AN ABSTRACT OF THE THESIS OF

Kathryn S. West for the degree of Master of Science in Animal Science presented on April 16, 1990.

Title: Effects of Differential Ewe Body Condition at Mating and Early Post-mating Nutrition on Embryo Survival

Abstract approved: Redacted for Privacy
Howard H. Meyer

Two trials were conducted over consecutive years to examine the effects of ewe body condition and post-mating nutrition on ovulation rates and embryo survival. Trial 1 used 146 Polypay ewes ranging in age from 5 to 8 years in a 3 x 2 factorial array of pre-mating (high-H, low flushed-LF and low unflushed-L) and post-mating nutrition (high and low) treatments. Trial 2 was a 2 x 2 x 2 factorial array of 60 Polypay and 60 Coopworth x Polypay (C x P) three year old ewes, two pre-mating (H and LF) and two post-mating (high and low) treatments. To estimate timing and extent of embryo loss, two methods of embryo detection were employed during Trial 2. Real-time ultrasound was performed on all ewes at 21, 28, 34 and 45 days post-mating. Blood samples were also collected on these days for analysis of Pregnancy-specific Protein B (PSPB) levels. There was no effect of pre-mating treatment or ewe age on ovulation or conception rates for Trial 1. Pre-mating treatment was significant in Trial 2, with H ewes having higher ovulation rates than LF ewes. Pre-mating treatment, post-mating treatment, ewe age (for Trial 1) and genotype (for Trial 2) had no effect on mean litter size in either trial. Analysis of litter size among ewes with twin and triple ovulations showed pre-mating treatment to be significant among triple ovulators in Trial 1, where L ewes had much lower litter size (1.86) than LF (2.37) or H (2.60) ewes. In Trial 2, post-mating treatment was significant among the ewes with triple ovulations, with ewes on low nutrition having lower litter size (2.01) than ewes on high nutrition (2.59). Two-way interactions were significant among twin and triple

ovulators in Trial 2. Breed x pre-mating interaction among twin ovulating ewes showed C x P LF ewes to have lower litter size (1.43) than the H ewes (1.87), while the opposite was true for Polypay ewes. Pre- x post-mating treatment interaction among triple ovulators showed LF ewes on low post-mating nutrition had lower mean litter size. This effect is largely due to lower litter size in the C x P triple ovulators on low post-mating nutrition. Data available from the ultrasound diagnosis showed little indication of treatment effects on amount or time of embryo loss. Ewes bearing single or multiple embryos differed in PSPB level at day 45, but not at earlier times. However, there were no differences in PSPB levels in ewes with twin or triple embryos at any of the trial days. Assay for PSPB failed to facilitate detection of the amount or time of embryo loss, as determined by ultrasound.

Effects of Differential Ewe Body Condition at Mating and Early Post-mating Nutrition on Embryo Survival

by

Kathryn S. West

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Completed April 16, 1990

Commencement June 1991

Approved:

Redacted for Privacy

Professor of Animal Science in charge of major

Redacted for Privacy

Head of department of Animal Science

Redacted for Privacy

Dean of Graduate School

Date thesis presented April 16, 1990

Typed by Kathryn S. West

ACKNOWLEDGEMENTS

First and foremost, I wish express my deepest gratitude to Dr. Howard H. Meyer for his guidance, patience and concern, without which I probably would not have survived the last months of my graduate program. A special thanks to Dr. R. G. Sasser and his staff at the University of Idaho for their help in running the necessary assays.

I would also like to thank Karen Swanson for hugging all those sheep during sample collection, and my fellow graduate student, Mohammed Nawaz, and Bob Klinger and the staff of the OSU Sheep Center for assistance in laparoscopies and other procedures.

Thanks go to Roswitha Herzog for her help in the entire process and for her unbridled curiosity which was an inspiration to me; to Rob and Sarah Lewis for all the sound advice and sympathy; to Dr. Dave Thomas for consultations regarding the SAS program; to Brad Wilkin for listening to hours of sheep talk; to Tom Utterback for the ear and the shoulder and for super-human patience; and to Apple Computers, Inc. for inventing the Macintosh--if you have to ask why, you wouldn't understand.

Lastly, and most importantly, I thank my family, especially my parents, Jim and Joyce, for their undying love and support. They may not always understand everything I talk about, but without their help and confidence in me, I would never have come so far. Everything I am I owe to them, which, to date, comes to about $1,758,978.42.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

EFFECTS OF DIFFERENTIAL EWE BODY CONDITION AT MATING AND EARLY POST-MATING NUTRITION ON EMBRYO SURVIVAL

CHAPTER 1

INTRODUCTION

Increasing ewe productivity has long been a major concern for producers in the sheep industry. The profits of a sheep operation are largely dependent on the number of lambs born per ewe mated. In 1988, the United States Department of Agriculture estimated that the average national lambing rate was only one lamb per ewe mated. Since the first lamb may pay for yearly maintenance costs of the ewe, profits are derived from subsequent lambs, and it is easy to understand the large amounts of research devoted to increasing ewe productivity.

Lambing rate is dependent on three factors; ovulation rate, fertilization rate and embryo survival. Much research has explored ways of increasing these factors, especially ovulation rate and embryo survival.

The genotype of the ewe has an effect on both ovulation rate and embryo survival. Meyer (1985) found higher ovulation rates in Booroola Merino and Finn-sired ewes than Romney straight-bred ewes. This investigator also found higher uterine efficiency rates (defined as the marginal response in litter size from one more egg ovulated among multiple ovulators) in Border Leicester x Romney and Booroola x Perendale-sired ewes than in the Romney straight-bred ewes.

Nutrition of the ewe before and during gestation has also been considered for its effects on ewe productivity. Flushing is defined as increasing energy feeding level of ewes in moderate or poor body condition for a short period immediately prior to mating to increase ovulation rate. Bramley et al. (1976) used flushing to increase mean ovulation rates for ewes on low nutrition and found the rates nearly equal to those of ewes on high nutrition. Pre-mating nutrition, as reflected by live weight and condition score has also shown an effect on embryo survival, with ewes in higher condition and/or live weight at mating having better embryo survival rates (Guerra et al., 1971; Doney et al., 1975; Gunn and Maxwell, 1989).

Post-mating nutrition may also have an effect on embryo survival. Nutrition levels of < 25% recommended maintenance energy levels have been shown to decrease embryo survival (Cumming, 1972b; Edey, 1966). Studies using nutrition levels higher than 25% recommended maintenance energy levels have reported no effects (Bellows, 1974; Parr et al., 1982).

The intent of this study was to examine the effects of pre-mating nutrition (as measured by body condition) and post-mating nutrition on embryo survival. Effects of breed of the ewe on embryo survival were also considered. The difference between ovulation rates and litter size was used to estimate embryo survival rates.

CHAPTER 2

LITERATURE REVIEW

Ewe reproductive performance may be the single most important factor determining the profitability of the sheep industry. It is estimated that a single lamb may pay for the yearly maintenance costs of the ewe, but that any profits come from multiple births. With a national lambing rate estimated near 100% (USDA, 1988), or one lamb born per ewe over one year of age, it is not difficult to understand why so much research has focused on ways to increase ewe productivity.

Factors that influence litter size can be divided into three groups--those which influence ovulation rate, those which act on ova fertilization rate, and those which influence embryo survival. Due to lack of information on fertilization rates, this review will focus on studies of ovulation rate and embryo survival.

Potential lambing rate is set by ovulation rate. Many factors can have an influence on ovulation rate, such as season of breeding, breed and age of the ewe, ewe live weight, body condition and pre-mating nutrition level.

Hulet et al. (1974) looked at effects of season of breeding on ovulation rates of mature Panama ewes. They used three seasons of breeding--September, November and January, and found mean ovulation rates of 2.05, 1.98 and 1.78, respectively. It was concluded that ovulation rate was at a peak from September through early November, followed by a general decline through the end of the study on February 4.

Newton et al. (1980) found a similar pattern for litter size in Masham ewes mated in September, October or December. Mean litter size gradually declined as the season passed (2.4 for September mating and 2.2 for December mating). Because ovulation rates were not determined, it is not possible to determine if the change in litter size was due to declining ovulation rate or some other factor.

Age of the ewe has a considerable influence on ovulation rate. When Fahmy and Dufour (1988) compared ovulation rates of Finn-cross ewes ranging in age from 1.5 years to 3.5 years, they found ovulation rates increased from 2.36 for the 1.5 year-olds, to 2.53 for the 2.5 year-olds and 2.90 for the 3.5 year-olds. Meyer (1985) also found ovulation rates to increase with age of ewe in 1100 ewes of widely different breeds. The mean ovulation rates were 1.35 for 1.5 year-olds, 1.56 for 2.5 year-olds and 1.64 for 3.5 year-olds, with nearly all genotypes showing an increase in ovulation rate with each increase in age.

Genotype of the ewe can also have a significant effect on ovulation rate. This is most readily observed as differences among breeds, but can also be detected between lines selected for differing levels of reproductive performance. Meyer (1985) compared ovulation rates of ewes from Romney dams and eight breeds of sires. Ovulation rates were highest in Booroola Merino (2.06) and Finn (2.04) sired ewes, with Romney straight-bred ewes having the lowest rates (1.06).

Fahmy and Dufour (1988) studied the cumulative effect of Finnsheep breeding in ewes ranging from 1/8 to 7/8 Finn, and found that ovulation rates increased linearly with increased percentage of Finn breeding, from 1.78 for 1/8 Finn to 3.20 for 7/8 Finn breeding. Hanrahan (1985) also examined ovulation rates of mature Finn-cross ewes. In Finn, Galway and Fingalway (Finn x Galway) ewes, he found ovulation rates of 3.46, 1.67 and 2.43, respectively.

Meyer and Clarke (1982) looked at differences in ovulation rates among Romney, Border Leicester and Border Leicester x Romney ewes. Mean ovulation rates were 1.74, 2.14 and 1.99, respectively. They also examined ovulation rates in three lines of Romney ewes that differed greatly in reproductive performance due to selection for litter size over a 20 year period. Three lines were used in the study--a line selected for high reproductive performance, a line selected for low reproductive performance and a line randomly selected (control). Mean ovulation rates of lambing ewes were 2.06 for ewes from the high group, 1.48 for the control group and 1.26 for the ewes from the low group.

Nutrition is a large cost for most producers and, of all factors affecting litter size, it is the one most easily controlled by the producer. Nutrition affects ovulation rates through several closely linked factors--live weight, body condition and pre-mating nutrition level. Live weight of the ewe is often used to estimate nutritional status; however, due to difference in skeletal size, nutritional state may be highly variable among individuals of the same weight.

Cumming (1972a) studied two groups of Border Leicester x Merino ewes, one of high initial live weight and one of low initial live weight. The low group was fed to increase live weight to time of mating and the high group was fed to lose weight to time of mating. The groups achieved nearly the same mean live weight at time of mating. Ewes on the decreasing nutritional plane had lower ovulation rates (1.59) than the ewes on the increasing plane (1.74).

Using a single flock of Merino ewes, Guerra et al. (1971) arbitrarily grouped them according to live weights for comparison of ovulation rates relative to weight. Groups with mean weights of 25.6kg, 29.7kg, 35.0kg and 40.3kg had mean ovulation rates of 1.53, 1.77, 1.75 and 2.06, respectively. In another study, Guerra et al. (1972) examined a flock of Merino ewes which ranged in weight from 27 to 64kg. Ewes were arbitrarily grouped for data analysis into five subsets according to weight; 27-34kg, 35-39kg, 40-44kg, 45-49kg and 50-64kg. Mean ovulation rates increased steadily from 1.04 for the lowest weight group to 1.54 for the highest weight group. In a study by Bramley et al. (1976) using mature Masham ewes, those fed to achieve mean live weights of 49.3kg had ovulation rates of 1.53, while ewes fed to achieve mean live weights of 70.6kg had mean ovulation rates of 2.27.

