
	25	50	100	250	500	750	1000	1500	2000
	Percent bound[a]								
Progesterone	82.3	71.2	58.2	34.3	24.1	20.0	18.2	14.3	11.0
17$_{\alpha}$OH progesterone	93.2	83.2	--	88.4	75.3	--	71.0	--	62.1
Pregnenolone	92.9	88.9	--	88.2	76.6	--	74.4	--	72.4
Corticosterone	95.6	94.3	--	92.8	83.0	--	73.1	--	49.3
Cortisol	99.9	99.8	--	97.5	88.8	--	99.7	--	99.3
Andosterndione	99.2	99.5	--	99.4	94.1	--	99.5	--	96.3
Andosterone	99.6	98.7	--	96.3	98.0	--	98.0	--	98.5
Testosterone	99.2	99.5	--	99.8	99.3	--	99.6	--	98.2
Estradiol-17B	99.8	99.6	--	99.4	99.5	--	99.4	--	99.0

[a] Zero concentration is considered to be 100% bound.

assay is, however, considered to be for total progestins due to the cross-reactivity with progesterone metabolites.

Plasma was also stripped of steroids as detailed under the estrogen assay and various quantities of progesterone added to the stripped plasma. The "spiked" plasma was then extracted and assayed. The results are listed in table 4. There was generally good agreement between progesterone added and progesterone measured.

A parallelism test was also conducted for progesterone using the procedure outlined in the estrogen analysis. The slope of the lines for the standard set and for various quantities of unknown plasma was comparable.

TABLE 4. ABILITY OF ASSAY TO MEASURE PROGESTERONE ADDED TO FLORSIL STRIPPED PLASMA

No. samples	Progesterone added ng/ml	Progesterone measured ng/ml	Corrected progesterone ng/ml	SE
5	0	.0400	.0400	.003
5	.05	.0864	.0464	.005
5	.10	.1425	.1025	.021
5	.25	.2776	.2367	.019
5	.50	.5170	.4770	.036
5	.75	.7820	.7420	.089

Standard curves for both the estrogen and progesterone analyses were plotted by regressing percent bound on concentration. Percent bound was transformed by a logit transformation (logit Y = $\log_n$ Yxl/(1-Y)). Concentration was transformed by a $\log_{10}$ transformation. Statistical analyses of discrete variables were performed by the method of least squares. Continuous variables were analyzed by the method of multiple regression and/or least squares. Chi-square analysis was performed on dichotomous data.

RESULTS AND DISCUSSION

1973 Study

The 1973 study was designed to determine if PGF and/or flumethasone were effective agents for timing of parturition in the ewe at various levels and routes of administration.

Percent ewes lambing within 72 hours when given the various treatments is listed in table 5. Chi-square analysis indicated that there was a significant difference between treatments in the percentage of ewes lambing within 72 hours post-treatment ($P<.01$). The medium level of PGF given IM and the high level of flumethasone given either IM or intravenous (IV) resulted in 78% of the ewes lambing within 72 hours post-treatment. These results are in agreement with the findings of Adams and Wagner (1970) and Bosc (1972). They found that glucocorticoids would induce parturition in the ewe if given near term. PGF-treated ewes also had a shorter interval from treatment to lambing than saline control ewes which indicated that PGF was also effective for the induction of parturition in the ewe. Diehl et al. (1974) have shown PGF to be effective for induction of parturition in the cow. Lauderdale (1972) has shown that PGF will induce premature parturition in the cow. There have been no previous data published indicating that PGF will induce lambing.

Analysis of variance for hours from treatment to parturition (table 6) indicated a difference ($P<.01$) between treatment groups. Flumethasone treatment resulted in a shorter ($P<.01$) interval from treatment to lambing than saline control treatment (table 7). PGF

TABLE 5. PERCENT EWES LAMBING WITHIN 72 HOURS POST-TREATMENT AND THEIR AVERAGE INTERVAL FROM TREATMENT TO LAMBING - 1973 STUDY

Treatment	Number	Number induced	Percent induced	Mean hours	SE
Low saline IM	9	2	22	40.00	6.08
Low flumethasone IM	9	2	22	28.00	27.02
Low PGF IM	9	5	56	45.20	12.97
Low saline IV	9	1	11	48.00	0
Low flumethazone IV	9	4	44	31.33	10.52
Low PGF IV	9	0	0	--	--
Medium saline IM	9	4	44	29.80	11.86
Medium flumethasone IM	9	4	44	29.80	6.08
Medium PGF IM	9	7	78	32.79	8.22
Medium saline IV	9	2	22	41.00	15.03
Medium flumethasone IV	9	5	56	37.00	11.44
Medium PGF IV	9	2	22	23.50	18.53
High saline IM	9	1	11	22.00	0
High flumethasone IM	9	7	78	42.00	3.12
High PGF IM	9	5	56	25.80	8.45
High saline IV	9	0	0	--	--
High flumethasone IV	9	7	78	42.93	6.71
High PGF IV	9	3	33	36.00	16.54

TABLE 6. LEAST SQUARES ANALYSIS OF VARIANCE FOR HOURS FROM TREATMENT TO PARTURITION - 1973 STUDY

Source of variation	df	MS
Breed	1	10716.48
Type of birth	2	17326.79
Hormone treatment	2	69150.78**
Route of administration	1	1885.56
Level of hormone	2	3923.38
Breed x type of birth	1	10021.83
Breed x hormone	2	12092.68
Breed x route	1	8025.53
Breed x level	2	20524.76*
Type of birth x hormone	4	12103.64
Type of birth x route	2	26891.10*
Hormone x route	2	8162.72
Hormone x level	4	6692.07
Route x level	2	2916.56
Higher interactions	23	8925.02
Residual	104	5735.32
Total	160	

* $P < .05$.
** $P < .01$.

TABLE 7. LEAST SQUARES MEAN HOURS FROM TREATMENT TO PARTURITION - 1973 STUDY

Parameter	No. ewes	Mean hours
Breed		
Finn	71	116.53
Non-Finn	89	150.15
Type of birth		
Single	85	83.22
Twin	69	124.37
Triplet	6	192.44
Hormone treatment		
Saline (control)	54	243.08[a]
PGF	54	116.94[b]
Flumethasone	52	40.01[c]
Route of administration		
IM	81	120.41
IV	79	146.27
Level of hormone		
Low	53	154.20
Medium	53	86.62
High	54	159.21

[a,b,c] Means within parameters with different superscripts are significantly different at $P < .05$ (a,b) or $P < .01$ (a,c).

treatment also resulted in a shorter ($P<.05$) interval from treatment to parturition than the control treatment. Data in table 7 also indicate that breed of ewe, type of birth (single, twin or triplet), route of administration and level of hormone treatment did not significantly affect ($P>.05$) hours from treatment to parturition.

