MELENGESTROL ACETATE AND NORGESTOMET FOR THE INDUCTION OF SYNCHRONIZED ESTRUS IN SEASONALLY ANOVULAR EWES

by

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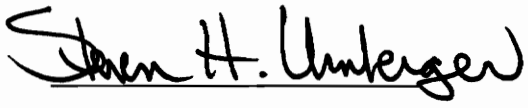

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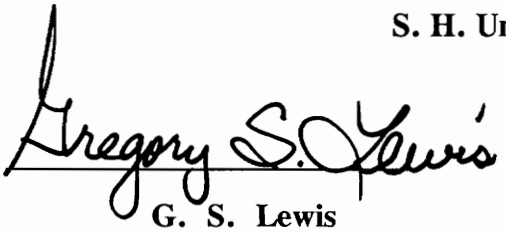

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THE INDUCTION OF SYNCHRONIZED ESTRUS IN SEASONALLY ANOVULAR EWES

by

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(ABSTRACT)

Two commercially available progestogen products for cattle, melengestrol acetate (MGA) and norgestomet (SMB), were evaluated for their ability to induce synchronized estrus in anovulatory ewes. Seasonally anestrous ewes (n=232; determined by blood serum progesterone concentration) of mixed breeding were randomly assigned within broad age groups to one of seven treatments: 1) control (C); 2) MGA only (OMGA); 3) MGA + zeranol (RMGA); 4) MGA + PG-600 (PMGA; 400 IU pregnant mare's serum gonadotropin + 200 IU human chorionic gonadotropin in a 5 mL dose); 5) SMB only (OSMB); 6) SMB + zeranol (RSMB); and 7) SMB + PG-600 (PSMB). Beginning 10 d before breeding, OMGA, RMGA, and PMGA ewes were fed .3 mg MGA/d provided through a mixture of shelled corn and a commercially prepared pelleted supplement containing MGA. Concomitantly, OSMB, RSMB, and PSMB ewes were given a 3 mg norgestomet implant inserted subcutaneously on the back of the ear. Immediately preceding initiation of the MGA and SMB treatments, RMGA and RSMB ewes were given a single i.m. injection of 2.5 mg zeranol. At the end of the 10-d treatment period, MGA feeding was discontinued and the norgestomet implants were removed. Concomitantly, PMGA and PSMB ewes were given a single i.m. injection of PG-600 (5 mL). All treatment groups were combined into one breeding group on May 4, 1992, with a ram to ewe ratio of 1:17 for a 30-d breeding

period. Mating to synchronized estrus was greater ($P < .0001$) for progestogen-treated ewes. Within progestogen treatments, more ($P < .0001$) SMB ewes were marked within the first 5 d of breeding than MGA ewes. Overall, there were no treatment differences in estrus response for the 30-d breeding period. Blood serum samples collected during the first 14 d of breeding were analyzed for progesterone as an indicator of corpora lutea formation. Even though a large proportion of C ewes displayed luteal activity, only 12 % exhibited behavioral estrus within the first 17 d of breeding. Progestogen treated ewes exhibited a shorter mean interval ($P < .0001$) from ram introduction to lambing. Fertility and prolificacy were not different for C, MGA, or SMB ewes. Of the two progestogen treatments used alone, lambing rate was 85 and 59 % ($P < .03$) for OMGA and OSMB ewes, respectively. Ewes primed with zeranol before MGA or SMB treatment exhibited similar levels of fertility and intervals from ram introduction to lambing compared with ewes receiving an injection of PG-600 after progestogen treatment. These data indicate that progestogen products commercially available for cattle may be useful in enhancing out-of-season breeding performance in sheep.

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Chapter 1. Introduction

In general, ewes initiate estrus in late summer and continue sexual activity through mid-winter. During the spring, most ewes exhibit an anestrous period when ovulation is absent and no behavioral estrus occurs. Also, rams show some seasonal fluctuation in libido and semen quality, but the overall seasonal effect is less drastic than with the female.

The seasonality of breeding inherent in sheep (Hammond, 1944; Hafez, 1952; Bittman et al.,1983a) hinders producers efforts to utilize spring breeding for once-a-year lambing in the fall or as a part of an accelerated lambing program. If genetic differences among ewes in their ability to breed out-of-season were identified and exploited, circumvention of the anestrous period could be possible through selection to produce a line of domestic sheep which show either a limited, short anestrus period or no anestrous period at all. Alternatively, ewes can be stimulated to breed during the period of anestrus with light control and (or) exogenous hormonal treatment, but these practices require confinement of animals and increased labor. However, even though hormonal treatment is labor intensive, it could be cost effective as a result of increasing the number of lambs produced per ewe per year.

The objective of this study was thus to compare and evaluate the use of melengestrol acetate (MGA) or norgestomet (Syncro-Mate-B; SMB) used alone and in combination with zeranol or pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG) on spring breeding performance of crossbred ewes.

Chapter 2. Literature Review

Domestic sheep (Ovis aries) exhibit a seasonal pattern of breeding behavior. Fertility is greatest during the fall, with a subsequent period of anestrus occuring in the spring. The actual time and duration of the breeding season is influenced by breed.

