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Alteration of the Anestrous Period in Targhee Ewes

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ALTERATION OF THE ANESTROUS PERIOD IN TARGHEE EWES

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ALTERATION OF THE ANESTROUS PERIOD

IN TARGHEE EWES

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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INTRODUCTION

Under natural conditions, sheep are considered polyestrous seasonal breeders. Estrus occurs as day length starts to decrease in the fall. The ability to extend this restricted breeding season to a year-round breeding season would make it possible for sheep to produce two lamb crops per year. This offers many advantages to the industry. Economic pressure could be spread over two lamb crops per year instead of one. Year-round lambing would even out high and low price fluctuations caused from seasonal production. Packing plants would be spared the enduring effects of over and under supply. Producers could better budget and utilize their manpower inventories by planning the breeding and lambing periods to fit their labor availability.

The following experiment was initiated to gain insight into the effects of light regimens and/or hormone manipulation on the onset of estrus in anestrous ewes.

REVIEW OF LITERATURE

Sheep are seasonally polyestrous animals that begin their sexual activity during the period of the year of decreasing day length. The question of inducing estrus in the anestrous ewe has brought about many theories. A series of previous experiments have been conducted observing the effect of controlled photoperiods and hormone stimulation. A review of the effect of artificial light and hormone manipulation on induction of estrus in anestrous ewes is presented in the following sections.

Types of Stimuli Affecting the Occurrence of Estrus

Photoperiod. Photoperiod is thought to be the dominant factor regulating sexual activity in ewes (Yeates, 1947). Attempts by Newton and Betts (1972) to influence estrus revealed that the type of light made no difference. However, artificial photo patterns caused estrus to occur earlier. When ewes were subjected to an abrupt increase in light, 16 hr artificial light for 42 days followed by an abrupt decrease to 8 hr of light, sexual activity of treated ewes occurred 35 days earlier than normal. Walton et al. (1977) exposed ewes to 6 hr light and 18 hr darkness with the change being made abruptly on the longest day of the year. Ewes subjected to short days expressed an ovulatory estrus 20 days earlier than control ewes on natural day length. Experiments of Ducker and Bowman (1970b), Ducker et al. (1970a) and Newton and Betts (1972) support this finding.

Grocock and Clarke (1974) postulated that it is not length of light or dark periods but rather the time when these effects occur that has the greatest altering force. They suggested there exists an endogenous circadian rhythm where animals are sensitive to light at different times of the day. This circadian rhythm has been revealed in work with the vole. The normal breeding season for the vole is spring and summer. During fall and winter, vole testes hypotrophy and sperm counts decrease. In spring, the reverse occurs. Voles kept under artificial light similar to a normal breeding season, i.e., 15L:9D (lights on 0900 to 2400 hr), had normal testes weights of approximately 304 milligrams. Another light pattern, 3L:9D:3L:9D (lights on 0900 to 1200 hr, 2100 to 2400 hr), resulted in testes weights of 334 milligrams. This lighting pattern, 3L:9D:3L:9D, had 60% less light hours than the control, 15L:9D, but maintained testes weights greater than the controls.

While 6 hr of light in the 3L:9D:3L:9D pattern maintained testes weight greater than the controls, 6 hr of light in the 6L:18D regimen did not. The pattern of 6L:18D (lights on 0900 to 1500 hr) resulted in testes weights of 133 milligrams. A lighting regimen of 3L:2D:2L:16D (lights on 0900 to 1200 hr and 1400 to 1700 hr) resulted in smaller testes weights (128 mg). This was different ($P<.05$) from the control (15L:9D) or the 3L:9D:3L:9D regimens. It appears that length of light stimulus is not as important as when the stimulus occurs. Perhaps the stimulant in the treatment of 3L:9D:3L:9D was not the two 3-hr light periods but rather the 9-hr dark stimulus between the lighted times. Hart (1950) reported

that a pattern of 4L:2D:4L:14D was more advantageous than 4L:8D:4L:8D in inducing early estrus in Suffolk ewes.

Pineal Gland. Rats, when exposed to constant light, experience hypotrophy of the pineal gland; darkness causes hypertrophy (Fiske et al., 1960). Wurtman et al. (1964) observed continuous light to stimulate incidences of increased estrus. However, darkness suppressed the occurrence of estrus. Extraction of the eyes and superior cervical ganglia blocked the effect of continuous light. Pinealectomy of rats caused ovarian and anterior pituitary hypertrophy (Wurtman et al., 1959). Protein-free pineal extract (PPE) administered to pinealectomized rats prevented hypertrophy of both the ovaries and anterior pituitary. Thus, it appears that the pineal gland has a stimulatory effect on the ovary and pituitary, while itself being stimulated by light. Roche et al. (1970c) investigated the effects of pinealectomy on ewes and found no difference in the time of estrus. Grocock and Clarke (1974) found that the inhibitory lighting pattern on voles referred to earlier, 6L:18D, could be blocked by pinealectomy. It should be noted those animals revealing the most effects from pinealectomy were nocturnal animals (Roche et al., 1970c). Perhaps these effects are due to an endogenous circadian rhythm.