Table 6 also contains analysis of variance of the two-way interactions for breed of ewe, type of birth, hormone treatment, route of administration and level of hormone as they influence hours to lambing. There was a significant ($P<.05$) breed of ewe x level of hormone interaction as well as a type of birth x route of administration interaction ($P<.05$). No significant ($P>.05$) effects were found for pooled three- to five-way interactions.

A summary of the multiple regression analysis of hours from treatment to lambing is presented in table 8. Lamb birth weight accounted for 1.7% of the variation in hours from treatment to parturition ($P<.05$). Type of birth included with lamb birth weight accounted for 4.6% of the variation. The 2.9% increase in R^2 was significant ($P<.05$). The addition of ewe age and ewe weight to the multiple regression equation did not contribute significantly ($P>.05$) to an increase in R^2 and therefore could be ignored when selecting an induction treatment for the ewe.

Although statistically significant in their contribution to variation in hours from treatment to parturition, lamb birth weight and type of birth are of little value for the prediction of hours from time of treatment to time of lambing. They accounted for only 4.6% of

TABLE 8. MULTIPLE REGRESSION ANALYSIS OF HOURS FROM TREATMENT TO LAMBING AS INFLUENCED BY LAMB WEIGHT, TYPE OF BIRTH, EWE AGE AND PRE-LAMBING EWE WEIGHT - 1973 STUDY

Step	Intercept	Lamb weight	Type of birth	Ewe age	Ewe weight	R^2
1	79.7706	2.4457	--	--	--	.017*
2	85.7457	6.3164	-40.2385	--	--	.046*
3	90.2446	6.7777	-41.3464	-4.4785	--	.051
4	89.0171	6.7416	-41.2709	-4.5304	.0114	.051

* $P < .05$.

the variation and, in addition, lamb birth weight and type of birth would not be known at the time of treatment.

In the 1973 study only one ewe (control) exhibited a retained placenta. This agrees with the findings of Bosc (1971) and Adams and Wagner (1970) who found no evidence of retained placentas in ewes in which parturition was prematurely induced with glucocorticoids.

Lambing difficulty score was not affected by either flumethasone or PGF treatment when compared to the control ($P > .05$).

Although both PGF ($P < .05$) and flumethasone ($P < .01$) significantly reduced hours from treatment to parturition, the maximum percentage of ewes lambing within 72 hours post-treatment was 78%. This percentage is lower than the approximately 90% of ewes lambing within 72 hours post-treatment found in the studies of Adams and Wagner (1970), Bosc (1972) and Fylling (1971).

1974 Study

The results of the 1973 study indicated that both flumethasone and PGF were effective for the induction of parturition in the ewe if given on day 141 of gestation. It was felt that a higher percentage of treated ewes lambing within 72 hours post-treatment would be necessary if either treatment was to be of importance to producers. Other researchers' data indicated that glucocorticoids would effectively induce parturition within 72 hours in approximately 90% of treated ewes (Adams and Wagner, 1970; Bosc, 1972; Fylling, 1971). This author felt that sufficient flumethasone might not have been administered in the 1973 study to obtain comparable results. Therefore, 2.0 mg flumethasone was chosen as the treatment level of flumethasone for the 1974 study. This represented a .5 mg increase over the highest level administered in the 1973 study. The high level (15 mg) of PGF was also chosen over the medium level (10 mg). It was felt that the extra 5 mg of PGF would not be detrimental and would insure an adequate level for maximum induction success. The IM route of administration was more effective than the IV route for PGF treatment during the 1973 study. IV administration showed no advantage over IM for flumethasone treatment. IM administration was easier and required less skill than IV and was therefore chosen as the route of administration for all treatments for the 1974 study.

The results of the 1974 study followed the same general pattern as seen in the 1973 study, although the absolute values differed. Eighty-nine percent of the flumethasone- and 33% of the PGF-treated ewes lambed within 72 hours of treatment. This compares to only 8% for the saline control ewes (table 9). The percentage of both flumethasone- and PGF-treated ewes lambing within 72 hours was significantly ($P<.01$) greater than the percentage of saline control ewes lambing within the same time period. The mean interval from treatment to lambing for those ewes lambing during the 72-hour period following treatment was 50.0 $\pm$ 2.3 and 41.7 $\pm$ 5.9 hours for flumethasone- and PGF-treated ewes, respectively. Dr. James Lauderdale (personal communication) indicated that the same level (15 mg) of PGF administered in split dosage 1 day apart might result in a higher percentage of PGF-treated ewes lambing within 72 hours post-treatment.

TABLE 9. PERCENT EWES LAMBING WITHIN 72 HOURS POST-TREATMENT AND THEIR AVERAGE INTERVAL FROM TREATMENT TO LAMBING - 1974 STUDY

Treatment	Number	Lambed within 72 hours		Mean hours	SE
		%	No.		
Flumethasone	39	89[a]	35[a]	50.9	2.3
PGF	39	33[a]	13[a]	41.7	5.9
Saline (control)	39	8	3	38.5	10.0

[a] Data within columns with superscripts differ significantly from those without superscripts, $P<.01$.

Analysis of variance for all ewes in each treatment and control group indicated a significant ($P < .01$) difference between treatments (table 10). Least squares means listed in table 11 indicate that flumethasone treatment resulted in a shorter ($P < .01$) mean interval from treatment to lambing than for control ewes. PGF treatment also resulted in a shorter ($P < .05$) average time from treatment to parturition than for control ewes. Hours from time of treatment to time of lambing was not affected ($P > .05$) by the breed of ewe, sex of lambs or type of birth (single, twin or triplet). No significant ($P > .05$) effects were found for pooled three- to five-way interactions. Lambing difficulty score was not affected by treatment ($P > .05$).

Multiple regression analysis of various factors that influenced hours from treatment to lambing is presented in table 12. Initial progestin level, lamb birth weight, sex of lambs and initial estrogen level accounted for 10.6, 7.0, 3.6 and 2.2%, respectively, of the variation in hours from treatment to parturition. These parameters were all significant ($P < .05$). The addition of age of ewe, weight of ewe and type of birth to the multiple regression equation did not significantly affect R^2 ($P > .05$). The variables which made a significant contribution to variation in hours to lambing are ones that a producer would not know at the time of treatment and therefore are of little practical importance when selecting an induction treatment in the ewe.

TABLE 10. LEAST SQUARES ANALYSIS OF VARIANCE FOR HOURS FROM TREATMENT TO PARTURITION - 1974 STUDY[a]

Source of variation	df	MS
Treatment	2	45336.14**
Breed of ewe	4	3531.37
Sex of lambs	2	9125.38
Type of birth	1	32.50
Treatment x breed	8	5247.10
Treatment x sex	4	1271.13
Treatment x type	2	9903.90
Breed x sex	7	4285.30
Breed x type	4	721.78
Sex x type	1	1589.23
Higher interactions	19	7671.48
Residual	55	4184.74
Total	110	

[a] Ewes giving birth to triplets not included in the analysis.

