AN ABSTRACT OF THE THESIS OF

Bronwyn B. Fackrell for the degree of Master of Science in Genetics presented on April 13, 2005.

Title: Effects of Genotype, Nutrition, and Progesterone on Uterine Efficiency in the Ewe.

Redacted for privacy

Abstract approved: ____________________

Howard H. Meyer

Embryonic loss of potential lambs resulting in partial failure of multiple ovulations is a significant factor in the reproductive rate of prolific breeds. The roles of genetic and nutritional variation (and their possible interaction) on embryonic loss were studied by mating blackface and whiteface ewes of either high or low body condition to either Suffolk or Polypay rams. Variation in body condition was created by placing ewes on either high or low planes of nutrition for 13 weeks prior to breeding and the first 30 days of pregnancy with the goal of achieving mean body condition scores of 4.0 and 2.5, respectively. Low ewes were flushed to overcome expected depression in ovulation rate due to poor body condition. The actual difference achieved was 1.0 condition score units (and 11 kg) at the commencement of mating. Embryo loss was defined as difference between ovulation rate as measured by laparoscopy shortly post-mating, and litter size at term. Mean ovulation rate for ewes conceiving to first estrus was 2.34 for

whiteface ewes and 2.02 for blackface ewes ($p<.05$). Mean ovulation rates for High and Low ewes were 2.10 and 2.20, respectively ($p=.32$). The potential role for progesterone insufficiency during critical times in early gestation was assessed from plasma samples collected at days 13 and 20 of gestation, and analyzed from ewes exhibiting embryo loss and contemporary ewes without loss. There was no evidence that genotype of either ewes or embryos, nutritional status, or progesterone levels had an effect on uterine efficiency in either twin- or triple-ovulating ewes. Plasma progesterone concentrations were affected by a genotype by ovulation rate interaction at both day 13 ($p=.04$) and at day 20 levels ($p<.01$). Day 20 concentrations were also affected by a genotype by litter size interaction ($p<.01$).

Effects of Genotype, Nutrition, and Progesterone on Uterine Efficiency in the Ewe.

by

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CONTRIBUTION OF AUTHORS

Dr. Howard Meyer was involved in experimental and statistical design, with data collection and surgical procedures, and with the writing of this manuscript.

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CHAPTER 1: INTRODUCTION

Issues in Sheep Production

Sheep productivity is generally measured by weight of lamb produced per ewe mated. Several factors influence this measurement, including conception rate, litter size, lamb survival, nutrition for both ewes and lambs, ewe milk production, and lamb growth rate. While all of these are major contributors to the final estimate, one source of reproductive wastage that is often overlooked is that of embryos lost soon after conception. Other factors are easier to see and measure: conception rate by the number of "dry" ewes, litter size by the number of lambs born, nutrition and milk production by general body condition of both ewes and lambs, and lamb growth rate by weight of lambs. Conversely, embryonic loss is an event that, in a production situation, will go entirely unnoticed in multiple ovulating ewes which carry one or more remaining embryos to term. What is even more obscure is any one definite factor influencing embryonic loss. It is likely a combination of determinants. Some of the possibilities are ewe genotype, fetal genotype, ewe body condition and nutrition, age, ovulation pattern, and hormone levels. This study focused on breed of ewe, fetal genotype, nutrition and body condition of ewe, and progesterone levels in early pregnancy.

CHAPTER 2: REVIEW OF LITERATURE

In a review, Edey (1976) stated that reliable estimates place normal pregnancy loss rates, those occurring in the absence of identifiable stress, at 20 to 30% and that over half of these losses occur in the first 30 days following mating. It is estimated that in a well managed flock, mating to a single estrus should result in 75% of ewes conceiving and progressing to parturition. Of the remaining 25%, it is estimated that half of these ewes, rather than having failed to conceive, were carrying viable embryos that were subsequently lost (ASI). Long and Williams (1980) collected embryos 2 to 3 days post-mating and through karyotyping determined that 6% of all embryos had chromosomal abnormalities or other factors that render the embryo unviable. Restall *et al.* (1976) determined through slaughter data that fertilization of multiple ova is an "all or none" event, i.e., either all ova are fertilized or none are. This supports the idea that "lost" embryos are not the result of fertilization failure.

Estrous Cycle of the Ewe and Effects of Progesterone

The ewe is seasonally polyestrous and has a cycle length of approximately 17 days from one period of estrus to the next. Ovulation occurs near the beginning of the cycle, triggered by a surge in luteinizing hormone which stimulates both the release of ova and the luteinization of thecal and granulosa cells that once surrounded each oocyte. The resulting corpora lutea secrete progesterone to maintain pregnancy until placentae are formed. Bindon (1971) determined that levels of plasma progesterone fluctuate throughout the cycle, the lowest concentrations occurring on the days immediately surrounding estrus and the highest between 11 and 14 days following estrus, corresponding to the time of maternal recognition of pregnancy. The study also reported that progesterone levels do not differ between pregnant and non pregnant ewes until day 16 post-estrus. If the ewe was pregnant, progesterone levels continued to increase while in the cycling ewe they decreased in preparation for the next estrus. Spencer and Bazer (1995) showed similar results as illustrated in figure 2.1.

Pregnancy and timing of embryonic loss

Gestational length in the ewe is approximately 147 days. Maternal recognition of pregnancy occurs approximately 13 days post mating when signals from the embryo suppress immune response and trigger changes in uterine caruncles to facilitate implantation (Spencer, *et al.*, 2004). Signals also prevent corpora lutea from regressing, thus allowing continued secretion of progesterone to maintain pregnancy (ASI).