Endocrinology of the Seasonal Breeding Behavior in Ewes

Introduction

Neural control of the estrous cycle in sheep is manifested primarily through the secretion of gonadotropin releasing hormone (GnRH) from the hypothalamus. Exerting its action on the pituitary gland, GnRH is responsible for the synthesis and release of the gonadotropins: luteinizing hormone (LH) and follicle stimulating hormone (FSH). For ovulation to occur, the frequency of LH pulses must be high enough to provide gonadotropic drive to the developing ovarian follicle. As the follicle matures, a sustained increase in the level of estradiol release occurs. A rise in estradiol is required for both estrus behavior and the LH surge which causes ovulation (McNatty et al., 1981; McNeilly et al., 1982).

Changes in the tonic secretion of gonadotropins acts as a major factor mediating breeding behavior in sheep. The generally accepted model of endocrine control of sexual behavior in ewes involves a negative feedback effect of estradiol on LH pulse frequency during periods of anestrus. Secretion of LH is pulsatile with the pulses being more frequent during the breeding season than during anestrus. Increased frequency of LH pulses promote ovarian production of estradiol. Seasonal changes in the sensitivity of the hypothalamus to the negative feedback effects of estradiol affects the frequency of LH pulses and thereby determines whether the ovary is active or inactive (Legan et al., 1977).

The LH pulse generator is the focal point for regulation of the sexual state of the ewe. This hypothalamic generator controls the pulsatile release pattern of GnRH which in turn regulates the release of LH. The existence of a neural oscillator or pulse generator was first inferred from the pulsatile pattern of serum LH concentrations observed in blood samples collected at frequent intervals (Goodman and Karsch, 1981). Levels of LH increased abruptly within a few minutes and then decayed exponentially until the beginning of the next pulse. This pattern reflects discrete bursts of secretory activity by the hypothalamus followed by a relatively long (1 h) period of little or no secretion during which LH concentrations decline as the hormone is metabolized (Dierschke et al., 1970; Butler et al., 1972).

Belchetz et al. (1978) showed that intermitant but not continuous administration of GnRH was able to maintain typical patterns of LH secretion following destruction of the hypothalamus. Thus, the episodic nature of GnRH secretion and the consequent pulsatile secretion of LH may be required for normal functioning of the hypothalmo-hypophyseal axis. Generally, the pulse generator is believed to be located in the medial basal hypothalamus (Krey et al., 1975) and is assumed to act through GnRH release. The arcuate nucleus appears to be involved, since lesions in this area abolish LH pulses (Plant et al., 1978).

Effect of photoperiod on seasonal breeding behavior

Photoperiod is the major environmental effect regulating seasonal reproduction in sheep (Hafez, 1952). In ewes maintained under natural light conditions, onset of the breeding season is associated with decreasing day length in late summer and early fall. The onset of anestrus occurs with increasing day length in late winter and early spring. The pineal gland has been shown to mediate the photoperiodic occurence of seasonal reproduction in sheep (Bittman, 1983a). This mediation appears to be regulated

through the nocturnal release of melatonin by the pineal gland. Circulating levels of melatonin increase during hours of darkness and decline with daylight. Pinealectomy renders ewes insensitive to both the inhibitory effects of long days and the stimulatory effects of short days (Bittman et al., 1983b).

Legan and Karsch (1978) demonstrated that photoperiod governed the marked seasonal changes in pulsatile LH release in estradiol-implanted, ovariectomized ewes. In their study, LH was elevated during short photoperiods which promoted estrous activity, and LH was supressed to undetectable levels during long photoperiods resulting in anestrus. These findings indicate that the profound seasonal change in the negative feedback effects of estradiol on tonic LH secretion is induced by photoperiod. In order to test the hypothesis that photoperiodic signals are perceived by retinal photoreceptors and transmitted to the pineal gland and then to the hypothalamus, Legan and Karsch (1983) performed two studies to determine whether the eyes are necessary for photoperiodic control of reproduction in ewes. In one experiment, intact and estradiol-treated ovariectomized ewes were housed in each of two photoperiodically controlled rooms with a vasectomized ram and subjected to 90 d alterations between long (16 h of light) and short (8 h of light) days. Long days initiated anestrus in intact ewes and a suppression of serum LH levels in ovariectomized ewes. Short-day exposure caused the expected opposite result. After 1.5 yr of this photoperiodic scheme, all ewes were surgically blinded. Photoperiodic control was lost following blinding in ewes without a ram present but circannual alterations between cyclicity and anestrus or high or low LH levels were observed for the following 2.5 yr. In the group of estradiol-implanted ovariectomized ewes, serum LH levels remained synchronized to the 90 d shift in photoperiod as long as a sighted intact ram was present with the ewes. When the sighted ram was removed, these ewes began a circannual rhythm of LH

levels that was independent of photoperiod. This result suggested that the blind ewes were recieving photoperiodic cues from the sighted ram.

Hafez (1952) noted that the domestic sheep is a short-day breeder but its reproductive season is skewed with respect to photoperiod. During 8 yr of observations on Suffolk ewes, Robinson and Karsch, (1984) observed a mean date of September 3 (14.0 h of light per d) for initiation of estrus as measured by behavioral estrus and progesterone (P_4) profiles and a mean date of Febuary 15 (11.5 h of light per d) for the start of anestrus. Despite the fact that ewes are short day breeders, they began cycling when days were longer than when they stopped cycling.

Robinson and Karsch (1984) demonstrated that Suffolk ewes normally cease breeding because they are no longer able to respond to prevailing short days, not because they are actively inhibited by increasing photoperiod. Hence the asymmetry between the annual cycles of photoperiod and reproduction can be explained at least in part by photorefractoriness, or the loss of response to a day length which was once stimulatory.

