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Superovulation of Crossbred Targhee Ewes With Follicle Stimulating Hormone and Prostaglandin $F_2\alpha$: The Effect of Varying FSH Dosage.

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SUPEROVULATION OF CROSSBRED TARGHEE EWES WITH FOLLICLE STIMULATING HORMONE AND PROSTAGLANDIN $F_2\alpha$: THE EFFECT OF VARYING FSH DOSAGE

By

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ABSTRACT

SUPEROVULATION OF CROSSBRED TARGHEE EWES WITH FOLLICLE STIMULATING HORMONE AND PROSTAGLANDIN $F_2\alpha$: THE EFFECT OF VARYING FSH DOSAGE

By

Cynthia Lynne Smith

Six doses of Follicle Stimulating Hormone (FSH), combined with Prostaglandin F_2 (PGF_2) were evaluated for their effect on superovulation in 79 crossbred Targhee ewes. An effect of the timing of FSH treatment in midcycle of the estrous cycle was tested for four days using PGF_2 to induce estrus. Dependent variables evaluated were numbers of corpora lutea, follicles, embryos, degenerated embryos and unfertilized ova.

A significant effect of FSH dose was seen on corpora lutea (CL) response. A significant ($p<.05$) cubic regression curve for FSH dose effect for CL response and a significant ($p<.05$) linear regression curve for FSH dose effect for embryo response were produced. The dose of FSH had no effect on any other variables. The timing of FSH treatment in midcycle had no effect. An optimum dose range of 22 mg to 30 mg FSH was estimated from the analysis.

Dedicated to

Kit

whose steadfast devotion and unwavering cheerful presence were given freely in this and all my endeavors.

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GLOSSARY OF ABBREVIATIONS

AP	Anterior pituitary preparations
CL	Corpus luteum or Corpora lutea
Clo	Cloprostenol, a synthetic prostaglandin $F_2\alpha$
DOCI	Day of the cycle the FSH was initiated
DOSE	Dose of FSH in milligrams
FSH	Follicle stimulating hormone
FSHp	Follicle Stimulating Hormone-p, a commercial FSH made from pituitaries of porcine species
GnRH	Gonadotrophin releasing hormone
HAP	Horse anterior pituitary extract
hCG	Human chorionic gonadotropin
IU	International units
IV	Intravenous
LH	Luteinizing hormone
MG or mg	Milligrams
ml	Milliliters
PGF_2	Prostaglandin $F_2\alpha$
PLH	Purified luteinizing hormone extract from ovine pituitaries
PMSG	Pregnant mares serum gonadotropin
SAP	Sheep anterior pituitary extract
µgm	Micrograms

INTRODUCTION

Superovulation is the use of exogenous gonadotropins to increase the number of ovulations at a given estrus. The gonadotropins used are unpurified or semipurified preparations that contain follicle stimulating hormone (FSH) and luteinizing hormone (LH) or have FSH and LH activity. The mechanism by which these gonadotropins cause increased follicular growth, maturation of the oocytes within the follicles and ovulation is not understood. The superovulated animals are bred and their embryos are recovered by a uterine flush technique. These embryos can then be placed in a surrogate dam's uterus to be carried to term.

The limitations of superovulation treatments are many. The different gonadotropins have varying degrees and duration of FSH and LH activities resulting in varying effect between gonadotropins. Inconsistency of ovulation rates among individuals occurs with any of the gonadotropins. Degenerated embryos and unfertilized ova are observed when ewes are superovulated. Additionally, unovulated follicles and prematurely luteinized follicles are sometimes observed in high numbers on ovaries of superovulated ewes and this may contribute to a reduced number of fertile embryos collected.

Pregnant mares serum gonadotropin (PMSG) has been the most widely used gonadotropin to cause superovulation in the ovine. Along with PMSG, progesterone treatment has been used to synchronize the onset of estrus with peak follicular growth. Both of these products have

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been shown to cause fertilization failure in the ewe. Progesterone is thought to interfere with sperm transport through the cervix.[85] The mechanism by which PMSG depresses fertilization is not known. Degeneration of early embryos and lack of recovery from the uterine flush are thought to be due to alteration of the normal endocrine environment in the oviduct and uterine horn. Chromosomal abnormalities and altered substrate utilization of the early embryos have been observed in PMSG stimulated cattle.[70]

Follicle stimulating hormone has been widely used to superovulate cattle. This is because a lower incidence of embryonic loss is observed in cattle at superovulatory dose levels of FSH than to PMSG. This may be due to FSH's lower LH activity and short half life.[70] At present a dose regimen to cause superovulation with FSHp has not been developed for the ovine.

Along with FSH treatments, prostaglandin F_2 may be used to synchronize estrus in ewes. One research trial with PGF_2, in conjunction with superovulation with PMSG in ewes, did not show an altered fertilization rate.[121] Studies where PGF_2 has been used at the time of artificial insemination have shown increased fertility in ewes.[111] Controversy involving the fertilization rates following estrous synchronization with PGF_2 exists. Reports of normal[44,111] and subnormal fertility[37,47] can be found in the literature.

The purpose of this study was to evaluate the effect of FSHp and PGF_2 in superovulated ewes. Three objectives were proposed to evaluate this effect: first, to determine if FSHp and PGF_2 would superovulate ewes; second, to determine if the dosage of FSHp had an effect

on the superovulation response in ewes; and third, to determine if a dose of 22 mg FSH or above would maximize embryo response in superovulated ewes.

LITERATURE REVIEW

Reproductive Cycle of the Ewe

The normal reproductive cycle of the ewe will be reviewed to facilitate a discussion of exogenous hormones used to control reproduction and fecundity. It should be pointed out, however, that the interrelations of the endocrine, anatomical, and behavioral events that occur in the cycle of the ewe are complex and not completely understood. Sheep are seasonally polyestrous. The estrous season begins in the late summer to early fall, during which estrous cycles, 16-17 days in length, occur in succession until late winter. At this time, the anestrous season begins, characterized by reduction of both ovarian activity and behavioral estrus.[28,117] The transition from the estrous season to the anestrous season and vice versa is controlled by the change in the photoperiod. The progressive shortening of daylight hours initiates the onset of the estrous season in the fall. The opposite, lengthening daylight hours, initiates the onset of the anestrous season in the spring.[60]

The Estrous Cycle

The 16-day ovine estrous cycle is divided into the luteal and follicular phases. Throughout the luteal phase, 12-13 days in duration, progesterone is the predominant steroid secreted from the corpus luteum on the ovary. During the follicular phase, 3-4 days in duration, serum

progesterone levels drop rapidly and estrogen begins to rise. Large increases in secretion of LH and FSH follow the increased estrogen levels,[28,43,83] see Figure 1.

Regression of the corpus luteum (CL) is responsible for the sudden drop in serum progesterone.[80] Initiation of this regression is controlled by PGF_2, a luteolysin, secreted by the nongravid uterus beginning on day 12-14 and reaching maximum levels just prior to the onset of estrus.[6,11,41,60,94] There are two stages of luteal regression. The first stage is functional regression, during which progesterone production by luteal cells is significantly reduced, but no anatomical cell changes have occurred. The beginning of serum progesterone decline on day 12-14 corresponds with the functional regression. This regression is triggered by PGF_2 which is released by the uterus after 7-10 days of progesterone influence. It is independent of preovulatory estradiol concentration and is reversible. The second stage is structural regression, characterized by irreversible morphological change of the luteal cells which occurs by day 16 due to continued PGF_2 influence. Estradiol secretion and the decline of progesterone enhance PGF_2 secretion from the uterus during structural luteolysis.[6]

Estradiol begins to rise rapidly (14-27 pg/ml) at the time of luteolysis just prior to estrus. This rapid estradiol rise is responsible for the initiation of behavioral estrus. Additionally, when serum progesterone is at a low level (0.5 ng/ml), estradiol has a positive feedback effect on the hypothalamal-pituitary axis to

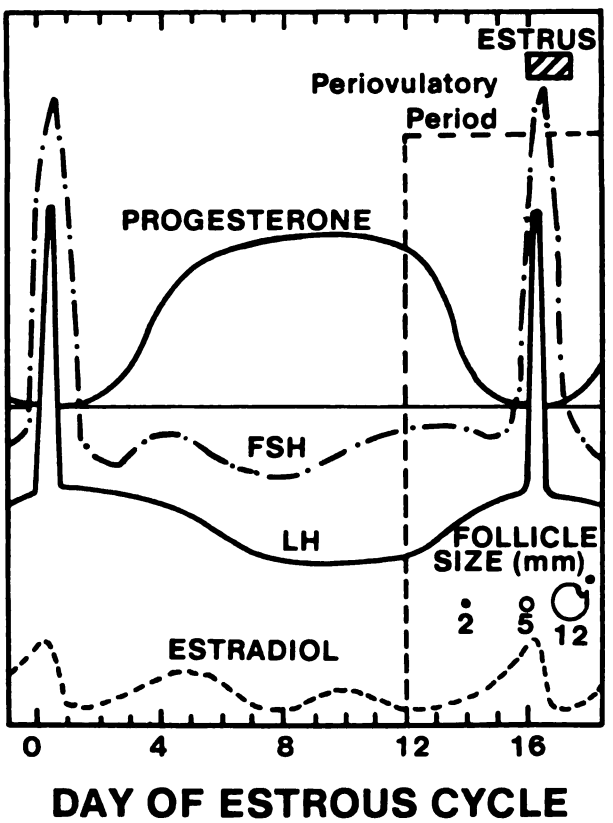

FIG. 1. Schematic representation of the 16-day estrous cycle of the ewe.

Adapted from S. J. LEGAN and F. J. KARSCH (1979)[60] and WARD (1980)[117]

increase frequency of tonic LH release (LH pulses).[6] The effect of estradiol is to increase the sensitivity of the pituitary to gonadotropin releasing hormone and increase the frequency of GnRH release from the hypothalamus.[54] The increasing pulses of LH over a 48-hour period result in a five-fold rise in LH above basal levels. The increasing LH has a positive feedback effect on the ovarian follicle(s) to increase estradiol secretion.[6] When the estradiol concentration reaches a threshold level for the hypothalamal-pituitary axis, surges of LH (up to 80 ng/ml)[28,83,117] and FSH (up to 170 ng/ml)[43] are released from the pituitary. These gonadotropins acting in concert and in the proper critical ratio trigger ovulation of the preovulatory follicle(s).[83] The LH and FSH surges occur 8-12 hours after the onset of behavioral estrus. Ovulation occurs 12-14 hours after the LH and FSH peaks or 24 hours after the onset of behavioral estrus.[117]

During the luteal phase of the cycle, LH remains at a low level (2-3 ng/ml)[6,28,83] due to negative feedback of progesterone on pituitary secretion.[59,60] A second FSH peak, occurring 24 hours after the first, has been reported by one group of researchers.[83] Other researchers have not measured this second FSH peak.[28] The relationship of this FSH rise to ovulation, if any, is not known. Some workers propose that this second FSH rise is the result of an increase in tonic secretion when inhibition by the preovulatory follicle(s) is removed.[44] Others speculate that the FSH rise initiates a second wave of follicular growth resulting in the increased serum estradiol seen at 3-4 days post estrus.[6,28,83,117] However, FSH is also elevated up to 80 ng/ml at day 3 and again at days 8-12 of the estrous cycle, then declines to

40 ng/ml until the next preovulatory surge. These two modest rises in FSH in the luteal phase are proposed to cause the two waves of follicular growth observed in sheep. These waves occur at days 3-4 and 6-9 of the cycle and correspond to undulating estradiol levels.[69,117] Other unknown factors may be responsible since the FSH rises do not directly precede the growth of follicle(s).[28,117] The decline of serum FSH just prior to estrus and ovulation may reflect an increase in FSH utilization by the follicle, rather than increased FSH secretion, promoting follicular growth. Other investigators suggest that the FSH surge may be more important in ovulation than in last-minute follicular growth.[83] After ovulation has occurred, granulosa cells under the influence of LH[106] proliferate, hypertrophy, and begin to secrete progesterone[66] and thus, the CL of the next estrous cycle is formed and progesterone rises from 0.5 ng/ml to 4 - 5 ng/ml by the third day post estrus.[83] This level of progesterone is maintained to day 12-13 when luteolysis begins and the estrous cycle repeats.[28,60,83]

Follicular Development and Ovulation

Superovulation involves the use of exogenous hormones to influence the complex hormonal environment that controls growth of follicles, oocyte maturation or resumption of meiosis, and ovulation. Undesirable effects have been observed as evidenced by increased chromosomal abnormalities in superovulated rabbits,[39] cattle,[68] and an increase in abnormal embryos from repeat breeder heifers.[61] Some of the aspects of follicular growth and development that apply to superovulation will be reviewed.