Luteinizing Hormone (LH) and Prolactin. Work done by Roche et al. (1970b) supported earlier findings that peak LH levels occur in the ewe at the onset of heat. They also reported LH concentrations decreased from May to July during anestrus. Denamur et al. (1973) and

Schroff et al. (1971) reported that LH and prolactin are both necessary for ovine corpus luteum (CL) maintenance. They found that 5 mg of Follicle Stimulating Hormone (FSH) per day was inadequate to prevent luteal regression in hysterectomized, hypophysectomized ewes. LH alone and mixtures of FSH and LH were insufficient, also. However, when a combination of 500 i.u. prolactin and .25 mg LH were injected intramuscularly daily, the CL was maintained comparable to that observed in the hysterectomized ewes prior to hypophysectomy. Niswender (1974) reported when prolactin levels were suppressed to approximately 1% of those observed in control ewes that no difference in luteal function was detected. This indicates that a very small quantity of prolactin is needed to maintain CL activity. Anestrous levels of LH in ewes were reported undetectable; but, when ovariectomized, ewe serum LH levels increased (Roche et al., 1970c). It has been reported ewe pituitary LH levels increased between days 1 and 15 of the estrous cycle, whereas serum LH levels remained constant (Roche et al., 1970b). These workers also found that during proestrus pituitary serum LH levels were depleted by 88%, supporting work reported by Robertson and Rakha (1966) and Dierschke and Clegg (1968).

Based on observed levels of FSH, LH, progesterone and prolactin, Walton et al. (1977) concluded that sexual activity during the fall breeding season in the ewe was brought about by the decreasing level of prolactin. In experiments in which 2-bromo-α-ergocryptine (CB-154) have been administered to ewes, serum levels of prolactin have been reduced ($P<.01$) when compared to controls receiving no treatment (Niswender, 1974). Results obtained by Schanbacker (1980) concurred with this response

to CB-154. In his experiment, ewes were injected intramuscularly daily with 1 to 2 mg of CB-154. Results showed prolactin levels to be equal to that of natural night time basal levels. He felt this decrease in antigonadotropic hormone (prolactin) was due to the decreased day length of the fall season. Walton et al. (1977) revealed an increase of prolactin in ewes during anestrus and a decrease prior to ovulation as determined via daily blood collections. They stated that the decrease was hastened in those ewes on short day treatments. This idea of decreasing prolactin in the fall was supported by work done by Schanbacker (1980) and Walton et al. (1977). However, Schanbacker acknowledged that Clun Forest ewes exposed to short day lengths or treated to depress prolactin synthesis did not express estrus any earlier than the controls.

Blask and Reiter (1975) found that blinding and anosmia resulted in depressed prolactin levels in female rats. They also reported that this dual sensory deprivation caused a decrease in body weight and a significant hypotrophy of the reproductive and accessory sex organs. When the pineal gland of these same animals was removed, the effects of blinding and anosmia were removed. Blinding alone had no effect on reproductive organ size (Reiter and Ellison, 1970). Work done by Relkin (1972) supported a pituitary prolactin decrease following blinding and anosmia.

Serum prolactin levels were reported to be more elevated during proestrus and estrus in the rat than during diestrus (Amenomori et al., 1970). Three weeks following ovariectomy rats were found to have significantly reduced serum prolactin levels. When 5 µg estradiol benzoate were

administered to these rats for 5 days, a significant increase in both pituitary and serum prolactin was seen. Ratner (1964) determined that estrogen caused an increase in prolactin by blocking the prolactin inhibiting factor. High levels of progesterone were correlated with low levels of prolactin during pregnancy in the rat (Amenomori et al., 1970). During anestrus, the ewe has no functioning CL, resulting in minimal progesterone production. As previously stated (Walton et al., 1977), there was an increase in prolactin in ewes during anestrus and a decrease prior to ovulation. Increased estrogen during estrus was thought to be responsible for increased prolactin synthesis in the rat (Ratner, 1964). Meites and Turner (1949) described progesterone as capable of inhibiting increased prolactin due to small doses of estrogen. Another interpretation is that the low levels of estrogen are responsible for low prolactin levels during pregnancy. These conflicting reports indicate a need for further investigation into the controlling mechanism of prolactin.

