Factors affecting ovulation rate, fertilization rate and ova normality in ewes managed under range conditions
by Clair C Engle

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Animal Science
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Abstract:
The trial reported herein represents four years of data collected to evaluate nutritional and other environmental effects on ovulation rate and fertilization rate in white-faced ewes managed under range conditions, A total of 817 ewes were randomly selected over the four-year period from a band of grade ewes of the Columbia, Targhee and Rambouillet breeds. Two age groups (mature--2-6 year olds, and yearlings) were selected in proportion to their numbers and breed in the grade band.

Each year, approximately 200 ewes were selected, and one-half were placed on a prebreeding treatment fourteen days pre-breeding and received a daily supplement of 227-grams of a protein pellet. The remaining one-half of the mature and yearling age group were not supplemented and were used for the controls.

A total of 100 mature ewes and 53 yearlings were slaughtered three days post-breeding (36 each year) during the four years. Data were collected on ovulation rate, fertilization rate, abnormal ova and any abnormalities observed that might interfere with subsequent reproduction.

The over-all ovulation rate for the supplemented and non-supplemented ewes was l.36 and 1.14, respectively. The total ovulation rate for the mature ewes, supplemented and non-supplemented was 1.53 and 1.17, respectively. The ovulation rate for the four years was 1.03 for the supplemented yearlings and 1.07 for the non-supplemented, a reverse response to prebreed ing treatment which also has been reported in earlier trials.

The recovery rate of the ova for the total period was 91.1 percent for the mature ewes and 92.9 percent for the yearling ewes.

The ewes fed supplements over the four years showed a 21.9 percent increase in ovulation rate, but the fertilization rate was 10.9 percent great er for the non-supplemented ewes than for the mature ewes. From the 52 ova recovered from the yearlings, 47 or 90.4 percent were fertilized.

The total abnormal ova for the four years was 9.0 percent for the mature ewes and 7.7 percent for the yearling ewes. The supplemented ewes had 4.4 percent more abnormal ova: than the non-supplemented ewes. The abnormal ova counted for the yearlings were 1.3 percent lower than that of the mature ewes, all of which had been fertilized.

Occlusion of the Fallopian tubes was a problem in five ewes, but it appeared not to occur in any particular age or pre-breeding treatment.

The supplementation period prior to breeding did insure body weight gain three out of the four years for both the mature and yearling ewes. Significant differences (P<01) in weight gain resulted due to treatment and supplementation for each year. Age and age X supplements were found to be significant

($P<.01$) in addition to treatment and supplementation for one year. The four-year analysis showed years and supplements to attribute the most significant results ($P<.01$). Significant results were also found for ages and the following interactions: Years X supplement and years X supplement X ages ($P<01$). Years X ages was found to be significant at the $P >.05$ level:

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## ABSTRACT

The trial reported herein represents four years of data collected to evaluate nutritional and other environmental effects on ovulation rate and fertilization rate in white-faced ewes managed under range conditions.

A total of 817 ewes were randomly selected over the four-year period from a band of grade ewes of the Columbia, Targhee and Rambouillet breeds. Two age groups (mature--2-6 year olds, and yearlings) were selected in proportion to their numbers and breed in the grade band.

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The total abnormal ova for the four years was 9.0 percent for the mature ewes and 7.7 percent for the yearling ewes. The supplemented ewes had 4.4 percent more abnormal ova than the non-supplemented ewes. The abnormal ova counted for the yearlings were 1.3 percent lower than that of the mature ewes, all of which had been fertilized.

Occlusion of the Fallopian tubes was a problem in five ewes, but it appeared not to occur in any particular age or pre-breeding treatment.

The supplementation period prior to breeding did insure body weight gain three out of the four years for both the mature and yearling ewes. Significant differences ($P < .01$) in weight gain resulted due to treatment and supplementation for each year. Age and age X supplements were found to be significant ($P < .01$) in addition to treatment and supplementation for one year. The four-year analysis showed years and supplements to attribute the most significant results ($P < .01$). Significant results were also found for ages and the following interactions: Years X supplement and years X supplement X ages ($P < .01$). Years X ages was found to be significant at the $P > .05$ level.

# INTRODUCTION

There is a great need for improved diagnosis of reproductive distrubances in males and females. Disturbances in fertility rate of large farm animals have been studied almost exclusively on males. With increasing use of artificial insemination, interest has been focused on quality of semen, and numerous investigations have been carried out on the physiology, morphology, and cytology of the spermatozoa. Ova are not as easily accessible for study as sperm, consequently, limited research has been conducted along this line.

It is well known that body condition of animals at mating has an important influence on the reproductive performance. Yet, there is little known as to how this influence is exerted. It is possible the results could be affected at any of the following times: estrus, ovulation, fertilization or implantation.

The reproductive processes of the ewe are influenced in some ways by the nutritive status. The influence of nutrition on age of puberty, the inverse effect on different age groups and their subsequent production has been established; however, it is not definitely known what causes these differences.

The predisposition for some reproductive disturbances may be due to endocrine imbalance or hereditary factors, but the manifestation depends usually on environmental factors, mainly stress of various kinds through malnutrition or over-feeding, management, climatic conditions and disease. The animal is conditioned to a certain extent against such stress factors; however, when the threshold of resistance is exceeded, the reproductive disturbances become evident.

The age of the animal may also have a considerable influence on the frequency and types of infertility.

Greater attention is now being focused toward the reproductive ability of our domestic animals. Increased knowledge of the fundamentals of reproductive physiology and their relationship to environmental factors could become an essential part in the improvement of reproductive efficiency in our domestic animals.

## REVIEW OF LITERATURE

### Factors Affecting Ovulation Rate

#### Nutrition and age

The practice of improving or increasing the nutrition level of livestock, sheep primarily, for a short time just prior to breeding (14-17 days) is often recommended as a method of increasing ovulation rate and subsequent lambing rate. Research of this nature conducted in many areas of the world indicate that this management practice more commonly called "flushing" results in a higher incidence of multiple births. Some of the early workers, Heape (1899), Darwin (1905), and Aristotle (1910) commented on the increased fertility of sheep under favorable environmental conditions. Work at Beltsville, Maryland, by Marshall and Potts (1924) indicated either concentrate feeding or grazing ewes on lush, green pastures prior to and during the breeding period increased lamb production. Nichols (1924) reported lambing rates of 150 and 125 lambs per 100 ewes supplemented and nonsupplemented, respectively. Clark (1934) observed an increased ovulation rate in ewes if they were below average condition prior to breeding, but found no evidence that supplementation increased ovulation rate in ewes of good condition prior to breeding. McKenzie and Terrill (1937) and Polovceva (1939) both concluded ovulation rates increased if ewes were supplemented prior to breeding. Brigg *et al*. (1942) reported no evidence during an eight-year study that a pre-breeding flushing period increased lambing percent. However, the management, rations and lengths of supplemental feeding periods varied from year-to-year. Also, yearling ewes were used some years as part of the experimental flock. Darroch *et al*. (1950), Wallace (1951), Hoversland (1958), Hoversland *et al*. (1958), Hoxsey (1959), Casida (1959), Hoxsey *et al*. (1960) Reid (1960) and Metianu (1963) have found an increased lambing rate in ewes supplemented prior to fall breeding and the difference was due to more multiple births. When yearling ewes were supplemented, El Sheikh *et al*. (1955) reported a higher ovulation rate but also a higher embryonic mortality rate as compared to ewes on a lower nutrient level when measured at forty days gestation.

Similar phenomena has been reported in dairy cattle by Reid (1963). Bedrak *et al*. (1964) working with yearling and two-year-old beef heifers recorded higher ovulation rate from feeding high levels of protein supplement prior to puberty. More normal embryos were found at forty-four days gestation than in the heifers fed the lower protein ration. This work supports earlier work done by Wiltbank *et al*. (1962).

Gossett and Sorensen (1956 and 1959), Haines *et al*. (1959), and Sorensen *et al*. (1961) found that gilts supplemented high energy level rations (70-93 therms per 100 pounds of feed) also had increased ovulation rates. However, the data did indicate that maximum ovulation rates and higher embryonic survival can be attained by reducing the energy content of rations fed to developing and gestating gilts.

Foote *et al*. (1959) studied the ovulation rate and embryo survival, over a three-year-period, on yearling ewes. Those animals that were better grown out as indicated by greater body weights had the higher ovulation rate in each of three years. Supplementing the ewes three weeks prior to breeding increased the ovulation rate in only one of the three years and no appreciable change in body weight was found. The year-to-year variation indicated that some environmental factor associated with years may be affecting embryo survival differently along with the variations in available and supplemented nutrients.

Joseph (1931) and Terrill and Stoehr (1942) reported that ewes of greater body weight at breeding tended to have higher reproductive rates. Tribe and Seeback (1962) reported that the increase in multiple births was a response to supplementing, but was not related to the live weight changes that occurred during the prebreeding treatment. However, live weights of the ewes at breeding, were related to subsequent lambing performance. The heavier ewes gave birth to more twins than did the lighter weight ewes.

Hulet _et al_. (1962) found that a seventeen-day supplementation period was an optimum length for increasing the multiple births in sheep. Two subsequent seventeen-day periods indicated an increase in costs and a decline in birth rate.

Ovulation rates for ewes supplemented prior to breeding have been compared to the non-supplemented group by some researchers. McKenzie and Terrill (1937) reported 1.52 ova per ewe for the supplemented group versus 1.06 for the controls, Wallace (1951) 1.30 versus 1.11, Hoxsey (1959) 1.31 versus 1.12, and Metianu (1963) 1.81 versus 1.51, respectively.

With few exceptions, evidence indicates that prebreeding supplementation will generally increase the number of multiple births born per ewe bred.

Age has been established to have a significant effect on ovulation rate and lambing performance. Carlyle and McConnell (1902) observed maximum lamb production between three and six-year-old ewes. Goot (1951), in evaluating the effects of year and age as a measure of fertility, found both highly significant; but age accounted for sixteen to seventy-two percent of the total variance in the fertility of ewes. The reproductive rate of sheep varies considerably from one year to another. Van Horn _et al._ (1952) reported that, over a four-year period, the percent of live lambs recorded per ewe bred varied from a low of 98.9 percent to a high of 125.1 percent. Asker and Ragab (1954) report lambing percentages in one flock as high as 143 percent and a low of 106 percent. Teodoreau and Rusu (1959) observed a greater percentage of four to eight-year-old ewes produced twins than did younger or older ewes. Bernoco (1961) reported the percentage of multiple births increased with age of the ewe up to six years.

William (1951), Hoversland _et al._ (1958), Hoversland (1958), Hulet (1959), and Bellows (1963a) have found a slightly increased ovulation rate in coming two-year old ewes as a possible result from the pre-breeding treatment. McKenzie and Terrill (1937) observed that lambs and yearlings had the lowest ovulation rate (mean = 1.21) and three and four-year-olds had a significantly higher rate (mean = 1.61). Five to seven-year-old ewes had a significantly lower ovulation rate than the three and four-year-old ewes (mean = 1.46). Edgar (1962) confirmed that the lower incidence of multiple births in eighteen month and two-year-old ewes was due to their lower ovulation rate; 1.10 as compared to 1.44 ova per ewe in the five-year old ewes.