Similarly, Robinson et al. (1985a, b) found that refractoriness to inhibitory day lengths initiates the breeding season of the Suffolk ewe. When ovariectomized estradiol implanted ewes were held at summer solstice (16.5 h daylength) conditions and compared to ovariectomized estradiol-implanted ewes held under natural lighting conditions, all ewes showed increasing LH levels at the same time.

Karsch et al. (1986) hypothesized that the photorefractoriness reflects a deficit in the post-pineal processing of the photoperiodic message since ovariectomized estradiol-implanted pinealectomized ewes held in either short or long photoperiods became photorefractory even though daily melatonin patterns remained unchanged. To check this Karsch et al., (1986) infused ovariectomized estradiol-implanted

pinealectomized ewes with short-day melatonin regimens which were initially reproductively stimulatory but later became unable to support reproduction at about the same time that intact control ewes were entering anestrus.

Bittman et al., (1983b) noted that while the pineal gland is related to seasonality of breeding in sheep, the persistence of seasonal breeding behavior in the absence of the pineal might result from annual changes in temperature, food quality, or other environmental cues. Alternatively, it might reflect persistence of some endogenous annual rhythm which remains intact and functional after photoperiodic synchronization to the environment has been eliminated. Robinson et al., (1985a) suggested that a changing day length does not normally drive either of the reproductive transitions of the Suffolk ewe, and that blinded or pinealectomized ewes (Bittman et al., 1983a, b; Legan and Karsch, 1983) show seasonal cycles which are no longer synchronized with animals perceiving day length. Thus, rather than actively driving reproductive changes, day length apparently ensures the appropriate timing of the breeding season of sheep in addition to an endogenous circannual rhythm of reproduction.

Malpaux et al., (1989) in a series of experiments using different lengths of photoperiod exposure in ovariectomized and estradiol-implanted ewes concluded that: 1) exposure to longer photoperiods between the winter and summer solstice is required for the occurence of the breeding season in the autumn; 2) the time of initial exposure to longer photoperiod provides an important cue for determining the breeding period; 3) the time of onset of breeding season neither depends upon the decreasing photoperiod after the summer solstice, nor requires the increasing photoperiod as the summer solstice approaches.

In summary, the pineal gland exerts its effects through the secretion of melatonin as a timer for breeding activity in sheep. Melatonin modulates a key

determinant of estrous cyclicity, the capacity of estradiol to inhibit tonic LH secretion. Melatonin is thus a blood borne photoperiodic hormone which drives seasonal changes of breeding activity in the ewe. The ability of melatonin to diminish the capacity of estradiol to inhibit gonadotropin secretion is critical in understanding the role of the pineal gland and the photoperiodic control of breeding activity in sheep. It is insufficient to describe the sheep as a short day breeder; rather, long days play a critical role in setting the breeding season to the autumn.

Hormonal Events that Effect the Onset of Estrus During the Breeding Season

Progesterone is the primary negative feedback hormone inhibiting LH secretion during the breeding season (Baird and Scaramuzzi, 1976). A decline in serum P_4 concentration during luteolysis initiates the sequence of events which culminates in the LH surge which produces ovulation. The sequence begins with low LH pulse frequencies during the luteal phase of the estrous cycle due to the inhibitory action of P_4 which is produced by the corpus luteum (CL). Estradiol which is present at ovulation and estrus appears to sensitize the LH pulse generator to P_4 and increase its ability to suppress the frequency of pulsatile LH release (Martin et al., 1983a). When the CL regresses, the progestational blockade of the LH pulse generator is lifted, and pulse frequecy increases markedly (Baird and Scaramuzzi, 1976; Karsch et al., 1983). Increased pulse frequency of LH stimulates increased estradiol secretion which elicits estrous behavior and a preovulatory surge of LH (Karsch et al., 1980). Thus, P_4 controls the timing of ovulation during the breeding season. When it is elevated, tonic LH secretion is held in check and ovulation does not occur. When P_4 levels fall, LH rises and ovulation follows within 4 d.

Goodman and Karsch (1981) showed that both P_4 and estradiol had an effect on LH secretion. Progesterone implants in ovariectomized ewes during the breeding season reduced the frequency of LH pulses and inhibited the LH surge whereas estradiol reduced only the amplitude of the pulses. Goodman and Karsch, 1980 suggest that P_4 acts on the hypothalamus to reduce the frequency of episodic GnRH release whereas estradiol acts upon the anterior pituitory to decrease its response to GnRH and thus to limit LH pulse amplitude. Estradiol appears to be able to limit LH pulse frequency only during anestrus but increases LH pulse frequency at the time of luteolysis or removal of P_4 implants (Karsch et al., 1983). Plasma FSH levels remain relatively constant throughout the luteal phase of the estrous cycle. During the follicular phase FSH falls gradually. At the time of the preovulatory LH surge there is a concomitant FSH surge (reaching levels of 80 to 150 ng/ml) and then a second FSH rise occurs 18-24 h later (Salamonsen et al., 1973).

Hormonal Events That Effect Onset of Estrus Out-Of-Season

During the anestrous season, LH pulses are very infrequent (approximately once every 10 h vs. once per h during the breeding season). This low pulse frequency occurs in the absence of P_4 and prevents follicular development thereby inhibiting the estradiol rise and subsequent LH surge which would occur during the breeding season. Seasonal changes in the ability of estradiol to elicit negative feedback on LH production have been shown in a number of breeds (Legan et al.,1977; 1985; Martin et al., 1983a). These seasonal changes in the negative feedback potency of estradiol are large and reflects a dramatic change in the role of estradiol in control of pulsatile LH secretion (Legan and Karsch, 1983; Goodman et al., 1982; Martin et al., 1983a).