Melatonin. Melatonin, a hormone synthesized in the pineal gland, has a greater productivity during natural darkness than during daylight (Rollag et al., 1976, 1978). Soon after the dark phase begins, melatonin concentrations increased eight- to tenfold in the ewe. This increased nighttime level coincides with the long nights, short days of the breeding season in the ewe. Possibly sheep monitor the longer length of fall darkness through the increased melatonin levels due to darkness (Rollag et al., 1978). Hydroxyindole-o-methyl transferase (HIOMT), the enzyme responsible for the biosynthesis of melatonin, was found in the

retina and pineal gland (Nagle et al., 1972; Ralph et al., 1971). The hypothalomo-hypophyseal axis was thought to be the target organ of melatonin (Rollag et al., 1978). A circadian rhythm in this enzymatic activity has been observed in the rat (Nagle et al., 1972; Axelrod et al., 1965; Rollag et al., 1976; Klein and Weller, 1970). Ralph et al. (1974) observed a similar rhythm of melatonin in the pineal bodies of White Leghorn cockerels. Maximum production in the retinal area occurred during light stimulation. However, the pineal reached greatest melatonin production during darkness. They also observed that constant light abrogated variations in both the retina and pineal gland. Ralph et al. (1971) deduced from in vitro studies that rat pineal glands produced more melatonin in darkness and less in light. This observation adds additional support to the circadian rhythm idea. Work done by Roche et al. (1970a) revealed again a circadian rhythm of melatonin. When melatonin was administered to golden hamsters, no effects on ovulation or the timing of LH release were observed (Bex et al., 1978). In pinealectomized rats, significant increases in pituitary LH were observed (Frashini et al., 1968a); but, when melatonin was administered, pituitary LH levels declined (Frashini et al., 1968b). Castrated ewes were described as producing more serum LH from days 15 to 30 postcastrated than from days 1 to 14 (Roche et al., 1970a). This indicates that the pituitary has the ability to adjust to the effects of castration. Castrated ewes continually infused with melatonin revealed an inhibitory effect on LH secretion (Roche et al., 1970a). Adams et al. (1965) reported that female rats receiving daily injections of melatonin had increased pituitary LH

stores. Roche et al. (1970a) found that castrated ewes with or without pineal glands experienced an increased serum LH level. They indicated that the pineal was not the dominant force regulating LH in the ewe. Pituitary LH stores were not affected by pinealectomy nor by the infusion of melatonin. Finally, ewes showed LH peaks after progesterone implants were removed while being infused continuously with melatonin.

Bex et al. (1978) have presented the idea that melatonin acts upon the hypothalmo-hypophysial axis to block prolactin release in male hamsters. Both exogenous melatonin and exposure to a short photoperiod suppressed gonadal function in the hamster (Tamarkin et al. (1976). This suggested that each has a common mediator, i.e., the pineal gland. An observation made by Reiter (1974) as interpreted by Tamarkin et al. (1976) indicated melatonin may act within the pineal gland and not as a pineal hormone. Tamarkin et al. (1976) found that pinealectomized female hamsters showed blocked antigonadal effects of administered melatonin. This observation is supportive of the thought that melatonin reacts within the pineal rather than as a pineal hormone.

Hart (1950) postulated that added hours of darkness during the fall result in more hours of increased melatonin production which overlap the sensitive periods of the circadian rhythm. Rollag et al. (1978) has indicated that sheep given endogenous melatonin during estrus will lengthen the cycle; but, when melatonin was given during anestrus, the reverse occurred. Melatonin given to rats inhibited ovarian growth (Wurtman et al., 1963a) and delayed the occurrence of the next estrus (Wurtman et al., 1963b).

The literature reveals different responses to melatonin between nocturnal and diurnal animals. Because of these differences, continued research is needed to determine the role of melatonin in initiation of estrus in the ewe.

Breed of Ewe. Genetic variation among breeds of sheep throughout the world affects major aspects of reproductive performance. Studies on the breeding season (Hafez, 1952a) have revealed significant breed differences among periods of maximum sexual activity. Dorsets were observed to maintain maximum sexual activity between September and December, while Suffolks experienced estrus October to February. Leicesters revealed maximum estrus to be November to December. Duration of breeding season was another observation differing among breeds as reported by Hafez (1952a). The shortest mean length of breeding season was observed in Leicester ewes (131 ± 11.7 days) and the longest found in Dorsets (223 ± 13.7 days). Within the breeding season, different breeds displayed considerable variation in the number of heat cycles per ewe. Hafez (1952b) reported a significant difference among breeds at the onset of first estrus. Cole and Miller (1935) reported Hampshires and Rambouillets to have an earlier breeding season than Southdown, Shropshires and Romneys.