** $P < .01$.

TABLE 11. LEAST SQUARES MEAN HOURS FROM TREATMENT TO PARTURITION - 1974 STUDY[a]

Parameter	No. ewes	Mean hours
Treatment		
Saline (control)	39	152.43[b]
Flumethasone	37	67.72[c]
PGF	34	115.86[d]
Breed of ewe		
Purebred	21	136.97
Suffolk	17	122.98
Targhee	16	97.47
Suffolk x Targhee	19	95.08
Finncross	37	107.53
Sex of lambs		
All female	37	94.68
One-half male	27	109.76
All male	46	131.58
Type of birth		
Singles	51	113.10
Twins	59	110.91

[a] Ewes giving birth to triplets not included in the analysis.

[b,c,d] Means within parameters with different superscripts are significantly different at $P < .05$ (b,d) or $P < .01$ (b,c).

TABLE 12. MULTIPLE REGRESSION ANALYSIS OF HOURS FROM TREATMENT TO LAMBING AS INFLUENCED BY INITIAL PROGESTIN LEVEL, LAMB BIRTH WEIGHT, SEX OF LAMBS, INITIAL ESTROGEN LEVEL, AGE OF EWE, WEIGHT OF EWE AND TYPE OF BIRTH

Step	Intercept	Progestins ng/ml	Lamb weight	Lamb sex	Estrogens pg/ml	Age of ewe	Ewe weight	Type of birth	R^2
1	74.9006	5.7519	--	--	--	--	--	--	.106*
2	-14.1220	6.0444	8.9796	--	--	--	--	--	.176*
3	-37.6556	6.0657	8.5035	8.6502	--	--	--	--	.212*
4	-12.2672	5.9045	8.4557	9.1422	-.1668	--	--	--	.244*
5	-21.6191	5.6269	7.7150	8.8789	-.1577	9.0429	--	--	.272
6	-44.0797	5.7895	6.8232	9.1428	-.1545	6.3637	.2277	--	.277
7	-17.8809	6.1121	4.3653	9.7916	-.1578	7.0671	.3282	-13.8996	.284

* $P<.05$.

Hormonal Data

There were no major differences ($P>.05$) indicated by analyses of variance for initial mean progestin levels between treatment groups, breed of ewe, type of birth or sex of lambs (table 13). There was a significant difference ($P<.05$) in initial estrogen levels for type of birth (singles *vs.* multiples) but no difference ($P>.05$) between treatment groups, breed of ewe or sex of lambs. Data in table 14 indicate that the difference in initial estrogen level for type of birth was due to ewes giving birth to singles having a higher ($P<.05$) initial estrogen level than those giving birth to multiples.

Bassett and Thorburn (1973) demonstrated in their study that mean progesterone concentration in ewes bearing twins was significantly higher than that for those bearing singles. The results of this study do not agree with their findings. Mean progestin levels between single bearing *vs.* multiple bearing ewes were not significantly different ($P>.05$). Although not statistically different, single bearing ewes had a higher pre-treatment progestin level than those bearing multiple fetuses (9.17 *vs.* 6.53 ng/ml, table 14).

The initial mean estrogen levels of 137.83 to 167.83 pg/ml for treatment groups are higher than the levels reported by Bassett and Thorburn (1973) and Liggins *et al.* (1972). They stated that the estrogen level was below 50 pg/ml until 24 hours pre-lambing. However, the levels reported in this study are in close agreement with the findings of Rawlings and Ward (1973). They reported estrogen levels in excess of 100 pg/ml 1 week prior to parturition. The present study

TABLE 13. LEAST SQUARES ANALYSIS OF VARIANCE FOR INITIAL AND FINAL CONCENTRATIONS OF TOTAL ESTROGENS AND PROGESTINS[a]

Source	df	Initial progestin MS	df	Initial estrogen MS	df	Final progestin MS	df	Final estrogen MS
Type of birth	1	39.65	1	27650.72*	1	.64	1	15533.00
Sex of lambs	2	5.57	2	7468.89	2	11.39	2	4892.96
Treatment	2	9.77	2	5323.38	2	8.71	2	24496.03
Breed of ewe	4	18.73	4	1461.32	4	1.51	4	18541.53
Type of birth x treatment	2	2.26	2	7214.31	2	.20	2	15488.18
Type of birth x breed	4	12.71	4	268.37	4	1.44	4	16549.55
Sex of lambs x treatment	4	13.75	4	4418.27	4	1.18	4	20956.42
Sex of lambs x breed	7	14.28	7	5645.26	7	1.12	7	19244.28
Treatment x breed	8	6.42	8	5226.33	8	4.44	8	23946.75
Higher interactions	16	11.47	15	6374.50	16	2.63	15	9249.87
Residual	43	25.85	47	6609.68	43	4.83	41	21467.49
Total	94		97		94		91	

[a] Ewes giving birth to triplets not included in the analysis.
* $P < .05$.

TABLE 14. LEAST SQUARES MEANS FOR INITIAL AND FINAL CONCENTRATIONS OF TOTAL ESTROGENS AND PROGESTINS[a]

Parameter	No. observations	Initial progestins ng/ml	No. observations	Initial estrogen pg/ml	No. observations	Final progestin ng/ml	No. observations	Final estrogen pg/ml
Type of birth								
Single	44	9.17	45	185.06[b]	42	3.25	39	216.83
Multiple	50	6.53	52	115.37[c]	52	3.56	52	269.21
Sex of lambs								
All female	33	7.51	29	127.12	30	3.10	28	231.55
50% female	21	8.73	25	176.44	23	4.60	23	234.86
All male	40	7.32	43	147.11	41	2.54	40	262.66
Treatment								
Saline (control)	37	7.86	34	145.01	35	2.97	32	250.34
Flumethasone	29	7.08	32	167.83	32	3.11	32	279.22
PGF	28	8.63	31	137.83	27	4.16	27	244.49
Breed of ewe								
Purebred	17	8.12	18	147.27	19	3.24	17	217.09
Finncross	34	7.90	34	168.94	32	3.00	31	258.00
Suffolk	15	7.18	17	151.06	15	3.97	16	280.40
Targhee	15	10.61	14	135.48	13	3.69	13	144.42
Targhee x Suffolk	13	5.46	14	148.39	15	3.16	14	315.17

[a] Ewes giving birth to triplets not included in the analysis.

[b,c] Means in columns within parameters with different superscripts differ significantly, $P < .05$.

also agrees with the findings of Thompson (1973) who reported estrone levels in excess of 100 pg/ml during the week prior to lambing.