<u>Seasons</u>

Early workers, Heape (1899) and Carlyle and McConnell (1902), tended to find increased twin births during the early part up to the middle of the lambing season and then a decline. More recent reports indicate that the highest frequency of multiple ovulations tended to be lower early in the breeding season, then rise to a peak during mid-season, and then decline toward the latter part of the season (Roberts, 1921; McKenzie and Terrill, 1937; Johnson, 1932; Asker and Ragab, 1954).

Reeve and Robertson (1953) postulated that the seasonal difference could attribute to differences between breeds and between regions. Environmental factors, climate and nutrition, could cause variation in ovulation rate between and within years. The months when ewes had the highest ovulation rates were September, October and November in the northern hemisphere and March, April and May in the southern hemisphere. Teodoreau and Rusu (1959) reported that ewes in Romania have a similar decrease in the percentage of multiple ovulations as the breeding season progresses. Nozdracer (1951) reported a 153 percent lamb crop from ewes bred in September, and 127 percent from ewes bred in November and December.

Radford (1959) studied the incidence of twin ovulations in Merino ewes held at a constant body weight, throughout the year. There was a statistically significant variation throughout the year in the ratio of twins to single ovulations. The major number of ovulations occurred in late autumn and the least during early spring.

The time of the season when first estrus occurs is commonly preceded by one or more cycles when ovulation is not accompanied by estrus, or commonly called "quiet heat" (Grant, 1933). Laing (1955) has reported a similar situation following the last estrus of the breeding season. "Quiet heat" may also be induced by stress conditions caused by extreme temperature variations or nutritional deficiencies (Hammond, 1955; Hafez, 1952; Braden and Moule, 1964; Bedrak et al., 1964).

Breeds

McKenzie and Terrill (1937) and Clark (1934) have reported breed differences in ovulation rate with the English breeds producing a greater number of ova than the fine wool breeds. Similar work by Bellows et al. (1963a) found a highly significant ($P < .01$) difference in ovulation rate in favor of the Hampshire ewe as compared to the mature Columbia ewe. However, the flushing period produced a significant ($P < .05$) increase in ovulation rate in the Columbia but not in the Hampshire ewes.

Climate

High and low atmospheric temperatures have been shown to exert a detrimental influence on reproductive performance in sheep. Dutt et al. (1959) reported high temperatures had no effect on ovulation rate or the occurrence or duration of estrus, but did result in longer intraestrual periods. Likewise, Ryle (1961) exposing Merino ewes to a $32^{o}$ C. temperature found no significant effect on the ovulation rate as compared to ewes kept at a normal daily temperature.

Unfortunately, only limited studies have been conducted on the effects of cold environmental temperatures on ovulation rate although indirect relationships may be developed from some research that has been conducted. Epaljevski (1934) observed low temperatures (below $15^{o}$ C.) and cold winds to reduce the number of ewes exhibiting estrus by 25.8 percent. Marshall and Hammond (1948) reported in England that any unfavorable climatic conditions such as a snow storm or drought during breeding will result in a corresponding reduction of multiple births the following lambing season.

Associated phenomena

It has become well established that ovulation rate increased with increased nutritive intake prior to and during the breeding season in sheep, but limited research is available explaining possible genetic, physiological or hormonal variations causing this effect.

Darlow (1942) suggested that some ewes may have a low genetic potential for twinning and will show no response to flushing.

More recent work by Bellows et al. (1963b) associated possible physiological mechanisms with the flushing effect. The results indicated that the amount of follicular development in the ovaries of flushed ewes is influenced by some component of carcass weight. As neither skeletal development nor fatness appeared to be implicated, it was implied that the flushing in ewes may be associated with the increased rate of protein synthesis.

Factors Affecting Fertilization Rate and Embryonic Mortality

Nutrition

Additional factors indicate in an indirect manner that nutrition plays an important role in influencing reproductive rates. Darroch et al. (1950) indicated that range ewes in good condition at breeding had 11 percent higher reproductive rates than ewes in poor condition. When thin ewes were flushed, it resulted in much greater increase in reproductive rates (15.1 percent) than when good conditioned ewes were supplemented (2.8 percent) prior to breeding.

Asdell (1949) stated that malnutrition may affect the reproductive system in a variety of ways, directly and indirectly, and it may take a wide variety of forms. A multiple deficiency usually exists and can be attributed to the imbalance or shortage of protein, minerals and vitamins.

Gilts fed on a continuously high plane of nutrition had a higher ovulation rate but frequently exhibited a higher percent of reproductive disorders and lowered embryo survival (Robertson et al., 1951; Christian and Nofziger, 1952; Casida, 1956). Similar results have been observed by El Sheikh et al. (1955) with sheep. Haines et al. (1959) found embryonic mortality to be higher in full-fed gilts than in the limited fed ones; the mortality percentage to twenty-five days being 27.1 and 11.6 respectively, and from twenty-five days to parturition, 9.7 and 8.5 percent respectively. Thus, it appears that high ovulation rate results from full feeding in swine, but a restricted diet is necessary for optimum embryonic survival.

Wiltbank et al. (1962) studying the reproductive performance with beef cattle reported that low energy levels prior to breeding lowered the conception rate. Reid et al. (1963) reported first calving dairy heifers fed high energy rations prior to puberty had a lower calving rate than that of heifers reared on lower energy levels. Yearling and two-year old heifers individually fed rations consisting of different levels of protein but similar amounts of energy, minerals and vitamins were used to study the effect of protein intake on reproduction. The high level protein rations produced a higher ovulation rate than the low level protein ration. Two-year old heifers on the higher intakes of protein had a greater number of normal embryos at forty-four days gestation than the heifers fed the lower protein ration. It was suggested that the lower protein ration in some instances may have caused ovulation without estrus to occur and therefore the heifers were not bred. However, Clawson et al. (1963) and Rippel et al. (1965)

reported neither the level nor the source of protein influences litter size, number of live pigs farrowed or birth weights in swine. Lodge and MacPherson (1963) indicated that the fertility rates in gilts on a low plane of nutrition from weaning to parturition was not significantly different from the gilts reared on a medium plane of nutrition.

Climate

High temperatures under controlled environmental conditions, show a detrimental effect upon reproduction. Dutt and Simpson (1957) studied the fertility rate and early embryonic death loss in ewes bred with semen from rams kept in an air-conditioned room at 7-9$^{\circ}$ C. during the summer, and semen from the rams kept at normal daily temperature. Thirty-eight percent more ova were fertilized by semen from the rams in the air-conditioned room. Subsequent lambing percentages showed the ewes bred to the rams from the air-conditioned room had 36.7 percent more lambs. Dutt et al. (1959) exposed sheared and unsheared ewes in a 32$^{\circ}$ C. temperature seven days prior to breeding. The sheared ewes had a higher fertility and embryonic survival rate, and lower percentage of abnormal ova. It was concluded from these results that high temperatures may affect the endocrine balance. Dutt (1960) and Alliston and Ulberg (1961) have reported that temperatures at 32$^{\circ}$ C. are detrimental to both ewe and ram fertility.

Prolonged estrus cycles, low fertilization rates and high embryonic mortality rates have also been associated with high summer temperatures in dairy cattle (Stott and Williams, 1962).

Dutt (1963) indicated that the critical period for the detrimental effects of temperature on fertilization is while the ova are still in the oviduct. Most of the abnormal ova and embryonic losses occurred within twenty-four hours after breeding. Similar work with gilts shows high ambient temperatures immediately post-breeding result in 1.9 less embryos when slaughtered twenty-five days later. Although, it appeared that a 32° C. temperature had less detrimental effect on fertility and embryo losses in gilts than in ewes (Warnick et al., 1965).

The fertilization rate and patterns of estrus activity have been shown to be correlated with precipitation. Suijdendorp (1959) observed that in years when there was a light rainfall breeding activity in sheep was greater, but this was offset by a lower fertilization rate. Similar work by Elpatjevskii (1934), Brezowsky and Haeger (1959) and Phillips (1960) shows that wet, cold or rapid changes in weather conditions may decrease breeding activity and subsequent reproductive rates. Shelton (1961) recording data on a flock of goats, reported a significant correlation ($P < .05$) between number of does which kidded (.39) or of kids born (.38) and total rainfall.

Factors Affecting Embryonic Mortality

Observed reproductive losses appear to occur soon after fertilization. In sheep and swine, the proportion of atrophic embryos increases with the number of ova shed per ovary. The production of a large number of ova per ovary frequently drains the nutrients available for them and leads to atrophy, either as follicles, newly fertilized ova or as partially developed embryos (Hammond, 1921).

Warwick (1928), Wilson et al. (1948), Squiers et al. (1952), Perry (1954), Self et al. (1955), Rathnasabapathy et al. (1956), Lerner et al. (1957) and Perry and Rowlands (1962) working with swine have found that a major proportion of embryo mortality occurring in the early stages of gestation, ranging from 20-85 percent of the original ova shed. Most of this work involved counting corpora lutea and live embryos on the twenty-fifth day of pregnancy.

Tanabe and Casida (1949) reported that cows of low fertility but with normal reproductive tracts had a satisfactory percentage of pregnancies (66.1 percent) when slaughtered on the third day postbreeding. Thirty-four days after breeding the cows were slaughtered and only 23.1 percent were pregnant. The embryonic death rate was 65.1 percent. Hulet et al. (1956) found embryonic losses greater during the first eighteen days postbreeding (20.4 percent) than during the remainder of gestation (9.4 percent). Similar work by Rathnasabapathy et al. (1956), with swine, showed a mortality rate of 31.3 percent on the fifty-fifth day of gestation and 32.8 percent at parturition, further substantiating that a bulk of the embryonic losses occur during the first trimester of pregnancy.

Dutt (1954) reported an embryonic death rate of 32.7 percent in sheep. The reproductive performance of the ewes as a group was classified as follows: 1) ova not fertilized 38.9 percent and 2) embryonic deaths 20.0 percent. Low fertilization rate in sheep has also been reported by Hoversland et al. (1958), Hoxsey (1959) and Hoxsey et al. (1960) to be the major factor reducing reproductive performance.

Pomeroy (1960) found that in sows the problem of reduced litter size was traceable to one of two main causes: 1) failure of fertilization or 2) early death of the embryos.

Lasley et al. (1963) associated embryonic mortality in swine to the degree of intra-uterine migration that might occur. Considerable migration was observed by comparing corpora lutea count to embryo present in the uterine horn. Where migration occurred, the litters contained 1.24 more pigs. It was suggested that intra-uterine migration may be important in preventing embryonic death losses by distribution of embryos between the uterine horns.