Variation in prolificacy can be seen among breeds caused from different ovulation rates during the same breeding season (Hammond, 1921; Henning, 1939). Age plays a role in prolificacy. Turner and Dolling (1965) reported that most ewes are fertile for at least 10 years but have the greatest potential for multiple births at 6 and 7 years of age.

Season and Temperature. Lifetime lambing performance is affected by the time of year at which parturition takes place. Lambs born early (January and February) will have more time to mature sexually before their first fall breeding season. Length of the breeding season is affected by latitude. Sheep originating in high latitudes have a shorter breeding season than those at the equator (Yeates, 1947; Hafez, 1952a). Continuous year-round breeding occurring in tropical regions may be due to selective breeding or photoperiod stimulation (Yeates, 1947).

In one study (Dutt and Bush, 1955) first estrus was 8 weeks earlier for ewes treated with cold temperature compared to controls exposed to natural day-to-day temperatures. Twenty-five ewes were contained in a 40.8 m^2 air-conditioned room maintained at 4.4 C. Control ewes were housed in the same size area but in the confines of a conventional barn with no temperature manipulation. Work done by Godly et al. (1966) confirmed that decreased temperature enhanced the onset of estrus.

Vesely (1975) detected a correlation in number of lambs born per ewe between breeds and the time of breeding. Ducker and Bowman (1970a) bred 100 Suffolk ewes during each of two periods (May to July and February to March). They found that for 100 ewes bred in May to July 62 lambs were born, whereas 113 per 100 ewes were born for those ewes bred in February and March.

Current literature leaves many questions unanswered and provides no precise mechanism responsible for estrus. In the hope of gaining

insight into this underlying mechanism, the study reported herein was designed to study the effectiveness of altered photoperiods, hormone manipulation and combinations of the aforementioned on inducing estrus in anestrous ewes.

MATERIALS AND METHODS

The study reported herein consisted of fifty anestrous 6-year-old Targhee ewes treated with artificial photoperiods and/or hormones to stimulate the onset of estrus. Ewes were randomly allotted to one of the following five treatment groups: (1) group 1 ewes served as controls and were maintained outdoors, exposed only to natural daylight throughout the study (ND), (2) group 2 ewes were maintained outdoors, exposed to natural daylight plus receiving 1 mg 2-bromo-α-ergocryptine (CB-154) and 12 µg melatonin per day (ND + CB-154 and Mel), (3) group 3 ewes were housed inside, treated only with artificially extended darkness, 8 hr of light and 16 hr dark (8L:16D), (4) group 4 ewes were housed inside and exposed to artificially extended darkness (8L:16D) plus receiving 1 mg CB-154 per day (8L:16D + CB-154) and (5) group 5 ewes received artificial extended light for 28 days (16L:8D) followed by extended dark (8L:16D) for the remaining 26 days (16L:8D-8L:16D).

This experiment started July 4, 1980, and concluded August 26, 1980. Ewes used in this experiment had been transported from the Antelope Range Field Station, Buffalo, South Dakota, to the SDSU Sheep Unit just prior to the start of the experiment. Prior to the start of this experiment, the ewes were paint branded for easy identification, their necks sheared to aid in venipuncture and their feet were trimmed.

Animals received 1.84 kg chopped hay and .45 kg cracked corn each morning from July 4 until July 31. Starting August 1 through August 24, each ewe received 2.27 kg chopped hay and .45 cracked corn each morning.

Intact, fertile Suffolk rams were used for breeding and were with the same group of ewes for the duration of the experiment. Rams were semen tested at the start of the experiment. A SPE Electro Ejaculator was used for collection. Semen was evaluated for motility, concentration, morphology and live-dead counts. Motility and concentration were evaluated by the hanging drop technique. Morphology was evaluated with the live-dead stain technique.

Rams were painted 7 to 10 cm ahead of the penis twice daily with a grease and wool paint mixture and exposed to the ewes continuously. Ewes were examined daily for breeding marks. Marks were recorded as good, fair or poor. A good represented a direct mark on the tail head, with a fair being less than 10 cm to either side. A mark more than 10 cm to either side of the tail head was designated poor. To maintain ram weight through this 53-day period, each ram received an additional afternoon feeding of .45 kg cracked corn.