The embryo enters the uterus on day 4 in the 16- or 32-cell stage and within a few days has progressed to the blastocyst stage. At this point, the embryo's outer cell layer develops finger-like villi which interdigitate with papillae on the uterine wall between days 15 and 18. These villi disappear by day

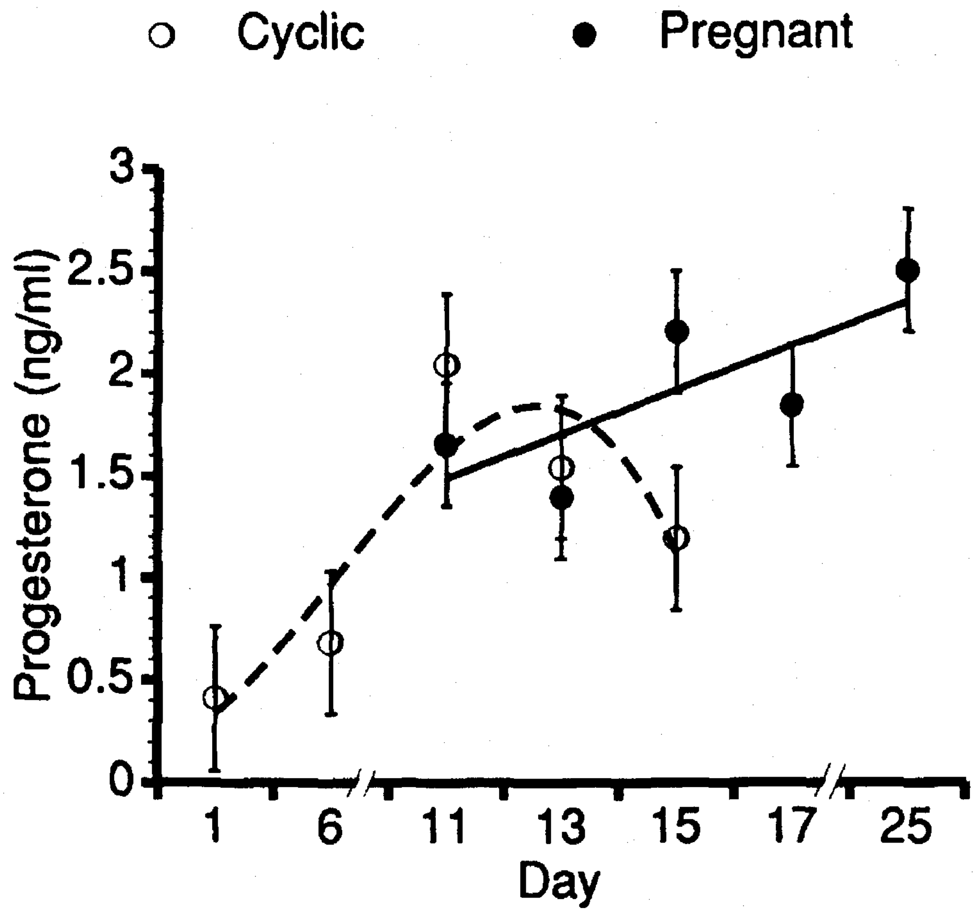

Figure 2.1 Concentrations of progesterone (ng/ml) in the peripheral plasma of cyclic and pregnant ewes. Concentration of progesterone in cyclic ewes was low on day 1, increased between days 1 and 13, and decreased to day 15 ($p<.08$, $Y=-.08x^3+.48x^2-.26x+.2$, $r^2=.68$). In pregnant ewes progesterone concentrations increased from days 11 to 25 ($p<.1$; $Y=.21x+.84$, $r^2=.43$). Reprinted from Spencer and Bazer, 1995

20, when more secure attachments, called placentome formations, have taken their place (Spencer, *et al.*, 2004).

Edey (1969) reviewed several studies involving the Merino, a typically single-ovulating breed. The review stated that over half of embryonic loss occurs before maternal recognition of pregnancy, allowing the ewe to return to estrus within the normal time frame of the estrous cycle. Such ewes would be perceived as failing to conceive.

In another review, Edey (1976) concluded that much of the remaining embryonic loss occurs between days 13 and 20. Edey (1967) found that if a ewe loses all embryos at this time, her next estrus will be delayed by as much as 20 days. Using injections of colchicine, embryonic death was induced on days 5, 7, 9, 11, 13, 15 and 19. Treatments on days 5 through 11 had no effect on subsequent cycle length. However, treatments on days 13, 15 and 19 resulted in cycles being lengthened by 2, 8 and 18 days, respectively. Using a similarly designed trial, Edey (1972) found that mating at an estrus following a cycle of 21 days or longer was about 34% less likely to result in pregnancy than mating of ewes exhibiting normal cycle length.

Uterine Efficiency

Meyer (1985) introduced the term "uterine efficiency" as a population estimate to describe the likelihood of one additional ovum becoming a lamb. Single ovulators which conceive and carry to term by definition have a uterine efficiency of 1.0, and are therefore the basis for comparison of higher ovulation rates. Each succeeding ovulation rate's uterine efficiency can be found by calculating the difference in average litter size due to the additional ovum. For instance, if twin ovulators in a particular flock have a mean litter size of 1.75, their uterine efficiency is then .75, found after subtracting the uterine efficiency of the next lower class, single ovulators. If in the same flock, triple ovulators have a mean litter size of 2.25, their uterine efficiency is then 2.25-1.75, or .50. This is a more useful method of measuring reproductive efficiency than overall lambing rate because it takes into account the reproductive wastage of lost embryos.

Nutritional Effects

It has long been known that poor body condition adversely affects ovulation rate and that increasing the plane of nutrition for two to three weeks prior to mating, a practice known as flushing, will substantially improve ovulation rate in thin ewes. However, multiple ova released by a thin ewe may not survive at the same rate as multiple ova in a ewe in good body condition. West *et al.* (1991) used either high and low nutritional planes to produce ewes of either good or poor body condition. Ewes were then randomly assigned within body condition to high or low planes of nutrition for the first 45 days after synchronized mating. Compared to ewes in good body condition, poor body condition at mating decreased litter size in twin ovulators by .15 lambs per ewe while among triple ovulators, litter size was reduced by .35 lambs per ewe. Thus, ewes at the higher ovulation rate were impacted more than the twin ovulators. In the same study, triple ovulating poor condition ewes assigned low post-mating nutrition had a negative uterine efficiency, i.e. actually produced fewer lambs per ewe than their twin-ovulating counterparts.

Changes in weight can also have an effect on embryonic survival. Cumming (1972a) used high and low planes of nutrition to generate a difference of 15.2 kg body weight between two groups of crossbred ewes. Groups were then treated so that higher weight ewes lost weight and low weight ewes gained weight during the three weeks prior to mating and the first 30 to 40 days of pregnancy. Among ewes lambing to twin ovulations, weight change had a significant effect on reproductive results. Of those which gained weight, 45% produced twins versus only 23% of twin-ovulating ewes in the group losing weight during this time. There were also about twice as many returns to estrus among ewes losing weight.

Cumming (1972b) showed that increasing length of nutritional stress had a proportionate effect on embryonic loss. Ewes were subjected to restricted feed intake for one, two or three weeks during the first three weeks of pregnancy. The specific week of restriction was not significant. However, the increasing length of time restricted led to a linear increase of twin-ovulating ewes with no embryos, as determined by slaughter data. The timing of specific week of restriction was not significant. There was little effect of restriction on single-ovulating ewes.