Associated Phenomena and Abnormalities

The evidence available tends to indicate that rising temperatures affect a variety of physiological functions such as respiration, circulation and possible metabolic and endocrine functions. Whether the reproductive functions are affected directly through the anterior pituitary gland or secondarily is not definitely understood (Andrews, 1953).

MacFarlane *et al*. (1957) cited by Ryle (1963) stated that adrenal gland hyperactivity in rats appeared to be the major factor in embryonic mortality in the acclimatized animal exposed to high temperatures, although after an adjustment period the adrenal gland was of little significance. McDonald (1962) associated a malnutrition and stress syndrome to early embryonic mortality in sheep. The prolonged stimulation of adrenal activity leads to adrenal hypertrophy and a greatly increased capacity to secrete cortisol. In the stress syndrome, the hypoglycemia alone is insufficient to provide for high cortisol secretion; however, the combination of a variety of stresses can add sufficient stimulus to cortisol secretion to produce an excess. The net effect would be much the same in the two syndromes, and between the two extremes various combinations of malnutrition and environmental stress may produce the same effect. Ryle (1961) injected two groups of Merino ewes with thyroxine and exposed one group to a $32^{\circ}$ C. temperature, the other group was kept at the normal daily temperature. The high temperature produced fewer ovulations and a smaller proportion of potential embryos continued to develop and, of these, a high percentage died within the first twenty-five days gestation. Both in the group on high temperature and in the control group, the proportion of potential embryos developing and the proportion of actual embryos surviving at twenty-five days of pregnancy were greater when thyroxine injections were given. However, in the ewes subjected to high temperatures and thyroxine injections there was a significant increase in number of live embryos at the first twenty-five days gestation. Ryle (1963) postulated that thyroxine may reduce embryonic mortality in ewes placed in a high temperature in two ways. In the absence of adequate maternal thyroid hormone, the trophoblast cells may enlarge too slowly to stimulate adequately some response in the uterine endometrium essential for maintenance of the embryo, or disproportionate growth in different parts of the fetus may result directly in embryonic losses. Ryle (1963) and Brooks et al. (1964) have reported a significantly greater lambing rate and birth weight from ewes with intact thyroid glands. On the basis of these observations, it was concluded that optimal reproductive ability in the ewe probably is dependent to a variable degree upon the thyroid hormones.

The presence of estrogens has also given some indication of possible effects on reproductive performance. Coop and Clark (1960) found a reduction in conception rate at first service of 10-15 percent and lambing percentage of 10-20 percent with ewes mated on lucerne. Although the factor in the lucerne responsible for these effects was not identified, it was presumed to be the presence of one or more estrogens.

Edgar (1962) found that in eighteen month to two-year old sheep that ova tend to pass more quickly through the Fallopian tubes, entering the uterus by the third day after mating versus the fourth day in older ewes. It was postulated that earlier relaxation of the uteral tubal junction in young ewes may be due to their having a lower level of estrogen secretion. Hawk et al. (1964) found that the injection of estrogen into immature or ovariectomized ewes resulted in rapid water and sodium uptake by the uterus, and young ewes had higher water content in the uterine tissue than did older ewes.

Some physiological factors have been studied which show change when an animal is exposed to low environmental temperatures. Hannon et al. (1950) measured an increase in plasma sodium concentration when rats were exposed to $5^{o}\pm 1^{o}$ C. temperature for one month.

Reid (1958) showed the response to stress by the adrenal gland in sheep is the greatest if it has been conditioned by prior malnutrition and then followed by a climatic stress factor such as cold, inclement weather conditions. Hess (1963) also detected an alteration in body fluid electrolytes in yearling sheep when placed in a fluctuating temperature from $-19^{o}$ C. to $+18^{o}$ C. At the lowest temperature, the sheep showed a significantly higher heart rate, lower rectal temperature, reduced water consumption, and decreased urine excretion than sheep maintained at the high temperature. Concentrations of sodium and potassium in the urine were also significantly increased by the cold temperature. Hawk et al. (1964) did not have temperature gradients, but found that sodium and chloride concentration in the uterine endometrium increased during the luteal phase to a greater degree in the eighteen month old ewe than in older ewes. Potassium concentration in each uterine tissue was highest in estrus ewes and lowest in ovariectomized ewes. Chloride content of the uterine myometrium was higher in the young ewes.

An over-fat condition of animals at breeding has been shown to cause an increased infertility. Hammond (1949) found that excessive fatness did not appear to cause sterility by mechanical obstruction of the Fallopian tubes, but suggested the possible cause of sterility in over-fat sows may be from the absorption of estrogens by the fat depots. Asdell (1949) reported that over-conditioned cows having excess fat deposits in the mesosalpinx probably prevented the close association of the oviduct to the ovary at ovulation.

Dutt (1954) reported an anatomical abnormality in the vagina of a ewe, which could have prevented fertilization if the semen had not been deposited at the cervix. Hoversland <u>et al</u>. (1958) and Hoxsey (1959) encountered such abnormalities as occlusions of the oviduct, cystic ovaries and infantile reproductive organs. Edgar (1962) reported one percent of the Fallopian tubes were occluded in young New Zealand Romney ewes.

Erb <u>et al</u>. (1958) postulated that environmental variations may sensitize or desensitize the target tissues to the hormones. This could be a major factor causing reproductive sterility in normal cows. Evidence for this was suggested from these reports that uteri of clinically normal cows contained culturable bacteria in a high percentage of cases and the recognition in this and other studies that abnormal ovaries and abnormal reproductive tracts are commonly associated. Erb et al. (1959) reported that cystic ovaries, short estrus cycles, estrus after conception and retention of placenta in dairy cattle may represent a genetically influenced syndrome of endocrine origin or hormonal imbalance.

Ova Collection

Ova recovery can be a valuable technique for obtaining estimates of fertilization rate and information on ovum morphology. As early as 1898, Assheton developed techniques for obtaining the ova from the Fallopian tube or uterus. The entire mucous membrane of the oviduct was scraped and placed on a slide and examined under a microscope. To obtain embryos from the uterus a ligature was placed at the cervical end and then filled with chromic acid to control the flow and then the uterine cavity was flushed with .25 percent chromic acid. It was also observed that the ovum generally reached the uterus early on the third day, when they are in the eight cell stage.

Clark (1933) secured tubal ova by directing a stream of Locke's solution through the Fallopian tube, collecting the flushings in a watch glass. The oviduct was dissected from the mesosalpinx, straightened and detached from the uterine horn. By manipulating the watch glass under the microscope, the ovum could be easily located. In flushing the uteri, a larger pipette tapering to a bore of 4 mm with a bulb of 20 cc capacity was used. The fluid was introduced at the tubal end of the cornu and the flushings caught in a large watch glass. An additional technique was described for taking photo-micrographs of the specimens.

Other researchers have followed a similar technique described by Clark

(1933), but most of them used different flushing solutions. Some of the flushing fluids used were as follows: 1) normal physiological saline (Hoxsey, 1959; Braden, 1964), 2) Krebs solution (Dutt, 1954), 3) sterile blood serum (Alliston and Ulberg, 1961), 4) equal parts Krebs solution and sterile blood serum (Hunter et al., 1955), and 5) Tyrodes solution (Hancock and Hovell, 1961; Vincent et al., 1964).

It has been reported that ovulation in the ewe occurs near the end of estrus or approximately twenty-three to thirty hours after the beginning of estrus (Green and Winter, 1935; McKenzie and Terrill, 1937).

The optimum time for the most efficient ova recovery appears to be two and one-half days after estrus has been detected.

Hunter et al. (1955) reported ova began entering the uterus between three and one-half and four days after the ewes were detected in estrus. At four and one-half days, all of the ova recovered were found in the uterus.

The relationship between time from first heat and cleavage stage is given in Table I (Hancock and Hovell, 1961).

TABLE I. RELATIONSHIP BETWEEN OVUM AGE AND CLEAVAGE STAGE. 1/

| Days from first observation of heat | Ovum Age (days) | Average No. of Cells | Range |
|---|---|---|---|
| 2½ | 1½ | 3.4 | 2-4 |
| 3 | 2 | 6.9 | 4-8 |
| 3½ | 2½ | 7.7 | 2-8 |
| 4 | 3 | 10.4 | 6-16 |

1/ Approximate ovum age was calculated from twenty-four hours after the first heat check at which estrus was observed.

Ova Classification

Criterion for determining fertilization are primarily 1) near equal and distinct cleavage or 2) numerous sperm present in zona (Hart, 1956; Hoxsey, 1959; Hancock and Hovell, 1961; Alliston and Ulberg, 1961; Vincent et al., 1964; Braden, 1964). However, Brock and Rowson (1952) used Evans Blue absorption method to determine fertilization. When the ova are alive, the stain will penetrate the zona pellucida but not the cytoplasm. When the ova are dead, the stain is taken up rapidly by the zona pellucida and cytoplasm which becomes a dark blue. Blandau (1952), cited by Hart (1956), working with rat ova found it possible to determine fertilization by identification of the male and female pronuclei within the nucleus.

An abnormal ovum refers to one with an indented or ruptured zona pellucida, degenerated blastomeres or vitelline membranes, large vaculoes in cytoplasm or similar abnormalities such as those described by Dutt (1954), Hart (1956), Averill (1958), Laffey and Hart (1959) and Braden (1964).

Braden (1964) postulates that ova recovered by a flushing technique may rupture the zona pellucida from excess pressures. Ova may vary in their sensitivity to mechanical rupture, and this sensitivity may be increased under certain environmental stress conditions.

The reports of Wilson et al. (1948), Dutt (1954), Hart (1956), Laffey and Hart (1959) and Braden (1964) indicate that the incidence of gross morphological abnormalities may range from 14-40 percent at certain periods during the breeding season.

Associated Phenomena

There is evidence that ova transport down the Fallopian tube is regulated by a constriction occurring at the uteral-tubal junction. Black and Asdell (1959) demonstrated in rabbits the existance of this constriction occurring at the time of estrus and for about three days thereafter. It prevented the entry of ova into the uterus until after the reproductive organs came under the influence of progesterone. It was also shown that the utero-tubal constriction was influenced by estrogen and progesterone, but were unable to demonstrate edema which they believed to be the cause of this block.

Similar work by Edgar and Asdell (1960), Black and Davis (1962), and Edgar (1962) reported this phenomena also occurs with ewes and cows for about three days after estrus and the valve-like action prevents fluid under pressure in the uterine tube from entering the uterus. Black and Davis (1962) found evidence that the controlling mechanism may be a circular musculature rather than vascular changes at the tubal uterine junction. This study was made on the cow oviduct.

It has also been reported that a higher number of ovulations occur in the right ovary of sheep and cattle (McKenzie and Terrill, 1937; Melton et al., 1951).