Treatment groups 3 (8L:16D) and 4 (8L:16D + CB-154) were housed together in a temperature controlled lightproof room. Temperature was maintained at 21.1 C by heating and cooling systems within the building. A Honeywell 153 multipoint fixed cycle recorder was used to monitor any changes in temperature. Maximum fluctuation was 4 C.

Ewes in groups 3 (8L:16D) and 4 (8L:16D + CB-154) were allowed 1.62 m^2 pen space per animal. The floor within the holding area was solid concrete except for a 60-cm wide grated gutter along the east wall. This floor was scraped daily and gutters flushed three times per week. Water was supplied to ewes from a stock tank via an automatic float valve.

Group 5 (16L:8D-8L:16D) ewes were allowed 2.34 m^2 per animal. The floors were comprised of two 2.43-m wide slotted areas with a 1.52-m wide center concrete alley. Water was supplied through automatic bowl fountains.

Artificial light sources in each room were provided by 660 watt, cool white fluorescent lights located 289 cm above the floor. Light schedules were controlled by Intermatic time switches.

Group 1 (ND) and 2 (ND + CB-154 and Mel) were kept outside without shelter in a pen providing 3.90 m^2 per ewe. Water was provided in the same way as for groups 3 (8L:16D) and 4 (8L:16D + CB-154). The pen area was dirt, shaded at one end by elm trees.

Groups 1 (ND) and 2 (ND + CB-154 and Mel) were maintained in natural daylight and ambient temperature. Groups 3 (8L:16D) and 4 (8L:16D + CB-154) were maintained under artificial light. Lights came on at 0930 hours. This 8-hr photoperiod was chosen as it represented the mean natural day length of the fall breeding season. Group 5 (16L:8D-8L:16D) was introduced to an abrupt increased artificial day length (16L:8D) for 28 days followed by a sudden reversal to 8L:16D for the remainder of the experiment.

Ewes in group 4 (8L:16D + CB-154) received injections right after the lights came on at 0930 hr and just prior to lights off at 1700 hours. The widest time span possible between injections was used in an attempt to suppress maximum prolactin production in both groups 2 (ND + CB-154) and 4 (8L:16D + CB-154). Injections of .5 mg of 2-bromo-α-ergocryptine (CB-154) per ml of carrier (60% ethanol and 40% saline) were administered

intramuscularly in the gluteal region of groups 2 (ND + CB-154 and Mel) and 4 (8L:16D + CB-154). To compensate for traveling time between light controlled laboratories and ewes at the farm, which were 1.609 km apart, CB-154 injections for group 2 (ND + CB-154 and Mel) were administered 1/2 hr later than group 4 (8L:16D + CB-154).

Group 2 (ND + CB-154 and Mel) ewes received daily subcutaneous melatonin injections at a level of 3 µg per ml in a 100% ethanol medium. Five ewes of the ten were given one 4-ml injection at 1800 hr daily. The remaining five received 1-ml injections each hour for 4 hr beginning at 1800 hr daily. It was decided that the injection volume was too large, so on July 12, the volume of carrier was reduced to one-fourth of the original levels. Those ewes receiving one 4-ml injection were given one 1-ml and individuals receiving 1-ml injections were reduced to .25 milliliter. The 1-ml injection was delivered in three to four different areas of the auxiliary space. All syringes used for both CB-154 and melatonin were labeled with a ewe's number to prevent a ewe from being injected twice or inadvertently missed. Inoculate was kept at 4 C in 10 cc bottles with rubber stoppers. During injections, containers were kept on ice.

Two 10-ml blood samples were collected from each ewe once weekly by jugular venipuncture, one for plasma and one for serum. Samples were collected between 1100 and 1200 hours. Plasma blood samples were collected in tubes containing 143 USP units sodium heparin. Plasma samples were kept on ice and serum samples were allowed to stand at room temperature. An International centrifuge, Size 1, Model SBV, located

in a walk-in refrigerator, was used to harvest plasma and serum. Heparinized plasma samples were immediately centrifuged upon completion of bleeding. Serum samples were allowed to stand at room temperature for an average of 70 min before spinning. All blood was harvested after spinning at 150 G for 25 min at 0 C. Sera and plasma were stored at -24 C for future assay.

Statistical analyses were performed on normally distributed data by least squares procedures (Steel and Torrie, 1980). Duncan's New Multiple Range Test was used to separate means for factors having significant F values. Binomial data were tested using Chi square analysis. Independent Chi square values were determined to identify significant treatment effects. A probability level of less than .05 was considered as the maximum level at which significance was accepted. Analysis of variance and Chi square values were determined from parame ers shown in appendix tables.