Meyer and Bradford (1973), using Targhee and Finn x Targhee ewes, examined the effect of pre-mating nutrition by feeding ewes to produce final weights of approximately 80, 90 and 105% of initial body weight. Ewes fed to reach 105% of initial weight had higher ovulation rates (1.94 for Targhee and 2.72 for Finn x Targhee) than ewes fed to reach 80% (1.62 for Targhee and 2.52 for Finn x Targhee) or 90% (1.71 for Targhee and 2.57 for Finn x Targhee) of initial weight .

Body condition score is a subjective estimation of fatness of an animal. It is commonly assessed on a 1 to 5 point scale, including half points, with a score of 1.0 being very thin and 5.0 being obese (Jefferies, 1961). Condition score may be a better estimator of nutritional state of the ewe than live weight, as skeletal size does not contribute as much to variation in nutritional state in ewes of the same condition score as it does in ewes of the same live weight. Ewes in higher condition (>3) generally have higher ovulation rates than ewes in lower body condition.

Rhind et al. (1984a) examined mature Greyface ewes in moderately fat condition (mean score 2.75) and fat condition (mean score 3.5). Mean ovulation rates were 2.33 for the moderately fat ewes and 3.36 for the fat ewes. Gunn et al. (1969) compared ovulation rates between Scottish Blackface ewes in condition score 1.5 or 3.0. Higher body condition at time of mating resulted in higher ovulation rates (2.07 vs. 1.15). Gunn and Doney (1979) compared ovulation rates from Cheviot ewes in mean condition score 2.0 or 3.0. Ewes in condition score 3.0 had significantly higher mean ovulation rates (1.61) than those of condition score 2.0 (1.19).

Both condition score and live weight are closely associated to pre-mating nutrition level. "Flushing" is the term used to describe the practice of increasing energy feeding level of ewes in moderate or poor body condition for a short period (about 3 weeks) immediately prior to mating in order to increase ovulation rate. Effects of pre-mating nutrition level on ewe production have been well explored, and the flushing effect is well documented (El Sheikl et al., 1955; Coop, 1966; Edey, 1966; Killeen, 1967).

Bramley et al. (1976) applied differential pre-mating levels of nutrition for a 10 week period immediately before mating. Ewes were on either high or low nutrition for 6 weeks, after which time half of the low ewes were switched to higher nutrition for the last 4 weeks prior to mating, while the remainder of the low ewes stayed on the low nutrition. Ovulation rates were 1.53, 2.13 and 2.27 for the LL, LH and HH groups, respectively.

Pre-mating nutrition effects were examined in a study by Gunn and Doney (1979) by differentially feeding Cheviot ewes to reach moderately good condition (mean score 2.5). Ewes of condition score 3.0 were fed low nutrition to drop to the desired condition score (2.5), while ewes which were in condition score 2.0 were fed to gain condition to the target condition score over the 5 week period before mating. Ovulation rates were higher in those ewes which were on an increasing plane of nutrition to reach the desired condition score (1.60) than those on a decreasing plane (1.23).

Cumming et al. (1972a) subdivided a flock of Border Leicester x Merino ewes into two groups which were fed either submaintenance (low quality pasture) energy levels or flushed on irrigated pasture for 10 days. Mean ovulation rates were 1.57 for the submaintenance group and 1.69 for the flushed group. Even this short flushing period resulted in a higher ovulation rate than the submaintenance diet.

Gunn et al. (1984) looked at the differential effects of duration and timing of high level feeding prior to mating. Scottish Blackface ewes were fed on maintenance (M) or high (H) level feed for each of four 9 day periods prior to mating, a total of 36 days in all. The treatment groups were: one period of maintenance followed by three periods of high feed (MH_3); two periods of maintenance followed by two periods of high feed (M_2H_2); two periods of high feed followed by two periods of maintenance (H_2M_2); and three periods of high feed followed by one period of maintenance (H_3M). Highest ovulation rates were found in MH_3 ewes (1.80) and H_3M ewes (1.73). A significant difference was noted between ewes of the M_2H_2 and H_2M_2 groups. Both received the same duration on each feed level, but the M_2H_2 group of ewes had ovulation rates nearly equal (1.79) to the MH_3 group of ewes, while the H_2M_2 ewes had much lower ovulation rates (1.48). It was concluded that high level feeding during the last two periods (18 days) prior to mating resulted in an increase in ovulation rates.

In another experiment exploring the influence of length of time of high energy feed on ovulation rate, Rhind et al. (1983) placed Scottish Blackface ewes on different durations of high level feed. Ewes which had only 4 weeks of high feed before mating had similar ovulation rates (1.47) to ewes which were given 8 weeks of high level feed (1.46).

Allen and Lamming (1961) flushed ewes on good pasture for 4, 8 or 12 weeks or fed them a submaintenance diet (low quality pasture) for the same time periods. A control group was fed maintenance for the entire period. Ovulation rates were considerably higher in flushed ewes (2.17) when compared to the control ewes (1.50). There was no difference in ovulation rate between the submaintenance group and the control group. There were also no benefits to extending flushing beyond 4 weeks, because ewes in all flushing groups had similarly high ovulation rates.

These reports demonstrate that a major role is played by pre-mating nutrition in determining ovulation rates. Higher rates are found in ewes which are in high body condition or live weight, or which have been exposed to high energy feed during the last 2-3 weeks before mating.

Another factor affecting litter size is failure of the fertilized ova, i.e. embryo loss. Many of the same factors which effect ovulation rates are thought to have an affect on embryo survival. There are also the added effects of post-mating stresses, such as disease, environment and nutrition. In examining post-mating stresses, this review will deal mostly with nutritional stresses, or lack thereof, as these are most easily controlled by the producer.

It is estimated that embryonic loss can account for about 20-30% of the failure of ova to become lambs (Edey, 1969). Much research has explored possible factors affecting this source of loss (Gunn et al., 1969; Killeen, 1967; Guerra et al., 1971; Cumming et al,. 1972ab; Gunn et al., 1972; Gunn et al. 1975; Bramley et al., 1976; Gunn et al. 1979; Newton et al., 1980; Rhind et al. , 1984ab; Hohenboken et al., 1988; Gunn and Maxwell, 1989; Rhind et al., 1989).

There has been some debate over the effect of number and site of ovulations on embryo survival rates. Doney et al. (1973) examined 467 mixed age Cheviot and Scottish Blackface ewes on the 20th day post-mating and classified ovulations as unilateral twin ovulators, bilateral twin ovulators, or single ovulators. Embryo success rate (estimated by the difference between ovulation rates and number of lambs born) was significantly lower in the unilateral twin ovulators (78%) compared to the bilateral twin ovulators (89%). Extensive work on this question was performed by Kelly and coworkers (1976ab, 1982, 1983, 1987). In 1987, 665 ewes were examined by laparoscopy on days 22 or 35 post-mating and sites of corpora lutea were compared to subsequent lambing rates and barrenness. Among multiple ovulators, there was no significant effect of site of ovulation on either proportion of barren ewes or the partial failure of twin ovulators. Earlier (Kelly et al., 1982), results of an exhaustive study of over 2500 ewes from New Zealand flocks showed no effect of site of ovulation on partial failure of twin ovulators. These results agree with the results from the later studies. White et al. (1981), after examining Merino ewes by laparotomy on day 20 post-mating, found no difference in average number of eggs ovulated from each ovary, and no significant difference in embryo survival rates (estimated by difference between ovulation rate and litter size) between unilateral (82%) and bilateral (84%) twin ovulators.

It could be that the difference shown between unilateral and bilateral twin ovulators in the report by Doney et al. (1973) was due in part to sampling error or interaction between site of ovulation and some other factor affecting embryo survival.

Embryo survival can also be affected by the age of the ewe. Hasnain (1964) compared survival rates of embryos between mature and maiden ewes. Percentage of corpora lutea represented by embryos at time of slaughter 18 days post-mating was 70.9% in mature ewes versus 61.5% in maiden ewes. Lahlou-Kassi et al (1981) compared embryonic survival between nulliparous (maiden) and multiparous (mature) ewes of the D'Man breed. They found 58% survival of ova in the mature ewes and 42% in the maiden ewes as measured by percentage of ovulations represented by lambs. The effect of age on embryo survival shows older ewes to have better embryo survival than maiden ewes.

Many studies have looked at the effect of breed on litter size. It has been shown that breed has an effect on ovulation rate, and genetic variation within breed may also have an effect on embryo survival.

Fahmy and Dufour (1988) examined effects of increased Finn breeding on embryo survival using ewes ranging from 1/8 to 7/8 Finn. Increased Finn breeding accounted for increased ovulation rates, but crosses with greater than 1/4 Finn breeding had nearly the same amount of embryonic survival (between 71 and 74%) when compared at the same ovulation rates. Ewes of less than 1/4 Finn breeding had embryo survival rates of about 80%. Higher litter size in ewes of greater percentage Finn breeding is, therefore, due to increased ovulation rates and not increased embryo survival.

Meyer (1985) used the term uterine efficiency to define the marginal response in litter size from one more egg ovulated, measured among multiple ovulators. He compared the uterine efficiency estimates of various breeds to the Romney. Mean uterine efficiency of twin ovulating ewes examined showed Border Leicester x Romney (.79) and Booroola Perendale (.77) to have the highest estimates and to be statistically different from Romney straight-breds (.59) which were used as the basis of comparison.

Meyer and Clarke (1982) also estimated uterine efficiency in Romney, Border Leicester and Border Leicester x Romney ewes as a way of comparing embryo survival between the genotypes. There was no significant difference between the genotypes, but Border Leicester x Romney ewes had slightly higher uterine efficiency estimates. These investigations also examined Romney ewes from lines selected for high or low reproductive performance over 20 years in a closed flock. Uterine efficiency in ewes from the high line did not differ from the control (randomly selected) group. Ovulation rates in ewes from the low line were too low to provide a reliable estimate of uterine efficiency.

Trounsen and Moore (1972) used Merino ewes from lines which had been selected over a long time for or against multiple births in an attempt to determine their ability to support multiple ova. Embryo transfer was conducted with donor and recipient ewes from the two selection lines. Eggs were transferred at rates of 1 to 3 eggs/ewe. When multiple eggs were transferred into the ewes, there was no effect of either origin of the egg or genotype of the ewe on survival of transferred ova. It was concluded that either group was equally capable of supporting multiple embryos.

The results from studies exploring the effects of genetic variability on embryo survival are not consistent and make drawing conclusions difficult. Effect of genotype on embryo survival may not be sorted out until a reliable method of measuring embryo survival is found.

Nutrition is another factor which may affect embryo survival. Effect of pre-mating nutrition on embryo survival is closely associated with the effects of live weight and body condition. Embryo survival can also be affected by post-mating nutrition levels.

Guerra et al. (1971) studied Merino ewes selected from a large flock and fed to achieve two different mean live weights. Ewes were subjected to laparotomy for estimation of ovulation rates on day 7 of gestation and autopsied on day 30 for embryo counts. Results showed ewes of both groups had similar mean ovulation rates (1.76 for the low group and 1.81 for the high group) but embryo wastage (measured as percentage ova shed not represented by embryos) was 70% for ewes with mean live weight of 20kg and 55% for ewes with mean live weights near 35kg. These are extremely low weights and are likely to be the cause of embryo loss rates being so high (average reported rates are around 20-30%).

Rhind et al. (1984b) studied Scottish Blackface ewes of two mean live weights (60kg and 40kg) and compared number of embryos recovered at slaughter with ovulation rates. Mean embryo survival rates were in the 80% range for both groups. It was concluded that live weight had no effect on embryo survival.