In sheep and cattle, waves of follicular growth have been documented throughout the estrous cycle. During a wave of follicular growth, several preantral follicles grow to large antral follicles and then undergo atresia.[65,101] If an antral follicle(s) is at a critical size at the time of the regression of the CL and the fall in progesterone, then its secretion of estradiol will have a positive feedback effect on the hypothalamus.[7,8,101] Gonadotropin releasing hormone (GnRH) will be released causing the increase in tonic LH release from the pituitary.[77] The tonic LH acts on the antral follicle(s) to promote growth and estradiol secretion which proceeds until an LH surge is achieved.[7] Shortly after the LH surge, the oocyte is observed to undergo resumption of meiosis (maturation) and ovulation follows.[70] These two events are reviewed by Hansel and Convey[54] and are believed to be under LH control which affect the pre-ovulatory size follicle and have no effect on small antral follicles. At this time the increased estradiol output promotes follicle vascularization and may thereby increase gonadotropin uptake into the follicle in the bovine. Additional observations indicate that the most active follicle stimulates its own growth while simultaneously inhibiting growth and differentiation of other follicles.[65]

Two waves of follicle growth have been observed,[7,101] corresponding with moderate increases in estradiol[69] during the luteal phase of the sheep's estrous cycle,[7,101] see Figure 1. It has been proposed for the bovine, that endogenous FSH is sufficient to stimulate follicle growth but is unable to sustain the large follicle resulting in atresia and growth of other small follicles.[55] The moderate increases seen in

the luteal phase of the ovine estrous cycle are believed to stimulate the growth of small antral follicles.[7,83]

The mechanism by which preantral follicles become antral is not known.[65,101] An intrafollicular polypeptide hormone called inhibin has been observed to suppress the release of FSH from the pituitary in the bovine and rat,[29,114] and inhibit oocyte maturation in the mouse, rat, pig, and sheep.[105,113,114] The suppression of oocyte maturation can be reversed by LH (5 mg/ml) in vitro.[26,53] LH binding inhibitory substance, that prevents LH binding to receptors on the granulosa cell, is thought to be inhibin. The proposed course of events suggests that as the follicle grows and matures, it releases less of the LH binding inhibitor and thus enables more LH to bind to granulosa cells. The LH bound to the granulosa cells in turn reduces the release of inhibitory substances and resumption of oocyte meiosis occurs.[23]

Hansel and Convey,[55] in their review, discuss the changes in LH and FSH receptors in preovulatory follicles. LH receptors and binding of LH in the theca and granulosa cells increase prior to ovulation in large follicles. These follicles become more responsive to LH and increase their estradiol secretion. In contrast, the numbers of FSH receptors decrease in ovulatory follicles just prior to ovulation.[54] Additionally, an apparent effect of the preovulatory follicle to inhibit growth of other follicles has been observed.[65] The control of this inhibition is not known.

In summary, many factors play a role in the control of follicular growth. Follicle stimulating hormone causes increased growth of antral follicles while the LH surge initiates oocyte maturation and is believed

to cause ovulation. Three waves of follicular growth occur in the cycle of the ewe, but only one gives rise to follicles destined to ovulate. Inhibin may be the controlling hormone that inhibits preantral follicular development and luteal follicle development by regulating gonadotropin release and receptor binding in the follicle.

Other Factors Affecting Ovulation Rates and Estrus

In the anestrous season, follicles grow and some ovulate, but in only a small percentage of sheep does behavioral estrus accompany ovulation.[21,60,117] Also, greater numbers of antral and atretic follicles have been observed in ovaries in the estrous as compared to the anestrous season.[21] Corpora lutea formed from induced ovulation by exogenous GnRH in the anestrous season, secrete inadequate progesterone even though LH receptors are in normal numbers.[67] Estradiol becomes a negative feedback hormone on the pituitary in the anestrous season.[42] Ewes in the anestrous season were observed to undergo luteal regression and show estrus but not develop mature follicles.[65] All of the above reports indicate an alteration in folliculogenesis in the anestrous season.[27,119] Diminished success of superovulation as ewes approach or enter anestrus may be a reflection of this altered folliculogenesis.

Variation between breeds has been reported for normal ovulation rates and superovulation response.[14,15,87] Other breed differences include, height of LH peak in response to GnRH,[52] timing of the spontaneous LH peak,[86] and duration of estrus.[14] Plasma progesterone has been shown to be higher in Finnish Landrace ewes compared to

breeds with lower fecundity.[86] Differences of fecundity have been observed within breeds when individuals are selected for litter size. One proposal for the mechanism for the observed breed difference is a genetically predetermined difference in sensitivity of the hypothalamal-pituitary-ovarian axis.[15,52]

A positive correlation between body weight and ovulation rate has been well documented.[26,38,90] Feeding high-energy rations prior to the breeding season to increase ovulation rate is routinely done.[65,117] The mechanism by which this occurs is not understood.[88] The role of FSH or LH in this phenomenon has been investigated and no differences in secretion or ovarian uptake were observed.[26,38,62] One proposed mechanism is that undernutrition changes the responsiveness of the ovary to gonadotropins.[62]

Superovulation

The aim of superovulation is to enhance folliculogenesis and ovulation by giving exogenous hormone preparations that have FSH and LH activity. Two commonly used gonadotropins, reviewed by Betteridge[13] and Seidel and Seidel,[100] are PMSG and FSH. Also, desiccated pituitaries of horses (HAP)[25,51,72,73] or sheep (SAP)[22] have been used. The gonadotropin preparations either contain FSH and LH, or they have molecules with FSH and LH activity. The combination of FSH and LH activity, reviewed by Moor,[70] affects antral follicles in the ovary causing growth, oocyte maturation, and ovulation of greater than the normal number of ova. The gonadotropin activity can be divided into two effects on the ovary. The FSH portion acts to increase the growth

rate of medium to large antral follicles and also rescues follicles undergoing early atresia resulting in increased population of large antral follicles. The LH portion acts to activate the oocyte to resume meiosis and to cause ovulation.[70] Betteridge,[13] in his review, cites the use of Human Chorionic Gonadotropin (hCG), in combination with PMSG, FSHp or HAP to imitate or heighten the preovulatory LH surge and increase the number of ovulating follicles.[13]

The importance of timing of the gonadotropin treatment within the cycle has been demonstrated.[79,103,116] The FSH and LH activity are thought to act optimally to cause superovulation when given during a wave of follicular growth.[79] Gonadotropin treatment during a wave of follicular growth is accomplished by two methods. One method is to initiate treatment in the animal's follicular phase. On the average, the individual's CL regression and resulting estrus and ovulation will coincide with the folliculogenic effect of the treatment; however, due to individual variation of cycle length, this does not always occur. The second method is to artificially control progesterone withdrawal so the ensuing estrus will coincide with the effects of the treatment. This is accomplished with either prolonged progesterone treatment followed by sudden withdrawal,[32,63,91] or by giving a luteolytic preparation such as PGF_2.[1,50,96,102,119] Both treatments have been shown to cause a rapid drop in plasma progesterone followed by an LH surge, estrus, and ovulation in a majority of animals treated.[1,31,32,36,94] Many factors are involved and interact in superovulation schemes to effect the results. These will be discussed in detail in the following sections.

Gonadotropins Used to Cause Superovulation

Pregnant Mares Serum Gonadotropin

Pregnant Mares Serum Gonadotropin (PMSG) is widely used to superovulate domestic species, and its use has been reviewed by Betteridge[13] and Seidel and Seidel.[100] Folliculogenic properties of PMSG were first used to cause ovulation in anestrous sheep in 1933, before its use for superovulation.[108] Dosage recommendations vary greatly within the literature from 100 iu used in the Rhesus monkey[12] to 3000 iu used to superovulate cattle.[19] Wide dose ranges have also been reported within species. The recommended dose range of PMSG for superovulation in swine is 750 iu - 1000 iu,[88,118] in cattle 500 iu - 3000 iu[19,97] and in sheep 500 iu - 2500 iu.[35,92,116] The most commonly reported dosages used for cattle range between 1500 iu - 2000 iu[2,97] and for sheep range between 1000 iu - 1500 iu.[17,121] A positive linear dose-response effect on follicular growth has been demonstrated in cattle and sheep for PMSG.[34,40,92,116] The low dose of 500 iu - 600 iu is used in sheep to produce 2-4 lambs, and high doses, 1000 iu or greater, result in up to 30 ovulations per ewe.[34,116].

Advantages of using PMSG as a superovulation gonadotropin are as follows: PMSG can be used fresh, freeze-dried for storage and later use, or purified to increase the concentration of FSH/LH activity.[79] The entire superovulatory dose can be given as a single injection because PMSG has a long half life.[79,92] One molecule of PMSG has 2 subunits with FSH and LH activity[84,92] and an interaction of these two gonadotropins is believed to be necessary for ovulation in sheep.[43,92]

Radioreceptor analysis of FSH and LH in several lots of PMSG showed a ratio that varied from 177.1:1 - 2:1 and a 5:1 ratio is considered to be close to optimal for follicular growth and ovulation.[75] Immunoassay measurements of FSH and LH content along with amino acid sequence indicate the ratio of FSH to LH may be 1:12.[84]

The LH activity or the long half life ($t_{\frac{1}{2}}$=40-50 hrs and 118-123 hrs for the rapid and slow component respectively[84]) may account for some of the undesirable effects of PMSG as a superovulatory hormone. A large degree of individual variation has been reported in cattle and sheep, ranging from zero to 30 ovulations.[19,20,34,121] This variation may account in part for the large dose ranges recommended in the literature. Significant variations in activity have been demonstrated between the different PMSG preparations, between harvested batches and between individual mares.[75] The dose reponse relationship of PMSG for total follicular development does not always reflect ovulation rate. At doses of 800 iu PMSG and above in sheep, the ovulation rate does not linearly increase and the percent of ovulations to total follicles decreases. In sheep and cattle, lack of ovulation of large follicles,[19,30,34,115,121] premature luteinization of follicles,[34,71,73] and reduction of recovery rate of ovulated ova,[92,93,115] as well as decreased conception rate of recovered ova[3,34,92,115,121] account for the decrease in percent ovulation.

Many mechanisms for these effects of PMSG have been proposed. High estradiol levels from the multiple follicles may feedback on the hypothalamus causing release of LH from the pituitary and result in premature luteinization of follicles before estrus.[34,93] A more

plausible explanation is a direct effect of the LH activity in PMSG.[34,70,75] The long-acting LH activity in PMSG has been suggested to inhibit ovulation of large follicles while FSH activity acts simultaneously to recruit more antral follicles to grow with a net result of many large unovulated follicles.[79] A comparison done in cattle where superovulation with PMSG or when purified LH (PLH) was added to FSHp superovulation treatments compared to FSHp alone showed that ovarian response (CL number) and ova number recovered was reduced by the former two treatments. The addition of PLH to FSHp also reduced the fertilization rate compared to FSHp alone.[70]

Oocyte activation in sheep was altered by PMSG. A trial comparing superovulation by PMSG or FSHp showed that both stimulated follicular development, but that PMSG caused premature activation in 33% of the oocytes. This resulted in retention of the oocyte in luteinized follicles or ovulation of aged eggs which contributed to the increased number of abnormal embryos and fertilization failure. These researchers concluded that a reduction of LH content of gonadotropin treatment may decrease the premature activation of oocytes during superovulation.[70]

The hormonal environment for capacitation, fertilization, and ova transport has been thought to be altered by excessive gonadotropins.[19,92,115] Moor recently demonstrated grossly altered nuclear and cytoplasmic substrate uptake in oocytes <u>in vitro</u> due to PMSG but not to FSHp.[70] Premature zona pellucida shedding has been reported in PMSG superovulated cattle and is speculated to be the effect of high progesterone and estrogens on the early embryo.[19,91] Rapid transport

of ova through the reproductive tract has been documented in sheep. This has been thought to be a result of increased oviduct motility due to high estrogen levels from the superovulated ovaries.[92,93,121] The rapid transport places the ova beyond the ampular-isthmal junction of the oviduct and in this way interferes with fertilization.[24,92,93] This phenomenon may also contribute to low recovery rates of ova seen with high PMSG dosages. Several authors suggested that the ova are either transported through the oviduct into the uterus rapidly and oviduct flushes do not recover them or retrograde movement of ova occurs and they are lost in the abdominal cavity.[19,92,93,121] Another contributing factor to fertilization failure was the occurrence of silent estrus observed in PMSG treated sheep.[92] The animals ovulated but did not show behavioral estrus, making natural breeding difficult and timing of artificial insemination inaccurate. One explanation for silent estrus was that progesterone from prematurely luteinized follicles inhibits estradiol's positive feedback on the hypothalamus and pituitary. Tonic LH did not increase rapidly and the rapid rise of estradiol, responsible for triggering expression of estrus, did not occur.[92] Progesterone has also directly interfered with sperm transport through the cervix in the ewe thereby interfering with fertilization.[85] Some ovulated ova that are not capable of fertilization may be due to either lack of maturation or premature activation of maturation prior to being ovulated.[70,92] PMSG has been reported to lengthen the estrous cycle[19,93] and hasten the onset of estrus post treatment.[93,120] High progesterone levels from the multiple CLs has been implicated in lengthening the cycle.[19]

Estradiol levels have been examined as a predictor of ovulation rate in cattle. Estradiol and progesterone levels were higher in animals with high ovulation rates, however, there was too much individual variation tc use them to predict ovulation rate.[107] Estradiol levels of superovulated prepuberal heifers remained high after treatment. High estradiol may deleteriously affect the ova and explain the high numbers of unfertilized and degenerated ova seen in these animals.[97,107]

Timing of the PMSG treatment to coincide with a wave of follicular growth affects follicular response and conception rate.[116] In earlier studies, PMSG was given in the follicular phase, day 16-17 in cattle[78] and days 12-14 in sheep.[92] PMSG alone given on days 9, 10, and 11 resulted in significantly reduced ovulation and conception rates in sheep.[116] PMSG has been given during midcycle in cattle to coincide with one of the midcycle waves of follicular growth.[79] This treatment was combined with estrous synchronization which will be discussed later. The timing of PMSG treatment in midcycle also has an effect on follicular growth. In cattle, treatment at days 9, 10, and 11 generally yields optimum ovulation rates.[78,79] This was thought to correspond with maximum population of medium size antral follicles.[70]

Anterior Pituitary Preparations

Several different anterior pituitary (AP) preparations have been used to superovulate domestic species. These preparations are desiccated anterior pituitary collected from sheep (SAP)[32] or horses (HAP)[25,51,72] and a commercial FSH, called Follicle Stimulating Hormone-p.[a] These

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[a]Burns Biotec Company, Chromally Pharmaceutical Inc., Omaha NE.

products contain a combination of FSH and LH and have a shorter half life than PMSG. These preparations will be discussed in the following sections and their properties described in comparison to those of PMSG.