Data in tables 13 and 14 indicate that mean progestin levels as well as mean estrogen levels of the final blood samples taken prior to lambing were not significantly different ($P > .05$) between treatments, type of birth, sex ratio of lambs carried or breed of ewe. Final progestin levels of all treatments averaged approximately 3 ng/ml. These findings are in agreement with those of Fylling (1971), Liggins et al. (1972), Thompson (1973) and Bassett and Thorburn (1973). They found that peripheral plasma progesterone levels were approximately 2 to 3 ng/ml just prior to parturition in the ewe.

Figure 1 is a graphic illustration of the mean estrogen level of flumethasone-treated ewes as compared to control ewes. Zero hours post-treatment represents the mean estrogen level of the ewes just prior to treatment. Control pre-treatment estrogen levels averaged approximately 135 pg/ml, while the level for flumethasone-treated ewes that did not lamb within 72 hours post-treatment (noninduced) was approximately 150 pg/ml. Flumethasone-treated ewes which lambed within 72 hours following treatment (induced) had a mean estrogen level of approximately 175 pg/ml. Examination of mean estrogen levels at 24 to 72 hours post-treatment for the three groups (control, induced and noninduced) reveals that induced ewes had a much higher estrogen level at the 48-hour post-treatment collection than did noninduced or control ewes. The 48-hour post-treatment collection corresponds to the 24-hour pre-lambing collection in the case of induced ewes and is therefore

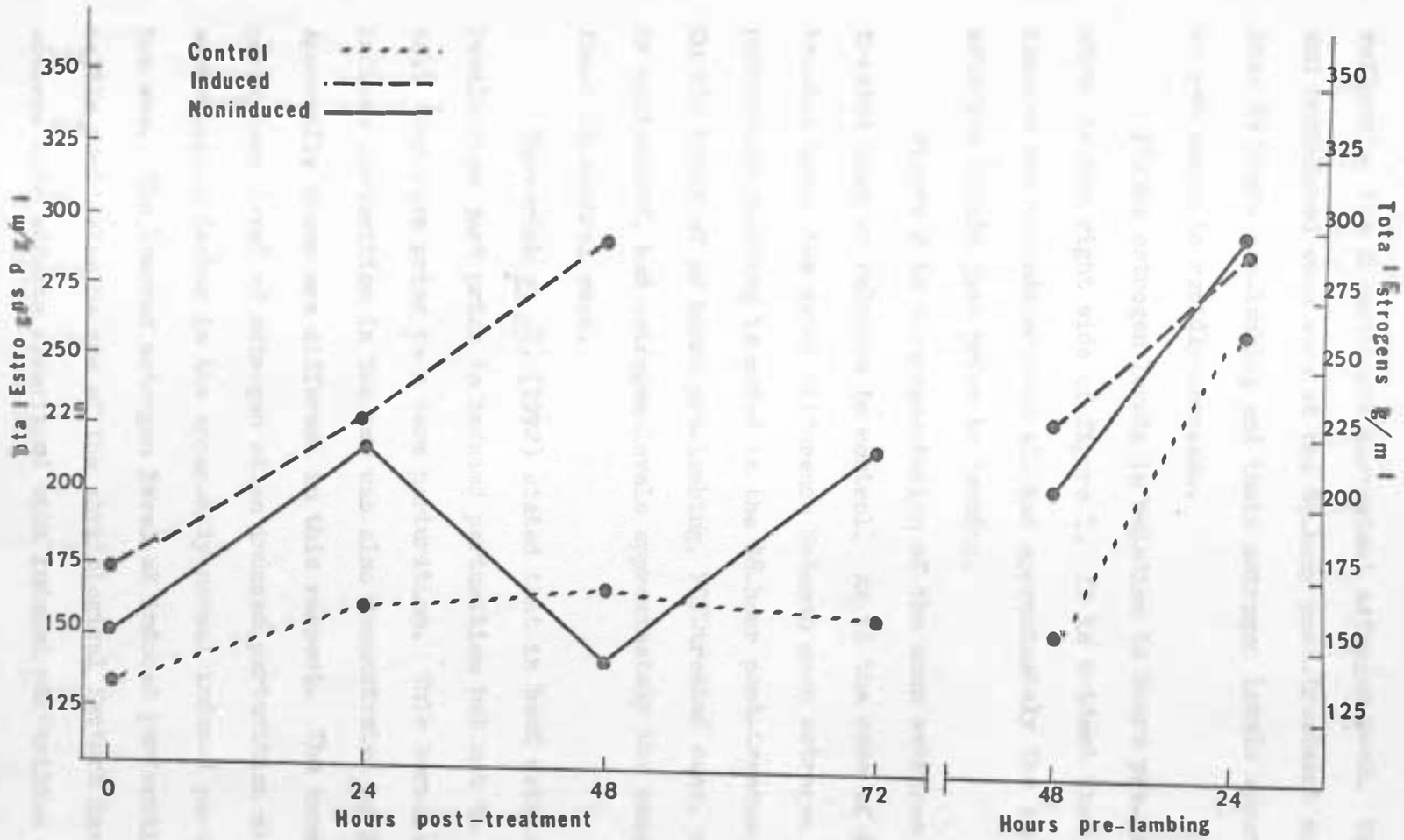

Figure 1. Mean estrogen level of flumethasone-treated ewes compared to mean level of control ewes.

reflecting the dramatic pre-parturient estrogen peak. The control ewes and noninduced ewes were at the 48-hour post-treatment collection more than 24 hours pre-lambing and their estrogen levels apparently had not as yet begun to rapidly increase.

Plasma estrogen levels in relation to hours pre-parturition are shown in the right side of figure 1. It is evident that control, induced and noninduced ewes all had approximately the same mean estrogen levels just prior to lambing.

Figure 2 is a representation of the mean estrogen level of PGF-treated ewes in relation to control. As in the case of flumethasone-treated ewes, the major difference between mean estrogen levels for a particular sampling is noted in the 48-hour post-treatment levels. On the basis of 24 hours pre-lambing, PGF-treated ewes, whether induced or noninduced, had estrogen levels approximately the same as those found in control ewes.