Gunn and Maxwell (1989) examined effects of live weight change on embryo loss to 30 days of gestation in two groups of Greyface ewes. Live weights of the groups were nearly equal at time of mating (69.2kg and 67.1kg) with one group having lost weight and the other having gained weight to time of mating. Ewes which lost weight had lower mean embryo numbers (1.58) than ewes which gained weight (1.96) to time of mating.

Body condition also has an effect on embryo survival. Guerra et al. (1971) studied Merino ewes ranging in condition score from .5 to 4.5 (on a scale from 0 to 5) . Mean embryo wastage rates, as measured at time of slaughter, ranged from 88% to 44%, with higher losses occurring in ewes with condition scores below 2.0. Guerra et al. (1972) examined effects of components of body weight on reproductive efficiency, using groups fed to high or low body condition score at mating. While condition score affected ovulation rates, there was no effect on embryo wastage (about 44% for all groups). This report may present some bias, as single ovulators were included in the analysis, and embryo wastage for a single ovulator is either 100% or 0%. Gunn and Doney (1975) compared ewes which had reached target condition score by gaining or losing condition. Scores were 2.5 and 1.5 at mating, with ewes gaining or losing half of a point to reach these levels. Results showed that ewes whose condition score decreased had lower embryo survival (51%) than those whose condition score increased (80%). Gunn and Doney (1979), in two experiments over two years with Cheviot ewes, examined embryo survival and condition score. Ewes with condition score 3.0 had mean embryo survival rates of 82.5% while ewes of condition score 2.0 had mean rates of 60.5%.

These studies indicate that high body condition scores result in higher embryo survival. The report by Guerra et al. (1972), which showed no difference in embryo wastage among ewes of high and low condition did not indicate actual condition scores. It may be that lack of effect in their study was due to condition scores not being different enough, or being above a certain threshold level.

Another factor to consider is post-mating nutrition levels. Results from studies examining this factor are not conclusive. Nutrition in late gestation is important for health of the ewe and fetus, and effects of differential nutrition levels during this time on production are well documented (Russel et al., 1981; Gunn et al., 1986; Wilkinson et al., 1988). Effects of differential nutrition in early pregnancy are less clear.

Parr et al. (1982) fed Merino ewes 100% maintenance energy requirements for the high group, or 25% of maintenance energy requirements for the low group, from mating to time of examination (day 11 or 21 post-mating). Ewes were examined by laparotomy and the reproductive tract was flushed to recover embryos. Embryo survival rates did not differ at either stage of pregnancy. All groups had mean embryo survival rates in the 80% range. Cumming et al. (1972b) randomly assigned ewes to a restricted feed level of 2kg of good hay per ewe per day for either one, two or three weeks during the first three weeks of gestation. The control group remained unrestricted the entire period. Three treatment groups were restricted for only one week (days 0-7, 7-14 or 14-21), three groups were restricted for two weeks (days 0-14, 7-21, or 0-7 and 14-21) and one group that was on the restricted feed for all three weeks (days 0-21). Ewes were slaughtered at day 28 post-mating for estimating numbers of embryos and corpora lutea. Results showed that increasing the length of nutritional restriction decreased embryonic survival; 56% embryo survival in the control (unrestricted ewes), 50% embryo survival in ewes restricted 14 or 7 days, and 43% embryo survival in ewes restricted all 21 days. In a similar study, Edey

(1966) restricted ewes to 15% of maintenance energy requirements for days 0-7, 6-13 or 13-20 of gestation. He also included two control groups which were unrestricted. Results showed a 10-12% decrease in number of lambs born for those ewes on restricted intake during any period of the first three weeks of pregnancy compared to ewes fed 100% maintenance requirements during the same time period. Bellows (1974) fed ewes on a high plane of nutrition for the first 140 days of gestation and found no effect on embryo survival compared with ewes on maintenance, but there was some interaction between breed of the ewes and level of nutrition. His suggestion was that some breeds may be more sensitive to levels of post-mating nutrition.

Part of the difficulty in assessing effects of post-mating nutrition on embryo survival stems from the difficulty in separating embryo loss from other parameters, such as conception failure. Most studies show no effects of post-mating nutrition levels above 25% maintenance energy requirements on embryo survival.

Extent and timing of embryonic loss is difficult to estimate. Over the years, several techniques have been used, each with its own advantages and disadvantages. Recent technological advances allow for better estimates than older experimental techniques and can lead to better understanding of factors which may play important roles in embryo survival.

The technique used most widely before the 1960's was serial slaughter. This involved slaughtering all or some of the ewes in the experiment and examining the reproductive tracts for corpora lutea (to estimate ovulation rate) and fertilized or unfertilized ova. Fertilized ova were then examined to determine viability of the embryo.

Braden (1971) used this technique in estimating embryo survival in Merino ewes. A few ewes were killed 2 days post-mating to estimate fertilization and ovulation rates, and the remainder of the experimental animals were killed on day 24 or 38 of gestation. Embryo survival was estimated by comparing numbers of viable embryos with ovulation rates. It was assumed that fertilization rates were equal in all groups and no difference was found among treatment groups in estimated embryo survival.

An advantage of the serial slaughter technique is that it permits direct examination of the reproductive tract and embryos, thus allowing estimation of both early losses and fertilization rates. The disadvantages include the expense in both labor and animals, if there are many treatment groups or if the experiment is repeated for several consecutive years, and inability to obtain repeat observations for any individual ewe.

Another technique involves using laparotomy or laparoscopy to obtain an estimation of ovulation rates. Laparotomy involves exposing the ovaries surgically, while laparoscopy, a recent development, uses a small fiber optic instrument inserted into the abdominal cavity to count corpora lutea. Ovulation rates are then compared to final lamb numbers or embryos recovered from autopsy of sacrificed ewes.

Parr et al. (1982) used laparotomy in 320 Merino ewes to estimate ovulation rates of ewes on differing planes of post-mating nutrition and compared ovulation rates with number of lambs born. Kelly et al. (1976b) used laparoscopy to estimate ovulation rates in over 2000 ewes and found that the technique had no adverse effects on embryo survival. Even repeated laparoscopy did not increase embryo loss.

Laparotomy and laparoscopy have the advantages of providing an estimate of ovulation and embryo survival rates without sacrificing the ewe, and allow for repeat observations on any individual ewe. However, fertilization failure and embryo loss cannot be separated by this method, and it is difficult to estimate the time of embryo loss.

Real-time ultrasound may also be used for estimating embryo loss, when coupled with estimations of ovulation rates. Haibel (1986) stated that the threshold for pregnancy detection with real-time ultrasound is around 25 days of gestation. He went on to say that fetal numbers are not easily diagnosed before 40 days of gestation. Most reports agree with the recommendation that fetal counts should be undertaken after day 40 (Wilkins, 1984ab; 1982; White et al., 1984).

A report from the Department of Agriculture, New South Wales, Australia (1985) discussed various techniques in using real-time ultrasound for pregnancy diagnosis. The report compared and contrasted the methods of transrectal scanning versus abdominal scanning. Transrectal scanning is used before day 35, while the uterus is still held very high in the body cavity. Abdominal scanning can be used for nearly any stage of gestation; however, before day 40 the uterus may be too high to permit a clear image, and after day 70 the uterus and lambs have dropped quite low and may be too large to distinguish without considerable movement of the probe. The conclusions of the report indicate that transrectal scanning is not as reliable as abdominal scanning for diagnosing embryo numbers after day 30 of gestation.

Another factor in the reliability of the ultrasound diagnosis is operator proficiency. The New South Wales report (1985) indicated as much as 5% difference in error rates between operators in detecting twins versus singles. Wilkins (1984a) showed an overall accuracy rate of around 95% for a single operator when diagnosing litter size after day 40 of gestation. This translates to 5% of ewes carrying twins being diagnosed as singles. Error for ewes carrying singles being diagnosed as twin bearers was very small (<1%).

Ultrasound has the advantage of allowing a non-invasive look at the uterus and its contents. The drawbacks are that the equipment is expensive and accuracy of diagnosis will depend largely on operator experience and stage of gestation. Also, the procedure can be fairly labor intensive, depending on the technique used.

In 1987, Ruder et al. reported that sheep sera contained a blood antigen which cross-reacts with Pregnancy-specific Protein B (PSPB), a protein used for pregnancy detection in cattle. When compared with ultrasound diagnosis on day 38 of gestation, PSPB was 99% accurate in diagnosing pregnancy, while ultrasound was 98% accurate (errors in pregnancy detection were in diagnosing pregnant ewes as open). It was concluded that PSPB is as accurate as ultrasound in diagnosing pregnancy at this stage. Comparing ultrasound detection and PSPB results at day 26 found PSPB to be more accurate than the ultrasound. Pregnancy-specific Protein B, therefore, is a good early test for pregnancy in sheep.

Because PSPB increases throughout pregnancy and because it is a product of the embryo tissues, it may be a convenient way of assessing litter size before parturition. Only one study has been published on this topic to date. Ruder-Montgomery et al. (1988) analyzed blood samples from 70 ewes of the Romney, Polypay and Targhee breeds 18, 25 and 37 days after the end of a 60 day breeding period. Pregnancy-specific Protein B results from these times were compared to embryo numbers recovered from slaughter 60, 90 and 120 days after the 60 day mating period, respectively. It was found that ewes bearing twins had significantly higher PSPB levels than ewes bearing singles after day 37 of gestation. Further studies are needed to verify these findings.

CHAPTER 3

THE EFFECTS OF NUTRITIONAL STATE OF THE EWE ON OVULATION RATE, CONCEPTION RATE AND EMBRYO SURVIVAL

K. S. West and H. H. Meyer

ABSTRACT

Two trials were conducted over consecutive years to examine the effects of pre- and post-mating nutrition on ovulation rates and embryo survival. Trial 1 used 146 Polypay ewes ranging in age from 5 to 8 years in a 3 x 2 factorial array of pre-mating (high-H, low flushed-LF and low unflushed-L) and post-mating nutrition (high and low) treatments. Trial 2 was a 2 x 2 x 2 factorial array of 60 Polypay and 60 Coopworth x Polypay (C x P) 3 year old ewes, two pre-mating (H and LF) and two post-mating nutrition (high and low) treatments. Pre-mating treatment had no effect on ovulation rate in Trial 1 but was significant in Trial 2, with ewes on H pre-mating treatment having higher ovulation rates than LF ewes. Ewe age (for Trial 1) and genotype (for Trial 2) had no effect on ovulation rate. Pre-mating and post-mating treatments, ewe age (for Trial 1) and genotype (for Trial 2) had no effect on mean litter size in either trial. Analysis of litter size among ewes with twin and triple ovulations showed pre-mating treatment effect to be significant among triple ovulators in Trial 1, where L ewes had much lower litter size (1.86) than LF (2.37) or H (2.60) ewes. In Trial 2 post-mating treatment was significant among the triple ovulators, with ewes on low nutrition having lower litter size (2.01) than ewes on high nutrition (2.59). Two-way interactions were significant among twin and triple ovulators in Trial 2. Breed x pre-mating treatment interaction among twin ovulating ewes showed C x P LF ewes to have lower litter size (1.43) than the H ewes (1.87), while the opposite was true for Polypay ewes. Pre- x post-mating treatment interaction among triple ovulating ewes showed LF ewes on low post-mating nutrition had lower mean litter size. This effect was largely due to lower litter size in the C x P triple ovulators on low post-mating nutrition.

INTRODUCTION

Many factors effect the prolificacy of ewes. Nutrition is one such factor and is most easily controlled by producers and has therefore received much study. The successful use of increased pre-mating nutrition (often referred to as "flushing") to increase ovulation rate is well documented (Rhind et al., 1983; Gunn and Maxwell, 1989).