Horse Anterior Pituitary

Horse anterior pituitary (HAP) was the most frequently used AP preparation from 1937 to 1955. Researchers harvested pituitaries from horses at the slaughter plant and prepared their own HAP. Lack of commercial availability and the labor to prepare the hormone are the main disadvantages of HAP.[25,51,72,73,73] The short half life of all the AP preparations is a disadvantage in that they must be given twice daily for several days. Anterior pituitary preparations are given for 3-4 days in sheep.[16,73,73] The interval from the last treatment to onset of estrus is correlated to ovulation and fertility. The shorter the interval the higher the ovulation rate and fertilization rate.[74] A linear dose-response for ovulation rate has been documented for HAP.[51,74] Doses have varied from 45 - 200 mg.[25,73,74] Decreased ovulation rates were reported at 200 mg HAP.[25] The wide variation in reported doses may reflect variation in the preparation and standardization of the HAP between research laboratories.

The advantages of HAP to be discussed will be in comparison to the previously reported effects of PMSG. Less individual variation of ovulation rates has been reported with HAP than with PMSG at their respective superovulation dosage levels. Stimulation of follicular growth is as good as with PMSG and ovulation rate is greater and more consistent.[92,115] Unruptured follicles, premature luteinization or

overstimulation of ovaries reported with PMSG occur less frequently with HAP.[74] Betteridge,[13] in his review, points out that simultaneous superovulation trials comparing PMSG to HAP or FSHp have not confirmed better ovulation rates, fertility or recovery rates for the anterior pituitary preparations.[13] Later, a comparative trial comparing PMSG to FSHp resulted in better ovulation and fertilization rates for FSHp.[70] Decreased recovery rates have been reported for HAP at 200 mg, but superovulation can be accomplished at a much lower dose and thereby avoid this.[25] Fertilization rates reported for HAP (65-90%)[16,72,73] are higher than for PMSG, 33-60%.[91] Anterior pituitary preparations shortened the onset of estrus and this may have a positive effect on fertilization.[74] As with PMSG, the timing of HAP treatment is considered important. Those days reported for optimal effect with PMSG are also used when HAP is given.[74] A survey of optimal days during midcycle to institute HAP treatment has not been reported for sheep.

Follicle Stimulating Hormone-p

Follicle Stimulating Hormone-p (FSHp) is prepared commercially and the dosages reported in the literature are less varied than with HAP. Doses for sheep and goats are 17 - 25 mg,[3,4,5] and cattle doses are 35 - 45 mg.[13,21] FSHp contains molecules of both FSH and LH with the half lives of 30 and 110 minutes for LH and FSH respectively,[104] requiring the drug be given twice a day for several days. The total superovulatory dose is divided over a 3-4 day period in sheep[17] and a 4-5 day period for cattle.[76,79,99] In cattle and sheep, several authors suggest that higher ovulation rates, higher fertility rates, less individual variation, higher recovery rates, and fewer unruptured

follicles occur with FSHp than PMSG.[3,17,79] Premature activation of oocytes, observed with PMSG treatment, has not been seen with FSHp.[70] Studies using FSHp noted lower estradiol serum levels around the time of ovulation than seen with PMSG, and high estradiol is implicated in fertilization failure.[4,5] Researchers recently observed that gonadotropins with high LH activity tended to adversely affect ovulation and fertilization to a greater degree than FSHp.[70,75]

The timing of FSHp treatment as observed for HAP and PMSG may have an effect on follicular growth rate in cattle. The days in the cycle that FSHp treatment is given are the same as enumerated for HAP.[99] The time in the cycle of the ewe to give FSHp has not been studied. Additionally, silent estrus has been reported in the ewe with FSHp alone or in combination with progesterone pretreatment.[17]

Human Chorionic Gonadotropin

Human chorionic gonadotropin (hCG) contains primarily LH activity and some FSH activity.[93] In sheep, hCG in combination with PMSG or HAP increases follicular growth but significantly reduces ovulation rate.[51,115] The usefulness of hCG to create an exogenous LH surge at the end of superovulation treatments of PMSG or FSH to enhance ovulation rate has been examined in sheep. The use of Prolan[b] with PMSG caused follicle development but ovulation rate was reduced due to premature luteinization attributed to the LH activity. Production of luteinized follicles by the LH activity and the progesterone secretion from them are believed to cause decreased fertility rates, recovery

[b]Commercial hCG available in Britain.

rates and prolonged estrous cycles (25 days) in sheep.[93] In FSH superovulated sheep, the addition of purified luteinizing hormone (PLH) on the last day of FSH treatment did not increase ovulation rates. The addition of hCG or LH to superovulation regimens was not beneficial and may be detrimental in the ovine.

Control of the Cycle: Estrous Synchronization

The interval between gonadotropin treatment and onset of estrus and ovulation has an effect on ovulation and fertilization rate. In the case of PMSG, the greater the interval the greater the number of fertilized ova.[40,93] For the anterior pituitary preparations, the shorter the interval the higher the ovulation and fertilization rates.[74] The average interval, cited by Betteridge,[13] when FSH is used for superovulation is 48 hours for cattle. The observed optimum interval to estrus in HAP superovulated sheep is 24-48 hours.[74] A great deal of individual variation in estrous cycle length exists and treatment with a gonadotropin on day 12-13 of the cycle of sheep can result in a variation from the end of treatment to onset of estrus[93] and therefore reduced ovulation rates.[34] Efforts to control the onset of estrus and ovulation have been attempted to reduce this variation. The methods used attempt to control the rapid drop in serum progesterone because the fall in progesterone removes the inhibition of progesterone on the estradiol induced LH release. The events that lead to estrus and ovulation as previously reviewed are then allowed to take place.[6,56]

An early method used was enucleation of the corpus luteum. This was done by removing the structure from the ovarian stroma by

rectal manipulation in cattle. This has proven unsatisfactory because all of the progesterone secreting tissue was not always removed in this manner and resulting hemorrhage would sometimes result in adhesions that would interfere with subsequent ovulations.[78]

The next method employed was progesterone treatment which has been reviewed by Thimonier.[109] The forms of progesterone that have been used are daily injections of progestogen,[17,32] daily oral feeding of progesterone salts,[32] intravaginal pessaries of progesterone salts,[32,34,63,91] and subcutaneous silastic implants containing progesterone.[28] These treatments are given or left in place for 10-14 days (average of 12 days), and when removed, estrus and ovulation occur in 36-48 hours in sheep.[91,109] The continuous progesterone inhibits the positive feedback of estradiol and blocks LH discharge and formation of a new CL. If a CL is present at the time of progesterone treatment, it regresses and secretes very little progesterone. Removal of the exogenous progesterone results in the sudden drop of serum progesterone and loss of inhibition of the estradiol-LH discharge.[32,56]

Although progesterone control of the cycle is an effective method to synchronize estrus and ovulation, fertility can be reduced.[110] Progesterone has been thought to cause faulty transport of sperm through the cervix,[57,85,110] reduced cervical mucus, and decreased duration of estrus in the ewe.[32] Surgical artificial insemination is described as one method to bypass the cervix. Placing fresh semen directly into the uterus of the ewe did improve fertility of superovulated ewes; however, recovery rate of ovulated ova was

reduced by the technique so the overall numbers of fertilized ova obtained were similar to naturally bred ewes.[110]

Progesterone is known to inhibit LH release by blocking the estradiol induced release of GnRH.[56] The LH rise observed following removal of progesterone treatment is delayed so that it does not coincide with the estradiol rise. Uterine contractions are also inhibited.[32] These effects may alter the uterine environment necessary for sperm transport, fertilization and development of the early embryo. Progesterone has been used in anestrous sheep to induce estrus with superovulation treatments. This remains the most effective method to induce estrus and ovulation in anestrous sheep. However, there is an even greater degree of variation in response to gonadotropin treatments than seen in sheep during the estrous season.[27,34,123] This will not be reviewed here as this thesis involves cycling ewes.

The third method of controlling the estrous cycle has been by using a PGF_2 called Lutalyse[c] or a synthetic prostaglandin, Cloprostenol sodium[d] (Clo). Both of these products are known to be luteolytic in cattle, sheep, and goats.[11,41,50,82,94] The hormonal changes following PGF_2 or Clo luteolysis are not different from those of naturally occurring luteolysis.[7,11] Prostaglandin F_2 and Clo reduced progesterone secretion (functional luteolysis) within 2 hours of administration.[36,93] This is believed to act intracellularly on

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[c]Lutalyse - Upjohn Company, Kalamazoo, MI.

[d]Estrumate - ICI 80996, ICI Pharmaceutical, Division At Kemix Inc., Mississauga, Ontario.

steroid synthesis since LH receptor numbers and activity are unchanged until structural luteolysis occurs.[31,35,36] Prostaglandin F_2 is thought to be the luteolysin of sheep and is secreted from the uterus into the uterine veins. A counter current exchange between juxtaposed ovarian artery and uterine veins transfers PGF_2 from the uterus to the ovary without reaching the systemic circulation.[41]

Prostaglandin F_2 and Clo have been used to synchronize estrus in cattle where superovulation was instituted in the luteal phase of the cycle.[2] Within the cycle of the sheep, PGF_2 has been an effective luteolytic drug between days 4-13. Prostaglandin F_2 does not improve ovulation, fertilization or synchronization when given on day 14 or later in the ovine estrous cycle.[121] Favorable ovulation and fertility rates as well as heat synchronization have been reported for cattle[19,102] and sheep[1,49,122] when used with superovulation. When PMSG was used, PGF_2 or Clo was given 48 hours after treatment in cattle[2,19] and 1-2 days after treatment in sheep.[122] The doses reported for cattle are 500 - 1000 µgm Clo[19,20,96] or 30 - 50 mg PGF_2 .[2,19,20,102] Doses reported for sheep are 100 µgm Clo or 15 - 20 mg PGF_2 .[50,122]

Seasonal variation in response to PGF_2 has been reported in sheep. In the mid-anestrous season, if a CL was present, it regressed following PGF_2 treatment; however estradiol and LH peaks were very low or absent and therefore no ovulation or estrus occurred. The use of PGF_2 to induce ovulation during anestrus has been limited.[119]

There have been reports of low fertilization rates from breedings subsequent to PGF_2 estrous synchronization.[37,47] One researcher measured the number of motile sperm in the anterior reproductive tract

and found sperm numbers in PGF_2 treated sheep to be lower than in untreated estrous ewes.[57] The majority of these studies compared a single appointed breeding time in the PGF_2 treated sheep to breeding of the untreated ewes at estrus, which may account for lower fertility if not all ewes were bred at estrus.[18] In other reports of PGF_2 estrous synchronization and subsequent fertilization rates, no reduction in lambing and fertilization rates were observed.[44,111] One group of researchers demonstrated increased transport of fresh and frozen ram spermatozoa, and correspondingly higher fertilization rates, with the addition of 100 µgm of Clo to semen or 2.5 mg Clo given intramuscularly to the ewe at the time of artificial insemination.[33,45,46] A controlled trial where PGF_2 treatment combined with progesterone synchronized was compared to non-PGF_2 treated ewes have shown no reduction in fertility[48] and no adverse effect on fertilization rate when used along with superovulation treatments.[121]

Other Problems Associated with Superovulation

A progressive reduction in ovulation rate occurs with repeated treatment with any of the superovulatory hormones.[64,95] This reduction was not observed in one study where cattle were superovulated 5 successive times at increased FSHp dosages each time.[70] Two explanations for the reduced response are currently being investigated. First, the hormones, as foreign protein, may induce an antibody response and become less effective as antibody titers increase. Efforts to produce and measure the antibody response to these gonadotropins have been unsuccessful.[12,68] Second, studies involving repeated

superovulation revealed that a rest period between treatments of one to two cycles would regain the original ovulatory response. Investigators proposed the reduced ovulation rate was due to either too short a time between treatments for follicular development,[64] or exhaustion of the proliferating follicular pool.[95] In sheep, repeated superovulation has had limited success because these animals entered their anestrous period. Poor results obtained during this time were reversed with the following breeding season.[34]

Large follicles in the ovary may have an influence on superovulation response. Occasionally, the gonadotropin will be given when one large follicle is present in the ovary during midcycle. This follicle is stimulated by treatment to ovulate and luteinize, and then inhibits growth of other follicles, either by a direct intraovarian influence, or because the progesterone secretion inhibits LH release and prevents ovulation of those follicles that do grow.[93]

Effect of Breed, Season, Plane of Nutrition, Age

As previously discussed, ovulation in the ewe is affected by breed, nutrition, age, and season. These factors are also thought to have an effect on ovulatory response to superovulation in ewes. Breeds that have high lambing rates tend to respond with greater ovulation rates than less prolific breeds, for example Finnish Landrace versus Suffolk breeds. English breeds that have long, deep anestrous seasons have a poor response to superovulation when in anestrus and during the transitional periods of their breeding season.[34,112] The level of nutrition in sheep has been shown to delay onset of cycling

by a low plane of nutrition as well as reduced ovulatory rates in severely thin animals.[116,117] However, the effect of nutrition on superovulatory response has not been demonstrated by a well controlled study at the present time.