As the transition to anestrus starts, estradiol has an increasing ability to inhibit tonic LH secretion. During the anestrous period estradiol has a powerful negative feedback effect on LH secretion (Goodman et al., 1982; Martin et al., 1983a). The existance of this highly effective negative feedback loop between estradiol and LH explains why LH pulse frequency remains minimal during anestrus and why follicular estradiol production is suppressed. Hence during anestrus, rising LH causes increased estradiol secretion which inhibits further LH release. Once estradiol levels have dropped, LH rises again, continuing a futile loop of rising and falling levels. However, as the ewe begins transition back to the normal breeding season, the negative feedback effect of estradiol weakens, allowing for an increase in LH pulse frequency, a preovulatory rise in estradiol, and estrus.

Hormonal Manipulation of Breeding Activity in Sheep

Introduction

Hormonal manipulation of reproduction gives producers control over breeding and lambing management which would otherwise be difficult to attain. Synthetic hormones have been used in the livestock industry as growth promotants, for estrous synchronization and for induced lambing. Following is a review of the different products and techniques used to manipulate breeding activity in sheep.

Progesterone for estrous synchronization in sheep

The secretion of progesterone (P_4) by the CL plays an important role in inhibiting positive feedback of estradiol on LH production (Baird et al., 1975). Regression of the CL results in the fall of serum P_4 and the progestational blockade of tonic LH pulses is lifted, resulting in a preovulatory surge of LH which is followed by

ovulation within 4 d. Hormonal techniques used for estrus synchronization are based upon the observation that P_4 inhibits the end of follicular development and ovulation. Its withdrawal leads to the development and maturation of preovulatory follicles and their subsequent ovulation. Controlling the life span of the CL helps to synchronize estrus and ovulation (Hansel and Convey, 1983).

Exogenous administration of P_4 for 10 to 14 d mimics the normal luteal phase of the estous cycle with its withdrawal resulting in increased pulse frequency of LH and ovulation. Makepeace et al. (1937) first reported that the administration of exogenous P_4 blocked postcoital ovulation in rabbits. Dutt and Cassida (1948) were the first to demonstrate the inhibition of estrus and ovulation in ewes by administering exogenous P_4. Upon withdrawal of the treatment, ewes exhibited estrus within 2 to 3 d, followed by ovulation. Foote and Waite (1965) studied the effects of P_4 injection on estrous behavior and fertility in ewes during the breeding season. Ewes (n=70) were randomly divided into 4 treatment groups. Group 1 acted as control and recieved no treatment. Groups 2, 3, and 4 were injected with 10 mg of P_4 for 17 d. Ewes were bred at their first, second or third post-treatment estrus period for treatment 2, 3 and 4 respectively. The results revealed that P_4 was effective in synchronizing estrus and ovulation. The first post-treatment estrus was significantly lower in fertility (28%) than the control (66.7%) or those in the second (67.9%) or third (86.4%) post-treatment periods. Foote and Bennett, (1968) evaluated P_4 injections in combination with 17-β estradiol and (or) PMSG injections in prepuberal ewes at 5 or 8 mo of age. Ewes (n=74) were divided into 4 groups: 1) control; 2) 12 mg P_4 injections for 14 d plus 1 mg 17-β estradiol injection on the first d and the second d after P_4 withdrawal; 3) 12 mg P_4 injection for 14 d plus 600 IU PMSG injection 2 d after P_4 withdrawal; 4) 12 mg P_4 injections for 14 d plus 600 IU of PMSG injected 2 d after P_4 withdrawal plus 1

mg of 17-β estradiol on the first day of P_4 injection. At the age of 5 mo, no ewes in the control group showed estrus whereas 45, 50, and 50 % of the ewes showed estrus in groups 2, 3 and 4 respectively. None of the ewes in the control group ovulated as compared to 50, 82, and 96 % of the ewes in groups 2, 3, and 4, respectively. At 8 mo of age, none of the control ewes exhibited estrus as compared to 53, 82 and 96 % of the ewes in groups 2, 3, and 4, respectively. All ewes showing estrus ovulated and 60 % of the ewes in group 4 were pregnant at d 25 post-breeding as compared to 0, 10, and 23 % of the ewes for groups 1, 2, and 3, respectively.

Progestogens

The exogenous administration of naturally occuring hormones has little practical value because of their relatively short half-life. Modification of the basic structure of a compound, such as the addition of an ester in the case of steroids, often delays their absorption from an injection site. Delayed absorption extends half-life and thus increases potency. In early work, P_4 was dissolved in oil and administered over 8 to 10 d as a series of injections. However, this was too labor-intensive for practical application. Presently, different commercial progestogen products are available. Progestogens are synthetic compounds with properties of P_4 and have a greater potency and are more easily administered in the form of intravaginal pessaries / sponges, implants, and oral products administered through the feed. Each delivery system has its own advantages and disadvantages. Rapid removal of the source of progestogen from the body is essential to achieve close synchrony of estrus.