Coop (1966) stated that nutritional effects on ovulation rate and litter size are exhibited as both dynamic and static effects. Ovulation rate and litter size are closely correlated with body condition, i.e. the static effect. Flushing leads to an increase in ovulation rate and litter size over what is expected due to body condition alone, i.e., a dynamic effect of short term good feed.

It has been estimated that nearly 30% of ova fail to become lambs (Edey, 1966). Pre-mating and post-mating nutrition, ewe age and breed of the ewe can all contribute to this loss (Edey, 1966; Gunn and Doney, 1975; Gunn and Doney, 1979; Meyer, 1979; Meyer, 1985; Fahmy and Dufour, 1988).

The objectives of this study were to assess the effects of differential pre-mating and early post-mating nutrition and ewe genotype on ovulation rate and embryo survival.

MATERIALS AND METHODS

Two trials were conducted over consecutive years. Trial 1 consisted of 146 white-faced, 5 to 8 year old ewes of >3/4 Polypay breeding (hereafter called Polypay), allocated to a 3 x 2 factorial array of pre-mating treatments and post-mating nutrition levels. Ewes were placed on similar pasture for the 4 months preceding trial initiation in late June. At trial initiation they were condition-scored on a 1 to 5 point scale, including half points, as described by Jefferies (1961), and allocated randomly within age and condition score to one of three pre-mating treatments 15 weeks prior to mating. Treatment 1 (high = H) ewes (n = 48) grazed pasture supplemented with alfalfa pellets while treatment 2 (low flushed = LF) ewes (n = 50) and treatment 3 (low unflushed = L) ewes (n = 48) were on limited, sparse, dry pasture for 12 weeks prior to mating.

The LF and L ewes were drafted off as they reached condition score 2.0 and placed on a maintenance diet. All groups of ewes were fed the differential nutrition levels until 6 weeks prior to mating and then fed maintenance energy levels based on body weight until either flushing (for the LF ewes) or mating (for the H and L ewes).

At 3 weeks prior to mating, the LF ewes were placed on a flushing diet to increase ovulation rates to levels comparable to ewes on high nutrition. The flushing diet consisted of alfalfa pellets fed to provide 150% of NRC maintenance energy requirements based on body weight. The L ewes were immunized with Fecundin (ovandrotone), a commercial steroid immunization product used for increasing ovulation rate, at six and three weeks prior to mating, as per manufacturer's directions.

At 2 weeks prior to mating, vaginal progestagen pessaries were placed in all ewes to synchronize estrus. Ewes were checked daily for device loss and lost devices were immediately replaced. Pessaries were removed 48 hours prior to introduction of the rams.

Semen-tested harnessed rams were placed with the separate treatment groups at a ratio of 1 ram per 10 ewes for a 3 day mating period. Crayon marks were recorded daily.

Following the end of the mating period, ewes were fasted for 24 hours and then examined by laparoscopy to count corpora lutea as an estimate of ovulation rates.

After laparoscopy, ewes were placed on one of two pre-allocated post-mating treatment levels (high or low). High nutrition level was defined as 150% of NRC maintenance energy requirements for LF and L ewes, and 100% of NRC maintenance energy requirements for H ewes. The low post-mating nutrition level was defined as 80 % of NRC maintenance energy requirements based on body weight for H ewes, and 100% NRC maintenance energy requirements for LF and L ewes (Figure 1).

Rams were placed with all treatment groups two weeks after initial mating to check for returns to service; returning ewes were dropped from the study.

Ewes remained on assigned nutrition levels for 45 days post-mating, then were combined and managed as a single group through parturition when litter size was recorded for each ewe. Ewes which failed to lamb to the synchronized mating were dropped from the study.

Trial 2 consisted of 60 Polypay and 60 Coopworth x Polypay (C x P) three year old ewes. Treatments consisted of a 2 x 2 factorial array of pre-mating and post-mating nutrition levels balanced across the two genotypes. Ewes were managed on similar pasture for the 4 months preceding the initiation of the trial in late June. All ewes were weighed and condition-scored before assignment to the high (H) treatment (good pasture +alfalfa pellets) or low flushed (LF) treatment (sparse pasture) as outlined in Trial 1. Low nutrition ewes were managed as a single group until all members were condition-score 2 or lower. At six weeks prior to mating the LF group was placed on a maintenance diet. High ewes remained on the high nutrition until time of mating.

At 3 weeks prior to mating, the LF group was placed on the alfalfa pellet flushing diet of 150% maintenance energy requirements as described in Trial 1. Synchronization of estrus, breeding and assignment to post-mating treatments occurred as outlined in Trial 1 (Figure 2).

Litter size was recorded for each ewe lambing to synchronized mating.

Ovulation rates and litter size were analyzed by the analysis of variance procedure of the Statistical Analysis System of the SAS Institute Inc. (1985), using the general linear model with all main effects regarded as fixed. Main effects for Trial 1 were age, pre-

Figure 1 **TRIAL 1 DESIGN**

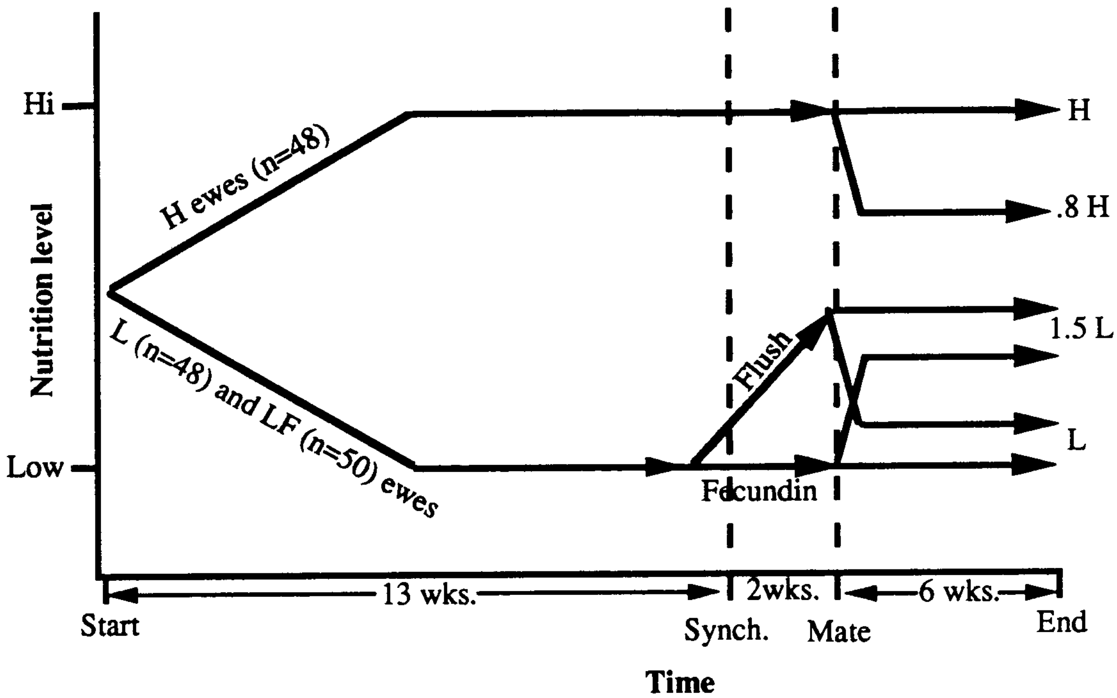
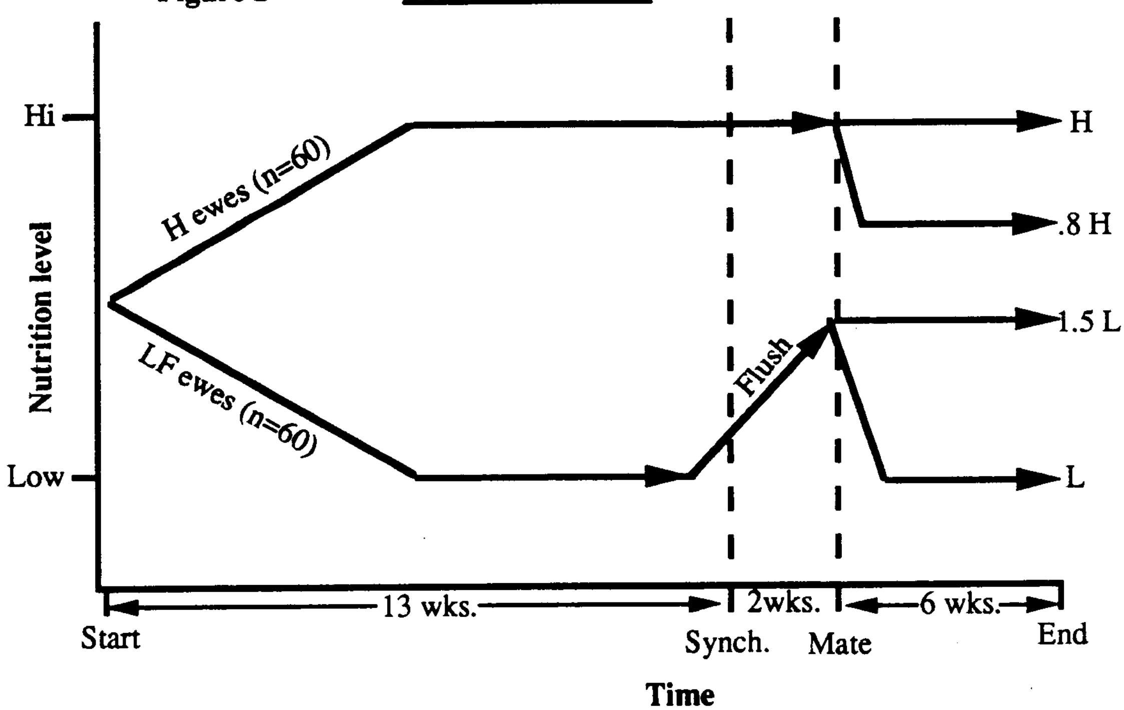


Figure 2 **TRIAL 2 DESIGN**



mating treatment and post-mating treatment. The Trial 2 model included breed, pre-mating treatment and breed x pre-mating treatment for analysis of ovulation rates, and breed, pre-mating treatment, post-mating treatment and all two-way interactions for analysis of litter size. Differences between ovulation rate and litter size in ewes lambing to the synchronized mating were designated as embryo loss.

RESULTS

Trail 1: Data were obtained from 111 ewes lambing to synchronized mating.

Condition score and body weight. Body weights at time of mating were heaviest in the H group (73.8 kg) and lowest in the L group (62.7 kg), with the LF group being intermediate (67.3 kg) (Table 1). Changes in body weight and condition score through the end of the study are shown in Figures 3 and 4. Condition score rose steadily for the H group from 2.7 to about 3.8 at flushing and stayed relatively constant through mating. Condition score stayed about the same from trial initiation to mating for both the LF and L groups (about 2.7). Ewes on high post-mating treatment gained condition, while ewes on low post-mating nutrition either lost condition or did not gain a significant amount.

Ovulation and conception rates. Mean ovulation rates and effects of age and pre-mating treatments are shown in Table 1. Mean ovulation rate was 2.62, and did not differ significantly between pre-mating treatment groups. Age had no significant effect on mean ovulation rates.

Conception rates, estimated as proportion of ewes lambing to synchronized mating, averaged 76% and were similar for all treatments. Age did not show a significant effect on conception rate, although 8 year old ewes had somewhat higher rates (88%) compared with the other ages (74%). Conception rates increased with increasing ovulation rates, from

TABLE 1: LEAST SQUARES PERFORMANCE MEANS AND STANDARD ERRORS(NO. OBS.)