Too high a plane of nutrition may also have a detrimental effect on embryo survival. Cumming *et al.* (1975) conducted trials to assess optimum diet for embryo survival. Merino and Merino/Border Leicester ewes were assigned randomly within condition score and live weight gradations to 25%, 100% and 200% maintenance diet during a three week mating period. Embryo survival was determined by hysterotomy on day 26-30 of pregnancy. For twin-ovulating ewes, embryo survival was highest in the 100% group for both Merinos and Merino crossbreds, followed by the 25% treatment in Merinos and the 200% treatment in crossbred ewes. Overall, crossbred ewes had higher survival rates than straightbred ewes. Again, there was little effect on reproductive success among single-ovulating ewes.

Nutritional restrictions appear to have less effect if imposed later in gestation. Edey (1976) reported that reducing intake for 5 to 8 weeks after the third week of pregnancy, resulting in ewes losing about 5kg live weight, had little or no effect on reproductive performance, lending credence to the idea that the vast majority of loss occurs prior to this time.

Plasma Progesterone Levels

In a study of nutritional effects on plasma progesterone levels, Cumming *et al.* (1971) assigned ewes to diets of 25%, 100% or 200% of maintenance during days 2 through 16 of pregnancy. Throughout the daily experimental bleeding period, ewes on 25% diet had significantly higher plasma progesterone than ewes on the 100% diet, which in turn were higher than those on the 200% diet, thus eliminating low progesterone levels as a likely cause of embryonic loss in underfed ewes.

Parr, *et al.*, (1987) conducted a similar study, and added within each nutritional treatment a group of ewes supplemented with exogenous progesterone. For unsupplemented ewes, plasma progesterone level at day 12 post mating for ewes fed 200% diets was less than half that for ewes fed 25% diets and slightly less than ewes fed 100% diets. Ewes on the 200% diet had significantly lower pregnancy rates than those on 25 or 100% diets. However, when ewes were given exogenous progesterone, pregnancy rates were comparable among all groups. It was theorized higher planes of nutrition caused greater metabolic clearance rate of hormones. Parr, *et al.* (1993) later confirmed this theory using ovariectomized ewes given infusions of progesterone and then randomized into 50%, 100% and 200% maintenance diets. Metabolic clearance of progesterone was significantly greater in 200% ewes than in 50% ewes, though neither was significantly different from 100% ewes, which fell between the two.

Miller, *et al.*, (1999) reported a similar relationship in swine. In a 2X2 factorial design, gilts were raised to be either fat or lean and then assigned to 115% or 230% maintenance diets. Previous body condition had little if any effect on progesterone clearance rate; however, the 230% diet did produce a significantly greater clearance rate than did the 115% diet.

Genetic Effects

<u>Uterine Efficiency</u>

Several studies have shown breed differences in both ovulation rate and uterine efficiency. Meyer *et al.* (1983) compared six different genotypes in several related studies over several years, using laparoscopic and lambing or slaughter data to determine uterine efficiency. In comparing twin-ovulators, straightbred Romneys showed the lowest success with an average uterine efficiency of .59 while Romney/Border Leicester crosses had the highest value at .79. Meyer *et al.* (1994) reported cumulative data from several trials over several years comparing Booroola Merinos, control Merinos, and their crosses with Dorset and Border Leicester. Results comparing twin-ovulators showed control Merinos with the lowest uterine efficiency at .58 while crossbred ewes usually exhibited higher uterine efficiency than straightbred ewes.

Using a matrix of Polypay and Coopworth ewes and Polypay, Coopworth and Suffolk rams, Nawaz and Meyer (1991) studied uterine efficiency in the array of resulting genotypes. Ewes from Polypay dams had significantly higher mean uterine efficiency than those from Coopworth dams, .90, vs .76. This superiority may have been due to the genetic background of the Polypay. The breed was developed using Finnsheep as one of a four breed cross. Finnsheep are highly prolific, partially due to higher ovulation rate and partially due to superior uterine efficiency. Meyer and Bradford (1973) reported that Finn/Targhee crosses had an ovulation rate approximately .6 higher than Targhee ewes, and uterine efficiency estimates of .82 vs. .56 among twin-ovulators and .55 vs. .27 for triple-ovulators.

West *et al.* (1991) showed that nutritional effects on uterine efficiency are more pronounced in some breeds than others. Uterine efficiency estimates for twin-ovulating Coopworth/Polypay crosses dropped from 0.71 in good condition ewes to 0.38 in poor condition ewes. By comparison, twin-ovulating Polypay ewes in the same trial had uterine efficiency estimates of 0.75 and 0.80, respectively. Among triple-ovulators, low nutritional plane reduced litter size by 0.6 lambs per ewe in Coopworth/Polypay ewes compared to only 0.3 lambs in Polypay ewes.

Embryonic genotype

The genotype of the embryo, as determined by both the sire and the dam, may also have an affect on uterine efficiency of the dam. Using the technique of embryo transfer to test this theory, Bradford *et al.* (1974) collected purebred embryos from each of seven breeds of varying prolificacy and mature body weight and placed them in Welsh Mountain, Border Leicester, or Finnish Landrace ewes. Each recipient received four embryos of a single breed. Donor breed had little effect on embryonic survival; however, recipient breed did affect survival and eventual litter size. Mean litter sizes were 2.58 for Welsh Mountain, 2.91 for Border Leicester, and 2.89 for Finnish Landrace.

CHAPTER 3: MATERIALS AND METHODS

Experimental Animals

The "Matrix" flock maintained at Oregon State University was created through various crosses to study the effect of ewe and fetal genotype on uterine efficiency. The original flock consisted of Polypay (P), Coopworth (C), Hampshire (H), CxP, HxP, HxC, and Hx(CxP) ewes. Subsequent generations were created by mating these ewes to either Polypay or Suffolk rams, yielding the experimental flock of approximately 180 ewes born 1996-1999. In this experiment, ewes are designated either white-faced (at least three-fourths Polypay breeding) or black-faced (three-fourths or more Suffolk breeding).

Treatment Groups

After weaning lambs in June, 2003, ewes were weighed and scored on a 5-point body condition scale, (1=emaciated, 3= well fleshed, 5=grossly fat) including half points, as outlined by Jeffries (1961). Ewes were randomized within genotype, weight/condition score, and age to one of four groups within a two x two factorial design: High vs. Low plane of nutrition and mating to either Suffolk or Polypay rams.