Intravaginal sponges

Synthetic analogues of progesterone such as medroxyprogesterone acetate (MAP), fluorogesterone acetate (FGA), chlorgesterone acetate (chloromadione), and fluorogesterone acetate (cronolone) administered by intravaginal sponges (Robinson, 1964) are commonly used for estrus synchronization outside of the United States. A high concentration of progestogen with a short duration of activity is most suitable (Robinson 1976). Deweese et al., (1970) compared MAP orally and in vaginal sponges for synchronization of estrus in ewes. Ewes (n=92) were randomly alloted within age groups to 4 treatments: control, vaginal sponges containing 40 or 60 mg of MAP and 60 mg of MAP fed per head daily for 14 d. A significant number of ewes ($P<.01$) were in estrus within 5 d after treatment as compared to control. No difference in reproductive performance of ewes fed MAP or treated with vaginal sponges were reported. Lewis and Inskeep (1971) investigated the reproductive performance of ewes primed for 13 d with 20 mg FGA sponges during the summer and either teased by vasectomized rams or without teaser rams. They reported that teasing increased the number of ewes showing behavioral estrus; 93.3% compared to 50.5% for the non-teased ewes ($P<.01$). Teasing had no effect on conception rate or lambing rate. Comparable studies conducted in Ireland showed that MAP and FGA sponges gave equal results when natural mating was used, but a significant advantage was found for the 30 mg FGA sponge when ewes were artificially inseminated at a fixed time (Gordon, 1983). This may be due to the fact that synchronization of estrus is more precise following FGA sponges (Pearce et al., 1984). Robinson et al. (1968), Gordon, (1971) and Colas (1975) suggested that a high level of progestogen, followed by rapid withdrawal and adequate ovarian stimulation, is a necessary prerequisite for acceptable fertility. Although cyclic ewes do come in estrus shortly after progestogen withdrawal

in the absence of exogenous gonadotropins, a low dose of PMSG results in a more predictable and precise synchronization.

Progestogens in Combination With Injection of PMSG

Endocrine abnormality has been reported following progestogen treatment. The pre-ovulatory LH surge occurs significantly earlier in relation to the onset of estrus in an induced cycle vs. a natural cycle (Cumming et al., 1973; Baumgartner et al., 1974). Results suggest an asynchrony of estrus and ovulation in progestogen-treated ewes. Further, follicular growth in anestrous, progestogen primed ewes must be stimulated to a state of maturity so that the natural surge of LH may occur to cause ovulation. In some cases, it can be achieved by the positive feedback of estradiol secretion of the follicle(s) but, in most cases an injection of gonadotropin releasing hormone (GnRH) or hormones having FSH like activity are required. PMSG, having FSH-like activity, and a longer half-life than FSH or GnRH can be administered to achieve greater follicular development and an increased ovulation rate. The injection of PMSG at the time of progestogen withdrawal improves conception rates (Colas, et al., 1973; Colas, 1975) and promotes an earlier onset of estrus and a better degree of synchrony for both estrus and ovulation (Signoret and Cognie 1975). Colas, (1975) reported that an i.m. injection of 375 to 750 IU PMSG at the end of the progestogen treatment advanced the onset of estrus, increased ovulation rate and induced a closer synchronization of ovulation leading to higher fertility in artificially inseminated ewes. Cognie and Mauleon, (1983) reported that injection of PMSG 2 d before progestogen sponge removal advanced the onset of estrus and increased ovulation rate without any improvement in litter size, but reduced fertility as compared with ewes treated with PMSG at the end of progestogen treatment.

Melengesterol acetate

Melengesterol acetate (MGA; Upjohn, Kalamazo, MI; 6-methyl-17-alpha-acetoxy-16 methylene-pregn-4,6-diene-3,20-dione), is an orally active progestational steroid developed in 1962 which is capable of maintaining pregnancy and suppressing estrus and ovulation in cyclic cows and heifers (Zimbelman and Smith, 1966). Melengestrol acetate is used commercially in feedlot heifer diets to increase feed efficiency and average daily gain by inhibiting estrus and ovulation without inhibition of follicular development. Estrus and ovulation are suppressed in cattle when MGA is fed at a dose of .5 mg / head / d, with a minimal effective dose of .42 mg /d (Zimbelman, 1963; Zimbelman and Smith, 1966). Structurally, MGA is closely related to naturally-occurring progesterone and is biologically characterized as an analog of MAP. Biological differences are evident among the three compounds when administered orally. Progesterone is essentially orally inactive, whereas MGA is 300 to 900 times more potent than MAP. Zimbelman (1963) compared feeding of 0.4 mg, 0.8 mg or 1.6 mg MGA for 15 d to cyclic ewes to synchronize estrus. All three levels of MGA were effective. When fed for 14 d and followed by an injection of zeranol, MGA improved the spring-breeding performance of anestrous Hampshire ewes (Burke and Keisler, 1988). Umberger and Lewis (1992) studied the effects of MGA fed in the spring, with and without zeranol or teaser rams and recorded the subsequent reproductive performance of virgin yearling ewes ($n=128$). Results from the 30 d breeding period indicated that ewes fed MGA had higher ($P<.01$) fertility rates than control or ewes kept with vasectomized rams. A greater proportion (over 66 %) of MGA treated ewes lambed within the first 10 d of lambing compared to 33 % for control ewes and none for ewes kept with vasectomized rams. No statistical difference was reported among MGA treatments, but MGA + zeranol tended to increase the

fecundity and decrease the percent of ewes lambing. They concluded that ewes can be successfully synchronized and induced to ovulate during the anestrous season when fed MGA before breeding. Safaranski et al., (1992) evaluated the effectiveness of MGA and (or) PG-600 (a combination of 400 IU of PMSG and 200 IU of hCG) for inducing and synchronizing a fertile estrus in seasonally anestrous ewes. MGA increased ($P < .001$) the proportion of ewes mated during the 9 d estrus-detection period whereas PG-600 alone had no significant effect. Percentage of ewes that ovulated was greatest for ewes that received the combined MGA and PG-600 treatments ($P < .01$). The ovulation rate of ewes ovulating was enhanced by PG-600 ($P < .01$), whereas MGA alone had no effect. In fact, MGA administration alone tended to decrease ovulation rate ($P < .10$). Lambs born per ewe exposed were approximately three times more in the MGA treated group than with control or PG-600 alone ($P < .001$).