Factors that reduce the number of fertilized embryos recovered from superovulated ewes have been attributed to undesirable effects of PMSG and progesterone. Because of these effects, this study was begun to determine the effects of FSHp and PGF_2, as an alternative for superovulation of ewes. The objectives of this research were: first, to determine if FSHp and PGF_2 would superovulate ewes; second, to determine if dosage of FSHp had an effect on the superovulation response in ewes; and third, to determine if a dose of 22 mg FSH or above would maximize embryo response in superovulated ewes.

MATERIALS AND METHODS

Animals

Seventy-nine crossbred Targhee ewes, between 2 and 4 years of age, and 100-110 lbs were used in these experiments. Thirty ewes were used from September to December 1981 and 49 ewes were used from September to December 1982. The ewes were kept on pasture and fed alfalfa hay and corn as a maintenance diet. Three weeks prior to being used in the experiment, the ewes were fed an increased energy ration of corn (3/4 lb per ewe per day).

Estrous Detection

All ewes were detected for estrus prior to initiation of treatments to determine the day of estrus. A painted vasectomized marker ram was turned in with the ewes twice a day and observed for 30 minutes. Ewes were defined as being in estrus when they would stand stationary when mounted by the marker ram.

Breeding

Rams used for breeding underwent a breeding soundness exam and semen testing as outlined by the Society of Theriogenology.[10,98] Based on this exam and previous fertile breedings in each year used, the rams were determined to be fertile. Two and 4 rams were used for breeding in 1981 and 1982, respectively. All ewes were exposed to two painted fertile rams from the time of the last hormone treatment to the end of

estrus. These rams were painted different colors to facilitate confirmation and identification of the two rams that bred each ewe.

Estrous Synchronization

In 1981, all ewes were allowed to cycle naturally and were estrous detected and selected in groups of 2 to 4 for treatment. In 1982, as a larger number of animals was used, the ewes were estrous synchronized in groups of 10 to 15 with 15 mg PGF_2.[c] Those ewes not showing estrus within 3 days of the PGF_2 were given 15 mg PGF_2 5 to 7 days after the first treatment.

Treatment Methods

All ewes were given injections of either FSH or saline intramuscularly. The total dose was divided into a descending mg dose schedule and given at 12 hour intervals for 3.5 days. The divisions of FSH dosages are listed in Appendix Table A1. Ten mg PGF_2 was given intramuscularly in conjunction with both treatments on the third day of this schedule. Ewes were bred at the ensuing induced estrus 12 to 36 hours after the last FSH or saline treatment. All treatments were initiated on an assigned day of the ewe's cycle. This was based on the previous estrus assigned to be day 0.

Experimental Design

1981 Experiment

Prior to testing for effect of FSH dosage on superovulation response we were interested in testing 4 days of the estrous cycle for the effect of initiating FSH treatment on these days. This is based on

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[c] Lutalyse - Prostaglandin F_2 - Upjohn Company, Kalamazoo, MI.

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observations in cattle where the days of the cycle that FSH is given has an effect on ovulatory response.[78,79,99] In cattle, the range of days when optimum ovulatory response occurs is thought to correspond to a midcycle follicular growth wave.[65,79,99] When PMSG is given outside the range of optimum days immediately preceding estrus in cattle, ovulation rates are reduced.[78,79,103,116] An optimal range of days in midcycle of the ewe has not been tested. The 4 days tested were selected from a period corresponding to a rise in the ewe's estradial levels (Figure 1 - literature review) which is to occur when a number of antral follicles would be growing (a follicular growth wave)[60,65,101] and are thought to be susceptible to superovulation treatment.[70]

Part I

This experiment was designed to evaluate the effect of the day of the cycle when the FSH treatment is initiated (DOCI) on response, for 4 days of the estrous cycle. This was done by determining the effect of 17 mg FSH initiated on days 7, 8, 9, and 10 of the estrous cycle. We assumed that the effect of DOCI was the same for all FSH dosages and that 17 mg FSH would produce a representative superovulation response for the effect of DOCI. Three ewes were assigned to each treatment group. The statistical model used was

$$Y_{ij} = \mu + DOCI_i + E_{ij}$$

where Y_{ij} is the dependent variable, μ is a constant, $DOCI_i$ is the i^{th} DOCI effect, and E_{ij} is random experimental error.

Part II

This experiment was designed to evaluate the effect of FSH dosage from 12 mg to 22 mg. This was done by determining the effect

of 6 FSH dosages: 0.0 mg, 12.0 mg, 14.5 mg, 17.0 mg, 19.5 mg, and 22.0 mg. All treatments were initiated on day 8 of the estrous cycle. Day 8 was selected because no difference based on visual observation of the data from Part I was observed, and it was intermediate in the 7-10 day interval. Three ewes were assigned to each treatment group. The treatment cells tested are listed in Appendix Table A2. The statistical model used was:

$$Y_{ijk} = \mu + DOCI_i + FSH_k + E_{ikj}$$

where Y_{ijk} is the dependent variable, μ is a constant, $DOCI_i$ is the i^{th} DOCI effect, FSH_k is the k^{th} FSH dose effect, and E_{ikj} is the random experimental error.

1982 Experiment

An apparent positive dose response relationship was seen from the 1981 experiment. Based on this, the 1982 experiment was designed to evaluate the effect of FSH dosage from 19.5 mg to 30 mg. Additionally, two days of the cycle were evaluated at each dosage level. The reason for testing FSH dosage at two days of the cycle was that the effect of FSH may vary for different days of the estrous cycle and this interaction was not tested in the 1981 experiment. This was done by determining the effect of 4 FSH dosages: 0.0 mg, 19.5 mg, 22.0 mg, and 30.0 mg, initiated on day 8 and day 10 of the estrous cycle. Four ewes were assigned to treatment with 0.0 mg, with 2 of the ewes on treatment day 8 and 2 ewes on treatment day 10. Fifteen ewes were assigned to each of the remaining FSH treatments, with 5 of the ewes on treatment day 8 and 10 ewes on treatment day 10. The treatment cells tested are listed in Appendix Table A3. The statistical model used was:

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$$Y_{ikj} = \mu + DOCI_i + FSH_k + DOCI\text{-}FSH_{ik} + E_{ik\,j}$$

where Y_{ikj} is the dependent variable, μ is a constant, $DOCI_i$ is the i^{th} DOCI effect, FSH_k is the k^{th} FSH dose effect, $DOCI\text{-}FSH_{ik}$ is the interaction of $DOCI_i$ and FSH $dose_k$, and $E_{ik\,j}$ is the random experimental error. Using this data and a second model, a multiple regression was done to calculate a polynomial or line of best fit for the effect of FSH dosage on corpora lutea and embryo number. This second model included linear, quadratic, and cubic regression to determine the line of best fit. These models are, first:

$$Y = a + bx$$

where Y is the dependent variable, a is a constant, b is a constant, and x is the FSH dosage. Second:

$$Y = a + bx + cx^2$$

where Y is the dependent variable, a, b, and c are constants, and x is the FSH dosage. Third:

$$Y + a + bx + cx^2 + dx^3$$

where Y is the dependent variable, a, b, c, and d are constants, and x is the FSH dosage.

Hormones Used

Follicle Stimulating Hormone

Follicle Stimulating hormone-P was used which consists of porcine pituitaries that are desiccated and freeze-dried by the manufacturer and FSH content is expressed in milligram equivalents of the Armour Standard as described by Steelman and Pohley.[104] Follicle stimulating hormone-P is

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[a]FSHp - lot number 550C81 - Burns Biotec Company, Chromally Pharmaceutical Inc., Omaha, NE.

sold in 50 mg vials of freeze-dried product to be diluted with 10 ml sterile water to a 5 mg/ml concentration. In these experiments the 50 mg vial was diluted with 50 cc of sterile water to a 1 mg/ml concentration to facilitate more accurate delivery of dosages.

Prostaglandin F_2

The PGF_2 used was Lutalyse[c] which contains 5 mg/ml of clinoprost tromethamine, a natural PGF_2. In these experiments 20 mg (4 ml) of clinoprost tromethamine was given intramuscularly to the ewes during the FSH treatment to synchronize estrus. In the 1982 experiment 15 mg (3 ml) of clinoprost tromethamine was also given to the ewes to synchronize estrus prior to the FSH treatments.

Data Collection

Laparotomy Technique

The sheep were given 1300 mg (13 ml) Ketamine HCl[f] and 10 mg (0.5 ml of 20 mg/ml concentration) Xylazine[g], intramuscularly. Following shearing and clipping of wool of the ventral abdomen, the sheep were placed in dorsal recumbency on a table slanted at a 30° angle with the ewe's head faced downhill. A surgical scrub was performed on the skin of the ventral abdomen and aseptic technique was maintained throughout the procedures. A ventral midline laparotomy was done immediately anterior to the ewe's udder. The uterus and ovaries were elevated out of the laparotomy incision and examination of the ovaries, followed by flushing of the uterine horns was done.

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[c]Lutalyse - lot numbers 010JH and 572JH - Upjohn Company, Kalamazoo, MI.

[f]Vetalar - Parke Davis, Warner Lambart, Morris Plains, NJ.

[g]Rompun - Haver Lockard, Miles Laboratory Inc., Shawnee, KS.

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The Ovarian Structures

The ovarian structures were counted, diameter measured in millimeters and recorded for right and left ovary, separately. The structures recorded were corpora lutea and follicles. Any abnormal findings such as poorly luteinized Cls, edema of the bursa or oviduct, and adhesions were recorded. Corpora lutea were defined as those structures that upon gross observation were greater than 5 mm in diameter and were comprised of luteal tissue with or without an ovulatory papilla. Follicles were defined as structures that upon gross observation were greater than 5 mm and fluid-filled with no evidence of an ovulation point.

The Uterine Flush

An antegrade uterine flush was done to collect ova and embryos from each horn (Appendix B). The technique is described below. An intravenous (IV) catheter was placed in the tip of the horn and directed toward the oviduct. A 14-gauge, Foley catheter was placed into the uterine lumen and the cuff inflated to occlude the lumen and hold the Foley catheter in place. A sterile syringe, containing 60 cc of Dulbecco's phosphate-buffered saline with 2% ovine serum and 5% glucose, was attached to the IV catheter and media were infused. The outflow of media, through the Foley catheter, was collected into separate, labeled petri dishes. The flushing procedure was done on each uterine horn. The uterus was then rinsed with sterile saline solution and replaced into the abdomen and the abdomen was closed. The ewes were given 1,000,000 iu Benzathine Penicilline[h] and 150 iu of Tetanus Antitoxin[i] intramuscularly after surgery.

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[h] Benzathine Penicilline - Beecham Inc., Bristol, TN.

[i] Tetanus Antitoxin - Jensen Salsbery Laboratory, Div. Richardson Merrell, Inc., Kansas City, MO.

In order to determine the volume of media to use for the uterine flush, 120 ml was used in 30 ewes. This volume was divided equally into two petri dishes. No ova were found in the second 60 ml volume. Sequentially, a 60 ml flush volume was used and divided equally into 2 petri dishes. Ova were found in the second petri dish from 3 of the 11 ewes. Based on these findings, a 60 ml flush volume was used for the remaining ewes in these experiments.

Because adhesions or damage to the reproductive tract has been reported[9,68] the uteri of 50 of the ewes were examined. A laparotomy was done at 30-60 days after the uterine flush. Also, 30 of the ewes were given prostaglandin F_2 immediately after the flush and housed with fertile rams for 30 days. This was done to assess adverse effects of the uterine flush technique on fertility based on gross anatomy of the reproductive tract and subsequent pregnancy rate in the ewes.

Embryo Counting

The petri dishes containing the uterine flushings were allowed to settle at 25° C for 30 minutes. Using a stereoscope at 40X magnification, the petri dishes were systematically scanned three times to locate the ova. The ova were then examined for fertilization, stage of development and degeneration, and the number recorded by horn for each ewe. Evaluation of embryos was done according to the criteria described by Seidel and Seidel.[100]

Statistical Analysis

An analysis of variance was used to determine the treatment effects on corpora lutea, embryos, follicles, unfertilized embryos, and degenerated embryos.[j] The treatments tested were FSH dosage, day of the

[j]Version SAS 79.5 of the GLM series of Statistical Analysis System,[59] Wayne State University, Detroit, MI.

cycle the FSH was initiated (DOCI) and interaction between FSH dosage and DOCI. The statistical model used was

$$Y_{ikj} = \mu + DOCI_i + FSH_k + DOCI\text{-}FSH_{ik} + E_{(ik)j}$$

where Y_{ikj} is the dependent variable, μ is a constant, $DOCI_i$ is the i^{th} DOCI effect, FSH_k is the k^{th} FSH dosage effect, $DOCI\text{-}FSH_{ik}$ is the effect of the interaction between $DOCI_i$ and FSH dose k, and $E_{(ik)j}$ is the random experimental error. A one-way analysis of variance was done to determine the effect of year of the experiment on corpora lutea and embryos[k]. The model used was

$$Y_{ij} = \mu + A_i + an_j/A_i$$

where Y is the dependent variable, μ is a constant, A_i is the i^{th} year effect, and an_j/A_i is the j^{th} animal within year and used as the year error term.

In order to determine a trend for the effects of FSH dosage on corpora lutea and embryos, multiple regression of the data was done[j]. The statistical models used were

$$Y = a + bx,\ Y = a + bx + cx^2,\ \text{and}\ Y = a + bx + cx^2 + dx^3$$

where Y is the dependent variable, a, b, c, and d are constants, and x is the FSH dosage. Significant ($P<.05$) polynomial regression equations were determined for the change in corpora lutea and embryos response over increasing FSH dosage. In order to estimate the optimum FSH dosage at which maximum CL response would occur, the derivative of the polynomial regression equation for CL response by FSH dose was solved. An estimate for the optimum FSH dosage at which maximum embryo response would occur was not done because the regression equation was linear and therefore had no optimum.