In mid-July, ewes were divided into their respective nutritional treatment groups, the goal being a mean condition score at mating of 4.0 for the High group and 2.5 for the Low group. All High ewes were maintained until the start of mating on pasture supplemented with cull cannery green beans. Low ewes were placed on one of three nutritional regimens depending on their initial condition score: ewes of condition score 2.0 or lower were pastured with the High ewes to bring them up to 2.5; ewes at either 2.5 or 3.0 were grazed on sparse summer pasture; ewes of condition score 3.5 or above were kept in the barn at the OSU sheep center and fed limited amounts of round bale grass hay. Ewes were weighed and condition scored every two weeks, and Low ewes were relocated to the appropriate feeding groups as needed. High ewes were kept on the same plane of nutrition until removal from rams. Low ewes flushed two weeks prior to ram introduction and were then returned to a low plane for the duration of ram exposure. All ewes were brought into the barn prior to mating. Rams were introduced on October 20 and removed December 2.

In order to counteract the expected depressing effects of poor body condition on ovulation rate, low ewes were flushed with supplemental hay beginning three weeks prior to ram introduction. Ewes were synchronized to standardize length of flushing using two 10 mg injections of $PGF_{2\alpha}$ (Lutalyse, Pharmacia & Upjohn Co., Kalamazoo, MI). The first injection was given eight days before introduction of rams and the second was given 24 hours before ram introduction. To spread demands of labor at lambing, ewes were randomized into two groups and synchronized one week apart.

Two semen-tested rams were placed with each of the four mating groups. All rams were fitted with marking harnesses and crayon marks were recorded daily.

Laparoscopy

Each ewe underwent laparoscopy approximately two to six days post mating. Prior to laparoscopy, ewes were fasted for 24 hours and removed from water overnight. Each ewe was sedated with Rompun (.15 mg/kg body wt.)(Bayer Corporation, Toronto, Canada.), and restrained in a cradle, and prepped by close shearing followed by scrubbing of the abdomen with an iodine solution. The ewe was then tilted at a 45 degree angle with the head down, her abdomen inflated with CO_2, and an endoscope was inserted through the abdominal wall to view the ovaries and determine ovulation rate by counting corpora lutea. Ewes were given feed and water and kept under surveillance in recovery pens for several hours before rejoining their mating groups.

Plasma Collection

Blood samples were collected from each ewe on days 13 and 20 post mating using 10 ml Vacutainers. The chosen collection times correspond to maternal recognition of pregnancy and implantation, respectively. Samples were placed on ice and within one hour were centrifuged at 4 degrees C at 2500 rpm for

20 minutes. Plasma was pipetted into 4 ml polypropylene storage vials and stored at -20 degrees C until analysis.

Sample analysis

Following lambing, each multiple-ovulating ewe's litter size was compared with her ovulation rate to identify ewes producing fewer lambs than their number of corpora lutea. Each such ewe was then matched by genotype, ovulation rate, treatment group, and ram breed to a ewe whose litter size indicated no lost ova. All plasma samples from selected ewes and their contemporary controls were analyzed for progesterone levels using radioimmunoassay as described by Paslay *et al.* (2003). Mean extraction efficiency was 89.1% and mean intra- and interassay coefficients of variation were .112 and .181, respectively.

Statistical Analyses

All data were analyzed using S-Plus 6 for Windows (Insightful Corporation, Seattle, WA). Bodyweight, condition score and ovulation rate were analyzed by ANOVA, with ewe genotype (blackface vs. whiteface) and treatment group (High vs. Low) as main effects. Conception rate and litter size were analyzed as per ovulation rate, with the inclusion of fetal genotype.

Day 13 and day 20 plasma progesterone levels were analyzed by ANOVA initially fitting genotype, treatment group, ovulation rate, and ova success (no loss vs. partial loss of ova) as main effects, all two-way interactions, and bodyweight at mating as a covariate. Interactions and the covariate were absorbed into the model when $p>.5$. Day 20 progesterone levels were also analyzed with day 13 level as a covariate. The final model for day 13 levels included bodyweight, genotype, treatment, ovulation rate, ova success, and genotype by ovulation rate interaction. The day 20 model was the same except for the inclusion of day 13 levels as a covariate. Both models were also refitted to include litter size instead of ovulation rate and ova success.

CHAPTER 4: RESULTS

Condition Score and Body Weight

Initial, mating, and postmating bodyweights and condition scores are shown in Table 4.1. Mean condition score at the beginning of the trial was 3.0 and a mean body weight 69 kg. Throughout the trial, blackface ewes remained significantly heavier than whiteface ewes, as expected due to their larger frame. Blackface ewes started out in poorer condition, with a mean condition score of 2.8

Table 4.1. Least squares means and standard errors for body weights (BW; kg) and body condition scores (BCS) at trial initiation and at the commencement and conclusion of mating

		Start		Mating		Postmating	
Item	No.	BW	BCS	BW	BCS	BW	BCS
Treatment*							
H	57	68.9	3.0	77.6^{a}	3.8^{a}	79.8^{a}	3.9^{a}
L	88	69.4	3.0	68.0^{b}	2.8^{b}	74.8^{b}	3.2^{b}
Genotype							
BF	83	72.1^{a}	2.8^{a}	75.7^{a}	3.2	80.7^{a}	3.5
WF	62	65.8^{b}	3.2^{b}	66.7^{b}	3.2	71.7^{b}	3.4
(Ave SE)	--	(1.13)	(.08)	(1.04)	(.06)	(1.13)	(.05)

*H=high plane of nutrition; L=low plane of nutrition; BF=blackface; WF=whiteface.

a,b Means in the same column within categories without common letters in their superscripts differ (p<.05).

vs. 3.2 for whiteface ewes. However, by mating, there was no significant difference in condition score between breeds. High ewes gained an average of 9 kg and increased by .8 condition score units between trial initiation and mating, followed by increases of 2 kg and .1 unit during the 6 week mating period. Low ewes lost one kg and .2 condition score units before mating, and gained 7 kg and .4 units during mating. High ewes were heavier and in better condition both at the beginning and the end of mating ($p<.01$).

Reproductive Performance

Means adjusted by group for conception rate, both at first estrus and overall, ovulation rate, and litter size within treatment group, ewe and fetal genotype are given in Table 4.2. Conception rate to first estrus was .72 and overall was .83. High ewes had the highest first-estrus conception rate at .81, while Low ewes had a mean of .67 ($p=.07$). Ewe and fetal genotype were not significant in first estrus conception rate. Overall conception rate was .83, with no significant differences between any of the groups.