For every corpus luteum, it is generally accepted that one ova is expelled although Dutt (1954) collected two normal-appearing ova and one with shrunken cytoplasm from the oviduct of a ewe having only two recent ovulation points; it was postulated that the ovum with shrunken cytoplasm had been ovulated during the previous estrus. Arthur (1956) observed the left ovary of an Ayrshire cow to contain only one corpus luteum, but two embryos were present. By using dyes, it was possible to establish that the extra-embryonic membrane development was similar to that which occurs with a dizygotic condition. Hoxsey (1959) reported collecting two ova from a ewe where only one rupture point was observed on the ovary. Hafez (1962) reported this phenomena to be rare, but it is possible for polyovular follicles to contain double or multiple oöcytes.

# EXPERIMENTAL CONDITIONS

## Location and Description of the Range

The range land used for this experiment is located about thirty-four miles southwest of Bozeman, Montana on the Montana Agricultural Experiment Station Red Bluff Research Ranch. The terrain is of a foothill type, located on the Eastern side of the continental divide. The Tobacco-Root mountain range lays in a Western direction from the ranch with the Eastern boundary of the ranch being the Madison River. The elevation varies from approximately 1402 to 1652 meters.

The range grazed by the experimental animals was of good quality. The vegetation consisted primarily of grass with a few sedges, forbs, and browse plants. Bearded bluebunch wheatgrass (*Agropyron spicatum*), western wheatgrass (*Agropyron smithii*), needle and thread (*Stipa comata*), junegrass (*Koeleria cristata*) and sandberg bluegrass (*Poa secunda*) were the most common grasses (Hoxsey, 1959).

The average annual precipitation, at the ranch, is thirty-six centimeters with the major rainfall occurring from April through September (United States Department of Commerce, 1958-1963).

The steeper regions of the range land used for the experiment were of the Shallow Range Soil Group. The flatter areas were classified in the Silty Range Group, by Dyksterhuis (1964).

The sheep were periodically relocated to provide fresh feed, and they were attended by a herder at all times. A source of fresh water was provided by developed springs and spring-fed creeks also supplying an abundance of water at other locations.

Experimental Animals

Ewes on this experiment varied from yearlings to six-year-olds. They were of Rambouillet, Targhee and Columbia breeding. All were grade ewes which had been managed under range conditions during their life span. No unsound ewes were included in this experiment.

The ewes (202-207), for each of the four years, were randomly selected within breed and age groups, in proportion to their numbers in the grade band of approximately 890 ewes. Nutritional levels were varied for two pre-breeding and three post-breeding treatments.

Thirty-six ewes, eighteen from each pre-breeding treatment, were randomly selected and identified for slaughter. The ewes randomly assigned to the three post-breeding feed treatments were equally divided by breed, age and pre-breeding treatment. These remaining ewes (164) were placed on the post-breeding treatments during the gestation period.

## EXPERIMENTAL METHODS AND PROCEDURE

### Pre-breeding

The pre-breeding treatment began fourteen days prior to breeding. At this time, for each of the four years, the (202-207) ewes were weighed, and one-half of the ewes were marked with a paint brand to designate the feed treatment. Body weights were taken again approximately fourteen days later or just prior to the breeding period. Prior to the weighing, all ewes were held overnight in a dry lot.

Blue plastic ear tags were placed in the right ears of the thirty-six ewes designated for slaughter, for easy identification when they were separated at the sorting chute. Additional paint brands were used to identify the three different breeds; the Rambouillets having a paint brand over the shoulder, Targhees were marked near the middle of the back and the Columbias were marked over the rump.

One-half of the ewes in 1961 were supplemented with 227 grams of a protein pellet (MSC Formula 209) per head per day in addition to range forage. In 1962 and 1963 one-half of the ewes were fed 227 grams of a protein pellet (MSC Formula 367) per head per day in addition to range forage. The following year (1964), the supplement was changed to 227 grams of 11.8 percent protein pellet (MSC Formula 366) per head per day for the first five days and then changed to the 1963 ration. During the supplementation period for the four years, the other group received only range forage. The two groups were managed as one band except during the feeding of the supplement. The ingredients of the protein pellets fed in 1961, 1962, 1963 and 1964 are shown in Table II.

TABLE II. FEED COMPOSITION OF THE PRE-BREEDING SUPPLEMENT.

| | Years | | |
|---|---|---|---|
| | 1961 | 1964 | 1962-1963-1964 |
| MSC Formula No. | 209 | 366 | 367 |
| | % of Ration | % of Ration | % of Ration |
| Ingredients: | | | |
| Barley | 47.50 | 90.75 | ----- |
| Oats | 10.00 | ----- | ----- |
| Soybean meal | 22.00 | ----- | 50.00 |
| Cottonseed meal | ----- | ----- | 20.00 |
| Linseed meal | 10.00 | ----- | 12.50 |
| Alfalfa, dehydrated | ----- | ----- | 5.00 |
| Wheat millrun | ----- | ----- | 5.00 |
| Molasses | 7.50 | 5.00 | 5.00 |
| Salt | .50 | 1.00 | 1.00 |
| Monosodium phosphate | ----- | 3.00 | 1.25 |
| Dicalcium phosphate | 2.40 | ----- | ----- |
| Trace minerals | 0.10 | 0.25 | 0.25 |
| Vitamin A 1/ | X | X | X |
| Vitamin A & D 2/ | X | ----- | ----- |
| | 100.00 | 100.00 | 100.00 |
| Calculated Protein, % | 20 | 12 | 30 |

1/ Vitamin A added to provide 10,000 I.U. per pound of feed.
2/ Pellets were fortified with Vitamin A to provide 5,000 I.U. per pound with Vitamin D providing 1,000 U.S.P. units per pound.

Breeding

The breeding period began about November 20th for each of the four years studied and lasted for seventeen days.

Breeding pens for the three different breeds were established adjacent to a sorting chute. Each night, the ewes were separated into the pens and exposed to rams of their respective breed. Three fertile rams were provided for every 100 ewes or in that ratio nearest to this figure. The rams were kept freshly ochred on the brisket to facilitate detecting ewes bred during the night.

Each morning, the rams were separated from the ewes and placed in a dry lot with fresh water and feed. The ewes bred during the night were identified and given a special chalk mark so they could be removed to their predesignated, post-breeding treatment, three days post-breeding. Any slaughter ewes that were bred during the night were also identified and chalked so they could be removed for transport to the meats laboratory on the Montana State College campus, two days post-breeding.

Each day, the supplemented ewes which had not come into estrus were separated from the non-supplemented ewes and fed before the band was returned to the range.

Thirty-six ewes were slaughtered each year, two and a half days post-breeding; the reproductive tracts were removed, wrapped in wet towels and placed in a cooler. The reproductive tract was trimmed of all excess fat and connective tissue and was carefully examined for any anatomical abnormalities or characteristics which might hinder normal reproductive performance. The ovaries were examined with respect to the follicular or corpora lutea development; and, after flushing the oviduct, measurements and weights were taken. Additional information was collected on number of ovulation points and location, ova collected and ova classification. These data were recorded the same day the ewes were slaughtered.

The tubal ova were recovered by dissecting the oviducts from the mesosalpinx, severing the tube just above the constricted region at the uterine-horn junction. The dissected oviducts were then flushed from the fimbriated end to the isthmus region, with 3 cc normal physiological saline. The flushing equipment was a 5 cc syringe, a blunt hypodermic needle and a watch glass.

If ova were not found in three flushings, the uterine horn was severed at the external bifurcation; and reverse flushing was carried out until the ova were located, or for at least three flushings. The flushings were then examined by use of a binocular-dissecting microscope. With the aid of a micro-pipette connected to a small rubber bulb, the ova were then transferred to a hanging drop slide for further examination under the microscope and photography. The flushing procedure used was similar to that described by Dutt (1954).

Fertilized ova were identified by:

1) numerous sperm found adhering to, or embedded in the zona pellucida,

2) presence of sperm in the zona with no evidence of cleavage but with one or two polar bodies observed in perivitelline spaces,

3) any degree of cleavage that contained near equal sized blastomeres and no indication of fragmentation.

Non-fertilized ova were identified by:

1) no visible sperm embedded in the zona pellucida,

2) no polar bodies or distinct cleavage furrows present.

Abnormal ova were identified by:

1) shrunken or involuted cytoplasm,

2) ruptured zona pellucida,

3) indistinct, large vaculoes in the perivitelline space and cytoplasm with indistinct cleavage furrows,

4) extremely unequal division and signs of blastomere fragmentation.

The above criterion for fertilized, non-fertilized and abnormal ova classification were based on previous work done by Dutt (1954), Hart (1956), Averill (1958) and Braden (1964).

## RESULTS AND DISCUSSION

Effects of Supplementation on the Reproductive Performance

The data collected for each of four years have been analyzed for two age groups within two pre-breeding treatments. One group of ewes was bred as yearlings and the other group of ewes, up to six-years-old, were designated as mature ewes.

### Results of 1961 Trial

Ovulation Rate, Fertilization Rate and Abnormalities

Mature ewes

All of the twenty-five mature ewes in the slaughter group had at least one ruptured graafian follicle. A total of thirty-six ovulation points were observed with an average ovulation rate of 1.72 for the supplemented ewes and 1.22 for the non-supplemented group.

Four abnormal ova, with unequal sized blastomeres were observed in the supplemented group and was the most common abnormality. Two ova with ruptured zona pellucidas and one ova with a shrunken cytoplasm were also found in the non-supplemented ewes. Of the thirty-four ova recovered, seven or 20.6 percent of the ova were classified abnormal (Table III).

Occlusion of the right Fallopian tube was present in one ewe from the non-supplemented group.

The number of ova recovered was two less than the total number of ovulation points located (Table III). The recovery rate represented 94.4 percent of the thirty-six observed rupture points of which twenty-seven or 79.4 percent were fertilized.

Yearling ewes

The fifteen yearling ewes had fewer multiple ovulations than the

TABLE III. OVULATION RATE, FERTILIZATION RATE AND ABNORMAL OVA IN THE MATURE AND YEARLING SLAUGHTER EWES (1961).

| Feed Treatment | Age of ewes | No. of ewes | No. of ovulation points | % ovulation points | Ova Recovered Fert. | Ova Recovered Unfert. | Ova Recovered Total | Abnormal Ova Fert. | Abnormal Ova Unfert. | Abnormal Ova Total |
|---|---|---|---|---|---|---|---|---|---|---|
| Supplement | Mature | 11 | 19 | 172.7 | 13 | 5 | 18 | 2 | 2 | 4 |
| Non-supplement | Mature | 14 | 17 | 121.4 | 14 | 2 | 16 | 3 | -- | 3 |
| Sum | Mature | 25 | 36 | 144.0 | 27 | 7 | 34 | 5 | 2 | 7 |
| Percent | | -- | -- | ----- | 79.4 | 20.6 | 94.4 | 14.7 | 5.9 | 20.6 |
| Supplement | Yearlings | 7 | 9 | 128.6 | 8 | -- | 8 | 1 | -- | 1 |
| Non-supplement | Yearlings | 8 | 9 | 112.5 | 9 | -- | 9 | 1 | -- | 1 |
| Sum | Yearlings | 15 | 18 | 120.0 | 17 | -- | 17 | 2 | -- | 2 |
| Percent | | -- | -- | ----- | 100.0 | 0.0 | 94.4 | 11.8 | 0.0 | 11.8 |
| Sum - Supplement slaughter ewes | | 18 | 28 | 155.6 | 21 | 5 | 26 | 3 | 2 | 5 |
| Percent | | -- | -- | ---- | 80.8 | 19.2 | 92.6 | 11.5 | 7.7 | 19.2 |
| Sum - Non-supplement slaughter ewes | | 22 | 26 | 118.2 | 23 | 2 | 25 | 4 | -- | 4 |
| Percent | | -- | -- | ----- | 92.0 | 8.0 | 96.2 | 16.0 | 0.0 | 16.0 |

mature ewes. Eighteen ruptured ovulation points were found and the ovulation rate for the supplemented and non-supplemented ewes was 1.29 and 1.13, respectively.