Syncro-Mate-B

Syncro-Mate-B (SMB; Sanofi Animal Health, Overland Park, KS) (17α-acetoxy-11β-methyl-19-nor-pregn-4-ene-3,20-dione; SC-21009) consists of a polyethacrylate polymer ear implant impregnated with 6 mg of norgestomet. It is used commercially for synchronization of estrus in beef heifers (Miksch et al., 1978; Brown et al., 1988) and cows (Miksch et al., 1978; Kiser et al., 1980). Boland et al., (1979) compared three treatments consisting of 60 mg MAP sponges, 30 mg FGA sponges and 3 mg norgestomet (NOR) implants in 234 cycling Galway sheep. The occurrence of estrus was greater (95%) in ewes treated with FGA ($p < .01$) than in ewes treated with either NOR (74%) or MAP (71%). Ewes treated with NOR had a shorter estrus by 7.6 h than ewes treated with sponges. In contrast to the findings of Boland (1979), Spitzer and Carpenter (1981) reported that 96 % of cronolone treated and 92 % of NOR treated

cycling ewes were in estrus within 5 d after the removal of the pessary or implant. First service pregnancy rates for ewes which mated during the 5 d synchronized period were 80% for cronolone and 59% for NOR treated ewes. Alifakiotis et al., (1982) compared MAP FGA, and NOR and two levels of PMSG (500 or 1000 IU) in 600 lactating anestrous ewes for estrus synchronization and prolificacy. There were no treatment differences in the percentage of ewes exhibiting estrus. However, ewes treated with NOR exhibited estrus 7 h earlier. Woody et al., (1983) studied the influence of progestogen dose and day of cycle at treatment. Ewes were implanted either on d 4 or 13 of the estrous cycle for 13 d with 2 mg or 3 mg NOR implants. Fewer ewes were in estrus within 5 d after implant removal ($P<.01$) for ewes implanted on d 13 than on d 4 of the cycle. In another experiment ewes received 3 mg or 6 mg NOR implants for 13 d on day 4 or 13 of the estrous cycle. Conception rate was not influenced by day of treatment but was higher in ewes treated with 6 mg than ewes treated with 3 mg NOR. Tritschler et al., (1991) investigated the effectiveness of 60 mg MAP sponges and 2 mg NOR implants with the injection of 500 IU PMSG on d 14 (withdrawal d), to induce estrus and conception in anestrous ewes. There were no differences in estrus rate, conception rate or number of lambs born per ewe. Youngs et al., (1990) compared the efficacy of FSH and PMSG used in conjunction with either MAP sponges or NOR implants. Rambouillet ewes ($n=91$) were treated for 13 or 14 d in early July with either a 60 mg MAP intravaginal pessary or 1/2 of a standard bovine 6 mg NOR implant inserted into the ear. Twenty four hours before progestogen removal ewes recieved either a single i.m.injection of 400 IU of PMSG or 3 mg s.c. injections of FSH given 3 times at 12 h intervals. Ewes were laproscopically inseminated with frozen semen 48 to 55 h after progestogen removal. Type of progestogen had no effect on pregnancy rate.

Webel and Walker (1993) investigated the effectiveness of NOR implants in combination with an injection of PG-600 (a combination of 400 IU of PMSG and 200 IU of hCG) for induction of fertile estrus in anestrous Suffolk ewes during the spring. Treatments were: 1) control; 2) NOR + 2.5 ml PG-600; 3) NOR + 5 ml PG-600; 4) NOR + 7.5 ml PG-600. The results indicated that more ($P < .005$) treated than control ewes were in estrus and lambed. No control ewes were observed in estrus during the 30-d observation period. No significant difference was reported between the three PG-600 treatment groups for number of ewes in estrus or number of ewes lambing; however, the number of lambs born per ewe was greater ($P < .1$) for ewes recieving 5 ml of PG-600 than for ewes receiving the 2.5 ml and 7.5 ml dose.

Zeranol

Zeranol is a derivative of a natural metabolite of the mold, Gibberella zeae originally found on corn and has 30% of the estrogenic activity of estradiol 17-β (Elsasser et al. 1983). Zeranol is marketed under the trade name of Ralgro (Pitman-Moore, Inc., Mundelein IL 60060) and is a non-steroidal growth promotant (Stob et al., 1962; Perry et al., 1970) that alters pituitary hormone secretion through an estrogen-like action on the pituitary (Peck and Chesworth 1977). Elsasser et al., (1983) studied the effects of different doses of zeranol administered to ovariectomized ewes. Injections of .333, 1 or 10 mg caused dose-related increases ($P < .01$) in plasma prolactin (PRL) peak levels at 12 to 18 h and increased LH peak levels at 12 to 20 h. FSH levels were significantly decreased 4 to 8 h after zeranol injection. Minimal surges of FSH were reported at times similar to those of LH, but the peak level was never greater than control levels.