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[k]Version SAS 82.3 of the GLM series of Statistical Analysis System,[89] Wayne State University, Detroit, MI.

RESULTS AND DISCUSSION

Effect of Treatments on Corpora Lutea

To determine whether the year of the experiment had an effect on the CL response, a one-way analysis of variance was done and the results are summarized in Table 1. A significant effect of the year of the experiment on the CL response was not demonstrated (P>.05), therefore the data of both years were pooled and an analysis of variance was done across years.

TABLE 1

Summary of the ANOVA for year effect on corpora lutea response

Source of Variation	df	ss	ms
Year	1	127.3	127.3
Animals within year	77	2977.3	38.7

The results of the analysis of variance for the effect of treatments on day of the cycle the FSH is initiated (DOCI), FSH dosage, and the interaction of DOCI and FSH dosage are listed in Table 2. From the analysis, three conclusions regarding the effect of the treatment can be made. First, no significant (P>.5) effect of DOCI on corpora lutea response was seen. This indicates that for 17 mg FSH the effect of initiating FSH on days 7, 8, 9, and 10 of the cycle had no significant (P>.5) effect on CL response. Therefore it appears that any one of these

TABLE 2

Summary of the ANOVA of the
treatment effects on corpora lutea

Source of Variation	df	ss	ms
DOCI	3	19.4	6.3
DOSE	6	991.5	165.3
DOCI-DOSE	4	259.0	64.8
Error	65	2845.5	43.8

4 days of the cycle would be equally suitable to do the FSH dosage testing on. Secondly, a significant effect ($P<.01$) of FSH dosage on number of corpora lutea was found. The main effects of FSH dosage on CL number are summarized in Table 3. The treatment means are: 1.86 ± 2.50, 6.67 ± 3.82, 6.67 ± 3.82, 8.40 ± 1.71, 12.06 ± 1.56, 14.39 ± 1.56, and 10.73 ± 1.71 CL/ewe for FSH dosage 0.0, 12.0, 14.5, 17.0, 19.5, 22.0, 30.0 mg FSH/ewe, respectively. A multiple regression of the data was done to determine a regression equation that best fit the effect of FSH dose on CL numbers. A significant ($P<.05$) cubic regression equation was calculated. The equation was:

$$Y = 218.088 - 36.354x + 1.937x^2 - 0.0319x^3$$

where Y was CL numbers and x was FSH dosage. The coefficient of determination is $r^2 = .31$. Taking the derivative of the regression equation and solving for zero estimates the optimum dose of FSH to maximize CL response. This dose is 25.8 mg FSH.

A graph of the cubic regression along with the treatment means is shown in Figure 2. The cubic regression is the best fit of the data

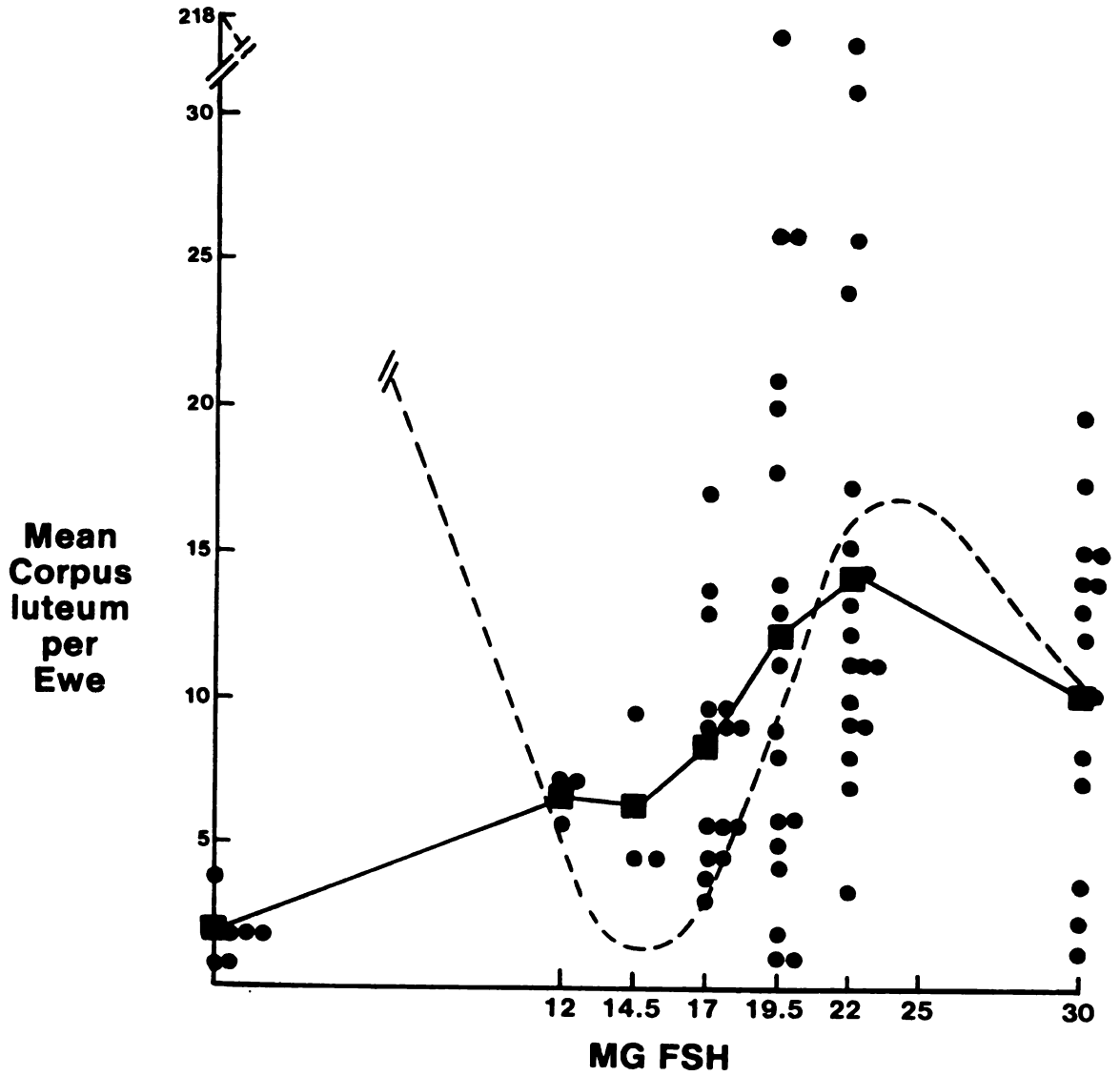

Figure 2. Graph of cubic regression curve and mean corpora lutea/ewe by FSH mg.

------- Cubic regression of mean corpora lutea/ewe by dose.

——— Mean corpora lutea/ewe by dose.

■ Treatment means.

● CL per individual ewe

TABLE 3

Main Effects of FSH Dose
on Corpora Lutea[a]

FSH mg	Mean Adjusted CL*/ewe ±SE	Total CL	No. Ewes
0.0	1.86 ± 2.50	13	7
12.0	6.67 ± 3.82	20	3
14.5	6.67 ± 3.82	20	3
17.0	8.40 ± 1.71	126	15
19.5	12.06 ± 1.56	217	18
22.0	14.39 ± 1.56	259	18
30.0	10.73 ± 1.71	161	15

*CL = Corpora lutea

[a]Significant ($P<.05$) cubic regression for change in number of corpora lutea over change in mg of FSH.

and a polynomial estimate of a dose-response curve. The calculated optimum dose 25.8 mg is relatively close to the observed optimum of 22 mg. From this, we can conclude that an optimum CL response will occur between 22 - 30 mg FSH and appears to be near 25.8 mg. The 25.8 mg dose is an extrapolated optimum and the best estimate based on the change in CL response from 22 mg to 30 mg. The real optimum may or may not be near the estimate, and more data between 22 - 30 mg are needed to determine this.

The positive dose-response effect seen in these experiments is similar to those seen with other gonadotrophins. Positive linear dose-response curves have been determined for both HAP[51,74,93] and PMSG[35,40,93,116] effect on follicular growth. Decreased ovulation rates were observed at high doses of HAP[25,93] and PMSG.[35,51,93] The results of these experiments are similar in that CL response decreased at 30 mg versus 22 mg. This may indicate that an increased CL response to

FSH dose is seen up to a point, after which more FSH may decrease CL response.

The cubic regression may reflect the way the ovary responds to exogenous gonadotrophins. The gradual slope of the curve at 14.5 mg FSH may reflect a response threshold for the follicle population to FSH. Below a certain threshold dose the amount of FSH may not be sufficient to stimulate FSH receptors, bind to them and effect follicular growth and ovulation in greater than 2 - 4 follicles. The steep slope between 14.5 - 25.8 mg may reflect the optimal dose range to stimulate these events. Higher levels of gonadotrophins, as reported in other studies[18,19,28] and as seen in these experiments at 30 mg, may alter an optimal relationship between receptor binding, FSH present, and FSH utilized to stimulate follicle growth. The result of excess FSH or LH activity is thought to be overstimulation of follicular growth and is reflected as unovulated follicles and prematurely luteinized follicles and thereby decreased corpora lutea numbers. More investigation toward understanding the mechanism and control of follicular growth is necessary to determine the action of these gonadotrophins.

A large degree of individual animal variation has been reported.[93] In these experiments, the coefficient of determination (r^2 = .31) reflects the wide degree of individual variation seen within all animals. The graph of adjusted treatment means with the 95% confidence intervals shows the variation around each mean (Figure 3). These experiments also have observed a wide degree of variation within animals as has been previously reported.

Thirdly, no significant (P>.2) interaction of DOCI and FSH dose on CL response was seen in these experiments. Two days, 8 and 10, of

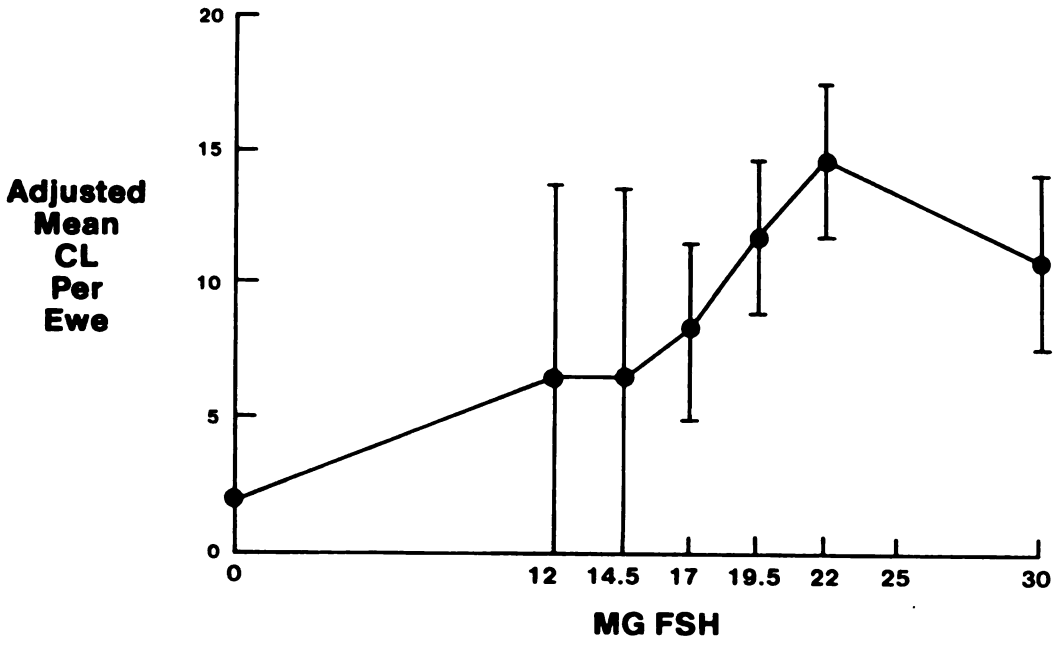

Figure 3. Graph of adjusted mean corpora lutea/ewe by dose of FSH. Each vertical bar represents the 95% confidence interval around the adjusted treatment means.

the estrous cycle were tested over four doses to determine whether an interaction existed. This had not been tested previously when DOCI testing was done, so the possibility of an interaction was evaluated by testing these data. Because no significant (P>.2) interaction was seen, the conclusion was that the effect of the FSH dosage did not differ between days 8 and 10 of the cycle.

Effect of Treatments on Embryos

A one-way analysis of variance was done to determine the effect of year of the experiment on embryo response and the results are summarized in Table 4. A significant interaction was not found (P>.2) and the data from both years were pooled and an analysis of variance was done across years.

The results of the analysis of variance for treatments, day of the cycle the FSH was initiated (DOCI), FSH dosage and the interaction of DOCI and FSH dosage are listed in Table 5. From the analysis, three conclusions regarding the effects of the treatments can be made. First, no significant (P>.5) effect of DOCI on embryo response was seen. This indicates as seen for CL in the previous section, that for days 7, 8, 9 and 10 of the cycle the embryo response was not significantly (P>.5) different between days. The rationale for testing the effect of DOCI on embryo response is the same as discussed for CL.