Average ovulation rate was 2.15 and was not significantly different between treatment groups. Whiteface ewes averaged 2.34 ova and blackface ewes 2.02 ($p<.05$). Whiteface ewes also had significantly higher litter sizes than blackface ewes: 2.12 vs. 1.86 ($p<.05$). Litter size did not differ significantly between treatment groups or fetal genotypes.

Table 4.2. Least squares means and standard errors for reproductive traits.

Item	No.	Conception rate First estrus	Conception rate Overall	Ovulation rate	Litter size No.	Litter size Mean
Treatment*						
H	57	.81	.86	2.10	49	2.03
L	88	.67	.81	2.20	71	1.92
Ewe Genotype						
BF	83	.73	.83	2.02[a]	69	1.86[a]
WF	62	.71	.82	2.34[b]	51	2.12[b]
Fetal Genotype						
BF	73	.70	.78	--	57	2.01
WF	72	.75	.88	--	63	1.92
(Ave SE)	--	(.05)	(.04)	(.08)	--	(.08)
Overall	145	.72	.83	2.15	120	1.97

*H=high plane of nutrition; L=low plane of nutrition; BF=blackface; WF=whiteface.

a,b Means in the same columns within categories without common letters in their superscript differ ($p<.05$).

Litter size was examined for ewes conceiving to twin and triple ovulations separately to assess effects of treatment and genotype on embryonic success. Results for twin-ovulators and triple-ovulators are presented in Table 4.3. Mean uterine efficiency was .82 for twin-ovulators and .83 for triple-ovulators. Neither litter size nor conception rate differed significantly between the different treatment groups or the different genotypes.

Table 4.3. Least squares means and standard errors for conception rate (CR) and litter size (LS) of twin- and triple-ovulating ewes and group uterine efficiency (UE) estimates.

	Twin-ovulators					Triple-ovulators				
	CR		LS			CR		LS		
Item	No.	Mean	No.	Mean	UE	No.	Mean	No.	Mean	UE
Treatment*										
H	37	.89	33	1.85	.85	11	.82	9	2.60	.75
L	57	.86	49	1.79	.79	17	.82	14	2.69	.90
Ewe Genotype										
BF	60	.90	54	1.80	.80	11	.82	9	2.67	.87
WF	34	.82	28	1.86	.86	17	.82	14	2.64	.78
Fetal Genotype										
BF	45	.82	37	1.82	.81	18	.77	14	2.64	.82
WF	49	.92	45	1.82	.82	10	.90	9	2.67	.85
(Ave SE)	--	(.05)	--	(.06)	--	--	(.11)	--	(.17)	--
Overall	94	.87	82	1.82	.82	28	.82	23	2.65	.83

*H=high plane of nutrition; L=low plane of nutrition; BF=blackface; WF=whiteface.

Plasma Progesterone Levels

Plasma progesterone was measured in 61 ewes for day 13. For four of these ewes, no data was available for day 20. Ewes chosen included all single- and triple-ovulators, and a subset of twin-ovulators so that all combinations of genotype and treatment groups were well represented. Adjusted mean progesterone levels at day 13 and day 20 are given in Table 4.4.

Treatment Effect

Treatment groups did not differ for progesterone levels measured at either day 13 (p=.97), or day 20, both with (p=.11), and without day 13 as a covariate (p=.13).

Genotype Effect

White face ewes exhibited higher progesterone levels than blackface ewes at both sampling times. The difference at Day 13 approached significance (P=.09) and the difference was significant at Day 20 (P=.04). When Day 13 level was included as a covariate in the analysis of Day 20 level, the genotype effect was reduced to P=.12, indicating the difference observed at Day 20 was largely due to a carryover effect rather than a genotype difference in degree of progesterone increase between the two samplings.

Table 4.4. Least squares means and standard errors for plasma progesterone (P_4; ng/ml) at day 13 and at day 20 with day 13 as a covariate (C) and without.

	Day 13 P_4		Day 20 P_4		
Item	No.	Mean	No.	(C)Mean	Mean
Treatment[a]					
H	23	3.84	21	3.76	3.74
L	38	3.90	36	4.22	4.20
Ewe Genotype					
BF	31	3.59	28	3.81	3.69[b]
WF	30	4.18	29	4.28	4.38[c]
Ovulation rate					
1	6	4.36	5	3.42[b]	3.52[b]
2	33	3.78	31	3.88[b]	3.83[b]
3	22	3.90	21	4.64[c]	4.46[c]
Litter size					
1	20	4.12	20	3.63	3.70[b]
2	27	3.56	27	4.20	4.08[b]
3	14	4.16	14	4.30	4.39[c]
Ova success					
Full	39	3.88	37	4.06	4.05
Partial	22	3.88	20	4.04	4.01
(Ave SE)	--	(.15)	--	(.21)	(.21)
Overall	61	3.88	57	4.05	4.03

[a]H=high plane of nutrition; L=low plane of nutrition; BF=blackface; WF=whiteface
[b,c]Means in the same column within categories without common letters in their superscripts differ ($p<.05$).

Corpora Lutea Effect

Ovulation rate was not found to be significant for day 13 levels ($p=.72$) and only approached significance for day 20 ($p=.07$, with day 13 as a covariate, $p=.10$ without). However, there was a significant interaction between genotype and ovulation rate for both day 13 ($p=.04$, Figure 4.1) and day 20 with day 13 as a covariate ($p<.01$, Figure 4.2). The most significant difference at day 13 was between blackface and whiteface triple-ovulators. At day 20, the most significant difference was between blackface and whiteface twin-ovulators.

Litter Size and Embryonic Loss

Litter size did not significantly affect progesterone at day 13 ($p=.34$) or at day 20 ($p=.20$ with day 13 as a covariate, $p=.29$ without). Interactions with genotype were not significant for day 13, but were significant for day 20 ($p<.01$ with day 13 as a covariate, Figure 4.3). The most significant difference was between blackface and whiteface ewes lambing twins.

Ova success was not related to progesterone levels at either day 13 ($p=.97$) or day 20 ($p=.92$, with and without day 13 as a covariate).