The effects of zeranol and estradiol on the secretion patterns of LH, FSH and PRL are similar. It is presumed that both zeranol and estradiol interact through a common mechanism on estrogen receptors in the hypothalamus and pituitary. It is also presumed that zeranol acts on the pituitary to secrete somatotrophin, the animal's own growth promoting agent. Keisler (1992) while studying the effect of MGA on synchronization of estrus in seasonally anovular ewes evaluated 0, 0.3125, 1.25 or 5 mg doses of zeranol to optimize synchrony of estrus. The results suggested that 1.25 mg of zeranol was most effective. In another study, Keisler (1992) studied and evaluated the optimal time of injection of zeranol after MGA withdrawal. Ewes (n=253) were fed 0.125 mg of MGA for 8 d morning and evening and injected with 1.25 mg zeranol at 30, 42, or 54 h after the last feeding of MGA. No significant difference between the hour of treatment (30 h vs. 42 h vs. 54 h) was reported. Umberger and Lewis (1992) reported that injection of 2.5 mg zeranol suspended in 2 Ml corn oil given once to ewes fed MGA for 10 d had higher lambing rates compared to control ewes or ewes kept with vasectomized rams for 7 d in a spring breeding trial. Lewis and Karen (1993) reported that injection of 2.5 mg zeranol prolonged the duration of estrus in ewes both in spontaneous estrus or estrus induced by progestogens ($P<.001$). Ewes treated with zeranol were in estrus an average of 102 h as compared to 55 h for control ewes. They concluded that zeranol can be used to prolong estrus in ewes, which will help reduce the number of ewes required for serving capacity tests of rams by 50 %.

Use of Ram-Effect for Induction of Estrus in Sheep

Introduction

Through the acute introduction of a ram, anestrous ewes previously isolated from rams for at least 1 mo can be stimulated to ovulate. This phenomenon, referred to as the "ram-effect" results in a synchronization of estrus (Underwood et al., 1944). Anestrous ewes generally ovulate in response to acute ram introduction within 54 h. Two peaks of estrus activity are usually observed in the flock, the first at d 18 and the second at d 24 of ram introduction (Oldham et al., 1978). The first ovulation after introduction of rams is usually not accompanied by behavioral estrous. This appears to be due to the absence of prior exposure to progesterone (Robinson, 1954). After ovulation some ewes develop a normal CL while other ewes will exhibit premature regression of the CL within 6 d (Oldham and Martin, 1978; Knight et al., 1981). A large proportion of ewes may not respond to the ram effect and may return to anestrus after one or two ovulations without mating (Oldham and Cognie, 1980). Ewe response to the ram effect is dependent on the time of ram introduction relative to the normal breeding season (Oldham and Cognie, 1980).

Physiology of the ram-effect

Within minutes of ram introduction, a pulse of LH is observed (Martin et al., 1980; Poindron et al., 1980). The rhythm of pulsatile secretion of LH remains accelerated and a preovulatory surge of LH similar to that observed during the estrous cycle takes place at around 36 h, after ram introduction, although the timing is highly variable, ranging from 6 to 54 h (Oldham et al., 1978; Knight et al., 1978). The capacity of a flock to respond to the ram effect is related to the percentage of females ovulating spontaneously, which is referred to as "depth" of anestrus (Lindsay and

Signoret, 1980). Ewe response to the ram effect increases with postpartum interval (Geytenbeek et al., 1984).

The rapid rise in LH levels in response to ram introduction suggests a direct effect that is indepedent of a change in the sensitivity of the hypothalamus to estradiol. The estrogen-induced pre-ovulatory surge of LH may be replaced by a ram-mediated response. Martin et al., (1983b) suggested that ram pheromones influence LH in the ewe through neural pathways that connect the accessory olfactory bulbs and anterior hypothalamus. Knight et al., (1978) stimulated 39 to 70% of anestrous Romney ewes to ovulate with acute Dorset ram exposure at the begining of the natural breeding season. No rise in estrogen levels were found prior to 9 h before the LH surge, indicating that initial rise in LH levels above baseline were independent of estrogen stimulation of the hypothalamus.

There are conflicting reports regarding isolation of ewes from rams prior to exposure in order to express the ram effect. Oldham (1980) has shown that 34 d is sufficient and possibly a period as short as 17 d is sufficient for seasonally anovular Merino ewes during October in western Australia. By contrast, Cushwa et al., (1992) reported that the ram effect can be achieved in a flock of ewes without prior isolation from rams. The four teatments (isolated vs. adjacent) and date of joining with novel breeding rams (May 15 vs. June 15) differed with respect to ewe proximity to rams before mating . All groups responded to the introduction of novel breeding rams. About 86% of the ewes responded to the ram effect. A period of isolation before mating did not increase response compared with ewes that remained adjacent to, or in contact with, rams (86 vs. 85%). Response was greater ($P<.05$) in June and in the second yr ($P=.05$).

Tervit and Peterson (1978) studied breed differences in teasing ability. Romney ewes were exposed to either Dorset or Romney rams in early summer. Testosterone levels were determined in the rams and all mating marks were recorded. The Romney ewes joined with Dorset rams showed estrus earlier than those with Romney rams. Romney rams had higher mean testosterone levels in the latter part of the study even though they were less effective than Dorset rams in stimulating ewes to cycle.