TABLE 4

Summary of the ANOVA for year effect on embryos

Source of Variation	df	ss	ms
Year	1	59.0	59.0
Animals within year	77	2920.4	37.9

TABLE 5

Summary of the ANOVA for
treatment effect on embryos

Source of Variation	df	ss	ms
DOCI	3	23.5	7.8
DOSE	6	425.7	71.0
DOCI-DOSE	4	59.3	14.8
Error	65	2920.2	44.9

Second, a significant ($P<.05$) linear regression for the effect of FSH dosage on embryo response was determined. The main effects of FSH dosage on embryos are listed in Table 6. The overall FSH dose effect was not significant ($P>.05$) and this may be because of the wide degree of individual variation in each treatment group. More ewes per treatment group may demonstrate a FSH dosage effect. The linear relationship of FSH dosage on embryo response was significant ($P<.05$) however; and this is the best fit of the treatment effects. The regression model used linear, quadratic and cubic regression to determine the line of best fit. Because the coefficients of determination (r^2) of the quadratic and cubic polynomials did not improve the fit of the regression to the data, the linear model was selected as the polynomial of best fit. The regression equation was:

$$Y = 0.708x - 6.213$$

where Y is embryo response, and x is FSH dosage. The coefficient of determination is $r^2 = .15$. This again reflects a wide degree of individual animal variation and is illustrated by the 95% confidence intervals in Figure 4.

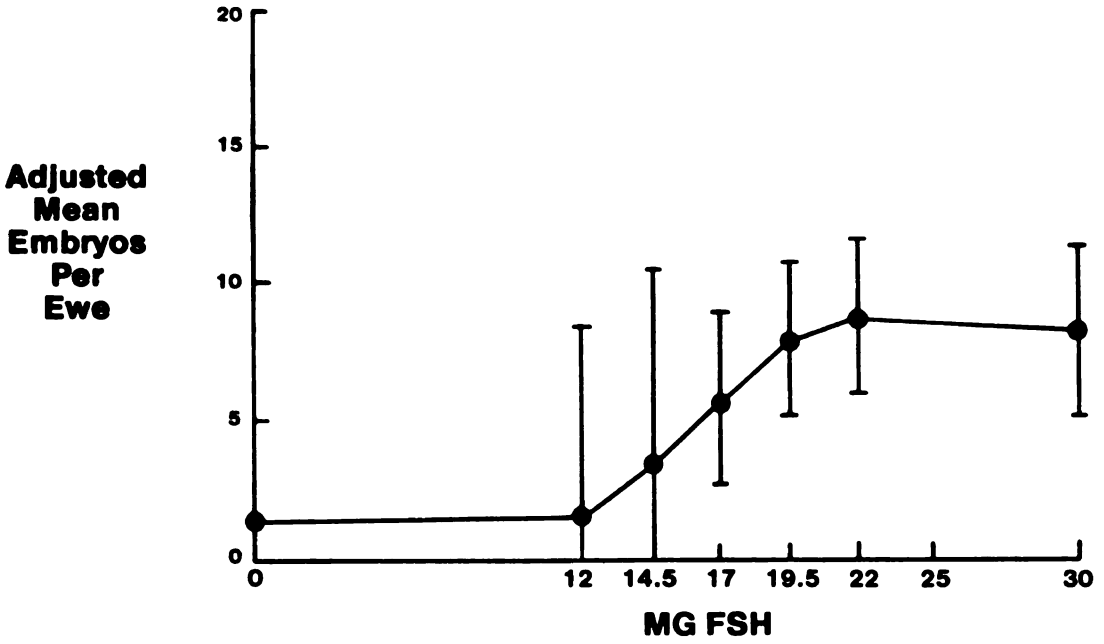

Figure 4. Graph of adjusted mean embryos/ewe by dose of FSH. Each vertical bar represents 95% confidence intervals around the adjusted treatment means.

TABLE 6

Main effects of
FSH dose on embryos[a]

FSH mg	Mean Adjusted embryos/ewe ± SE	Total embryos	No. of ewes
0	1.14 ± 2.53	8	7
12.0	1.33 ± 3.87	4	3
14.5	4.33 ± 3.87	13	3
17.0	5.87 ± 1.73	88	15
19.5	7.72 ± 1.58	139	18
22.0	8.44 ± 1.58	152	18
30.0	7.93 ± 1.73	119	15

[a]A significant ($P<.05$) linear regression for change in number of embryos over change in mg of FSH

A graph of the linear regression and treatment means is shown in Figure 5. A positive linear trend exists for both curves at dose 19.5 mg and below. Above the 19.5 mg level the treatment means and regression curve diverge. Because a greater number of ewes (N = 64) have a strong linear relationship at 22 and below, their data have more weight. Likewise, the fewer ewes (N = 15) at dose 30 mg do not have enough weight to alter the slope of the regression. Therefore, a linear regression remains the best estimate from the data, although the treatment means level off. An optimum FSH dose for embryo numbers cannot be estimated from these data. Because the number of embryos is a function of the number of CLs (ovulations), the maximum number of embryos should not exceed the number of CLs. One would expect then that the optimum dose of FSHp for embryos would be equal to or less than the optimum dose for CLs. However, a divergence from the linear trend cannot be proven from these data and may be due to the amount of variation around the treatment means.

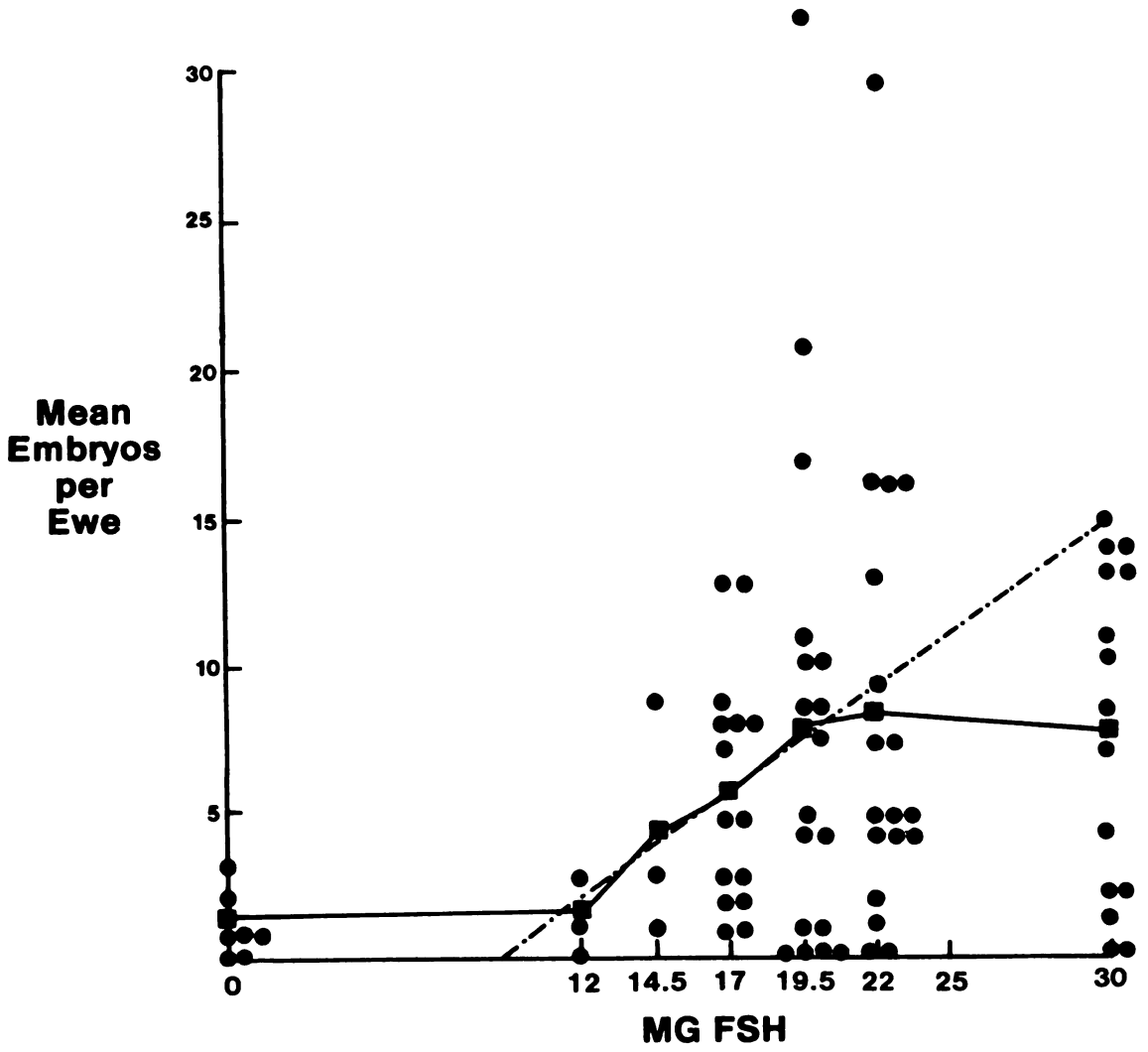

Figure 5. Graph of linear regression curve and mean embryos/ewe by FSH mg.

------- Linear regression of mean embryos/ewe by dose of FSH.

——— Mean embryos/ewe by dose of FSH.

■ Treatment means.

● Embryos for individual ewes.

More data in the dose range of 19.5 - 30 mg are needed to demonstrate a leveling of the linear dose response curve to determine the optimum dose for embryos.

Reduction of embryos/corpora lutea (ovulation ratio) has been documented by others at high doses of PMSG.[3,19,92] Reduction of the embryo/CL ratio has been attributed to several factors. At high doses of PMSG, fertilization failure has been observed.[3,92,121] Progesterone pretreatment as a synchronizing method has resulted in fertilization failure due to interference with sperm transport.[84] Fertilization failure was not observed to a great degree in this experiment and would not explain the reduction of embryo/CL ratio seen at 22 mg and 30 mg.

Highly stimulated superovulated ovaries produce high levels of estradiol.[96,97,107] The high estradiol is thought to increase the motility of the oviduct, thereby rapidly moving ova out of the oviduct. Rapid transit of ova has been reported in cattle and sheep.[19,24,34,92] The ova are then missed by oviduct flush techniques. Recovery rates reported for uterine flush techniques are lower (38-60%)[9] than those for oviduct flush (60-70%).[110] With the uterine flush method the ova are thought to be "lost" within the uterus, degenerate due to an unfavorable environment for the early embryo, or be flushed retrograde into the oviduct, preventing retrieval.[78] The observation that embryo numbers did not increase above 19.5 mg FSH cannot be attributed solely to fertilization failure because the fertilization rates (reported as unfertilized ova/total ova) did not differ between treatment groups. Tests for FSH dosage effect on unfertilized ova are discussed later in this thesis (page 52). Also, recovery rates did not appear to be different between FSH treatment groups (See Table 14). These effects were not tested for significant differences.

Factors other than either fertilization rates or recovery rates may play a role in embryo response but have not been identified in these experiments.

The third conclusion is, no significant (P>.5) effect of the interaction of DOCI and FSH dosage on embryo response was seen. As discussed previously, the effect of the interaction of FSH dosage and DOCI was considered. In these experiments a significant interaction was not seen (P>.5), indicating that the effect of FSH dosage on embryo response was equivalent for days 8 and 10 of the cycle.

Effect of Treatments on Follicles

The results of the analysis of variance for treatments, day of the cycle the FSH was initiated(DOCI), FSH dosage and the interaction of DOCI and FSH dosage are listed in Table 7. From the analysis three conclusions regarding the effect of the treatments can be made. First, no significant (P>.5) effect of DOCI on follicle response was seen. Second, no significant (P>.5)effect of FSH dosage on follicles was

TABLE 7

Summary of the ANOVA of
treatment effects on follicles

Source of Variation	df	ss	ms
DOCI	3	7.3	2.4
DOSE	6	10.1	1.7
DOSE-DOCI	4	9.8	2.5
Error	65	252.1	3.9

seen. Table 8 shows the main effects of FSH dosage on follicles and a lack of difference between the treatment means can be seen. Thirdly, no significant (P>.5) effect of the interaction of DOCI and FSH dosage was seen.

The mean number of follicles observed was 1.90 ± 0.22 and the treatment means ranged 1 ± 1.12 - 2.67 ± 1.12 (Table 8). Control ewes had an average of 2.14 ± 0.73 follicles. Seven ewes had 5 to 10 follicles on their ovaries (Appendix Table C1). These individuals appeared to be evenly distributed between treatment groups but statistical testing for homogeneous distribution was not done.

TABLE 8

Main effects of
FSH dose on follicles

FSH mg	Mean Follicles ± SE	Total Follicles	No. of Ewes
0.0	2.14 ± 0.73	15	7
12.0	2.67 ± 1.12	8	3
14.5	1.0 ± 1.12	3	3
17.0	2.13 ± 0.50	32	15
19.5	1.83 ± 0.46	33	18
22.0	2.11 ± 0.46	38	18
30.0	1.4 ± 0.50	21	15

The reason for interest in a treatment effect on follicles is based on reports of increased numbers of unovulated follicles and luteinized follicles with superovulatory dosages of PMSG.[2,4,34] High LH activity of PMSG due to a long half life, may be responsible

for this effect on the follicle.[70] Follicle stimulating hormones used in these experiments has a reported LH:FSH ratio of 1:3.[75] Perhaps at higher doses of FSHp, an increase in number of unovulated follicles would be seen. In these experiments, the number of follicles was not significant (P>.5) between treatment groups.