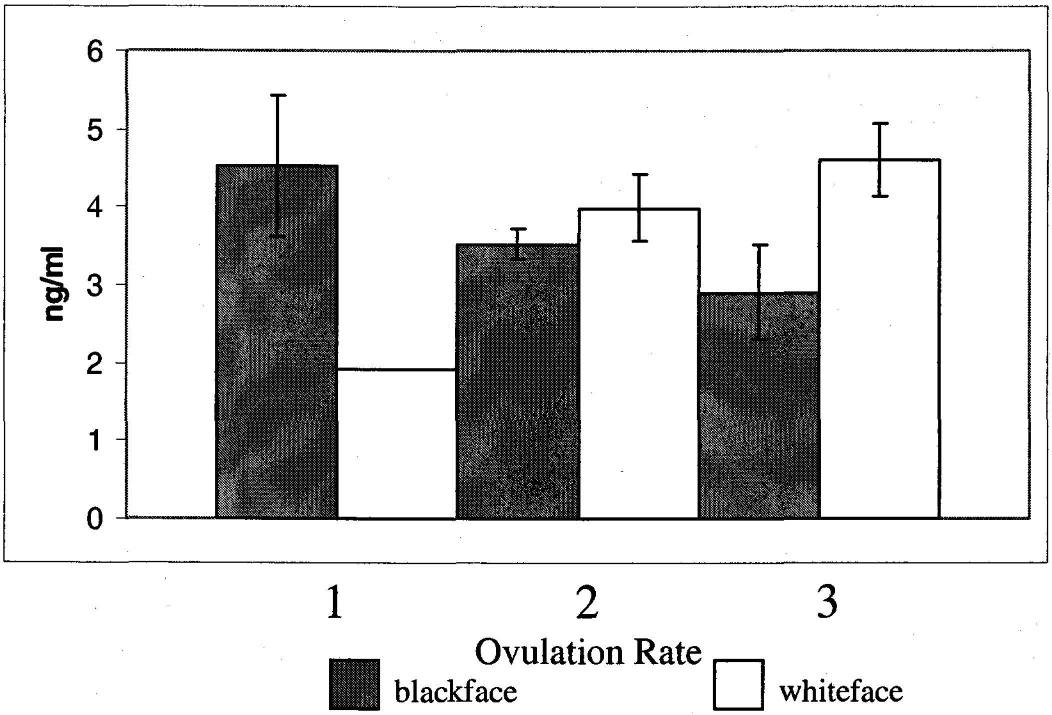

Figure 4.1 Plasma progesterone concentrations (ng/ml, mean ± SE) at day 13 of gestation in ewes having ovulation rates of one, two or three.

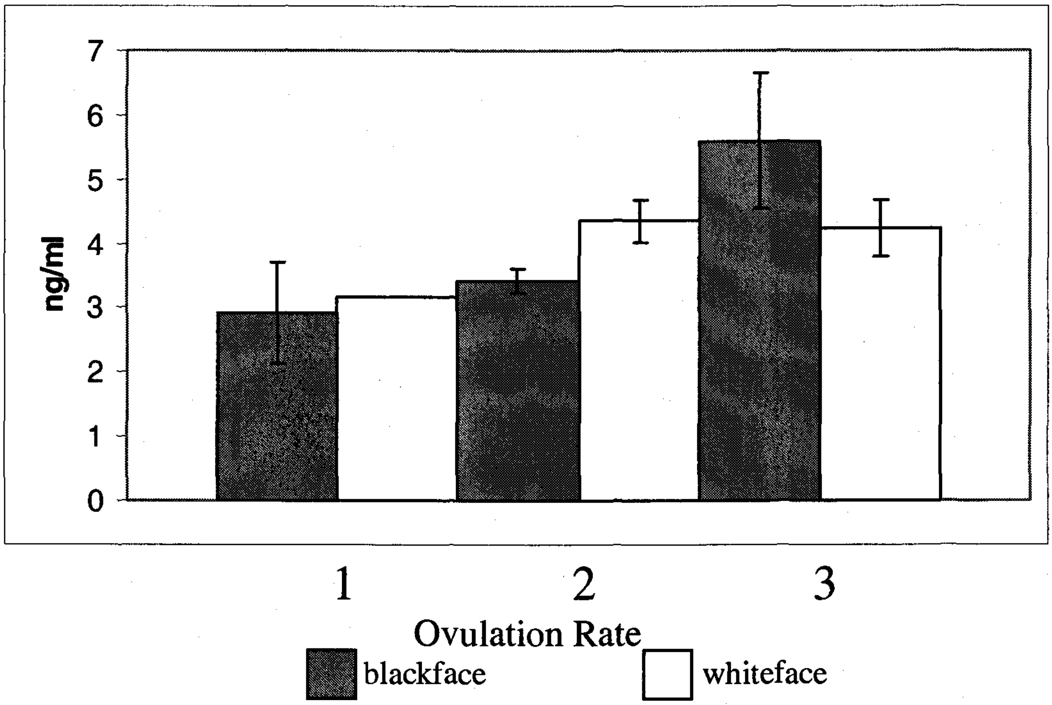

Figure 4.2 Plasma progesterone concentrations (ng/ml, mean ± SE) at day 20 of gestation for ewes having an ovulation rate of one, two, or three.

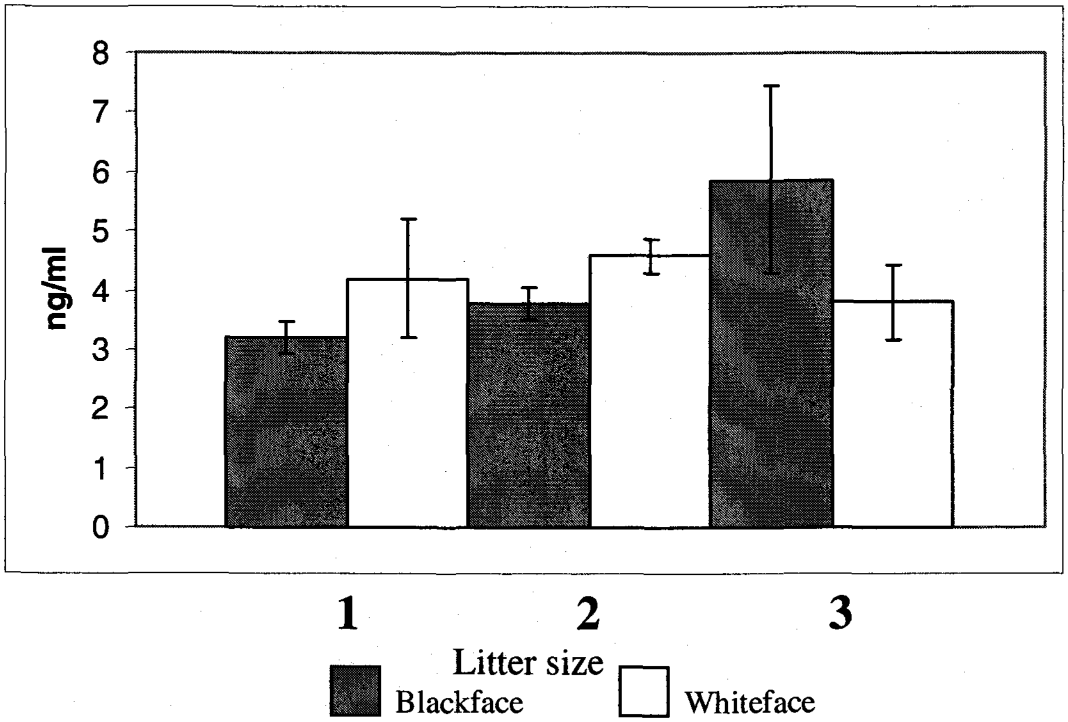

Figure 4.3. Plasma progesterone levels (ng/ml, mean ± SE) at day 20 of gestation for ewes having litter sizes of one, two, or three.