Pre-mating treatment	Ovulation rate	Condition score[a]	Body weight[a]	Conception rate	Litter size
Trial 1					
High	2.62±.11 (47)	3.80±.14	73.8 kg±2.1	79 %	2.22±.18 (37)
Low Flushed	2.71±.10 (51)	2.73±.13	67.3 kg±2.0	76 %	2.18±.18 (39)
Low Unflushed	2.52±.10 (48)	2.77±.14	62.7 kg±2.1	73 %	1.86±.19 (35)
Trial 2					
High	2.76±.12(55)[b]	3.85±.13	55.3 kg±2.1	89 %	2.11±.16(48)[d]
Low Flushed	2.36±.12(56)[c]	1.46±.13	45.8 kg±2.1	95 %	1.81±.15(52)[e]
Age Trial 1					
5	2.63±.09 (67)	3.15±.13	67.2 kg±1.8	76 %	2.00±.16(51)
6	2.79±.12 (34)	3.03±.17	66.7 kg±2.5	71 %	2.42±.23(24)
7	2.50±.14 (28)	3.09±.18	68.8 kg±2.7	75 %	2.00±.24(21)
8	2.41±.18 (17)	2.94±.23	71.5 kg±3.5	88 %	2.00±.29(15)
Breed Trial 2					
Polypay	2.62±.12(51)	2.60±.13	50.9 kg±2.2	92 %	2.02±.16(46)
Coopworth x Polypay	2.50±.11(60)	2.68±.12	50.1 kg±2.0	92 %	1.98±.15(54)
Post-mating treatment					
Trial 1					
High					2.09±.15(59)
Low					2.10±.16(52)
Trial 2					
High					1.96±.15(50)
Low					1.94±.15(50)

[a] At time of mating.
[bc] Means differ at P<.01.
[de] Means differ at P<.07.

Figure 3 **CHANGE IN WT. OVER TIME**
TRIAL 1

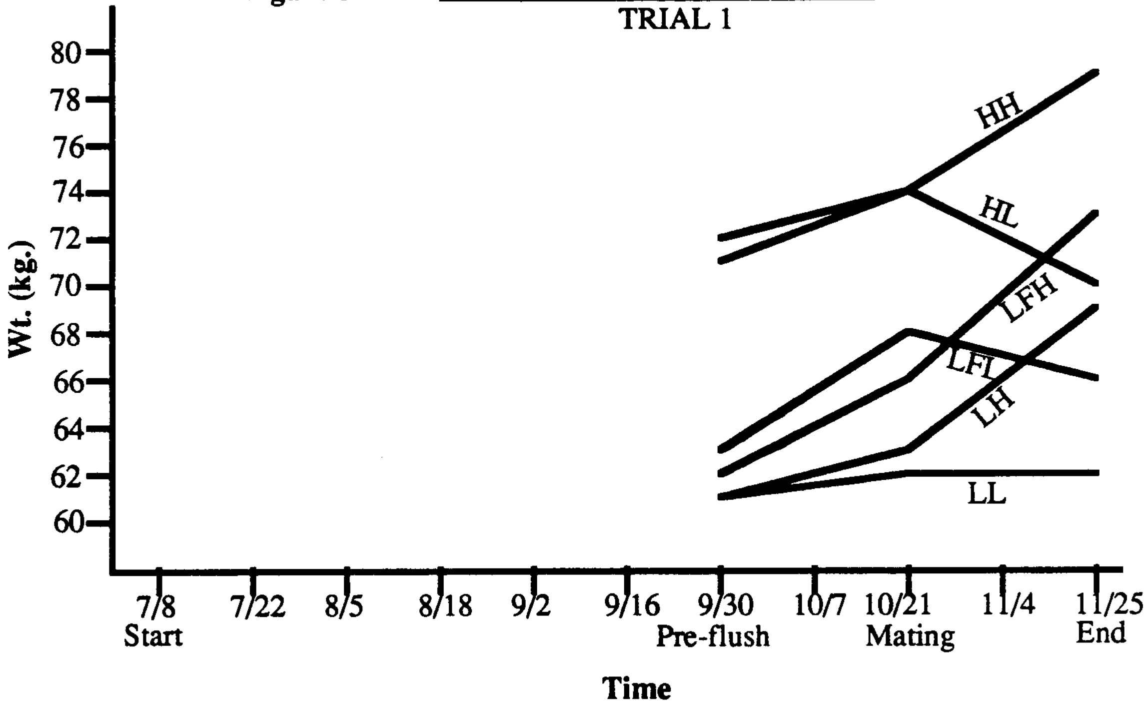
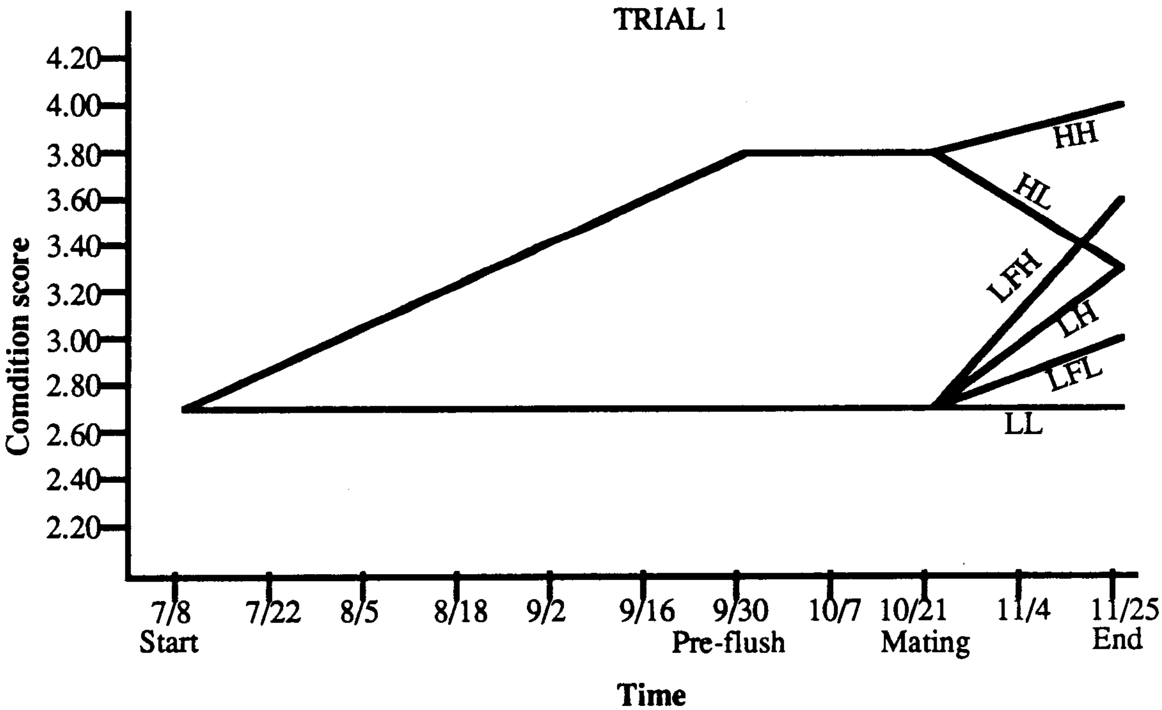


Figure 4 **CHANGE IN C.S. OVER TIME**
TRIAL 1



71% for single ovulators to 74% for twin ovulators to 78% for triple ovulators to 83% for quadruple ovulators.

Lambing data. Mean litter size is presented by treatment and ewe age in Table 1. Although ovulation rates and conception rates did not differ for the pre-mating treatment groups, the L ewes had somewhat lower mean litter size (1.86) compared to the H (2.22) and LF (2.18) ewes. The difference was not significant.

Looking specifically at litter size for twin and triple ovulators (Table 2), no main effects were significant for the twin ovulators, but pre-mating treatment was significant for litter size in the triple ovulators ($P<.05$). This is largely the result of the L group having much lower litter size (1.86) than the H (2.60) or LF (2.37) groups. The L group did not seem to gain any advantage in litter size from the third egg ovulated.

There was no effect of age or post-mating treatment on litter size (Table 2).

<u>Trial 2:</u> Data were obtained from 100 ewes lambing to synchronized mating.

Condition score and body weight. Change in condition score and body weight over the time of the trial are shown in Figures 5 and 6. Mean condition scores at time of mating were 3.85 for the high group and 1.46 for the low group .

Ovulation rate and conception rate. Table 1 shows mean ovulation rates for the pre-mating treatments and breed effects. Mean ovulation rates were high for both groups (2.56 overall), with LF ewes significantly lower (2.36) than the H ewes (2.76). Genotype had no significant effect on ovulation rates, although Polypay ewes were slightly higher (2.62) than C x P ewes (2.50).

Conception rates were high for all groups and averaged 92% overall, with no significant differences between pre-mating treatment groups or between breeds.

Lambing data. Mean litter size for all treatment groups is shown in Table 1. Pre-mating treatment was significant at $P<.07$ with H ewes having a mean litter size of 2.11

TABLE 2: CONCEPTION RATE AND LEAST SQUARES MEANS AND STANDARD ERRORS FOR LITTER SIZE IN TWIN AND TRIPLE OVULATORS (NO. OBS.)

	Conception rate		Litter size	
	Twin	Triple	Twin	Triple
Pre-mating treatment				
Trial 1				
High	79 %	75 %	1.80 (15)±.13	2.60 (15)±.18[a]
Low flushed	75 %	76 %	1.67 (15)±.13	2.37 (19)±.16[b]
Low unflushed	71 %	82 %	1.73 (15)±.13	1.86 (14)±.19[c]
Trial 2				
High	75 %	89 %	1.69 (15)±.13	2.39 (25)±.15
Low flushed	94 %	91 %	1.62 (31)±.09	2.22 (20)±.16
Age Trial 1				
5	71 %	78 %	1.70 (20)±.12	2.14 (21)±.15
6	58 %	76 %	1.57 (7) ±.20	2.62 (13)±.20
7	71 %	73 %	1.80 (10) ±.16	2.13 (8) ±.25
8	89 %	100 %	1.88 (8) ±.18	2.33 (6) ±.29
Breed Trial 2				
Polypay	91 %	88 %	1.66 (21)±.11	2.37 (21)±.16
Coopworth x Polypay	93 %	92 %	1.65 (25)±.10	2.23 (24)±.15
Post-mating treatment				
Trial 1				
High			1.83 (24)±.11	2.17 (24)±.14
Low			1.62 (21)±.11	2.42 (24)±.14
Trial 2				
High			1.56 (24)±.10	2.59 (20)±.16[d]
Low			1.75 (22)±.11	2.01 (25)±.15[e]

[abcde] Means with superscripts that do not have a common superscript letter differ ($P<.05$)