Garverick et al. (1972) stated that in beef cattle estrogen levels rise just prior to induced parturition but not to the extent that they rise prior to a term parturition. This hormonal pattern at induced parturition in the cow was also demonstrated by Evans (1973). Apparently sheep are different in this respect. The normal pre-parturient level of estrogen at an induced parturition might be a contributing factor in the apparently normal induced parturition in the ewe. The lowered estrogen level at induced parturition in beef cattle could also be one of the physiological factors involved in adverse side effects associated with induced parturition that have been

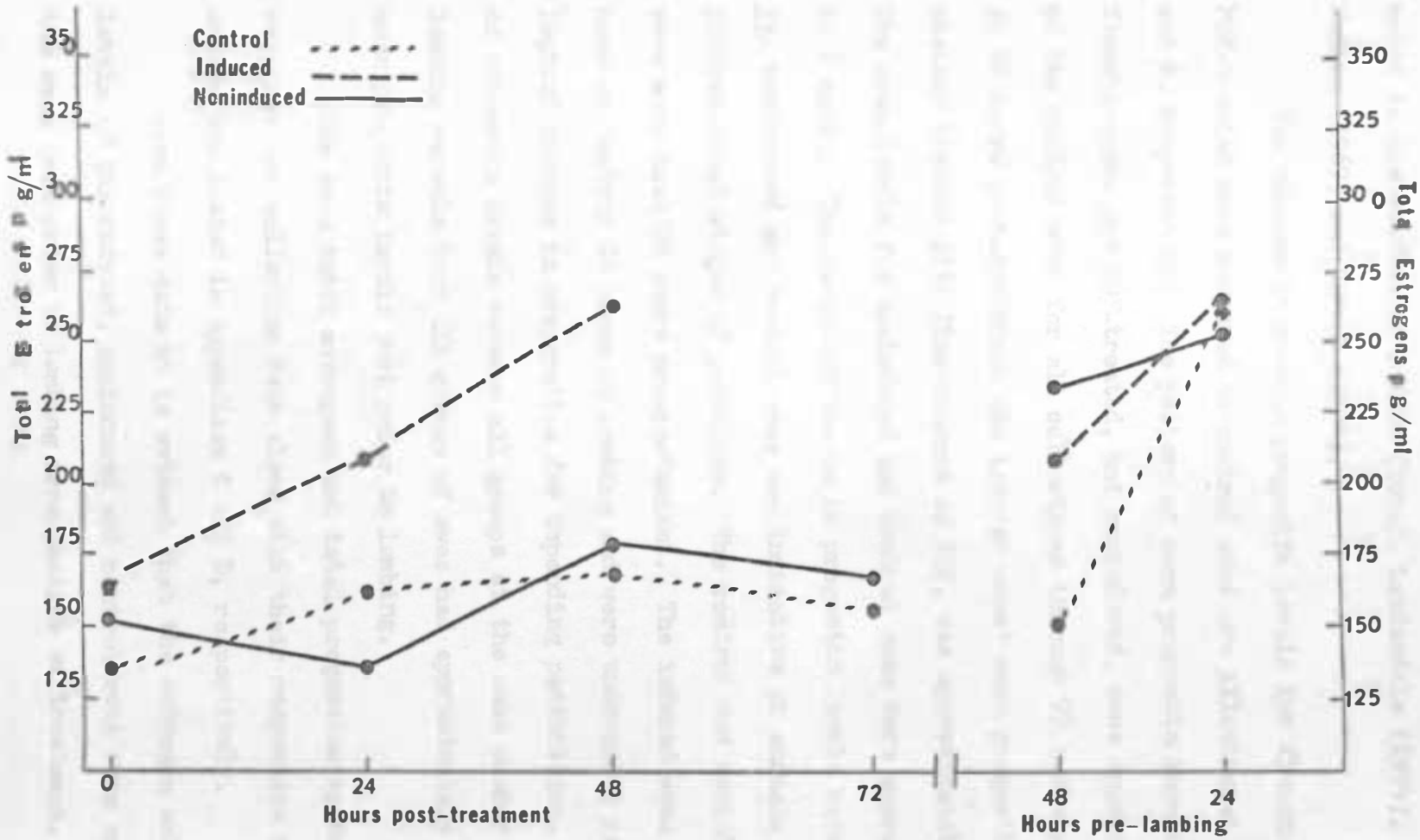

Figure 2. Mean estrogen level of PGF-treated ewes compared to mean level of control ewes.

noted in the studies of Jochle (1971), Lauderdale (1974), Adams and Wagner (1969) and Carrol (1974).

The changes in average progestin levels for flumethasone- and PGF-treated ewes compared to control ewes are illustrated in figures 3 and 4, respectively. The pattern of mean progestin levels of both flumethasone- and PGF-treated, but noninduced, ewes approximated that of the control ewes for all collections through 72 hours post-treatment. At 48 hours post-treatment the induced ewes' mean progestin level, whether treated with flumethasone or PGF, was approximately 3 ng/ml. The mean levels for noninduced and control ewes were approximately 7 to 8 ng/ml. The large difference in progestin levels between induced vs. noninduced and control ewes was indicative of animals at different physiological stages of gestation. The control and noninduced ewes were more than 24 hours pre-parturient. The induced ewes were, however, within 24 hours of lambing and were undergoing rapid physiological changes in preparation for impending parturition. A comparison of progestin levels between all groups at the same number of hours pre-lambing reveals that all groups of ewes had approximately the same mean progestin levels just prior to lambing.

The mean total estrogens and total progestins by treatment, response and collection date along with their respective standard errors are listed in appendixes C and D, respectively.

From these data it is evident that the estrogen and progestin levels of the induced, noninduced and control ewes were approximately the same just prior to lambing irrespective of treatment. The major