CHAPTER 5: DISCUSSION

Reproductive Efficiency

Uterine efficiency in the experimental flock was high but consistent with the findings of Nawaz and Meyer (1991), a study which used ewes of similar breeding. In both studies, all ewes had at least some Polypay breeding. Additionally, this reproductive trait might be benefiting from hybrid vigor, as none of the ewes are straightbred. Meyer *et al.* (1994) reported that crossbred ewes usually have higher uterine efficiency than their straightbred counterparts.

The nutritional treatment groups generated an average difference of 11 kg and 1.0 condition score units between groups. Despite these differences, litter size within twin- and triple-ovulators did not differ significantly between groups. West *et al.* (1991) reported substantial differences in uterine efficiency between treatment groups for triple-ovulators. Weights of high and low ewes in two separate trials differed by 10 and 18 kg and 1.1 and 2.2 condition score units. The differences in bodyweight and condition score in the present study may be on the edge of the range which will induce embryonic loss. Cumming (1972a) concluded that nutritional status per se did not have as much effect as changes, especially loss, in bodyweight. Ewes in the present study did not lose weight between the beginning of mating and removal of rams. In fact, some in the low group gained weight during this time. Cumming *et al.* (1975) also concluded that extremes of nutritional plane, either high or low, had a detrimental effect on embryo survival.

Interestingly, in the present study, Low triple-ovulators had higher uterine efficiency than High triple-ovulators. The High ewes may have been feeling the effect of their rich diet.

There is anecdotal evidence that breeding to blackface rams depresses uterine efficiency. The present study showed no difference in ova success between ewes bred to blackface rams and ewes bred to whiteface rams. This is consistent with findings by Bradford *et al.* (1974) using embryo transfer to determine that the genotype of the embryo has little effect on the uterine efficiency of the recipient dam.

Plasma Progesterone Levels

The overall plasma progesterone means for day 13 and day 20 were 3.88 and 4.05 ng/ml, respectively. Spencer and Bazer (1995) reported day 13 levels to be 1.5 ng/ml, and while day 20 was not included, days 17 and 25 were, at 1.75 and 2.25 ng/ml, respectively.

Day 13 levels observed in this trial were also higher than those reported by Parr *et al.* (1987). In that study, ewes were assigned to 25%, 100%, or 200% maintenance diets, and half were supplemented with exogenous progesterone. Unsupplemented ewes had mean progesterone levels of 3.2, 2.0, and 1.5 ng/ml, respectively. Interestingly, the highest pregnancy rate occurred among ewes who had hormone levels in the 4-5 ng/ml range, which is close to the mean of 3.88 found in the present study.

Progesterone concentrations in the present study were closer to those reported by Kimya *et al.* (2004), who conducted two trials involving control and fasted ewes. Serum progesterone levels measured on day 11 were approximately 3.0 and 3.5 ng/ml for control ewes and 8.0 and 7.0 ng/ml for ewes which had been fasted for four days.

Treatment Effect

Parr *et al.* (1987) reported an inverse relationship between plane of nutrition and levels of progesterone at day 12 based on data collected from ewes fed a 25%, 100%, or 200% maintenance diet. Cumming *et al.* (1971) reported the same relationship using data from days 12, 15, and 16, although specific progesterone values were not included. The present study found no difference between High and Low group mean plasma progesterone levels at day 13. Day 20 levels approached significance and were consistent with previous findings in that the Low group had a higher mean than the High group. While there are no reports in the literature for nutritional impact on progesterone during implantation, results from Parr *et al.* (1993), Miller *et al.* (1999), and Kimya *et al.* (2004) all show that the effects of feed restriction on metabolic clearance rate of hormone levels continue for as long as they are imposed. Given this information, it was expected that an inverse relationship between feed intake and progesterone levels would exist at day 20, as the present study suggests.

Genotype Effect

Whiteface ewes had higher progesterone levels at both collection times. The difference was significant at day 20 when day 13 was excluded from the model. Whiteface ewes also had higher ovulation rates and litter sizes, which may be at least partially related to their higher progesterone levels.

Interesting variations occurred within the blackface breed when ovulation rate was taken into account. At day 13, hormone level and number of CL's were inversely related but at day 20 they were directly related. A pattern may also exist for whiteface ewes, but is more difficult to judge from the present study which only included one whiteface single-ovulator. Progesterone was higher in whiteface triple-ovulators than twin-ovulators at day 13. However, at day 20, twin-ovulators had a higher concentration of progesterone. Between breeds, whiteface ewes within ovulation rate usually had higher progesterone levels, except in the case of triple-ovulators at day 20, which is interesting considering that blackface triple-ovulators had greater uterine efficiency than whiteface triple-ovulators but blackface twin-ovulators had lower uterine efficiency than their whiteface counterparts.

Reproductive Efficiency

In terms of the industry, predicting litter size would be very useful to producers who could then sort ewes and feed accordingly, giving those carrying three or more lambs adequate nutrition without overfeeding twin and singleton carriers. To this end, Miller *et al.* (2000) studied fecal progestins in early gestation ewes. Hormone levels at days 5, 19 and 30 of gestation were not correlated litter size. However, some progestins, albeit not progesterone, were higher in triple-ovulators than in twin- or single-ovulators at day 19. In the present study, the significance of ovulation rate on progesterone levels is due entirely to triple-ovulators having a much higher mean than the two lower ovulation classes. However, in both Miller's study and the present study, there is enough variation in reported hormone levels to make reliable litter size predictions from these data guarded at best.