One ovum with a ruptured zona pellicuda was found in each pre-breeding group, but no other abnormalities were found that might have prevented normal reproduction.

The fertilization rate and recovery rate were considerably higher in the younger ewes compared to the mature, 100.0 percent and 94.4 percent, respectively. However, the number of ovulation points for both the supplemented and non-supplemented ewes was nine each (Table III).

By applying the Chi-square method of analysis to the fertilization rate between the two pre-breeding treatments combined age groups, the probability level was $<.25$ that the observed difference was due to chance. Statistical methods used were those of Snedecor (1964).

## Results of 1962 Trial

### Ovulation Rate, Fertilization Rate and Abnormalities

#### Mature ewes

A total of twenty-six or 81.3 percent of the ova were recovered from the twenty-four mature slaughter ewes. Thirty-two recently formed corpus lutea were found and the average ovulation rate for the supplemented and non-supplemented ewes was 1.50 and 1.17, respectively.

One fertilized ova with unequal sized blastomeres was recovered from a non-supplemented ewe.

Twenty-one or 80.8 percent of the ova recovered were fertilized (Table IV).

TABLE IV. OVULATION RATE, FERTILIZATION RATE AND ABNORMAL OVA IN THE MATURE AND YEARLING SLAUGHTER EWES (1962).

| Feed Treatment | Age of ewes | No. of ewes | No. & of ovulation | % points | Ova Recovered Fert. | Ova Recovered Unfert. | Ova Recovered Total | Abnormal Ova Fert. | Abnormal Ova Unfert. | Abnormal Ova Total |
|---|---|---|---|---|---|---|---|---|---|---|
| Supplement | Mature | 12 | 18 | 150.0 | 12 | 2 | 14 | -- | -- | -- |
| Non-supplement | Mature | 12 | 14 | 116.7 | 9 | 3 | 12 | 1 | --- | 1 |
| Sum | Mature | 24 | 32 | 133.3 | 21 | 5 | 26 | 1 | -- | 1 |
| Percent | | -- | -- | ---- | 80.8 | 19.2 | 81.3 | 3.8 | -- | 3.8 |
| Supplement | Yearlings | 9 | 9 | 100.0 | 6 | 3 | 9 | -- | -- | -- |
| Non-supplement | Yearlings | 9 | 9 | 100.0 | 6 | -- | 6 | -- | -- | -- |
| Sum | Yearlings | 18 | 18 | 100.0 | 12 | 3 | 15 | -- | -- | -- |
| Percent | | -- | -- | ----- | 80.0 | 20.0 | 83.3 | 0.0 | 0.0 | 0.0 |
| Sum - Supplement slaughter ewes | | 21 | 27 | 128.6 | 18 | 5 | 23 | -- | -- | -- |
| Percent | | -- | -- | ----- | 78.3 | 21.7 | 85.2 | 0.0 | 0.0 | 0.0 |
| Sum - Non-supplement slaughter ewes | | 21 | 23 | 109.5 | 15 | 3 | 18 | 1 | -- | 1 |
| Percent | | -- | -- | ----- | 83.3 | 16.7 | 78.3 | 5.6 | 0.0 | 5.6 |

Yearling ewes

All of the eighteen yearling ewes that were slaughtered had one ovulation point for each ewe or an ovulation rate of 1.00 for supplemented and non-supplemented ewes. Three ova were not recovered which gave an 83.3 percent recovery rate for both groups.

No abnormal ova were reported in either group and of the fifteen ova collected twelve or 80.0 percent were fertilized (Table IV).

The fertilization rate between the two pre-breeding treatments and combined age groups was tested by the Chi-square method and the probability level calculated was $<.75$.

Results of 1963 Trial

Ovulation Rate, Fertilization Rate and Abnormalities

Mature ewes

The ovulation rate for the twenty-seven mature ewes was nearly equal for both the supplemented and non-supplemented groups, 1.35 and 1.38, respectively. Of all the thirty-seven ruptured follicles, thirty-two or 86.5 percent of the ova were recovered. One ewe from the supplemented group appeared to have a developed graafian follicle which had not ruptured.

One unfertilized ovum was classified as abnormal from a supplemented ewe when it appeared to have a ruptured zona pellucida.

Occlusion of the oviduct was present in one ewe from each treatment group. Excess pressure caused the Fallopian tube to rupture in one instance, and the ova was not recovered. The other required extra force although the saline passed through and the ovum was recovered on the third flushing.

Cystic type transparent growths (approximately .75 cm in diameter) filled with a clear solution and opaque granular objects were located on the mesosalpinx at the medial area of both oviducts in two mature ewes. However, the cysts did not interfere with flushing as normal ova were recovered from both oviducts. Another ewe from the supplemented group contained a white appearing nodule (.5 cm in diameter) on the left ovary and the ovary appeared to show signs of atrophy.

The cervix, uterine body and both uterine horns were observed to contain pigmentation in one of the ewes. This has been reported by Grant (1933) and is not considered to be an abnormal condition.

Of the thirty-two ova collected, twenty-two or 68.8 percent of the ova were fertilized (Table V).

<u>Yearling ewes</u>

All ova were collected for every developed corpus luteum in the yearling ewes slaughtered in 1963, and all the ova were fertilized (Table V).

One ovum appeared to be abnormal that was collected from a supplemented ewe. The zona pellucida contained numerous spermatozoa although the eight blastomeres appeared to contain large vacuoles and granular material, giving indication of fragmentation (Hart, 1956).

The Chi-square test on fertilization rate between combined age groups and comparing the two pre-breeding treatments had a probability level of $<.90$.

TABLE V. OVULATION RATE, FERTILIZATION RATE AND ABNORMAL OVA IN THE MATURE AND YEARLING SLAUGHTER EWES (1963).

| Feed Treatment | Age of ewes | No. of ewes | No. & % of ovulation points | | Ova Recovered | | | Abnormal Ova | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Fert. | Unfert. | Total | Fert. | Unfert. | Total |
| Supplement | Mature | 14 | 19 | 135.7 | 10 | 7 | 17 | -- | 1 | 1 |
| Non-supplement | Mature | 13 | 18 | 138.5 | 12 | 3 | 15 | -- | -- | -- |
| Sum | Mature | 27 | 37 | 137.0 | 22 | 10 | 32 | -- | 1 | 1 |
| Percent | | -- | -- | ----- | 68.8 | 31.3 | 86.5 | 0.0 | 3.1 | 3.1 |
| Supplement | Yearlings | 4 | 4 | 100.0 | 4 | 0 | 4 | 1 | -- | 1 |
| Non-supplement | Yearlings | 4 | 4 | 100.0 | 4 | 0 | 4 | -- | -- | 0 |
| Sum | Yearlings | 8 | 8 | 100.0 | 8 | 0 | 8 | 1 | -- | 1 |
| Percent | Yearlings | -- | -- | ----- | 100.0 | 0.0 | 100.0 | 1.3 | 0.0 | 1.3 |
| Sum - Supplement slaughter ewes | | 18 | 23 | 127.8 | 14 | 7 | 21 | 1 | 1 | 2 |
| Percent | | -- | -- | ----- | 66.7 | 33.3 | 87.5 | 4.8 | 4.8 | 9.6 |
| Sum - Non-supplement slaughter ewes | | 17 | 22 | 129.4 | 16 | 3 | 19 | 0 | 0 | 0 |
| Percent | | -- | -- | ----- | 84.2 | 15.8 | 86.4 | 0.0 | 0.0 | 0.0 |

## Results of 1964 Trial

### Ovulation Rate, Fertilization Rate and Abnormalities

#### Mature ewes

Of the twenty-four mature ewes, thirty ovulation points appeared on the ovaries and thirty-one ova were recovered. Two ova were flushed from the left oviduct of a mature non-supplemented ewe and the left ovary appeared to have only one rupture point. The phenomena just mentioned has been reported to occur occasionally in cattle as well as in sheep (Arthur, 1956; Hoxsey, 1959; Hafez, 1962).

The ovulation rate for the supplemented and non-supplemented ewes was 1.58 and .91, respectively (Table VI).

Two abnormal ova from one mature ewe of the supplemented group appeared to have ruptured zona pellucidas and vitelline membranes. A major portion of the cytoplasm had disappeared giving the ova a halo appearing effect when observed under the dissecting microscope.

The left oviduct in one of the supplemented ewes was obstructed and three flushings were required before the ovum was recovered.

Of the thirty-one ova recovered, twenty-four or 77.4 percent of the ova were fertilized.

#### Yearling ewes

Of the four years data reported, the 1964 yearling slaughter ewes from the supplemented group responded differently by having a lower ovulation rate than the non-supplemented ewes, .83 and 1.16, respectively. All ova were recovered for every corpus luteum observed.

An abnormal fertilized ovum, with a ruptured zona pellucida, was found

TABLE VI. OVULATION RATE, FERTILIZATION RATE AND ABNORMAL OVA IN THE MATURE AND YEARLING SLAUGHTER EWES (1964).

| Feed Treatment | Age of ewes | No. of ewes | No. of ovulation points | % ovulation points | Ova Recovered Fert. | Ova Recovered Unfert. | Ova Recovered Total | Abnormal Ova Fert. | Abnormal Ova Unfert. | Abnormal Ova Total |
|---|---|---|---|---|---|---|---|---|---|---|
| Supplement | Mature | 12 | 19 | 158.3 | 13 | 6 | 19 | -- | 2 | 2 |
| Non-supplement | Mature | 12 | 11 | 91.7 | 11 1/ | 1 | 12 | -- | -- | 0 |
| Sum | Mature | 24 | 30 | 125.0 | 24 | 7 | 31 | -- | 2 | 2 |
| Percent | | -- | -- | ----- | 77.4 | 22.6 | 103.3 | 0.0 | 6.5 | 6.5 |
| Supplement | Yearlings | 6 | 5 | 83.3 | 5 | 0 | 5 | 1 | -- | 1 |
| Non-supplement | Yearlings | 6 | 7 | 116.7 | 5 | 2 | 7 | -- | -- | 0 |
| Sum | Yearlings | 12 | 12 | 100.0 | 10 | 2 | 12 | 1 | -- | 1 |
| Percent | | -- | -- | ----- | 83.3 | 16.7 | 100.0 | 8.3 | 0.0 | 8.3 |
| Sum - Supplement slaughter ewes | | 18 | 24 | 133.3 | 18 | 6 | 24 | 1 | 2 | 3 |
| Percent | | -- | -- | ----- | 75.0 | 25.0 | 100.0 | 4.2 | 8.3 | 12.5 |
| Sum - Non-supplement slaughter ewes | | 18 | 18 | 100.0 | 16 | 3 | 19 | 0 | 0 | 0 |
| Percent | | -- | -- | ----- | 84.2 | 15.8 | 105.6 | 0.0 | 0.0 | 0.0 |

1/ Two (2) ova were flushed from the left oviduct of a mature ewe and the left ovary appeared to have only one rupture point.

in a ewe from the supplemented group.