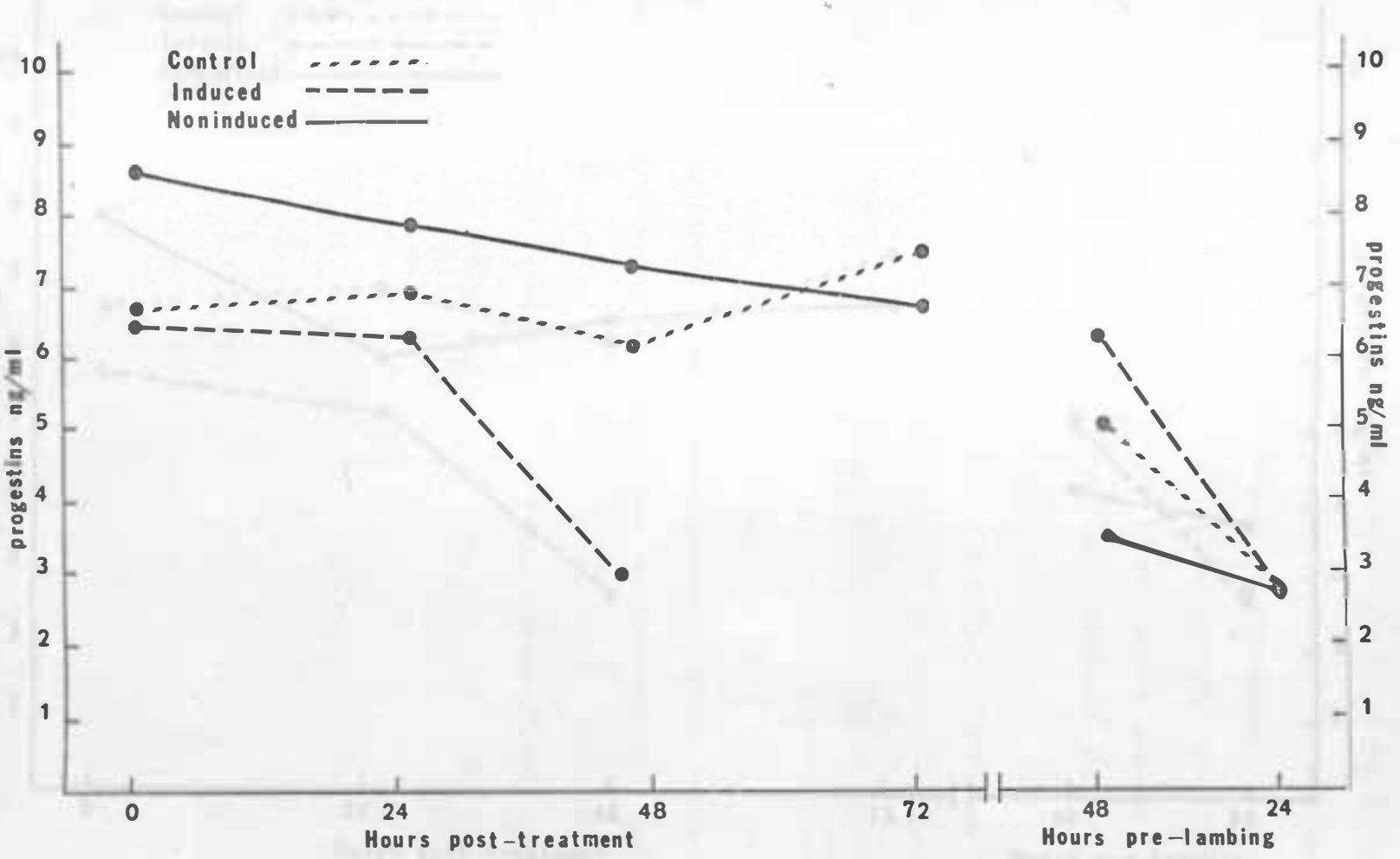

Figure 3. Mean progestin level of flumethasone-treated ewes compared to mean level of control ewes.

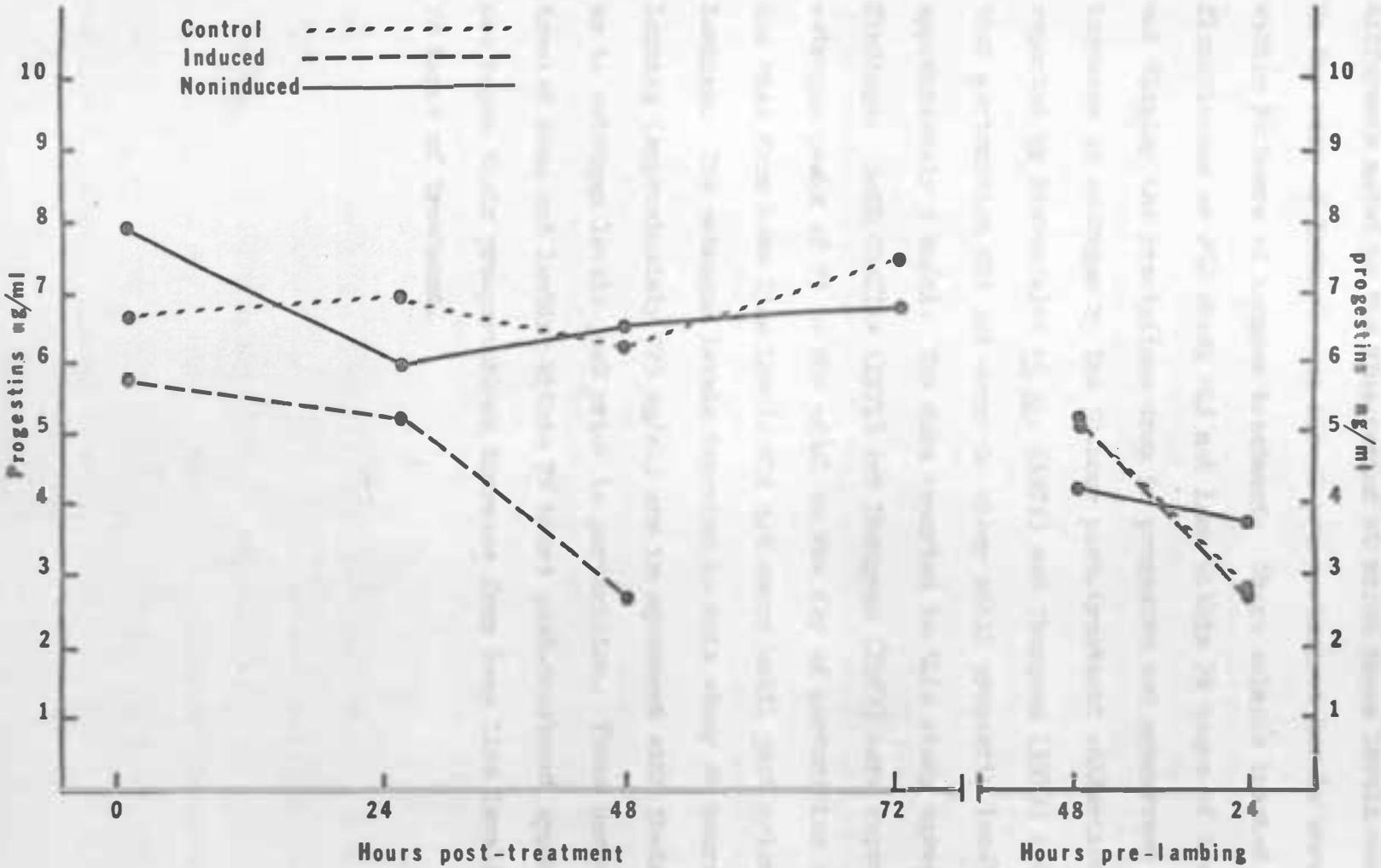

Figure 4. Mean progestin level of PGF-treated ewes compared to mean level of control ewes.

difference noted is the time period at which these levels were attained. In the case of induced ewes normal pre-parturient levels were obtained within 72 hours of hormone treatment. Those animals treated with either flumethasone or PGF which did not lamb within 72 hours of treatment did not display the precipitous drop in progestin and concurrent sharp increase in estrogen by the 72-hour post-treatment collection. Data reported by Stabenfeldt et al. (1972) and Thompson (1973) indicated that parturition did not occur in sheep until progestin levels were approximately 3 ng/ml. The data reported in this study agree with their findings. Both Challis (1971) and Thompson (1973) have reported estrogen peaks of 75 to 800 pg/ml on the day of parturition and that the rise from base line levels did not occur until just prior to lambing. The estrogen levels reported in this study 24 hours pre-lambing (approximately 275 ng/ml) are in agreement with their findings as to estrogen levels just prior to parturition. Those hormonally treated ewes not lambing within 72 hours post-treatment apparently had not begun their pre-parturient increase from base line levels within 72 hours of treatment.