Cumming *et al.* (1971) reported that plasma progesterone levels were lower in ewes which experienced embryonic loss, but that the difference did not become significant until day 15. That embryonic loss and levels of progesterone are apparently unrelated in the current study is therefore a little unexpected. A drop in day 13 levels could possibly be an indicator of things to come. At this point it might even be possible to give supplemental hormone to the ewe to ensure implantation. By day 20 however, lowered levels are more likely an indicator of what has transpired. By this time, the implantation process is well underway, and is quite likely that loss of the embryo has already occurred.

CHAPTER 6: CONCLUSION

Embryonic mortality was not affected by genotype of either the ewe or the ram, nor was it affected by plane of nutrition and body condition. The role of progesterone in the event of embryonic loss, either as a cause or an indicator, is unclear based on current data. Progesterone varied with ovulation rate within breed, but was erratic enough to be unreliable as an indicator thereof.

BIBLIOGRAPHY

American Sheep Industry Association, Inc. 1996. Sheep Production Handbook. C&M Press.

Bindon, B. M. 1971. Role of progesterone in implantation in the sheep. J. Reprod. Fert. 24:146

Bradford, G. E., St.C. S. Taylor, J. F. Quirke, R. Hart. 1974. An egg-transfer study of litter size, birth weight and lamb survival. Anim. Prod. 18:249-263.

Cumming, I. A. 1972a. The effects of increasing and decreasing liveweight on ovulation and embryonic survival in the Border Leicester x Merino ewe. Proc. Aust. Soc. Anim. Prod. 9:192-198.

Cumming, I. A. 1972b. The effect of nutritional restriction on embryonic survival during the first three weeks of pregnancy in the Perendale ewe. Proc. Aust. Soc. Anim. Prod. 9:199-203.

Cumming, I. A., B. J. Mole, J. Obst, M. A. de B. Blockey, C. G. Winfield, J. R. Goding. 1971. Increase in plasma progesterone caused by undernutrition during early pregnancy in the ewe. J. Reprod. Fert. 24:146-147.

Cumming, I. A., M. A. de B. Blockey, C. G. Winfield, R. A. Parr, A. H. Williams. 1975. A study of relationships of breed, time of mating, level of nutrition, live weight, body condition, and face cover to embryo survival in ewes. J. Agric. Sci. 84:559-565.

Edey, T. N. 1967. Early embryonic death and subsequent cycle length in the ewe. J. Reprod. Fert. 13:437-443.

Edey, T. N. 1969. Prenatal mortality in sheep: a review. Anim. Breed. Abstr. 37:173-190.

Edey, T. N. 1972. Fertility at the first oestrus following embryonic death. J. Reprod. Fert., 28:147.

Edey, T. N. 1976. Nutrition and ebryonic survival in the ewe. Proc. N. Z. Soc. Anim. Prod. 36:231-239

Jeffries, B. C. 1961. Body condition scoring and its use in management. Tasmanian J. Agric. 32:19.

Jindal, R., J. R. Cosgrove, F. X. Aherne, G. R. Foxcraft. 1996. Effect of nutrition on embryonal mortality in gilts: association with progesterone. J. Anim. Sci. 74:620-624.

Kimya, Z., B. M. Alexander, E. A. Van Kirk, W. J. Murdoch, D. M. Hallford, G. E. Moss. 2004. Effects of feed restriction on reproductive and metabolic hormones in ewes. J. Anim. Sci. 82:2548-2557.

Long, S. E., C. V. Williams. 1980. Frequency of chromosomal abnormalities in early embryos of the domestic sheep *(ovis arres)*. J. Reprod. Fert., 58:197-201

Meyer, H. H. 1985. Breed difference in ovulation rate and uterine efficiency and their contribution to fecundity. R. B. Land and D. W. Robinson (Ed.) Genetics of Reproduction in Sheep. p. 185. Butterworths, London.

Meyer, H. H., G. E. Bradford. 1973. Reproduction in Targhee and Finnish Landrace x Targhee ewes. J. Anim. Sci. 36:847-853

Meyer, H. H., J. N. Clarke, T. G. Harvey, I. C. Malthus. 1983. Genetic variation in uterine efficiency and differential responses to increased ovulation rate in sheep. Proc. N. Z. Soc. Anim. Prod. 43:201-204.

Meyer, H. H., L. R. Piper, B. M. Bindon, R. R. Woolaston. 1994. Litter size and uterine efficiency of Booroola Merinos, control Merinos and their crosses with Border Leicester and Dorset. Livest. Prod. Sci. 38:217-223.

Miller, C. W., H. H. Meyer, D. W. Holtan. 2000. Fecal progestins in th early gestation ewe monitored by gas chromatography/mass spectrometry. Proc., Western Section, ASAS. 51:79-82.

Miller, H. M., G. R. Foxcroft, J. Squires, F. X. Aherne. 1999. The effects of feed intake and body fatness on progesterone metabolism in ovariectomezed gilts. J. Anim. Sci. 77:3253-3261.

Nawaz, M., H. H. Meyer. 1991. Effects of genotype and mating weight on ovulation rate, litter size, and uterine efficiency of Coopworth, Polypay, and crossbred ewes. J. Anim. Sci. 69:3925-3930.

Parr, R. A., I. F. Davis, R. J. Fairclough, M. A. Miles. 1987. Overfeeding during early pregnancy reduces peripheral progesterone concentration and pregnancy rate in sheep. J. Reprod. Fert. 80:317-320.

Parr, R. A., I. F. Davis, M. A. Miles, T. J. Squires. 1993. Feed intake affects metabolic clearance rate of progesterone in sheep. Res. Vet. Sci. 55:306-310/.

Paslay, E. M., J. R. Jaeger, U. Sall, F. Stormshak. 2003. Ovarian function in ewes after treatment with mifepristone early during the oestrous cycle. Reproduction. 125:205-210.

Restall, B. J., G. H. Brown, M. A. de B. Blockey, L. Cahill, R. Kearins. 1976. Assessment of reproductive wastage in sheep. 1. Fertilization failure and early embryonic survival. Aust. J. Exp. Agric. Anim. Husb. 16:329-335.

Spencer, T. E., F. W. Bazer. 1995. Temporal and spatial alteration in uterine estrogen receptor and progesterone receptor gene expression during the estrous cycle and early pregnancy in the ewe. Biol. Reprod. 53:1527-1543.

Spencer, T. E., G. A. Johnson, F. W. Bazer, R. C. Burghardt. 2004. Implantation mechanisms: insights from the sheep. Reproduction. 128:657-668

West, K. S., H. H. Meyer, M. Nawaz. 1991. Effects of differential ewe condition at mating and early postmating nutrition on embryo survival. J. Anim. Sci. 69:3931-3938.