Effect of Treatments on Unfertilized Ova

The results of the analysis of variance for treatments, day of the cycle the FSH was initiated (DOCI), FSH dosage and the interaction of DOCI and FSH dosage are listed in Table 9. From the analysis three conclusions regarding the effect of the treatments can be made. First, no significant (P>.5) effect of DOCI on unfertilized ova response was seen. Second, no significant (P>.5) effect of FSH dosage on unfertilized

TABLE 9

Summary of the ANOVA of
treatment effects on unfertilized ova

Source of Variation	df	ss	ms
DOCI	3	4.6	1.5
DOSE	6	10.7	1.8
DOSE - DOCI	4	13.4	3.4
Error	65	190.3	2.9

ova response was seen. Table 10 shows the main effects of FSH dosage on unfertilized embryos and a lack of difference between the treatment means can be seen. Thirdly, no significant (P>.2) effect of the interaction of DOCI and FSH dosage on unfertilized ova was seen.

TABLE 10

Main effects of FSH dose
on unfertilized ova

FSH mg	Mean Unfertilized Ova ± SE	Total Unfertilized Ova	No. of Ewes
0.0	0.00 ± *	0	7
12.0	1.00 ± 1.02	3	3
14.5	0.33 ± 1.02	1	3
17.0	0.66 ± 0.46	10	15
19.5	0.83 ± 0.42	15	18
22.0	0.94 ± 0.42	17	18
30.0	0.06 ± 0.46	1	15

*Standard error was not applicable.

The mean number of unfertilized ova was 0.60 ± 0.20 and the treatment means ranged 0.00 * - 1.00 ± 1.02 (Table 10). Two ewes showed high numbers of unfertilized ova, 6 unfertile ova out of 10 ova and 12 unfertile ova out of 12 ova recovered (Appendix Table C1). The ewe with 12 ova did not exhibit estrus and did not allow breeding by the ram. The reason for 6 unfertile ova out of the other ewe is not known. Fifteen ewes showed 3 or less unfertilized ova and appeared to be evenly distributed through treatment groups, although statistical testing for even distribution was not done. The control group (0.0 mg FSH) had 0 unfertilized ova, however no significant (P>.5) difference between other treatments was seen.

Decreased fertilization (%) rate of recovered ova is reported at superovulatory doses of PMSG.[34,92,121] Higher fertilization rates have been reported for HAP (75 to 95%).[22,115,122] Data from these

experiments show a high fertilization rate (total fertilized embryos/ total ova = 91.75% ± 3.00) corresponding with other reports using anterior pituitary preparations. The depressed fertilization rates observed with PMSG indicate some adverse effect on those events leading to fertilization. The mechanism of action by which PMSG exerts this effect has been discussed by many authors but has not been determined. The difference in effect on fertilization rate between FSH, HAP, and PMSG also has not been determined. The high LH activity of PMSG has been implicated in depression of fertilization by causing premature activation of the oocyte. This may result in ovulation of aged ova that are not capable of fertilization.[70] Also, PMSG elevates estradiol and progesterone serum levels above normal and this may affect the uterotubal environment and interfere with sperm transport, capacitation, or fertilization.

Effect of Treatments on Degenerated Embryos

The results of the analysis of variance for treatments, day of the cycle the FSH was initiated (DOCI), FSH dosage, and the interaction of DOCI and FSH dosage are listed in Table 11. From the analysis

TABLE 11

Summary of the ANOVA of
treatment effects on degenerated embryos

Source of Variation	df	ss	ms
DOCI	3	1.1	0.4
DOSE	6	5.2	0.9
DOCI-DOSE	4	5.5	1.4
Error	65	77.0	1.2

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these conclusions regarding the effect of treatments can be made. First, no significant (P>.5) effect of DOCI on degenerated embryo response was seen. Second, no significant (P>.5) effect of FSH dosage on degenerated embryo response was seen. Table 12 shows the main effects of FSH dosage on degenerated embryos and a lack of difference between the treatment means can be seen. Thirdly, no significant (P>.2) effect of the interaction of DOCI and FSH dosage on degenerated embryos was seen.

TABLE 12

Main effects of FSH dose
on degenerated embryos

FSH mg	Mean Degenerated Embryos ± SE	Total Degenerated Embryos	No. of Ewes
0	0.00 ± *	0	7
12.0	0.00 ± *	0	3
14.5	0.00 ± *	0	3
17.0	0.33 ± 0.28	5	15
19.5	0.22 ± 0.25	4	18
22.0	0.72 ± 0.25	13	18
30.0	0.60 ± 0.28	9	15

*Standard error was not applicable.

One ewe had 8 degenerated embryos out of 29 (Appendix Table C1). The reason for this animal having a high number of degenerated embryos is not known. Seventeen ewes had 3 or less degenerated embryos and appeared to be evenly distributed throughout the treatment groups; however, they were not statistically tested for even distribution.

No degenerated embryos were observed in treatments of 14.5 mg FSH or less; however this difference was not statistically significant (P>.5).

Degenerated embryos have been observed by many workers with PMSG and FSHp used in certain cows. The high LH activity of PMSG and the high estrogen and progesterone levels seen in superovulated cows are thought to be involved in embryo degeneration. Moor et al observed altered substrate uptake into the nucleus and cytoplasm of embryos from PMSG but not FSHp superovulated cows.[70] There appears to be an effect on the metabolism of the early embryo due to PMSG, which may account for the reports of greater numbers of degenerated embryos seen. Neither a large number of degenerated embryos or a dose effect on number of degenerated embryos was seen in these experiments. The appearance of all the degenerated embryos above dose 17 mg may reflect a relationship between degree of superovulation on the ovary and degeneration of embryo.

Prostaglandin $F_2\alpha$

No significant difference (P>.05) of FSH dose or DOCI effect was seen between ewes given prostaglandin F_2 in 1982 to synchronize estrus prior to FSH treatments and those allowed to cycle naturally in 1981. This was tested by a one-way analysis of variance to determine if a significant difference between years on CL or embryo response existed. (pp 38,44) The results of the estrous synchronization are summarized in Table 13. The average onset of estrus following one treatment with PGF_2 was 44 hours and within a 24-72 hour range. Thirty-eight of 45 ewes (84%) came into estrus after one PGF_2 treatment. Five of 8 of the remaining ewes came into estrus after a second

TABLE 13

Estrous synchronization with PGF_2 : interval from treatment to estrus

Interval from PGF_2 Treatment to Estrus	Number of Ewes
24 hours	1
30 hours	13
43 hours	14
52 hours	13
65 hours	2
72 hours	2

PGF_2 injection given 5 to 7 days after the first injection. All 3 of the remaining 3 ewes came into heat after a third PGF_2 injection 5 days after the second PGF_2 treatment. Five ewes came into estrus naturally and are not included in these data. Forty of 45 ewes (89%) came into estrus within a range from 30 to 50 hours after PGF_2 treatment. (See Figure 6.) Analysis for the effect of multiple PGF_2 injections was not done.

Prostaglandin F_2 was selected to synchronize ewes in this study for several reasons. Prostaglandin F_2 is considered the luteolysin of the ewe and has been shown to cause similar luteal regression and resulting hormone profiles as normal regression. Prostaglandin F_2 causes rapid luteal regression with estrus occurring in 24-72 hours and in combination with previous superovulation regimes has not had an adverse effect on ovulatory response or fertility.[50] However, progesterone, an alternate synchronizing agent, has produced fertilization failure.[33,64,84]

In these experiments, one hundred percent of ewes (pre-FSH-treatment) were synchronized into 24 to 72 hour periods. Seventy-eight of 79 of FSH-treated ewes came into estrus within 48 hours of the last FSH treatment. Close synchrony of the last FSH treatment and estrus is desirable since higher ovulation rates and fertilization rates are seen when this interval is short.[74] Finally, since the fertilization rate was 91.75% ± 3.00 and no apparent effect on the number of unfertilized ova was observed, PGF_2 did not appear to reduce the fertilization rate.

Ova Recovery Rate

The ova recovery rates defined as total ova/corpora lutea are listed in Appendix Table C2. The recovery rates are summarized by FSH dose in Table 14. The arithmetic mean ova recovery rate was 65.3% ± 3.7. The mean recovery rate at 12 mg FSH appears to be much lower than the rest of the treatment means. This is because 1 of the 3 sheep had a recovery rate of 0% and in a treatment group of 3 ewes this lowered the mean recovery rate. The treatment mean recovery rates appear to be similar over the remainder of the treatment group; however data were not statistically tested for significant differences.

Distribution of ewes with low (0-33%), medium (34-66°C) and high recovery rates (67-100%) are illustrated in Figure 6. Forty-eight ewes had recovery rates above 67%, 17 ewes had recovery rates between 34-66%, and 14 ewes had recovery rates of 33% or less. The ova recovery rate in this report is higher than previously reported for uterine flush techniques. Reports of higher ova recovery rates are seen if the oviduct is flushed, but this may lead to adhesions or obstructions of the oviduct and adjacent structure.[16] In this experiment, based on

TABLE 14

Summary of mean ova recovery rate
in % by FSH dose

FSH Dose mg	Ova Recovery %
0	71.4 ± 12.4
12.0	36.7 ± 18.9
14.5	63.3 ± 18.9
17.0	69.5 ± 8.5
19.5	62.3 ± 7.7
22.0	63.6 ± 7.7
30.0	66.1 ± 8.5

laparotomy examination, no adhesions of oviducts, fimbriae or ovaries were seen and a subsequent 100% pregnancy rate in 30 ewes bred after the flush technique indicates patent and functional oviducts, fimbriae and ovaries. Pregnancy was determined either at lambing or at the time of the laparotomy examination.

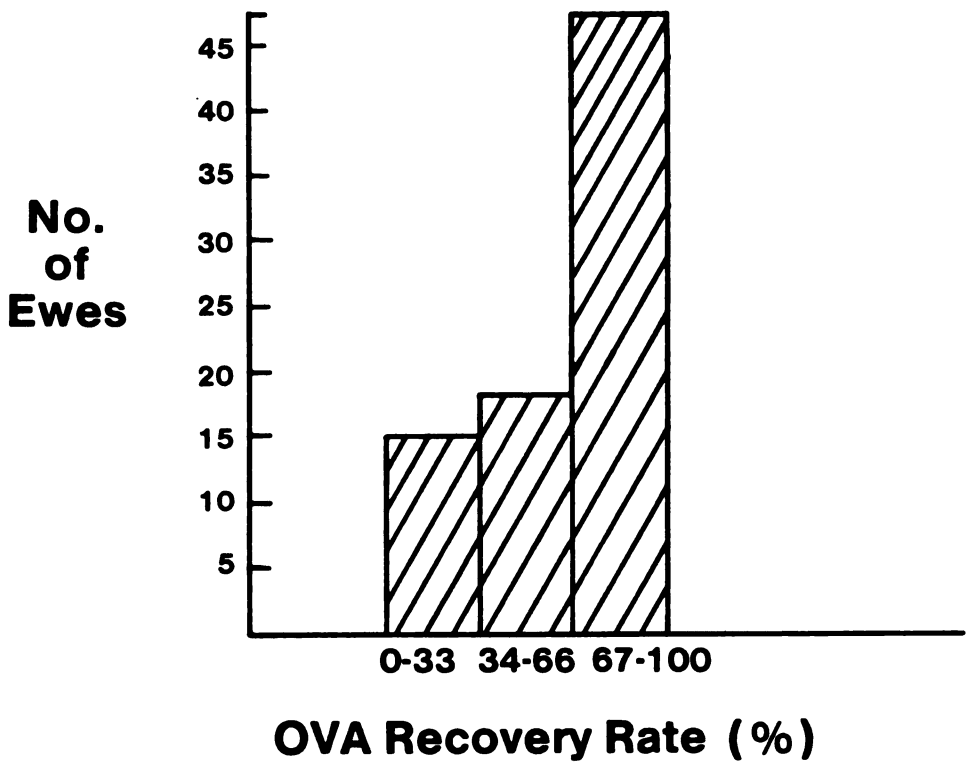

Figure 6. Graph of the distribution of ewes with low, medium, or high ova recovery rates (%).

SUMMARY AND CONCLUSIONS

The effect of FSHp and PGF_2 to induce superovulation in 79 crossbred Targhee ewes was determined. The variables measured were number of follicles, corpora lutea, embryos, unfertilized ova and degenerated embryos. A total of 570 ova were collected, of which 523 were fertilized and 31 degenerated. The fertilization rate was equal to 91.75% ± 3.0%. A total of 816 corpora lutea and 149 follicles were observed.

The experiments were done in the fall of 1981 and 1982. The 1981 experiment was designed to determine the effects of the time FSHp is initiated within 4 days of the estrous cycle of the ewe. Following this, a second experiment was done to determine the effect of 6 different doses of FSHp. The 1982 experiment evaluated the interaction of the day of the cycle FSHp was initiated and 3 FSHp doses based on results of the 1981 experiment.

The effect of the day of the cycle FSHp was initiated (DOCI) was examined for days 7, 8, 9, and 10 of the estrous cycle. Interaction between DOCI and FSHp dose was evaluated for days 8 and 10 and doses 0 mg, 19.5 mg, 22 mg, and 30 mg FSHp . No significant ($P>.9$) effect of DOCI was found and no significant ($P>.2$) interaction of DOCI with dose was determined. Therefore, initiation of FSHp treatment on day 7, 8, 9, or 10 of the cycle of the ewe has produced equivalent results.