SUMMARY AND CONCLUSIONS

The effectiveness of flumethasone and PGF for the timing of parturition was studied in 167 and 117 purebred and crossbred ewes during the 1973 and 1974 lambing seasons, respectively.

The treatments used in the 1973 study were three levels of flumethasone (.5, 1.0, 1.5 mg), three levels of PGF (5, 10, 15 mg) and a control group receiving 1, 2 or 3 cc of physiological saline. Each treatment and control dosage level were administered both IM and IV.

In the 1973 study 78% of the ewes receiving the high level of flumethasone by either the IM or IV route of administration lambed within 72 hours post-treatment. A similar percentage (78) of the ewes receiving PGF by the IM route lambed in the 72-hour period following treatment.

All ewes considered, mean hours from treatment to parturition were shorter for flumethasone-treated ewes ($P<.01$) and PGF-treated ewes ($P<.05$) than for saline control ewes.

Lamb birth weight and type of birth (single, twin or triplet) made significant contributions ($P<.05$) to variations in hours from treatment to lambing. However, they accounted for only 4.6% of the variation and therefore are of little practical importance when selecting an induction treatment in the ewe.

Only one ewe (control) exhibited a retained placenta. Lambing difficulty score was not affected ($P>.05$) by either flumethasone or PGF treatment when compared to the control.

Treatments used for the 1974 study were 2 mg flumethasone and 15 mg PGF given IM. Eighty-nine percent of the flumethasone-treated ewes lambed within 72 hours of treatment with an average interval from treatment to lambing of 50.9 ± 2.3 hours. Thirty-three percent of PGF-treated ewes lambed within 72 hours post-treatment with a mean interval of 41.7 ± 5.9 hours. The percentage of PGF- and flumethasone-treated ewes lambing within 72 hours post-treatment was higher ($P<.01$) than the 8% for the saline control ewes which lambed within a similar period.

Total ewes included, flumethasone-treated ewes had a shorter ($P<.01$) interval of hours from treatment to parturition than the saline control ewes. PGF treatment also resulted in a shorter ($P<.05$) interval from the time of treatment to lambing than the control treatment.

Initial progestin and estrogen levels of the treatment groups were not significantly different ($P>.05$) nor was there a significant difference ($P>.05$) in final progestin levels between treatment groups. The major difference in levels of estrogen and progestin was noted 48 hours post-treatment. At this collection period ewes lambing within 72 hours after treatment had much higher estrogen and much lower progestin levels than those not lambing within 72 hours of treatment.

Initial progestin level, lamb birth weight, sex of lambs and initial estrogen level accounted for 10.6, 7.0, 3.6 and 2.2% of the variation in hours from treatment to parturition. Lambing difficulty score was not affected by treatment ($P>.05$).

It is concluded that both flumethasone and PGF treatment will result in the induction of parturition in the ewe if given on day 141 of gestation. However, the percentage of ewes lambing within 72 hours of treatment was only 33% for PGF treatment. This percentage of ewes lambing within 72 hours of treatment indicates that PGF given at 35 mg per ewe in a single IM injection would not be of benefit to the producer as a method of timing parturition in a breeding flock.

Flumethasone treatment consisting of 2 mg per ewe given in a single IM injection was highly effective for the induction of parturition. Eighty-nine percent of the ewes treated with flumethasone lambed within 72 hours post-treatment. Therefore, it could be possible for parturition to be effectively timed in the ewe with flumethasone treatment on day 141 of gestation. Field trials need to be conducted to determine if the degree of success reported in this study can be obtained under conditions which prevail in a commercial operation.

Some of the questions that remain to be answered concerning induced and/or timed parturition in the ewe are:

1. Why is there a difference in response between ewes treated with the same hormone at the same stage of gestation?
2. Will once a week flumethasone treatment of ewes in the gestational range of 138 to 145 days result in effective induction and/or timing of parturition?
3. Will split dosages of PGF result in more effective induction and/or timing of parturition?

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APPENDIX A

Estrogen Assay Procedure

I. Extraction of blood plasma samples

1. Label 25 x 200 mm test tubes in duplicate.
2. Place 200 µl plasma in each tube and 200 µl glass distilled water in a duplicate set.
3. Add 3 ml anhydrous ether to each tube.
4. Place on metabolic shaker at a 45 degree angle and shake for 3 minutes.
5. Freeze aqueous phase in a dry ice-alcohol bath.
6. Decant ether phase into 15 x 85 mm test tube and transfer label.
7. Dry down in water bath at 45 C under a stream of nitrogen gas.

II. To test recovery

1. Place approximately 2,000 cpm ^{3}H-E-17β in extraction tube (duplicate; these are extracted recoveries).
2. Place same amount ^{3}H-E-17β into scintillation vial (duplicates; these are total recoveries).
3. Dry extraction tubes and scintillation vials in a water bath at 45 C under a stream of nitrogen gas.
4. Add 200 µl plasma to extraction tubes and shake on vortex mixer for 20 seconds.
5. Incubate extraction tubes in a water bath at 45 C for 5 minutes.
6. Incubate extraction tubes in ice bath for 10 minutes.
7. Carry extraction tubes through extraction procedure as outlined in I above starting at step 3 through step 5.
8. Decant ether phase into scintillation vial and dry down at 45 C under stream of nitrogen gas.
9. Add 10 ml scintillation cocktail to all scintillation vials.

III. Preparation of standards

1. Dilute unlabeled E-17β in ETOH such that 50 µl contains 0, 12.5, 25, 50, 100 and 200 ng E-17β.
2. Add to duplicate 15 x 85 mm test tubes 0, 12.5, 25, 50, 100 and 200 ng E-17β.
3. Label one duplicate set of tubes 'total counts' and carry through assay.
4. Dry down at 45 C under stream of nitrogen gas.

IV. Radioimmunoassay procedure

1. To all standard, water blank and unknown 15 x 85 mm tubes add 100 µl E-17β antibody which has been diluted to 1:60,000 in PBS.
2. To each of the above add 100 µl ^{3}H-E-17β (approximately 7,500 cpm).
3. Shake on vortex mixer for 20 seconds.
4. Incubate at 5 C for at least 2 hours.
5. Place all tubes in an ice bath and add 1 ml of dextran coated charcoal to each tube except total counts.
6. Add 1 ml PBS to total count tubes.
7. Shake all tubes on vortex mixer for 20 seconds.
8. Centrifuge at approximately 1,800 X G for 15 minutes at 5 C.
9. Aliquot 1 ml of supernatant into scintillation vials.
10. Add 10 ml scintillation fluid to each vial.
11. Shake on vortex mixer for approximately 3 seconds.
12. Count all standards, water blanks, recoveries and unknowns on a scintillation counter.