Occlusion of the oviduct caused some difficulty with one ewe from the non-supplemented group. Developing, but unruptured Graafian follicles appeared to be present on each ovary of a ewe from the supplemented group.

One ovary and a portion of the Fallopian tube on the left side in one yearling ewe appeared to have been removed accidently during slaughter, therefore no record was possible, although an ovum was recovered from the right oviduct.

The fertilization rate for the twelve yearling ewes was ten out of twelve ova recovered or 83.3 percent.

The Chi-square method for testing fertilization rate in relation to the combined age groups and pre-breeding treatments had a probability level of $<.75$.

Effects of Pre-breeding Treatment on Body Weight Change

The body weights as influenced by the pre-breeding treatment each of the four years have been analyzed as two age groups within two treatments. The age groups are designated as yearling ewes and the mature ewes from 3-6 years old.

### Results of 1961 Trial

Mature Ewes

Both the supplemented and non-supplemented mature ewes gained weight during the pre-breeding treatment (November 3--November 24). This resulted in a weight gain advantage of +3.26 pounds for the supplemented ewes as compared to the non-supplemented ewes (Table VII). This increase or weight change between the two treatments was found to be significant ($P<.01$)

TABLE VII. EFFECTS OF PRE-BREEDING TREATMENTS ON BODY WEIGHTS OF MATURE AND YEARLING EWES.

| Feed Treatment and Year | Age of Ewes | Avg. Body Weights kg. | Body Weight Change kg. | Weight Change Between Treatments kg. |
|---|---|---|---|---|
| | | Dates | | |
| 1961-62 | | Nov. 3 | Nov. 24 | |
| Supplement | Mature | 60.95 | +2.71 | 1.48 |
| Non-supplement | Mature | 60.44 | +1.23 | |
| Supplement | Yearlings | 55.82 | +2.07 | 1.09 |
| Non-supplement | Yearlings | 56.33 | +0.98 | |
| 1962-63 | | Nov. 13 | Nov. 24 | |
| Supplement | Mature | 56.15 | -1.10 | 2.50 |
| Non-supplement | Mature | 54.87 | -3.60 | |
| Supplement | Yearlings | 59.80 | -1.64 | 1.75 |
| Non-supplement | Yearlings | 59.81 | -3.39 | |
| 1963-64 | | Nov. 6 | Nov. 21 | |
| Supplement | Mature | 63.17 | +1.66 | 4.60 |
| Non-supplement | Mature | 61.66 | -2.94 | |
| Supplement | Yearlings | 58.02 | +0.24 | 2.09 |
| Non-supplement | Yearlings | 59.41 | -1.85 | |
| 1964-65 | | Nov. 3 | Nov. 19 | |
| Supplement | Mature | 64.15 | +1.87 | 1.09 |
| Non-supplement | Mature | 65.14 | +0.78 | |
| Supplement | Yearlings | 58.83 | +1.16 | 0.91 |
| Non-supplement | Yearlings | 57.17 | +0.25 | |

(Table VIII). Statistical methods were those of Steel and Torrie (1960).

Yearling Ewes

The body weight difference between the two groups for the yearling ewes compared closely to the mature ewes for the year 1961. The supplemented and non-supplemented ewes gained +4.56 and +2.16 pounds, respectively. Likewise the weight change was significant for the pre-breeding period ($P < .01$).

Although the weight change within the two age groups and pre-breeding treatments were highly significant, there was no significance found between the mature and yearling groups (Table VIII).

TABLE VIII. ANALYSIS OF VARIANCE OF WEIGHT CHANGE DURING THE PRE-BREEDING PERIOD (1961).

| Variations due to: | Degrees of freedom | Sums of squares | Mean square | s | F Value |
|---|---|---|---|---|---|
| Treatment | 3 | 607.81 | 202.60 | | 10.771** |
| Age | 1 | 49.30 | 49.30 | | 2.621 |
| Supplement | 1 | 558.29 | 558.29 | | 29.680** |
| Ages X Supplement | 1 | .22 | .22 | | .012 |
| Error | 200 | 3762.02 | 18.81 | 4.338 | |
| Total | 206 | 4369.83 | | | |

** ($P < .01$)

## Results of 1962 Trial

Mature Ewes

During the pre-breeding treatment (November 13 - November 24), the supplemented and non-supplemented mature ewes lost 1.10 and 3.60 kilograms, respectively. However, the supplemented ewes did have a weight gain of

2.50 kilograms over the non-supplemented ewes at the termination of the pre-breeding period (Table VII). There was a significant difference in body weights ($P < .01$) between the two treatments (Table IX).

Yearling Ewes

The yearling ewes responded very similarly to the mature ewes; however, they had a greater weight loss during the pre-breeding treatment (Table VII). The supplemented ewes showing a weight loss of 1.64 kilograms, and the non-supplemented ewes, 3.39 kilograms. The weight gain advantage of 1.75 kilograms between the two groups was found to be significant ($P < .01$) (Table IX). However, as reported in 1961 there was no significant difference between the age groups and body weight change.

TABLE IX. ANALYSIS OF VARIANCE OF WEIGHT CHANGE DURING THE PRE-BREEDING PERIOD (1962).

| Variations due to: | Degrees of freedom | Sums of squares | Mean square | s | F Value |
|---|---|---|---|---|---|
| Treatment | 3 | 1333.84 | 444.61 | | 15.087** |
| Age | 1 | 2.70 | 2.70 | | .092 |
| Supplement | 1 | 1299.45 | 1299.45 | | 44.094** |
| Ages X Supplement | 1 | 31.69 | 31.69 | | 1.075 |
| Error | 199 | 5865.39 | 29.47 | 5.429 | |
| Total | 205 | 7199.23 | | | |

** ($P < .01$)

Results of 1963 Trial

Mature Ewes

The body weight change for the supplemented and non-supplemented mature ewes was +1.66 and -2.94 kilograms, respectively, for the pre-breeding period (November 6 - November 21). This weight change was greater than for either of the two previous years (Table VII). The supplemented ewes had a weight gain advantage of 4.60 kilograms over the non-supplemented ewes, and this difference was found to be significant ($P < .01$).

Yearling Ewes

The yearling ewes for each treatment responded similarly to the mature ewes, although not to such an extreme degree of weight change. The supplemented ewes gained 0.24 kilograms while the non-supplemented group lost 1.85 kilograms (Table VII). The over-all weight change between the two groups was 2.10 kilograms which was found to be significant ($P < .01$) (Table X). This was the only year that a highly significant difference was found for age and age X supplement in the variance of analysis table. However, supplementation showed the greatest significance as it did in the previous years.

TABLE X. ANALYSIS OF VARIANCE OF WEIGHT CHANGE DURING THE PRE-BREEDING PERIOD (1963).

| Variations due to: | Degrees of freedom | Sums of squares | Mean square | s | F Value |
|---|---|---|---|---|---|
| Treatments | 3 | 2571.79 | 857.26 | | 59.040** |
| Age | 1 | 259.47 | 259.47 | | 17.870** |
| Supplement | 1 | 1627.12 | 1627.12 | | 112.061** |
| Ages X Supplement | 1 | 685.20 | 685.20 | | 41.190** |
| Error | 194 | 2816.49 | 14.52 | 3.811 | |
| Total | 200 | 5388.28 | | | |

** ($P < .01$)

## Results of 1964 Trial

### Mature Ewes

Both the supplemented and non-supplemented groups of mature ewes had a weight gain of 1.87 and 0.78 kilograms, respectively, during the pre-breeding period (November 3 - November 19) (Table VII). The weight gain difference of the supplemented ewes over the non-supplemented ewes was 1.09 kilograms and was found to be significant ($P < .01$) (Table XI).

### Yearling Ewes

The yearlings in both the supplemented and non-supplemented groups gained 1.16 and 0.25 kilograms, respectively (Table VII). The weight change of 0.90 kilograms within the two pre-breeding treatments was found to be significant ($P < .01$) (Table XI). There was no significant difference between the weight change in the mature and yearling ewes during the pre-breeding period.

TABLE XI. ANALYSIS OF VARIANCE OF WEIGHT CHANGE DURING THE PRE-BREEDING PERIOD (1964).

| Variations due to: | Degrees of freedom | Sums of squares | Mean square | s | F Value |
|---|---|---|---|---|---|
| Treatments | 3 | 343.67 | 114.56 | | 5.229** |
| Ages | 1 | 80.40 | 80.40 | | 3.670 |
| Supplement | 1 | 261.63 | 261.63 | | 11.941** |
| Ages X Supplement | 1 | 1.64 | 1.64 | | .075 |
| Error | 196 | 4294.98 | 21.91 | 4.681 | |
| Total | 202 | 4638.65 | | | |

** ($P < .01$)

## Effects of Supplementation on the Reproductive Performance

### Results of 1961 - 1964 Trials

### Ovulation rate, fertilization rate and abnormalities

Mature and yearling ewes

In summarizing the four years for the mature ewes, a total of one-hundred ewes were slaughtered. The ovulation rate for the supplemented and non-supplemented groups was 1.53 and 1.17, respectively. These ovulation rates compare to earlier work reported by McKenzie and Terrill (1937). From the total of 135 ovulation points found, 123 ova were collected and studied, giving a recovery rate of 91.1 percent for the four year period (Table XII).

The high number of ovulation points agrees with earlier work that reported ovulation not to be a major factor in lowering reproductive rates in sheep (Dutt, 1954; El Sheikh *et al*., 1955; Hoxsey, 1959).

The data compiled for the four years on the yearling ewes shows the ovulation rate to be 1.04 for the supplemented ewes and 1.07 for the non-supplemented ewes. Edgar (1962) confirmed that the lower incidence of multiple births in yearling and two-year-old ewes was due to their lower ovulation rate, 1.10 as compared to 1.44 ova per ewe in the five-year-old ewes. Fifty-six ovulation points were observed of which fifty-two ova were recovered for a recovery rate of 92.9 percent. There was no evidence of fertilization in five or 9.6 percent of the ova recovered. Also, the fertilization rate for the four years ranged from a six to thirty-two percent increase in the yearling ewes over the mature ewes. The over-all difference in fertilization rate between the yearling and mature ewes over the four years was 14.0 percent (Table XII).