Figure 5 **CHANGE IN WT. OVER TIME**
TRIAL 2

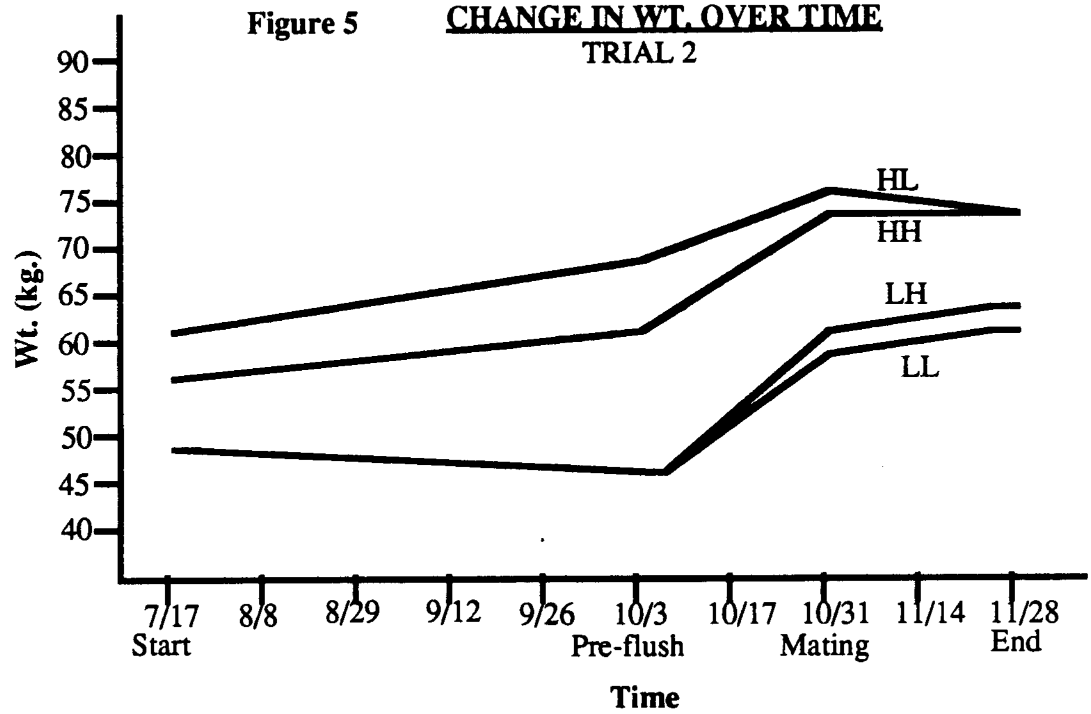
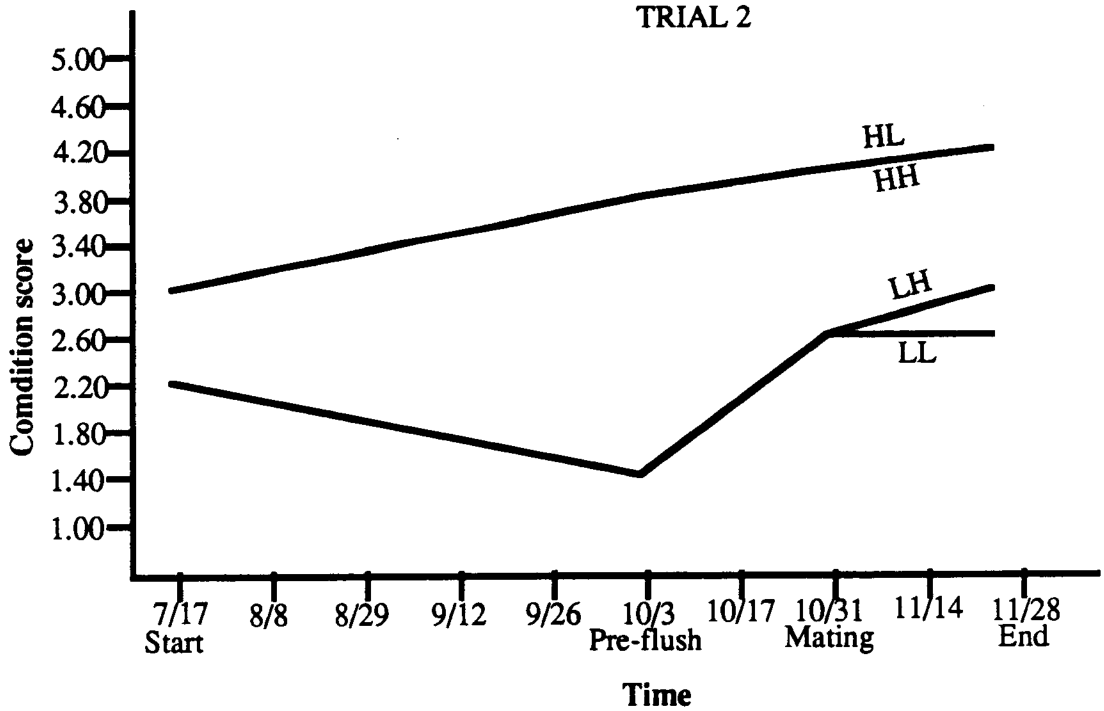


Figure 6 **CHANGE IN C.S. OVER TIME**
TRIAL 2



and LF ewes having a mean litter size of 1.81. Post-mating treatment also showed no effect on mean litter size (1.96 and 1.94). Examining twin and triple ovulators specifically showed post-mating treatment and two-way interactions to be significant ($P<.05$) (Tables 2, 3, 4 and 5). In the twin ovulators, breed x pre-mating treatment was significant ($P<.05$). Coopworth x Polypay ewes showed a considerable drop in mean litter size from high (1.87) to low (1.43) pre-mating nutrition, while Polypay ewes showed a higher litter size for ewes on low nutrition (1.80) compared to high nutrition (1.50).

Among triple ovulators, post-mating nutrition was highly significant ($P<.01$) and pre-mating x post-mating treatment interaction was significant ($P<.05$). The post-mating effects show a considerably lower mean litter size for ewes on low nutrition (2.01 for low vs. 2.59 for high), seen mainly in the C x P genotype (2.66 for high vs. 1.81 for low). Ewes on low nutrition both pre-mating and post-mating were at a disadvantage for embryo success in triple ovulators compared to ewes which had some exposure to high nutrition (Table 3).

DISCUSSION

The similarity in ovulation rates between high condition ewes and flushed low condition ewes observed in both trials agrees with previous studies on the flushing effect (Rhind et al., 1983; Gunn et al., 1984; Gunn and Maxwell, 1989). In Trial 1, Fecundin-treated ewes had high ovulation rates, which is similar to previous results. Meyer and Lewis (1988) found that Fecundin-treated ewes had mean ovulation rates significantly higher (.20) than ewes not treated. This is equivalent to an extra 20 eggs ovulated per 100 treated ewes. In Trial 1 there was no significant difference in ovulation rates between the H and LF ewes although mean condition score varied by about one entire scale point. In Trial 2 mean condition scores between the H and LF groups differed by nearly 2.5 scale

TABLE 3: PRE-MATING TREATMENT X BREED TWO-WAY TABLE OF MEAN LITTER SIZE AMONG TWIN OVULATORS IN TRIAL 2 (NO. OBS.)

	Pre-mating treatment	
Breed	High	Low flushed
Polypay	1.50 (6)	1.80 (15)
Coopworth x Polypay	1.87 (9) [a]	1.43 (16) [b]

[ab] Means with superscripts that do not have a common superscript letter differ ($P<.05$).

TABLE 4: PRE-MATING X POST-MATING TREATMENT TWO-WAY TABLE OF MEAN LITTER SIZE AMONG TRIPLE OVULATORS IN TRIAL 2 (NO. OBS.)

	Post-mating treatment	
Pre-mating treatment	High	Low
High	2.41 (11)	2.36 (14)
Low flushed	2.78 (9)	1.66 (11) [a]

[a] Differs significantly from all other means in table.

TABLE 5: BREED X POST-MATING TWO-WAY TABLE OF MEAN LITTER SIZE AMONG TRIPLE OVULATORS IN TRIAL 2 (NO. OBS.)

	Post-mating treatment	
Breed	High	Low
Polypay	2.52 (9)	2.21 (12)
Coopworth x Polypay	2.66 (11)	1.81 (13)

Difference between means was not significant.

points and ovulation rates showed a significant difference.

The slightly higher ovulation rate observed for Polypay ewes compared to the C x P ewes in Trial 2 is probably a reflection of greater percentage Finn ancestry. Other work has also found genotype of the ewe to be important in determining ovulation rates (Meyer and Clarke, 1982; Meyer, 1985; Hanrahan and Quirke, 1985). Fahmy and Dufour (1988) found ewes with higher percentage Finn breeding to have higher mean ovulation rates than other breeds.

Results of Trial 1 showed a trend in conception rate with pre-mating treatment. Ewes which were exposed to high level of nutrition, even for the short flushing period, had slightly higher conception rates than those which had no exposure (L group). This may indicate that nutrition is playing a role in conception rates, or may be due to the use of Fecundin to increase ovulation rates. The increase in conception rates with increasing ovulation rates suggests some marginal advantage for higher ovulation rates. This extends the findings of previous studies which have shown increased conception rates for twin ovulators compared to singles (Dolling and Nicolson, 1967; Allison, 1975; Meyer, 1985). There were no significant differences in conception rates between treatment groups in Trial 2, although twin ovulators on high pre-mating nutrition had somewhat lower rates (75%) than twin ovulators on pre-mating low nutrition(94%).

Meyer (1985) used the term uterine efficiency to describe the marginal response in litter size due to one more egg ovulated, which is measured in multiple ovulators. Using this means of comparison, uterine efficiency rates for twin ovulators in Trial 1 were .80, .67 and .73 for the H, LF and L pre-mating treatment groups, respectively, and .60, .37 and -.14 for the triple ovulators. One important difference between the L group and the other pre-mating treatments is lack of exposure to good feed for the L group, and the resulting litter sizes would suggest that pre-mating nutrition is important for embryo survival. However, use of Fecundin confounds the results and it cannot be determined whether the effect is due to lack of good feed or use of Fecundin, or both. Uterine efficiency rates in Trial 2 were .69 and .62 for the H and FL twin ovulators, and .39 and .22 for the H and FL triple ovulators.

Trial 1 results agree with those of several other experiments which showed no effect of age of the ewe past the yearling stage on embryo survival (Adalsteinsson, 1979; Thomson, 1988; Gunn et al., 1984).

Results from the twin ovulators in Trial 2 indicate an advantage in litter size for Polypay ewes on low pre-mating nutrition compared to ewes on high pre-mating nutrition. Litter size in the twin ovulating C x P ewes increased from low to high pre-mating condition, which agrees with work by Gunn and Doney (1979) who concluded that higher condition scores (>2.0) at time of mating gave higher embryo survival.

Results of Trial 1 show no significant effect of post-mating nutrition on embryo survival. This may be due in part to level of low nutrition being relatively moderate. Previous studies which have reported an effect of post-mating nutrition on embryo survival used very low nutrition levels of <25% NRC maintenance energy requirements (Edey, 1965; Edey, 1966; Cumming, 1972b; Gunn et al., 1986).

While effects of post-mating nutrition were not significant for litter size among twin ovulators in Trial 2, there was an increase in mean litter size for ewes on low post-mating nutrition compared to those on high post-mating nutrition. This is due mainly to an increase in litter size among the C x P ewes on low nutrition. While some research has reported no effect of low post-mating nutrition on embryo survival (Parr et al., 1982; Bellows et al., 1974) there have been no reports indicating an advantage of low level feeding. While reports on the sow (Toplis et al., 1983) show a decrease in litter size due to increased progesterone caused by high level feeding in early pregnancy, there is little evidence of detrimental effects due to high level post-mating feeding in the ewe.

Litter size in the triple ovulators for Trial 2 reflects the effects of post-mating nutrition seen in the twin ovulators. Ewes on low pre- and post-mating nutrition had significantly lower mean litter size than any other treatment group (Table 4). Nearly all of the decrease in litter size is due to a decrease in embryo survival in the C x P ewes on low nutrition compared to any of the other treatment groups (Table 5). Polypay ewes had nearly equal litter size for ewes on high and low post-mating nutrition (2.52 and 2.21, respectively). Cumming (1972b) also found low post-mating nutrition to decrease embryo survival.

Triple ovulators in Trial 2 had lower survival rates when both pre-mating and post-mating nutrition is low. This agrees with results from Trial 1, which indicate that embryo survival is better in ewes which have more exposure to high level feed.