RESULTS AND DISCUSSION

A difference ($P<.05$) in days to first breeding mark is shown in table 1. Based on breeding marks, group 5 (16L:8D-8L:16D) came into estrus 1 week earlier than group 3 (8L:16D), 2 weeks before group 4 (8L:16D + CB-154) and 3 weeks before groups 2 (ND + CB-154 and Mel) and 1 (ND). Testing among treatments using orthogonal contrasts revealed treatments 1 (ND) and 2 (ND + CB-154 and Mel) to be different ($P<.05$) from the other three groups. Although a difference was observed in the days to first marking, no difference was found in the average lambing date (table 2).

The number of days to lambing from the start of this experiment is defined as the latent period. There were no differences ($P>.05$) between latent periods for the five treatment groups (table 2). This study failed to support the hypothesis that decreased day length enhanced estrus as found in work done by Ducker et al. (1970b). While there was no absolute date for the start of the breeding season for Targhee ewes, control ewes (ND) bred earlier than expected. Perhaps first estrus for these ewes was abnormally early due to the cooler than normal temperatures recorded for South Dakota during the summer of 1980. Previous studies have shown ewes must adjust to treatment, i.e., artificial lighting regimens and hormone manipulation, before the latent period is decreased (Vesely, 1975; Schanbacker, 1980). To detect treatment response, it may be necessary to initiate the experiment earlier to allow sufficient time for such an adjustment period.

TABLE 1. LEAST SQUARES MEANS FOR DAYS TO FIRST BREEDING MARK FROM THE START OF EXPERIMENT

Treatment	No. ewes	Mean days to first mark
Natural daylight	10	33.1 ± 5.23[a]
Natural daylight plus CB-154 and melatonin	9	33.8 ± 4.61[a]
Controlled light 8L:16D	10	19.1 ± 4.61[b]
Controlled light 8L:16D plus CB-154	9	26.0 ± 5.65[b]
Controlled light 16L:8D for 28 days followed by 8L:16D for 26 days	10	12.2 ± 4.37[b]

[a,b] Means with different superscripts are different ($P<.05$).

TABLE 2. LEAST SQUARES MEANS FOR LATENT PERIODS AND LENGTH OF LAMBING PERIOD

Treatment	Latent period[a]	Average lambing date	Length lambing period
Natural daylight	179.3 ± 3.20	1/16/80	26
Natural daylight plus CB-154 and melatonin	186.1 ± 2.99	1/7/81	29
Controlled light 8L:16D	187.6 ± 2.99	1/9/81	9
Controlled light 8L:16D plus CB-154	181.8 ± 3.46	1/3/81	34
Controlled light 16L:8D for 28 days followed by 8L:16D for 26 days	178.6 ± 3.79	1/30/81	23

[a] Latent period is the days from the start of the experiment July 4 until lambing.

Associated with days to lambing was range of days each group required to lamb (table 2). Although not tested statistically, an observed difference was seen. Treatment group 3 (8L:16D) ewes receiving decreased daylight lambed within 9 days. The next shortest lambing period was in treatment group 5 (16L:8D-8L:16D) with 23 days.

A span of 43 days was observed between the first and last recorded breeding mark for group 3 (8L:16D). A 43-day breeding period would mathmatically contain two and one-half 17-day estrus cycles. Ewes bred over a 43-day time span should lamb in a similar time period. According to breeding marks, ewes recorded as early and late breeders within this 43-day range were only bred once. Perhaps ewes recorded as breeding early in the experiment did not conceive and were bred later without observable breeding marks.

No difference ($P>.05$) in the percentage of ewes lambing due to treatment was observed (table 3).

There was no difference ($P>.05$) in total kilograms (table 4) of lamb born per ewe lambed. On the basis of ewes that lambed, the overall mean weight of lamb born per ewe lambed was 7.49 ± 1.88 kilograms.

Sex ratios were not different ($P>.05$) among treatments (table 4).

Of the 34 ewes lambing, 32% had multiple births (table 5). As previously reported (Turner and Dolling, 1965), 6-year-old ewes were at peak potential to have multiple births. Terrill (1974) has reported that quite often less ova are shed during the first seasonal ovulation. Since only 32% of all ewes lambing had multiple births, this suggests these ewes could have conceived on first ovulation or at least at an early ovulation.