TABLE XII. TOTAL OVULATION RATE, FERTILIZATION RATE AND ABNORMAL OVA IN THE MATURE AND YEARLING SLAUGHTER EWES (1961 - 1964).

| Feed Treatment | Age of ewe | No. of ewes | No. & of ovulation | % points | Ova Recovered Fert. | Ova Recovered Unvert. | Ova Recovered Total | Abnormal Ova Fert. | Abnormal Ova Unfert. | Abnormal Ova Total |
|---|---|---|---|---|---|---|---|---|---|---|
| Supplement | Mature | 49 | 75 | 153.1 | 48 | 20 | 68 | 2 | 5 | 7 |
| Non-supplement | Mature | 51 | 60 | 117.6 | 46 | 9 | 55 | 4 | 0 | 4 |
| Sum | Mature | 100 | 135 | 135.0 | 94 | 29 | 123 | 6 | 5 | 11 |
| Percent | | --- | --- | ----- | 76.4 | 23.6 | 91.1 | 4.9 | 4.1 | 9.0 |
| Supplement | Yearlings | 26 | 27 | 103.8 | 23 | 3 | 26 | 3 | 0 | 3 |
| Non-supplement | Yearlings | 27 | 29 | 107.4 | 24 | 2 | 26 | 1 | 0 | 1 |
| Sum | Yearlings | 53 | 56 | 105.7 | 47 | 5 | 52 | 4 | 0 | 4 |
| Percent | | --- | --- | ----- | 90.4 | 9.6 | 92.9 | 7.7 | 0.0 | 7.7 |
| Sum - Supplement slaughter ewes | | 75 | 102 | 136.0 | 71 | 23 | 94 | 5 | 5 | 10 |
| Percent | | --- | --- | ----- | 75.5 | 24.4 | 92.2 | 5.3 | 5.3 | 10.6 |
| Sum - Non-supplement slaughter ewes | | 78 | 89 | 114.1 | 70 | 11 | 81 | 5 | 0 | 5 |
| Percent | | --- | --- | ----- | 86.4 | 13.6 | 91.0 | 6.2 | 0.0 | 6.2 |

In comparing the total supplemented mature and yearling ewes to the non-supplemented ewes, the supplemented ewes had a 21.9 percent increase in ovulation rate; but the fertilization rate was 10.9 percent greater for the non-supplemented ewes. This phenomena was also true for each year (1961-1964) with the 1963 non-supplemented ewes having the largest increase, in fertilization rate, of 17.5 percent over the supplemented ewes (Table V).

The fertilization rate between the two pre-breeding treatments and age groups was tested by Chi-square ($P < .90$).

No fertilization was evident in 23.6 percent of the ova collected from the mature ewes, and 9.6 percent showed no signs of cleavage in the yearling ewes. Dutt (1954) reported over a three-year period that 38.9 percent of the ova recovered were not fertilized. Low fertilization rate in sheep has also been reported by Hoversland et al. (1958) and Hoxsey (1959) to be the major factor reducing reproductive performance. Of the fertilized ova, 4.9 percent were abnormal and 4.1 percent of those not fertilized were abnormal. In the fall of 1961, the highest abnormality rate for the four years was reported. The highest number of abnormal ova was collected when the low and high temperature over a four-day period ranged from $-37^{\circ}$ to $-11^{\circ}$ C., respectively. Marshall and Hammond (1948) found that any unfavorable climatic conditions such as a snow storm or drought during breeding resulted in a reduction of multiple births.

The total abnormal ova for the four years was 9.0 percent for the mature ewes and 7.7 percent for the yearling ewes. The supplemented ewes had 4.4 percent more abnormal ova than the non-supplemented ewes. El Sheikh et al. (1955) has reported a higher ovulation along with a greater embryonic mortality rate in ewes that were supplemented prior to breeding as compared to the ewes on a lower nutrient level.

The abnormal ova for both the mature and yearling ewes were classified and ranked as follows: 1) ruptured zona pellucidas, 2) unequal sized blastomeres, 3) fragmentation and 4) shrunken cytoplasm.

On December 12th, in the fall of 1964, one yearling ewe and three mature ewes appeared to have unruptured follicles at slaughter time. There was a slight drop in temperature prior to and during this period though no adverse weather conditions or high amounts of precipitation were reported. Error in determining time of breeding may have attributed to this situation.

It was found that a major portion of multiple ovulations occurred at the middle of the breeding seasons and one to two days past the mid-point, before a decline began. Some researchers reporting similar results have been Roberts (1921), Johnson (1932), McKenzie and Terrill (1937), and Asker and Ragab (1954).

In the fall of 1961, from December 9 to December 12, the low temperatures were $-22^{o}$, $-36^{o}$, $-37^{o}$ and $-30^{o}$ C., consecutively. During the four-day period, a total of fifteen ova were classified abnormal, with nine abnormal ova from the non-supplemented group. From these fifteen abnormal ova it was found that ten ova were classified abnormal because of unequal sized blastomeres and five because of ruptured zona pellucidas.

Braden (1964) postulated that ova may vary in their sensitivity to mechanical rupture, and this sensitivity may be increased under certain environmental stress conditions and also, that the flushing technique may rupture the zona pellucida because of extreme pressures.

It has not been well established, but work by MacFarlane *et al*. (1957), Ryle (1963) and Brooks *et al*. (1964) has indicated that acclimatic conditions produce a variety of physiological changes that alter the respiration, circulation and possible metabolic and endocrine functions in the animal body. Whether the reproductive processes are affected directly is not definitely known at this time.

Microphotographs of sheep ova in Figures 1, 2, 3 and 4 are representative of normal unfertilized ova, fertilized ova, and an abnormal condition where unequal sized blastomeres are observed.

## Effects of Pre-breeding Treatment on Body Weight Change

### Results of 1961 - 1964 Trials

### Mature and yearling ewes

The influence of supplemental feeding fourteen days prior to breeding on body weight change for the four years is shown in Table XIII.

TABLE XIII. BODY WEIGHT CHANGE FOR THE TWO PRE-BREEDING TREATMENTS BY AGE GROUPS (1961-1964).

| Feed Treatment | Age of ewes | Avg. Body Weight (Before) | Body Weight Change (After) | Weight Change For Treatments |
|---|---|---|---|---|
| | | kg. | kg. | kg. |
| Supplement | Mature | 62.13 | +1.29 | +1.75 |
| Supplement | Yearlings | 58.12 | +0.46 | |
| Non-supplement | Mature | 60.53 | -1.13 | -2.13 |
| Non-supplement | Yearlings | 58.18 | -1.00 | |

A body weight gain was found for both the supplemented mature and yearling ewes for each year with the exception of 1962 when supplemented and non-supplemented ewes from both age groups lost weight (Table VII).

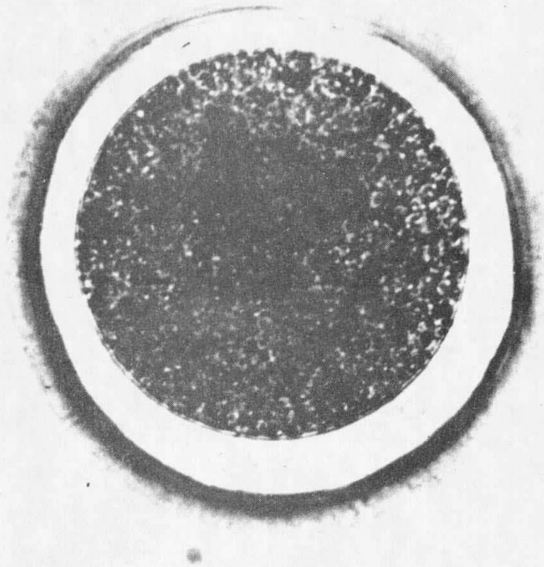

Figure 1. Unfertilized sheep ovum. X 870

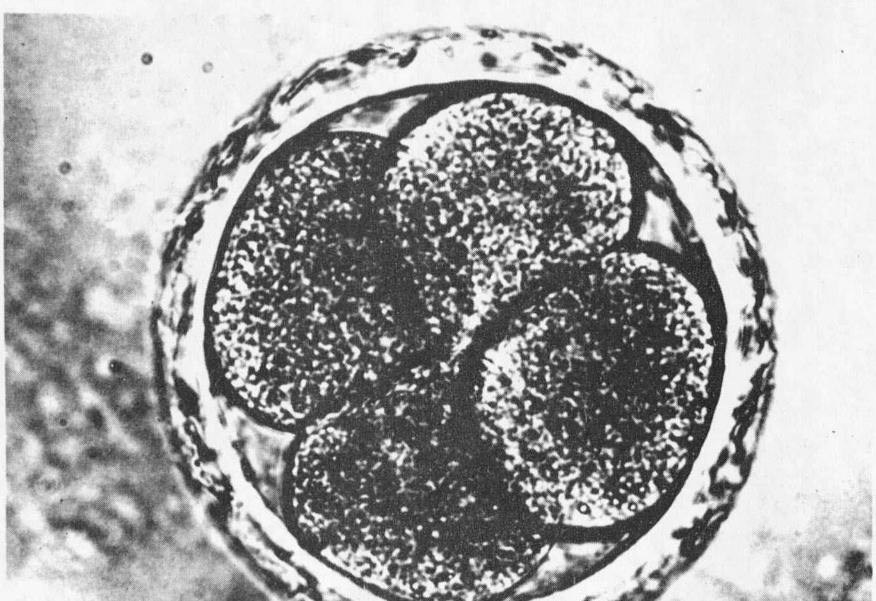

Figure 2. Fertilized sheep ovum; four-cell stage. X 870

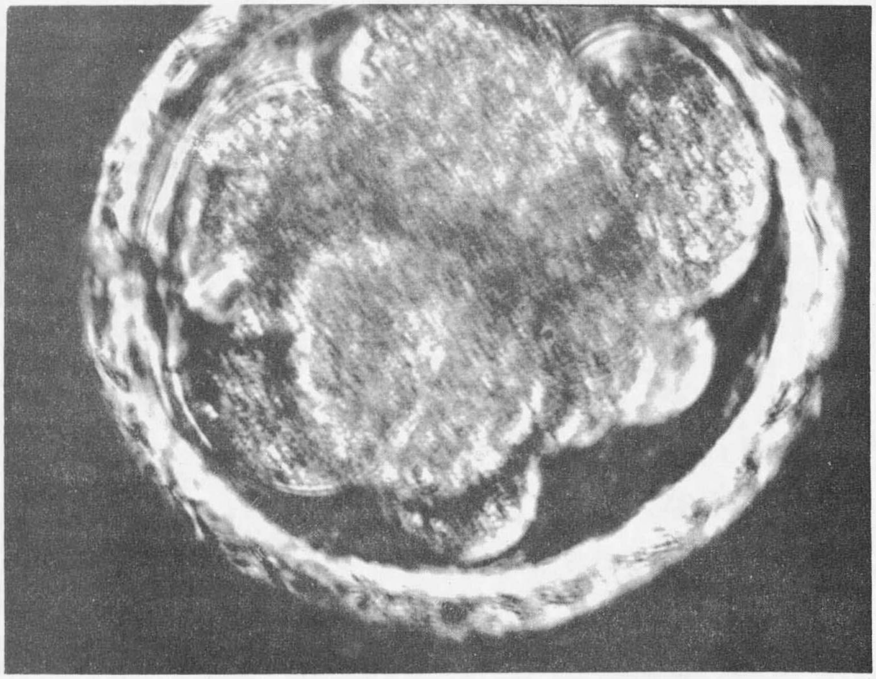

Figure 3. Fertilized sheep ovum with degenerating blastomeres; note the different sized blastomeres. X 950