CHAPTER 4

USE OF REAL-TIME ULTRASOUND AND PREGNANCY-SPECIFIC PROTEIN B TO ASSESS EMBRYO LOSS IN THE EWE

K. S. West and H. H. Meyer

ABSTRACT

Two methods of estimating embryo numbers were employed to estimate timing and extent of embryo loss in a trial to examine effects of pre- and post-mating nutrition on embryo survival. Sixty ewes of > 3/4 Polypay breeding (hereafter called Polypay) and 60 Coopworth x Polypay ewes three years of age were employed in a 2 x 2 factorial array of pre- and post-mating treatments. Pre-mating nutrition levels were high (H) and low-flushed (LF), and post-mating treatment levels were high and low nutrition planes. Real-time ultrasound diagnosis was performed on all ewes at 21, 28, 34 and 45 days post-mating. Blood samples were also collected on these days for assay of serum concentrations of Pregnancy-specific Protein B (PSPB) levels. Ultrasound data provided little indication of treatment effects on amount or time of embryo loss. Pregnancy-specific Protein B results showed a difference between ewes bearing singles and ewes bearing multiples at day 45, but were unable to detect a difference between twins and triplets. Comparing PSPB concentrations and ultrasound data, measurement of PSPB failed to provide any indication of amount or time of embryo loss.

INTRODUCTION

Nutrition has an effect on litter size and embryo loss in the ewe (Rhind et al., 1984a; Gunn and Maxwell, 1988). If litter size were known during gestation, ewes bearing multiple lambs could be preferentially fed to reduce the incidence of embryo loss. Knowing embryo numbers during gestation could also be useful in assessing effects of genotype or experimental treatments on embryo survival. For these reasons, much research has been aimed at finding an accurate and easy means of early diagnosis of embryo number in the ewe.

Embryo losses have been estimated by using serial slaughter (Braden, 1971), laparotomy (Parr et al., 1982) or laparoscopy (Kelly et al., 1982) to estimate ovulation rates and then comparing these estimates with embryos recovered (in the case of serial slaughter) or number of lambs born. These techniques are labor intensive and do not provide information on time of embryo loss.

Ultrasound has been shown to be useful for determining embryo numbers; however, accuracy of detection of embryos and accuracy of counts during early gestation depend greatly on operator experience (White, 1984; Wilkins, 1984; Wilkins, 1986). While the typical 95% accuracy level of diagnosing twins from singles at 50 days post-mating is acceptable, the cost in terms of both cash and labor to have a flock diagnosed may outweigh possible benefits derived.

Another recent development involves the use of a serum protein for detection of pregnancy. Pregnancy-specific Protein B (PSPB), a protein which is pregnancy specific in the bovine, shows cross-reactivity in the ewe and is an easy, accurate method of early detection of pregnancy in sheep (Ruder et al., 1986). Because PSPB is present throughout gestation and is a product of embryonic tissues, it may also be a useful method of assessing embryo numbers.

The purpose of this study was to use real-time ultrasound and PSPB to monitor incidence and timing of embryo loss relative to pre- and post-mating nutrition levels and breed effects.

MATERIALS AND METHODS

Ewes reported in this study were part of a trial to determine the effects of nutrition on embryo survival (West and Meyer, 1990). Sixty white-faced ewes of >3/4 Polypay breeding (hereafter called Polypay) and sixty Coopworth x Polypay crossbred 3 year-old ewes were used. Treatments consisted of a 2 x 2 factorial array of pre-mating (high and low-flushed) and post-mating (high and low) nutrition levels. Ewes in the low-flushed pre-mating treatment group were fed on limited sparse, dry pasture to reach a target condition score of 2.0, while ewes in the high pre-mating treatment were fed on good pasture and supplemented with alfalfa pellets as needed to reach a target condition score of 4.0. Condition scores were assessed by the method outlined by Jefferies (1961) on a 1 to 5 point scale, including half points, with 1 being very thin and 5 being obese. For three weeks prior to mating, the low-flushed pre-mating treatment group was flushed with alfalfa pellets to provide 150% of NRC energy requirements for maintenance based on body weight.

Vaginal progestagen pessaries were placed in all ewes two weeks prior to mating to synchronize estrus. Ewes were checked daily for pessary loss, and lost devices were immediately replaced. Pessaries were removed 48 hours prior to the introduction of rams.

Semen-tested, harnessed rams were placed with the two separate treatment groups at a ratio of 1 ram per 10 ewes for a 3 day mating period. Crayon marks were recorded daily.

Following the end of the mating period, ewes were fasted for 24 hours and examined by laparoscopy to count corpora lutea as an estimate of ovulation rates. Following laparoscopy, ewes were placed on pre-assigned post-mating nutrition levels. The high nutrition level was 150% of NRC maintenance energy requirements for ewes which had previously been on low nutrition, and 100% NRC maintenance energy requirements for ewes which had been on high nutrition. The low post-mating nutrition level was defined as 80% of NRC maintenance energy requirements for ewes which had previously been on high nutrition, and 100% NRC maintenance energy requirements for ewes which had previously been on low nutrition.

A single ram was placed with each group 2 weeks after the synchronized estrus to check for returns to service; returning ewes were dropped from the study.

Ultrasound imaging for embryo counts and blood collection for PSPB analysis were performed on days 21, 28, 34 and 45 of gestation. Ultrasound imaging was performed using a 3 MHz linear ray probe. Imaging was done transrectally on days 21 and 28, and transabdominally on days 34 and 45. Embryo counts were made by the same operator on all days.

Blood was collected via jugular venipuncture with standard vacuum tubes. Samples were allowed to coagulate for several hours in refrigeration before centrifuging for serum separation. Serum was suctioned off and stored at -20° C until time of assay. Serum was assayed by radioimmunoassay as described by Sasser et al. (1986) using antiserum for ovine PSPB.

At 45 days post-mating all ewes were combined and thereafter managed as a single group through parturition when litter size was recorded. Ewes which failed to lamb to synchronized mating were excluded from the study.

Ultrasound data on numbers of embryos were used only for those ewes from which clear images were obtained. Serum levels of PSPB were analyzed by analysis of variance using the Statistical Analysis System of the SAS Institute Inc. (1985). The model included ewe genotype, pre- and post-mating treatments and number of lambs born. Orthogonal contrasts were used to compare PSPB concentrations of ewes bearing single versus multiples, and twins versus triplets. Each day of gestation was analyzed separately.

RESULTS AND DISCUSSION

Ultrasound imaging proved difficult on days 21 and 28 due to interference which prevented clear imaging of the uterus. This obstruction had been previously encountered only in ewes which had been inadvertently fasted overnight. Cause of the obstruction was unclear; the occurrence among ewes appeared to be random and showed no connection with either time of day or feeding levels. Reliable data were defined as those which presented clear images on days 21 or 28 and had at least one subsequent clear observation. These data were obtained from only 38 ewes out of 100 pregnant to the synchronized mating.

Of those ewes with reliable data, 19 (50%) exhibited some degree of embryo mortality, defined as a difference between ovulation rate and number of lambs born. This is a random sample of the entire experimental group, which exhibited 48% embryo loss overall. With fertilization assumed to be an all-or-none event (Restall et al., 1976), 4 ewes lost embryos between mating and day 21; no ewes lost embryos between days 21 and 28; 6 ewes lost embryos between days 28 and 34; 8 ewes lost embryos between days 34 and 45; and 6 ewes lost embryos between day 45 and parturition. Six ewes each lost 2 embryos (Table 6).

There was little indication of treatment effects on either time or occurrence of embryo loss (Table 6), but losses were slightly higher for ewes on low nutrition both pre- and post-mating, compared to the other treatments.

Pre-mating nutrition level showed a significant effect ($P<.05$) on PSPB serum concentrations at day 21, with ewes on high pre-mating nutrition having higher levels of PSPB. Nutritional levels pre- and post-mating had no effect on PSPB levels measured later in gestation. At early stages of gestation (days 21 and 28), there was no significant difference in PSPB levels between ewes with singles or multiples. The difference in PSPB levels between ewes with twins and triplets was not significant at any stage of gestation (Table 7). At day 45, ewes bearing multiple fetuses had higher PSPB levels than those carrying singles ($P<.05$). This finding agrees with the study by Ruder-Montgomery et al.

TABLE 6: ULTRASOUND DIAGNOSIS OF TIME AND TREATMENT EFFECTS ON EMBRYO LOSS

	No. embryos lost / Total eggs ovulated	No. ewes exhibiting loss /[a] Total number of ewes obs.
Time period		
0 - 21 days	4	4
21 - 28 days	0	0
28 - 34 days [b]	6	6
34 - 45 days [b]	8	8
45 - lamb	6	6
Treatment group		
HH	4/19	2/7
HL	5/16	4/6
LH	6/28	6/12
LL	10/30	7/12

[a] Loss of two embryos was observed in six ewes during the time of the trial.

[b] One ewe lost an embryo between days 28 and 45, but ultrasound image was not clear on day 34.

TABLE 7: LEAST SQUARES MEAN PSPB CONCENTRATIONS AND STANDARD ERRORS BY PRE-MATING TREATMENT AND LAMB NUMBER

	PSPB level (ng/ml)			
Pre-mating treatment	Day 21	Day 28	Day 34	Day 45
High	$5.74^{a} \pm .82$	16.67 ± 2.54	48.78 ± 5.62	57.06 ± 3.93
Low flushed	$2.87^{b} \pm .74$	19.25 ± 2.20	44.78 ± 4.83	53.12 ± 3.66
Lamb number				
1	$3.98 \pm .95$	15.29 ± 2.94	43.45 ± 6.58	$46.39^{c} \pm 4.73$
2	$5.18 \pm .87$	18.27 ± 2.57	54.12 ± 5.62	$58.96^{d} \pm 4.15$
3	3.76 ± 1.06	20.02 ± 3.23	42.78 ± 7.12	$59.92^{d} \pm 5.24$

[abcd] Means with superscripts that do not have common superscript letters differ ($P < .05$).

(1988) in finding a significant difference between PSPB levels in singles and twins.

Accuracy of detecting singles or multiples was assessed by determining the point of least overlap between values of PSPB for ewes carrying singles versus ewes carrying multiples. Any values above this point would be diagnosed as multiples, and values below this point would be diagnosed as singles. Using this estimation, 30% of ewes bearing multiples would have been diagnosed as having singles, and 33% of singles would have been diagnosed as multiples.

Comparing PSPB levels from the 19 ewes for which ultrasound showed embryo loss to levels in ewes which showed no loss, PSPB levels did not decrease with embryo loss, nor was there any difference in titer levels between ewes exhibiting loss and those which exhibited no loss.

Neither pre- nor post-mating nutrition nor ewe genotype had any affect on extent or timing or embryo loss. Ultrasound may be an effective means of assessing timing and extent of embryo mortality if a clear image can be obtained. Pregnancy-specific Protein B may detect single bearers from multiple bearers, but accuracy is somewhat low and there was no evidence of timing or extent of embryo loss in the levels of PSPB up to 45 days of gestation.

CHAPTER 5

CONCLUSIONS

Results from Trial 1 show no effect of pre-mating treatment on ovulation rate or conception rate. There was no effect of pre-mating treatment, ewe age or post-mating treatment on embryo survival in Trial 1.

Results from Trial 2 show a significant effect of pre-mating treatment on ovulation rate, with H ewes having higher rates than LF ewes. Pre-mating treatment had no effect on conception rate. Pre-mating treatment, ewe breed and post-mating treatment had no overall effect on embryo survival in Trial 2.

Examining embryo survival among ewes with twin and triple ovulations found pre-mating treatment to be significant among triple ovulators in Trial 1, with L ewes having much lower embryo success than LF or H ewes. These results would indicate that some exposure to high nutrition is important in order to take advantage of high ovulation rates. Two way interactions for Trial 2 suggest that some breeds may be more sensitive to low nutrition levels both pre- and post-mating. Ewes with triple ovulations seem more sensitive than ewes with twin ovulations to post-mating nutrition effects and more likely to lose an embryo due to low nutrition.