TABLE 3. EFFECT OF TREATMENT ON PERCENTAGE OF EWES LAMBING

Treatment	No. ewes	No. ewes lambing
Natural daylight	10	7 (70)[a]
Natural daylight plus CB-154 and melatonin	9	8 (89)
Controlled light 8L:16D	10	8 (80)
Controlled light 8L:16D plus CB-154	9	6 (67)
Controlled light 16L:8D for 28 days followed by 8L:16D for 26 days	10	5 (50)

[a] Numbers in parentheses are percentages.

TABLE 4. WEIGHT OF LAMB BORN PER EWE LAMBED AND SEX RATIOS

Treatment	Lamb born/ewe (kg)	Sex ratios Male:female
Natural daylight	8.4 ± .67	5:7
Natural daylight plus CB-154 and melatonin	7.5 ± .84	5:1
Controlled light 8L:16D	6.3 ± .71	5:3
Controlled light 8L:16D plus CB-154	7.4 ± .77	5:3
Controlled light 16L:8D for 28 days followed by 8L:16D for 26 days	7.8 ± .67	7:4

TABLE 5. MULTIPLE BIRTHS PER GROUP

Treatment	Type of birth (number) Single:twin
Natural daylight	6:1
Natural daylight plus CB-154 and melatonin	4:4
Controlled light 8L:16D	5:3
Controlled light 8L:16D plus CB-154	4:2
Controlled light 16L:8D for 28 days followed by 8L:16D for 26 days	4:1

There was no difference ($P>.05$) in ewe weight among treatment groups at the start of this experiment (table 6). Mean ewe weights at the end of this study were different ($P<.05$). Orthogonal comparisons revealed groups 1 (ND) and 2 (ND + CB-154 and Mel) were different ($P<.05$) from the other three treatment groups. Mean weight of ewes in groups 1 (ND) and 2 (ND + CB-154 and Mel) was 7.1 kg lighter ($P<.05$) than that of ewes in the remaining three groups at the end of the experiment (table 6).

Weight change during the experiment was different ($P<.05$) among treatment groups (table 6). Treatment groups 3 (8L:16D), 4 (8L:16D + CB-154) and 5 (16L:8D-8L:16D) gained more weight ($P<.05$) during this study than groups 1 (ND) and 2 (ND + CB-154 and Mel). Treatment group 5 (8L:16D) was 2.8 kg heavier ($P<.05$) than group 4 (8L:16D + CB-154). This greater weight gain of groups 3 (8L:16D), 4 (8L:16D + CB-154) and 5 (16L:8D-8L:16D) may have been due to decreased maintenance requirements brought about by less exercise and decreased temperature. Although not

TABLE 6. LEAST SQUARES MEANS FOR BEGINNING AND ENDING WEIGHTS AND WEIGHT CHANGES DURING THE STUDY

Treatment	Beginning weight (kg)	Ending weight (kg)	Weight change (kg)
Natural daylight	57.4 ± 1.30	57.7 ± 1.51[a]	.3 ± .89[a]
Natural daylight plus CB-154 and melatonin	57.1 ± 1.30	54.1 ± 1.60[a]	-2.2 ± .93[a]
Controlled light 8L:16D	58.1 ± 1.30	66.2 ± 1.51[b]	8.1 ± .89[c]
Controlled light 8L:16D plus CB-154	56.7 ± 1.30	62.3 ± 1.60[b]	5.3 ± .93[b]
Controlled light 16L:8D for 28 days followed by 8L:16D for 26 days	55.2 ± 1.30	60.5 ± 1.51[b]	5.3 ± .89[b]

[a,b,c] Means in the same column with different superscripts are different ($P<.05$).

statistically different from control group 1 (ND), group 2 (ND + CB-154 and Mel) ended this study with a negative weight gain. As stated earlier, group 2 received injections in which a carrier consisting of 100% ethanol was used. It was observed that varying amounts of tissue damage resulted in the areas of injection. Therefore, the observed weight decrease of group 2 (ND + CB-154 and Mel) may have been the result of stress associated with injections.

In conclusion, no difference ($P>.05$) in mean lambing date was detected in these ewes due to treatment. These data suggest it may be necessary to initiate treatment earlier in order for the ewes to perceive the stimuli in time to respond before the natural breeding season starts.

SUMMARY

Fifty Targhee ewes were used to study the effects of artificial photoperiods and/or hormone manipulation for inducing estrus during the normal anestrous period. Ewes were randomly allotted to one of the five following groups: (1) natural daylight (ND); (2) natural daylight plus 2-bromo-α-ergocryptine and melatonin (ND + CB-154 and Mel); (3) controlled light, 8 hr light:16 hr dark (8L:16D); (4) controlled light, 8 hr light:16 hr dark, plus CB-154 (8L:16D + CB-154); (5) controlled light, 16 hr light:8 hr dark for 28 days followed by 8 hr light:16 hr dark for 26 days (16L:8D-8L:16D). Ewes were continually exposed to Suffolk rams which were painted daily with a grease paint mixture to aid in detecting sexual activity.