Seven doses of FSHp were evaluated, 0 mg, 12 mg, 14.5 mg, 17 mg, 19.5 mg, 22 mg, and 30 mg FSH. A positive effect of dose on

number of corpora lutea was determined. A cubic regression curve was calculated by method of least squares. The regression curve is the dose response curve for FSHp dose on CL number and can be used to predict response in areas of the curve not tested in these experiments. An optimum dose for maximum number of CLs was calculated to be 25.8 mg FSH. This dosage is close to the observed optimum of 22 mg, beyond which the response decreased. Therefore, an optimum dose to produce the maximum CLs (ovulations) was estimated to be between 22 and 30 mg. In addition, FSHp in excess of this dose may be detrimental to ovulation rate. Large variations in response existed within all treatments, and the coefficient of determination (r^2 = .30) for the regression curve reflected this degree of error. Large variation in individual response to superovulation have been reported frequently for other gonadotrophins. The variation in these experiments is similar to previous reports.

A linear regression curve for number of embryos by dose of FSHp was calculated by method of least squares. This curve showed a positive dose effect of FSH on embryo number. The regression curve and actual data closely agreed up to 19.5 mg FSHp. At this dose and beyond, the actual measured data leveled off while the regression curve continued to increase because the strong linear relationship below 22.0 mg exerts more influence on the curve than do data above 22.0 mg. This regression curve is the best predictor of embryo response from the data. Decrease in the embryo/CL ratio at high doses of PMSG and HAP has been documented by other researchers. The observation that embryos do not increase beyond 19.5 mg may or may not reflect a lower optimal dose than for CL numbers. More data at doses above 19.5 mg are needed to determine

the optimum FSHp dose for embryo response.

Three additional observations were made from the data of these experiments. First, PGF_2 was used for estrous synchronization prior to and in conjunction with the FSHp treatment with satisfactory induction of estrus and fertilization rates. Second, a modified antegrade uterine flush technique was employed which resulted in satisfactory ova recovery rates and minimal trauma to the reproductive tract. Third, high fertilization rates of the ova were seen.

In conclusion, FSHp and PGF_2 in combination induced superovulation in crossbred Targhee ewes. A significant positive dose response curve was determined for corpora lutea and embryos. An optimum dose for CL numbers was between 22 - 30 mg FSHp. No optimum dose for embryo number can be estimated from the data. The fertilization rate in this study indicates that FSHp and PGF_2 did not adversely affect fertilization to the degree reported for PMSG and progesterone. Although a small number of FSHp doses were evaluated, the information gained from this study may aid future research on superovulation of the ewe. A better understanding, however, of folliculogenesis, ovulation, fertilization and early embryo development is needed to manipulate these events.

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APPENDICES

APPENDIX A

TABLE A1

Descending dose regimes for
FSH doses: 0 to 30 mg

Total FSH mg		1st Treatment	2nd Treatment	3rd Treatment	4th Treatment
Control Saline	AM	5 ml	4 ml	3 ml + 10 mg PGF_2	2 ml
	PM	5 ml	4 ml	3 ml + 10 mg PGF_2	
12.0 mg	AM	3 mg FSH	1.5 mg FSH	1 mg FSH + 10 mg PGF_2	1 mg FSH
	PM	3 mg FSH	1.5 mg FSH	1 mg FSH + 10 mg PGF_2	
14.5 mg	AM	3 mg FSH	2 mg FSH	1.5 mg FSH + 10 mg PGF_2	1.5 mg FSH
	PM	3 mg FSH	2 mg FSH	1.5 mg FSH + 10 mg PGF_2	
17.0 mg	AM	4 mg FSH	3 mg FSH	1 mg FSH + 10 mg PGF_2	1 mg FSH
	PM	4 mg FSH	3 mg FSH	1 mg FSH + 10 mg PGF_2	
19.5 mg	AM	4 mg FSH	3 mg FSH	2 mg FSH + 10 mg PGF_2	1.5 mg FSH
	PM	4 mg FSH	3 mg FSH	2 mg FSH + 10 mg PGF_2	
22.0 mg	AM	5 mg FSH	3 mg FSH	2 mg FSH + 10 mg PGF_2	2 mg FSH
	PM	5 mg FSH	3 mg FSH	2 mg FSH + 10 mg PGF_2	
30.0 mg	AM	6 mg FSH	5 mg FSH	3 mg FSH + 10 mg PGF_2	2 mg FSH
	PM	6 mg FSH	5 mg FSH	3 mg FSH + 10 mg PGF_2	

TABLE A2

The frequency of observations
within the treatment cells
for the 1981 experiment

Total mg FSH	Day of Cycle Treatment Initiated (DOCI)			
	7	8	9	10
0.0		3 ewes		
12.0		3 ewes		
14.5		3 ewes		
17.0	3 ewes	6 ewes	3 ewes	3 ewes
19.5		3 ewes		
22.0		3 ewes		

Total: 30 ewes

TABLE A3

The frequency of observations
within the treatment cells
for the 1982 experiment

Total mg FSH	Day of Cycle Treatment Initiated (DOCI)	
	Day 8	Day 10
0.0	2 ewes	2 ewes
19.5	5 ewes	10 ewes
22.0	5 ewes	10 ewes
30.0	5 ewes	10 ewes

Total: 49 ewes

APPENDIX B

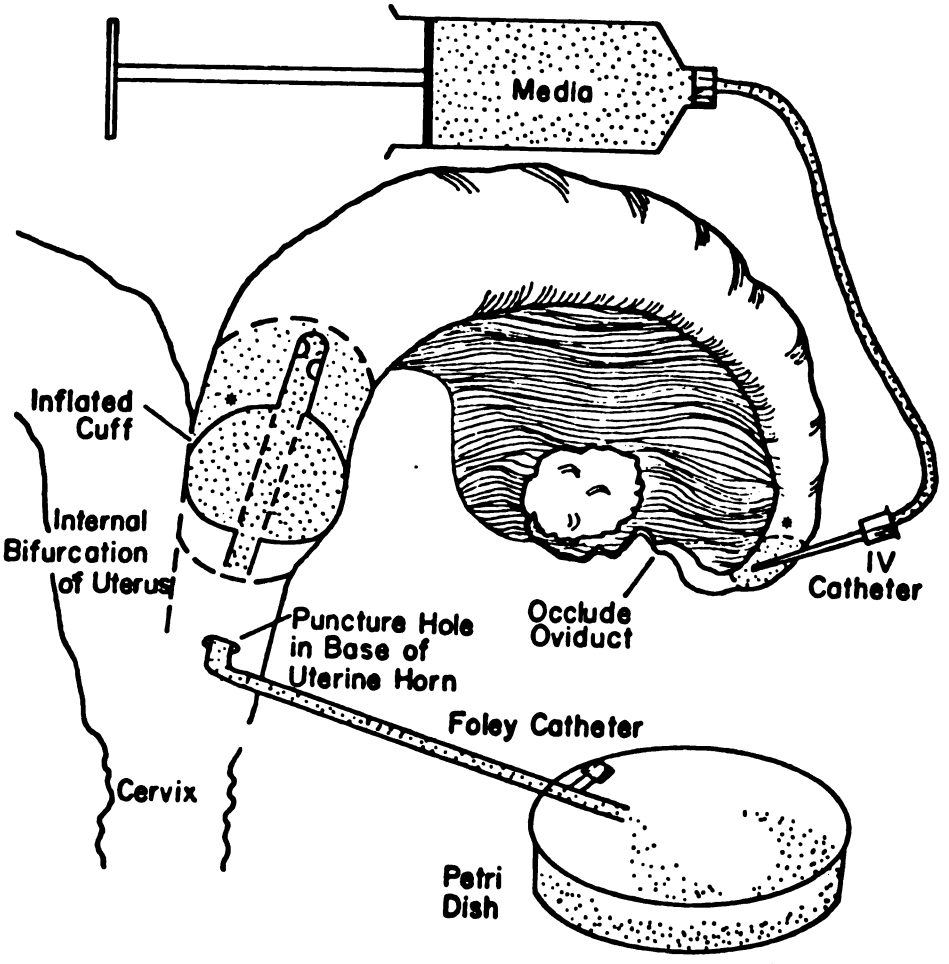

Figure B1. Schematic diagram of ovine uterine flush technique

*Cutaway view of position of catheters in lumen of uterine horn

APPENDIX C

TABLE C1

Results of superovulation: total CL, follicles, embryos, unfertilized ova, and degenerated embryos by FSH dose

Dose mg FSH	Sheep	Day of Cycle	Total CL	Total Follicles	Total Embryos	Total Unfertilized Ova	Total Degenerated Embryos
0.0	619	8	1	1	1	0	0
0.0	627	8	2	2	3	0	0
0.0	632	8	2	3	0	0	0
0.0	710	8	2	2	2	0	0
0.0	722	8	1	1	1	0	0
0.0	728	10	1	2	1	0	0
0.0	717	10	4	4	0	0	0
12.0	602	8	7	3	0	0	0
12.0	613	8	7	3	3	0	0
12.0	614	8	6	2	1	3	0
14.5	629	8	10	1	9	0	0
14.5	669	8	5	1	1	1	0
14.5	670	8	5	1	3	0	0
17.0	649	7	5	2	1	0	0
17.0	642	7	6	0	1	3	0
17.0	639	7	13	2	13	0	2
17.0	611	8	5	5	2	3	0
17.0	622	8	9	0	8	0	1
17.0	620	8	17	1	13	0	0
17.0	607	8	9	0	7	2	1
17.0	625	8	3	10	2	0	0
17.0	636	8	10	0	8	0	1
17.0	635	9	6	6	3	2	0
17.0	644	9	4	2	3	0	0
17.0	633	9	9	2	8	0	0
17.0	643	10	14	1	9	0	0
17.0	640	10	10	0	5	0	0
17.0	648	10	6	1	5	0	0
19.5	605	8	4	2	0	0	0
19.5	628	8	8	4	7	0	0
19.5	674	8	14	0	0	12	0
19.5	718	8	20	3	10	0	0
19.5	709	8	18	2	8	0	0
19.5	706	8	11	1	10	0	1
19.5	725	8	21	0	17	0	1
19.5	719	8	25	0	11	3	0
19.5	704	10	13	2	4	0	0
19.5	707	10	1	0	0	0	0
19.5	723	10	5	3	0	0	0
19.5	739	10	1	5	1	0	0

TABLE C1 (continued)

Dose mg FSH	Sheep	Day of Cycle	Total CL	Total Follicles	Total Embryos	Total Unfertilized Ova	Total Degenerated Embryos
19.5	735	10	32	0	32	0	1
19.5	714	10	21	1	21	0	0
19.5	747	10	9	3	8	0	0
19.5	726	10	6	5	5	0	0
19.5	750	10	2	2	1	0	0
19.5	702	10	6	0	4	0	0
22.0	612	8	7	6	5	0	0
22.0	617	8	11	2	10	0	0
22.0	630	8	9	4	4	0	0
22.0	749	8	24	4	17	3	1
22.0	729	8	10	0	7	0	0
22.0	738	8	12	1	0	0	0
22.0	732	8	11	1	7	1	0
22.0	716	8	8	1	0	2	0
22.0	721	10	11	2	9	0	0
22.0	724	10	13	0	1	1	1
22.0	743	10	3	7	2	1	0
22.0	736	10	25	2	17	0	0
22.0	734	10	17	3	17	0	0
22.0	737	10	31	0	29	1	8
22.0	730	10	9	1	5	0	0
22.0	731	10	14	3	5	2	2
22.0	742	10	29	1	13	0	0
22.0	727	10	15	0	4	6	1
30.0	711	8	20	1	15	0	0
30.0	701	8	2	0	2	0	0
30.0	720	8	7	2	4	0	2
30.0	715	8	10	0	7	0	0
30.0	744	8	14	4	14	0	2
30.0	712	10	15	0	8	0	3
30.0	708	10	10	1	10	0	1
30.0	713	10	1	1	0	0	0
30.0	748	10	3	3	1	0	1
30.0	705	10	15	0	15	0	0
30.0	740	10	14	2	14	0	0
30.0	703	10	17	0	16	0	0
30.0	746	10	13	1	2	0	0
30.0	733	10	8	2	0	0	0
30.0	741	10	12	4	11	1	0

TABLE C2

Ova recovery rate in %
(Total ova/corpora lutea)
by FSH dose

FSH Dose mg	Sheep	Recovery %
0.0	632	0
0.0	619	100
0.0	627	100
0.0	722	100
0.0	717	0
0.0	710	100
0.0	728	100
12.0	614	67
12.0	602	0
12.0	613	43
14.5	670	60
14.5	629	90
14.5	669	40
17,0	635	83
17.0	620	77
17.0	622	78
17.0	611	100
17.0	639	85
17.0	642	67
17.0	649	20
17.0	648	83
17.0	640	50
17.0	643	64
17.0	633	67
17.0	644	75
17.0	625	67
17.0	607	67
17.0	636	60
19.5	674	86
19.5	605	0
19.5	628	86
19.5	735	94
19.5	739	100
19.5	704	44
19.5	718	50
19.5	707	0
19.5	725	81
19.5	706	91
19.5	709	44

TABLE C2 (continued)

FSH Dose mg	Sheep	Recovery %
19.5	723	0
19.5	719	56
19.5	702	67
19.5	750	50
19.5	726	83
19.5	747	89
19.5	714	100
22.0	617	91
22.0	630	44
22.0	612	72
22.0	716	29
22.0	732	73
22.0	738	0
22.0	727	67
22.0	729	70
22.0	742	45
22.0	730	56
22.0	749	83
22.0	731	50
22.0	736	68
22.0	734	100
22.0	737	100
22.0	743	100
22.0	724	15
22.0	721	82
30.0	748	33
30.0	715	70
30.0	720	57
30.0	713	0
30.0	741	100
30.0	733	0
30.0	746	15
30.0	744	100
30.0	708	100
30.0	701	100
30.0	703	94
30.0	740	100
30.0	705	100
30.0	712	53
30.0	711	70