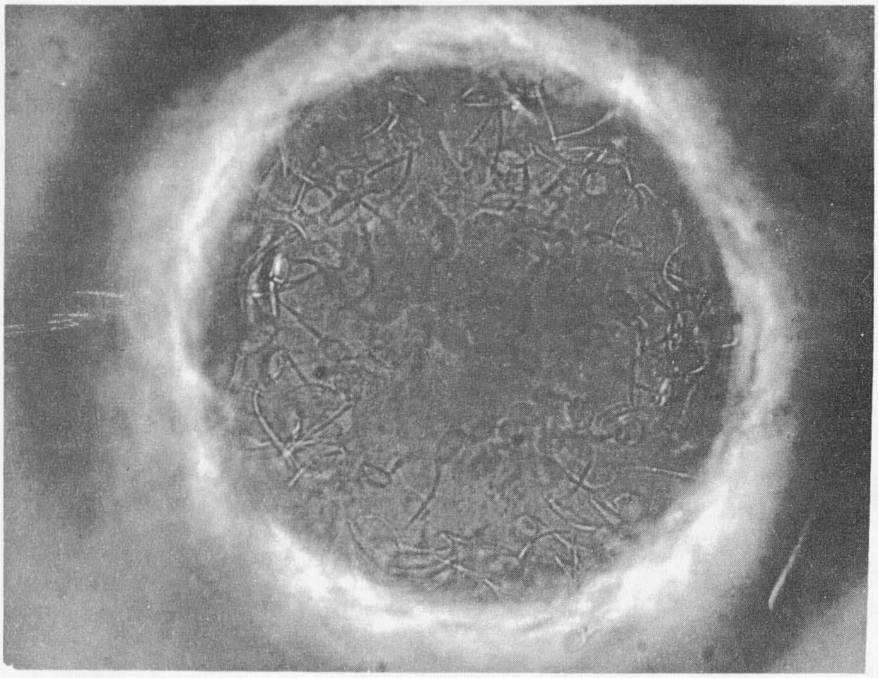

Figure 4. Sperm in zona pellucida of sheep ovum; polar-view. X 950

Supplementing during the pre-breeding period of 1962 did not produce a weight gain, but reduced weight loss by 2.50 kilograms for the mature supplemented ewes and 1.75 kilograms for the yearling supplemented ewes.

During the fall of 1963, the supplemented and non-supplemented ewes and the two age groups showed the greatest body weight variation. The weight change between the treatments being 4.60 kilograms for the mature ewes and 2.10 kilograms for the yearling ewes. At the same time, the supplemented ewes from the mature and yearling groups gained 1.66 and 0.24 kilograms, respectively, while the non-supplemented mature and yearling ewes lost 2.94 and 1.85 kilograms, respectively.

The mature ewes for the total of four years had a body weight change between the two treatments of 2.42 kilograms while yearling ewes had a difference of 1.46 kilograms (Table XIV).

TABLE XIV. EFFECTS OF PRE-BREEDING TREATMENTS ON BODY WEIGHTS OF MATURE AND YEARLING EWES (1961-1964).

| Feed Treatment | Age of ewes | Avg. Body Weight (Before) | Body Weight Change (After) | Weight Change Between Treatments |
|---|---|---|---|---|
| | | kg. | kg. | kg. |
| Supplement | Mature | 62.13 | +1.29 | 2.42 |
| Non-supplement | Mature | 60.53 | -1.13 | |
| Supplement | Yearlings | 58.12 | +0.46 | 1.46 |
| Non-supplement | Yearlings | 58.18 | -1.00 | |

The difference in weight gain or loss due to treatment was significant ($P < .01$) for all years. Analysis of variance table combining all years is shown in Table XV.

TABLE XV. ANALYSIS OF VARIANCE OF WEIGHT CHANGE DURING THE PRE-BREEDING PERIOD (1961-1964).

| Variations due to: | Degrees of freedom | Sums of squares | Mean square | s | F Value |
|---|---|---|---|---|---|
| Years | 3 | 10069.81 | 3356.60 | | 169.954** |
| Ages | 1 | 190.60 | 190.60 | | 9.650** |
| Supplement | 1 | 3244.57 | 3244.57 | | 164.282** |
| Years X Supplement | 3 | 597.12 | 199.04 | | 10.077** |
| Years X Ages | 3 | 183.45 | 61.15 | | 3.096* |
| Supplement X Ages | 1 | 135.66 | 135.66 | | 6.868 |
| Supplement X Ages X Years | 3 | 1517.13 | 505.71 | | 25.605** |
| Error | 801 | 15822.66 | 19.75 | 4.444 | |
| Total | 816 | 31665.73 | | | |

* $(P < .05)$
** $(P < .01)$

As noted in Table XV, combined data for all four years, in addition to finding a significant $(P < .01)$ supplement effect, there also occurred a significant $(P < .01)$ year effect. Also shown is a significant age effect and years X supplement and years X ages X supplement interactions. The years X age interaction was significant at the $(P < .05)$ level.

It is difficult to know what specific components of the environment were responsible for the different year effects and the interactions. It may be assumed that acclimatic variations and grazing conditions for the different years possibly exert a great influence on weight gain or loss during the experimental period.

Results for the pre-breeding treatment in body weight change during the four years, are similar to findings reported by Hoversland (1958).

The daily supplementation prior to breeding of ewes managed under range conditions did not insure body weight gain. The mature ewes showed a 0.96 kilogram increase above the yearling ewes for the four years studied. The difference between supplementing and non-supplementing did produce a significant ($P < .01$) weight advantage for each age group.

## SUMMARY

Data from four years work (1961, 1962, 1963, and 1964) were compiled and evaluated for nutritional and environmental effects on ovulation rate, fertilization rate and ova normality in white-faced ewes managed under range conditions.

The experimental animals were randomly selected from grade ewes of the Columbia, Targhee and Rambouillet breeds. From the total of 817 ewes, 576 three to six year olds were designated as mature ewes and 241 represented the yearling or coming two-year-old classification.

The pre-breeding treatment consisted of a fourteen-day period prior to breeding and during breeding, whereby one-half of the ewes (approximately 100 each year) were group-fed a pelleted supplement at the rate of 227 grams per head per day. The remaining one-half of each age group received no supplement and were used for the control. The supplemented ewes were separated each morning, fed, and then grazed on the range, together with the controls, during the day.

Body weight change between the two treatments and two age groups was recorded and analyzed for each year and for all four years. All the ewes were weighed just prior to the pre-breeding treatment and again approximately fourteen days later.

A total of 100 mature ewes and 53 yearlings were slaughtered three days post-breeding during the four years to collect data on ovulation rate, fertilization rate and any abnormalities observed that might hinder subsequent reproductive performance.

There was some variation in ovulation rates within years, but the over-all rate for the supplemented and non-supplemented ewes was 1.36 and

1.14, respectively. The total ovulation rate for the mature ewes, supplemented and non-supplemented was 1.53 and 1.17, respectively. The yearling ewes had an ovulation rate of 1.03 for the supplemented ewes and 1.07 for the non-supplemented ewes. This reverse response of the pre-breeding treatment to the ovulation rate in yearling ewes has been reported in earlier trials.

The recovery rate for the total period was 91.1 percent for the mature ewes and 92.9 percent for the yearling ewes.

The Chi-square method for testing fertilization rate between the two pre-breeding treatments and combined age groups showed varying levels of significance for each year, with the over-all test for the four years having a probability level of $\angle .90$. In comparing the total supplemented mature and yearling ewes to the non-supplemented ewes, the supplemented ewes had a 21.9 percent increase in ovulation rate; but the fertilization rate was 10.9 percent greater for the non-supplemented ewes. This phenomena was also true for each of the four years with the 1963 non-supplemented ewes having the largest increase of 17.5 percent over the supplemented ewes. No fertilization was evident in 23.6 percent of the ova recovered from the mature ewes, and 9.6 percent showed no sign of cleavage in the yearling ewes.

The highest incidence of abnormal ova were recollected when the low and high temperatures over a four-day period ranged from -37° to -11° C., respectively. The total abnormal ova for the four years was 9.0 percent for the mature ewes and 7.7 percent for the yearling ewes. The supplemented ewes had 4.4 percent more abnormal ova than the non-supplemented ewes.

Occlusion of the oviducts was a problem in five ewes, which was reported four times in mature ewes, two each from the pre-breeding treatments and once in a yearling from the non-supplemented group.

The body weight changes during the supplemental feeding period for the four years resulted in body weight gains for all but one year, 1963. The supplemental feeding did result in a significant ($P < .01$) weight difference between the supplemented and non-supplemented ewes in both age groups. In addition to all four years showing a significant ($P < .01$) treatment and supplement effect in 1963, age and age X supplement interaction were also significant ($P < .01$). In addition to a significant ($P < .01$) year effect occurring, there also was a significant ($P < .01$) treatment effect, years X supplements and years X ages X supplements interactions. The years X age interaction was also significant ($P < .05$).

APPENDIX

APPENDIX TABLE I. METRIC AND ENGLISH EQUIVALENTS AND TEMPERATURE CONVERSION.

| | | |
|---|---|---|
| **Linear Measure** | | |
| 10 millimeters (mm.) | = | 1 centimeter (cm.) |
| 10 centimeters | = | 1 decimeter (dm.) |
| 10 decimeters | = | 1 meter |
| 1 inch (in.) | = | 2.54 centimeters |
| 1 foot (ft.) | = | 0.3048 meter |
| 1 yard (yd.) | = | 0.9144 meter |
| **Square-Measure Equivalents** | | |
| 1 square inch | = | 6.452 square centimeters |
| 1 square foot | = | 0.0929 square meter |
| 1 square yard | = | 0.8361 square meter |
| **Weight-Measure Equivalents** | | |
| 1 kilogram (kg.) | = | 1000 grams (gm.) |
| 1 kilogram | = | 2.2046 pounds (Avoir.) |
| 1 quintal | = | 220.46 pounds |
| 1 metric ton | = | 2204.6 pounds |
| **Temperature Conversion** | | |
| Fahrenheit degrees (F.$^{\circ}$) | = | 1.8 X Centigrade degrees (C.$^{\circ}$) +32 |

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