Ultrasound data from Trial 2 suggested that neither pre- nor post-mating nutrition nor ewe genotype had any effect on extent or timing of embryo loss. Pregnancy-specific Protein B may be a useful method of detecting pregnancy, but accuracy in detecting litter size is not high. Further studies are needed to determine the best time for detecting litter size using PSPB and to determine if it is economically worthwhile to do so at that time. Because there was no indication of timing or extent of embryo loss in the serum levels of PSPB, it is not likely that it will be useful in studying causes of embryo loss.

LITERATURE CITED

Allen, D. M., and G. E. Lamming. 1961. Nutrition and reproduction in the ewe. J. Agric. Sci. 56:69.

Allison, A. J. 1975. Effect of nutritionally induced live-weight difference on the ovarian response and fertility in ewes treated with pregnant mare's serum gonadotropin. N. Z. J. Agric. Res. 18:101.

Braden, A. W. H. 1971. Studies in flock mating of sheep 3: Effect of undernutrition of ewes during joining. Aust. J. Exp. Agric. Anim. Husb. 11:375.

Bramley, P. S., H. L. Denehy and J. E. Newton. 1976. The effect of different planes of nutrition before mating on the reproductive performance of Masham ewes. Vet. Rec. 99:294.

Coop, I. E. 1966. The response of ewes to flushing. Wld. Rev. Anim. Prod. 4:69.

Coop, I. E. and V. R. Clark. 1969. The influence of nutrition level in early pregnancy of the ewe. J. Agric. Sci. 73:387.

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.

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

Cumming, I. A., M. A. Blockey, C. G. Winfield, R. A. Parr and A. H. Williams. 1975. A study of relationsheps 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.

Department of Agriculture, New South Wales, Australia. 1985. Developing a field technique for determining litter number during pregnancy in sheep. Bulletin #10. Agdex 433/47.

Dolling, C. H. S. and A. D. Nicolson. 1967. Vital statistics for an experimental flock of merino sheep IV: Failure in conception and embryonic loss as causes of failure to lamb. Aust. J. Agic. Res. 18:767.

Doney, J. M., R. G. Gunn and W. F. Smith. 1973. Transuterine migration and embryo survival in sheep. J. Reprod. Fert. 34: 363.

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

Edey, T. N. 1964. Prenatal mortality in sheep: A review. Anim. Breed. Abst. 37:173.

Edey, T. N. 1966. Nutritional stress and pre-implantation embryonic mortality in Merino sheep. J. Agric. Sci., Camb. 67:287.

El-Sheikl, A. S., C. V. Hulet, A. L. Pope and L. E. Casida. 1955. the effect of level of feeding on the reproductive capacity of the ewe. J. Anim. Sci. 14:919.

Fahmy, M. H. and J. J. Dufour. 1988. The accumulative effect of Finnsheep breeding in crossbreeding shcemes: Reproductive performance. Can. J. Anim. Sci. 68:69.

Guerra, J. C., C. J. Thwaites and T. N. Edey. 1971. The efects of live weight on the ovarian response to PMSG and on embryo mortality in the ewe. J. Agric. Sci. 76:177.

Guerra, J. C., C. J. Thwaites and T. N. Edey. 1972. The effects of components of body weight on reproductive efficiency in the Merino ewe. J. Agric. Sci. 78:245.

Gunn, R. G. 1983. The influence of nutrition on the reproductive performance of ewes. In: Sheep Production. W. Haresign, ed. pp. 99-110. Butterworths and Co. Ltd., Boston.

Gunn, R. G. and J. M. Doney. 1975. The interaction of nutrition and body condition at mating on ovulation rate and early embryo mortality in Scottish Blackface ewes. J. Agric. Sci. 85:465.

Gunn, R. G. and J. M. Doney. 1979. Fertility in Cheviot ewes. Anim. Prod. 29:11.

Gunn, R. G., J. M. Doney and A. J. F. Russel. 1969. Fertility in Scottish Blackface ewes as influenced by nutrition and body condition at mating. J. Agric. Sci. 73:289.

Gunn, R. G., J. M. Doney and A. J. F. Russel. 1972. Embryo mortality in Scottish Blackface ewes as influenced by body condition at mating and by post-mating nutrition. J. Agric. Sci. 79:19.

Gunn, R. G., J. M. Doney and W. F. Smith. 1984. The effect of different durations and times of high level feeding prior to mating on the reproductive performance of Scottish Blackface ewes. Anim. Prod. 39:99.

Gunn, R. G. and T. J. Maxwell. 1989. A note on the effect of the direction of live-weight change about the time of mating on reproductive performance of Greyface ewes. Anim. Prod. 48:471.

Haibel, G. K. 1986. Real-time ultrasound assessment of the uterus and fetus in small ruminants. Proc. Soc. Therio. 9:275.

Hanrahan, J. P. and J. F. Quirke. 1985. Contribution of variation in ovulation rate and embryo survival to within breed variation in litter size. In: Genetics of Reproduction in Sheep. pp. 193-201. Land and Robinson, ed. Garden City Press Ltd., Litchworth.

Hasnain, H. U. 1964. Embryonic mortality in sheep. Anim. Breed. Abst. 33:89.

Hohenboken, W. D., J. N. Clarke, P. V. Rattray, J. F. Smith and M. Wheeler. 1988. Responsiveness of ewe genotypes to varying nutrition management and conventional genotype x environment interactions in sheep. Anim. Prod. 47:253.

Hulet, C. V., D. A. Price and W. C. Foote. 19 . Effects of month of breeding and feed level on ovulation and lambing rates of Panama ewes. J. Anim. Sci. 39:73.

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

Kelly, R. W. 1987. Fertilization failure and embryonic wastage. In: Reproduction in Sheep. pp. 127-133. Lindsay and Pearce, ed. Cambridge University Press, Cambridge.

Kelly, R. W. and A. J. Allison. 1976b. Measurement of ovulation rates by laparoscopy and effects on reproductive performance. Proc. N. Z. Soc. Anim. Prod. 36:240.

Kelly, R. W. and P. D. Johnstone. 1983. Influence of site of ovulation on the reproductive performance of ewes with 1 or 2 ovulations. N. Z. J. Agric. Res. 26:433.

Kemp, J. D., R. J. Vinini and D. G. Ely. 1988. influence of maternal frame size and nutritional restriction on growth and development of the postnatal lamb. J. Anim. Sci. 66:3073.

Killeen, I. D. 1967. The effects of body wieght and level of nutrition before, during and after joining on ewe fertility. Aust. J. Exp. Agric. Anim. Husb. 7:126.

Lahlou-Kassi, A. and M. Marie. 1981. A note on ovulation rate and embryonic survival in D'Man ewes. Anim. Prod. 32:227.

Meyer, H. H. 1979. Ewe and teaser breed effects on reproductive behavior and performance. Proc. N. Z. Soc. Anim. Prod. 39:68.

Meyer, H. H. 1985. Breed differences in ovulation rate and uterine efficiency and their contribution to fecundity. In: Genetics of Reproduction in Sheep. pp. 185-191. Land and Robinson, ed. Garden City Press Ltd., Letchworth.

Meyer, H. H. and G. E. Bradford. 1973. Reporduction in Targhee and Finnish Landrace x Targhee ewes. J. Anim. Sci. 5:847.

Meyer, H. H. and J. N. Clarke. 1982. Effect of ewe ovulation rate and uterine efficiency on breed and strain variation in litter size. Proc. N. Z. Soc. Anim. Prod. 42:33.

Meyer H. H., R. D. Lewis and S. L. Berry. 1988. Increasing reproduction of commercial ewes by Fecundin (Ovandrotone) Immunization. Prof. Anim. Sci. 4:39.

Newton, J. E., J. E. Belts and R. Wilde. 1980. The effect of body condition and time of mating on the reproductive performance of Masham ewes. Anim. Prod. 30:253.

Parr, R. A., I. A. Cumming and J. J. Clarke. 1982. Effects of maternal nutrition and plasma progesterone concentrations on survival and growth of the sheep embryo in early gestation. J. Agric. Sci. 98:39.

Restall, B. J., G. H. Brown, M. Blockey, L. Cahill, and R. Kearins. 1976. Fertilization failure and early embryonic survival. Aust. J. Exp. Agric. Anim. Husb. 16:329.

Rhind, S. M., J. M. Doney, R. G. Gunn and I. D. Leslie. 1984a. Effects of body condition and environmental stress on ovulation rate, embryo sruvival and associated plasma follicle stimulating hormone, lutenizing hormone, prolactin and progesterone profiles in Scottish Blackface ewes. Anim Prod. 38:201.

Rhind, S. M., J. M. Doney, R. G. Gunn and I. D. Leslie. 1984b. A note on the reproductive preformance of Greyface ewes in moderately fat and very fat condition at mating. Anim. Prod. 38:305.

Ruder, C. A., R. G. Sasser, K. A. Ivani, P. M. Panlasugui, J. J. Dahmen and J. N. Stellflug. 1988. Diagnosis of pregnancy in sheep by measurement of a blood antigen that cross reacts in a radioimmunoassay for a bovine pregnancy-specific protein. J. Anim. Sci. Suppl. 1. 59:367.

Ruder-Montgomery, C. A., R. G. Sasser, J. Willard and J. N. Stellflug. 1988. A blood test to detect pregnancy and to determine ewes bearing single or twin fetus(es). In: Annual Research Project Report. U. S. Sheep Expeimental Station/Univeristy of Idaho Agriculture Experiment Station. pp.78.

Russel, A. J. F., J. Z. Foote, I. R. White and G. J. Davies. 1981. The effect of weight at mating and of nutrition during mid-pregnancy on the birth weight of lambs from primiparous ewes. J. Agric. Sci. 97:723.

Sasser, R. G., C. A. Ruder, K. A. Ivani, J. E. Butler and W. C. Hamilton. 1986. Detection of pregnancy by radioimmunoassay of a novel pregnancy-specific protein in serum of cows and a profile of serum concetrations during gestation. Biol. Reprod. 35:936.

Toplis, P. and M. F. J. Ginesi. 1983. The influence of high food levels in early pregnancy on embryo survival in multiparous sows. Anim. Prod. 37:45.

Trounson, A. O. and N. W. Moore. 1972. Ovulation rate and survival of fertilized ova in Merino ewes selected for and against multiple births. Aust. J. Agric. Res. 23:851.

United States Department of Agriculture, Agriculture Statistics Board. Statistics on Sheep and Goats. February, 1988. LvGn 1 (2-87).

White, D. H., D. J. Rizzoli and I. A. Cumming. 1981. Embryo survival in relation to number and site of ovulations in the ewe. Aust. J. Exp. Agric. Anim. Husb. 21:32.

White, I. R., A. J. F. Russel and D. G. Fowler. 1984. Real-time ultrasound scanning in the diagnosis of pregnancy and the determination of fetal numbers in sheep. Vet. Rec. 155:140.

Wilkins, J. F. and D. G. Fowler. 1982. Real time ultrasound for diagnosing litter number in naturally mated flocks. Proc. Aust. Soc. Anim. Prod. 14:638.

Wilkins, J. F. and D. G. Fowler. 1982. The accuracy of ultrasonic imaging with scanners in determining litter number in pregnant ewes. Proc. Aust. Soc. Anim. Prod. 14:636.

Wilkins, J. F. and D. G. Fowler. 1984. Ultrasound imaging for litter size diagnosis in breeding flocks. In: Reproduction in Sheep. pp. 182-184. Lindsay and Pearce, ed. Cambridge University Press, Cambridge.

Wilkinson, S. C. and D. M. B. Chestnutt. 1988. Effect of level of food intake in mid and late pregnancy on the preformance of breeding ewes. Anim. Prod. 47:411.