Days to first breeding mark were different ($P<.05$) among treatment groups. According to breeding marks, group 5 (16L:8D-8L:16D) bred 1 week earlier ($P<.05$) on the average than any of the other four groups.

There was no difference ($P>.05$) in the date when lambing occurred after the start of this experiment among treatment groups. Perhaps this was due to control ewes breeding earlier than normal as a result of abnormally cool summer temperatures.

While not tested statistically, there was an observed difference in the time required for each group to lamb. Group 3 (8L:16D) lambed in 9 days, whereas the mean lambing period for the remaining four groups was 28 days.

No difference ($P>.05$) was found in the percentage of ewes lambing per group due to treatment. Of the 48 ewes exposed to rams, 70.8% lambed.

There was no difference ($P>.05$) in total kilograms of lamb born per ewe lambed or in the ratio of male:female lambs born.

Mean ewe weight among treatment groups at the start of this study was similar ($P>.05$). At the end of this study, group 3 ewes (8L:16D) were heavier ($P<.05$) than ewes in the other treatment groups. There was a difference in weight change ($P<.05$) among treatment groups in that groups 3 (8L:16D), 4 (8L:16D + CB-154) and 5 (16L:8D-8L:16D) gained more weight than the control group (ND).

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APPENDIX

TABLE 1. LEAST SQUARES ANALYSIS OF VARIANCE FOR DAYS TO FIRST BREEDING MARK--TREATMENTS 1 THROUGH 5

Source of variation	df	SS	MS	F
Treatment	4	3067.212	766.803	4.01**
Error	36	6883.568	191.210	
Total	40	9950.780		

** P<.01.

TABLE 2. LEAST SQUARES ANALYSIS OF VARIANCE FOR LATENT PERIODS TREATMENTS 1 THROUGH 5

Source of variation	df	SS	MS	F
Treatment	4	449.346	112.336	1.56
Error	29	2084.212	71.869	
Total	33	2533.558		

TABLE 3. CHI SQUARE ANALYSIS FOR NUMBER OF EWES LAMBING PER GROUP--TREATMENTS 1 THROUGH 5

	Values determining χ^2 (=4.01)			
	Lambed		Did not lamb	
Treatments	Observed	Expected	Observed	Expected
1	8	6.38	1	2.63
2	5	7.08	5	2.92
3	7	7.08	3	2.92
4	6	6.38	3	2.63
5	8	7.08	2	2.92

P<.05, χ^2 value = 9.49 for four degrees of freedom.

TABLE 4. LEAST SQUARES ANALYSIS OF VARIANCE FOR KG OF LAMB BORN PER EWE LAMBED--TREATMENTS 1 THROUGH 5

Source of variation	df	SS	MS	F
Treatment	4	83.586	20.896	1.21
Error	29	501.399	17.289	
Total	33	584.986		

TABLE 5. CHI SQUARE ANALYSIS FOR SEX RATIOS OF MALE:FEMALE LAMBS BORN PER TREATMENT GROUP--TREATMENTS 1 THROUGH 5

	Values determining χ^2 (=8.22)			
	Male		Female	
Treatments	Observed	Expected	Observed	Expected
1	5	7.36	7	4.63
2	5	6.13	1	3.86
3	5	8.59	3	5.40
4	5	7.36	3	4.63
5	7	6.75	4	4.25

$P<.05$, χ^2 value = 9.49 for four degrees of freedom.

TABLE 6. LEAST SQUARES ANALYSIS OF VARIANCE FOR STARTING WEIGHT--TREATMENTS 1 THROUGH 5

Source of variation	df	SS	MS	F
Treatment	4	45.454	11.363	.68
Error	45	754.980	16.777	
Total	49	800.434		

TABLE 7. LEAST SQUARES ANALYSIS OF VARIANCE FOR ENDING WEIGHT--TREATMENTS 1 THROUGH 5

Source of variation	df	SS	MS	F
Treatment	4	795.503	198.875	8.68**
Error	43	985.740	22.924	
Total	47	1781.244		

** P<.01.

TABLE 8. LEAST SQUARES ANALYSIS OF VARIANCE FOR CHANGE IN WEIGHT DURING 53-DAY EXPERIMENT

Source of variation	df	SS	MS	F
Treatment	4	669.486	167.371	21.10**
Error	43	341.154	7.933	
Total	47	1010.640		

** P<.01.