V. Each run consists of

1. A duplicate set of standards.
2. A duplicate set of total counts.
3. A duplicate set of water blanks.
4. A set of 25 unknowns in duplicate.
5. A duplicate set of total recoveries.
6. A duplicate set of extracted recoveries.

APPENDIX B

Progesterone Assay Procedure

I. Extraction of plasma samples

1. Label 15 x 85 mm test tubes in duplicate.
2. Place 100 µl plasma in each tube and 100 µl water in a duplicate set.
3. Add 3 ml benzene:hexane to each tube.
4. Shake on vortex mixer at high speed for 30 seconds.
5. Place all tubes in freezer at -20 C for approximately 18 hours.
6. Decant benzene:hexane phase into 12 x 75 mm test tube and transfer label.
7. Dry down in water bath at 45 C under a stream of nitrogen gas.

II. To test recovery

1. Place approximately 3,000 cpm ^{3}H-progesterone in 15 x 85 mm test tube (duplicates).
2. Place same amount ^{3}H-progesterone into scintillation vial (duplicates).
3. Dry extraction tubes and scintillation vials in a water bath at 45 C under a stream of nitrogen gas.
4. Add 100 µl plasma to extraction tubes and shake on vortex for 20 seconds.
5. Incubate extraction tubes in a water bath at 45 C for 5 minutes.
6. Incubate extraction tubes in ice bath for 10 minutes.
7. Carry extraction tubes through extraction procedure as outlined in I above starting at step 3 through step 5.
8. Decant benzene:hexane phase into scintillation vial and dry down at 45 C under a stream of nitrogen gas.
9. Add 10 ml scintillation cocktail to all scintillation vials.

III. Preparation of standards

1. Dilute unlabeled progesterone in ETOH such that 50 µl contains 0, .025, .05, .1, .25, .50, .75, 1.0 and 1.5 pg of progesterone.
2. Add to duplicate 12 x 75 mm test tubes progesterone in the above concentrations.
3. Label one duplicate set 'total counts' and carry through assay.
4. Dry down at 45 C under a stream of nitrogen gas.

IV. Radioimmunoassay procedure

1. To all standard, water blank and unknown 12 x 75 mm tubes add 100 µl progesterone antibody which has been diluted to a concentration of 1:4,500 in PBS.
2. To each of the above add 100 µl ^{3}H-progesterone (approximately 10,000 cpm).
3. Shake on vortex for 15 seconds.
4. Incubate at 5 C for at least 2 hours.
5. Add .2 ml PBS to all tubes.
6. Place all tubes in an ice bath and add 1 ml dextran coated charcoal to each tube except total counts.
7. Add 1 ml PBS to total count tubes.
8. Incubate in ice bath for 15 minutes.
9. Shake on vortex for 15 seconds.
10. Centrifuge at approximately 1,800 X G for 15 minutes at 5 C.
11. Aliquot .7 ml of supernatant into scintillation vial.
12. Add 10 ml scintillation fluid to each vial.
13. Shake on vortex for approximately 3 seconds.
14. Count all standards, water blanks, recoveries and unknowns on scintillation counter.

V. Each run consists of

1. A duplicate set of total counts.
2. A duplicate set of standards.
3. A duplicate set of water blanks.
4. A set of 25 unknowns in duplicate.
5. A duplicate set of total recoveries.
6. A duplicate set of extracted recoveries.

APPENDIX C

Mean Total Estrogen by Treatment, Response and Collection Date Beginning on Day 141 of Gestation

Treatment	Lambed within 72 hours	Collection[a]	Mean estrogen pg/ml	SE	Number observations
Saline	Yes	1	206.15	46.79	3
	Yes	2,5[b]	102.86	9.62	2
	Yes	3,6[c]	352.56	28.70	2
Saline	No	1	132.27	10.42	29
	No	2	156.57	15.90	27
	No	3	172.40	23.86	23
	No	4	157.64	12.15	23
	No	5	158.36	17.41	23
	No	6	265.79	32.07	30
Flumethasone	Yes	1	177.60	20.02	31
	Yes	2,5[b]	221.58	19.12	18
	Yes	3,6[c]	288.29	18.89	29
Flumethasone	No	1	153.60	22.69	6
	No	2	213.58	49.88	6
	No	3	139.94	29.06	5
	No	4	224.46	79.46	4
	No	5	206.95	63.97	5
	No	6	321.05	44.80	5
PGF	Yes	1	161.36	24.46	9
	Yes	2,5[b]	204.27	40.50	4
	Yes	3,6[c]	260.70	34.22	10
PGF	No	1	144.57	14.38	26
	No	2	133.60	16.85	23
	No	3	178.86	21.56	21
	No	4	160.77	13.17	16
	No	5	230.34	35.63	21
	No	6	252.72	30.20	22

[a] 1 = before treatment, 2 = 24 hours post-treatment, 3 = 48 hours post-treatment, 4 = 72 hours post-treatment, 5 = 48 hours pre-lambing and 6 = 24 hours pre-lambing.

[b] Collection represents both 24 hours post-treatment and 48 hours pre-lambing.

[c] Collection represents both 48 hours post-treatment and 24 hours pre-lambing.

APPENDIX D

Mean Progestin by Treatment, Response and Collection Date Beginning on Day 141 of Gestation

Treatment	Lambed within 72 hours	Collection[a]	Mean progestin ng/ml	SE	Number observations
Saline	Yes	1	4.85	.89	3
	Yes	2,5[b]	10.14	3.34	2
	Yes	3,6[c]	4.34	1.51	2
Saline	No	1	6.73	.51	32
	No	2	7.00	.50	31
	No	3	6.26	.87	26
	No	4	7.53	1.45	13
	No	5	5.12	.48	29
	No	6	2.78	.28	31
Flumethasone	Yes	1	6.45	.57	30
	Yes	2,5[b]	6.39	.59	17
	Yes	3,6[c]	3.28	.40	32
Flumethasone	No	1	8.78	2.11	3
	No	2	6.01	1.56	4
	No	3	7.43	1.67	6
	No	4	6.80	3.00	3
	No	5	3.51	--	1
	No	6	2.85	.54	3
PGF	Yes	1	5.94	1.37	6
	Yes	2,5[b]	5.26	.82	5
	Yes	3,6[c]	2.64	.25	10
PGF	No	1	7.87	1.32	26
	No	2	5.89	.44	24
	No	3	6.43	.74	21
	No	4	6.66	.83	20
	No	5	4.13	.50	21
	No	6	3.80	.62	32

[a] 1 = before treatment, 2 = 24 hours post-treatment, 3 = 48 hours post-treatment, 4 = 72 hours post-treatment, 5 = 48 hours pre-lambing and 6 = 24 hours pre-lambing.

[b] Collection represents both 24 hours post-treatment and 48 hours pre-lambing.

[c] Collection represents both 48 hours post-treatment and 24 hours pre-lambing.