Nugent et al., (1988) evaluated the effects of ram pre-exposure and ram breed on ewe fertility in late summer. Ewes were randomly divided and kept isolated from rams for 2 wk or pre-exposed to yearling Dorset or Suffolk rams for 2 wk by penning rams adjacent to ewes. Teasing occurred through visual, olfactory, auditory and limited tactile contact with ewes. Post-teasing, all ewes were re-assigned randomly for breeding to Suffolk or Dorset rams for a 44-d breeding period. Lambing rates were 29% higher in 1984 and 13% higher in 1985 for ewes pre-exposed to Dorset rams compared to ewes pre-exposed to Suffolk rams or those kept isolated. They concluded that Dorset rams were superior to Suffolk rams in stimulating anovular ewes to cycle in spring or summer.

In another experiment, Nugent and Notter (1990) studied the effect of co-habitation with white faced ewes on estrous activity of Hampshire and Suffolk ewes exposed to rams in the summer. Ewes were isolated from mature rams for about 9 mo and were exposed to vasectomized rams plus 65 white-faced ewes (treated group) or kept with just vasectomized rams (control group) for 30 d. They reported a higher frequency of ovulation and mating ($P < .05$) for the treated group in the presence of white-faced ewes as compared to the control group. They concluded that cohabitation of anestrous black-faced ewe with white-faced ewes increased the response to the ram-effect.

Progestogens and the ram-effect

It is clear from the preceding review that anestrous ewes can be induced to ovulate during the non-breeding season through acute introduction of rams into the breeding flock. One of the problems with this technique is the variable response of ewes to exhibit behavioral estrus and the potential for pre mature regression of the CL. A more compact synchronization of estrus could be accomplished if pre mature regression of the CL was avoided.

Legan et al., (1985) observed that most ewes experience a transient increase in serum P_4 concentrations for 1 to 2 d just prior to their first estrous cycle of the new breeding season. Progesterone levels rise to about 1 ng/ml during this time. Adult Suffolk and Suffolk crossbred anestrous ewes were administered synthetic GnRH for 3 d in a pulsatile fashion which resulted in mimicking the pattern of LH secretion during the preovulatory period of the breeding season. As a further treatment, ewes received P_4 by injection or implant and subsequent luteal phase lengths were measured. The induced LH surges caused full length luteal phases in 10 of the 10 ewes pretreated with P_4 and in only 8 of 18 ewes that were not pretreated.

In the cyclic ewe, P_4 is provided by the regressing CL of the prior cycle. But, in the anestrous ewe, frequently a short, 6-d CL occurs. Though the short cycle is observed, it does not appear to be essential in all ewes. According to Martin et al., (1986) normal luteal function is ensured by P_4 injection which acts directly on the ovary and also delays the preovulatory LH surge. Similarly, Pearce et al., (1984) stated that injection of P_4 immediately before ram introduction delays the preovulatory surge of LH and extends gonadotropin priming of follicles.

Pearce et al., (1985) divided a flock of anovulatory Merino ewes into three treatment groups: 1) control; 2) ewes received a single 20 mg injection of P_4; 3) ewes

received an injection of P_4 plus a series of GnRH injections. All ewes were exposed to rams. The frequency of CL's with a short life span was 72% for control ewes, 58% for ewes treated with P_4 and GnRH, and 0 % for ewes receiving P4 alone. The injection of P_4 alone delayed the preovulatory surge of LH by 45.8 h. The progesterone alone mimicked a CL which inhibited a rise in LH until P_4 levels subsided. In ewes receiving P_4 and GnRH, the GnRH caused increased LH resulting in ovulation. The control group had many short CL's due to the lack of a prior progestational phase and its priming effect. This study supports the theory that P_4 assures normality of the CL by lengthening the period of gonadotropin priming of follicles before ovulation.

Cognie et al., (1982) isolated anestrous ewes from rams for 1 mo and treated them with intravaginal sponges impregnated with 30 mg FGA for 12 d prior to ram exposure (treatment 1); 15 d vasectomized ram exposure followed by joined with intact ram (treatment 2); 15 d vasectomized ram exposure and a single injection of 20 mg P4 before joining with intact rams (treatment 3). Of the 35 Merino ewes in each group 34, 27, and 32 ovulated in response to ram exposure, between d 0 to 9. However, 34 out of 35 ewes in treatment 1 exhibited estrus at the first ovulation while only 5 out of 27 and 7 out of 32 ewes showed estrus in treatments 2 and 3, respectively. During the second estous cycle, 33 and 31 ewes exhibited estrus in treatment 2 and 3 respectively.

Reeves and Chamely (1984) reported a treatment period of 6 d for progestogen treatment before the introduction of rams into a flock while Hunter et al. (1971), Oldham et al., (1980) and Pearce et al., (1984) reported a treatment period of 12 to 16 d. Six-day priming with progestogen-impregnated intravaginal sponges results in a higher proportion of ewes exhibiting estrus and ovulation in response to introduction of

rams. This method allows for increased ovulation rates and fertility associated with ram induced ovulation but spontaneously ovulating ewes are not synchronized. To synchronize cyclic ewes, 12 to 16 d treatment with progestogens is required. This is of great importance in Merino ewes where a proportion of ewes ovulating spontaneously can be high (Pearce et al. 1984).

In conclusion, the ram effect used in conjuntion with progestogen administration appears to be effective in stimulating anestrous ewes to exhibit behavioral estrus at first ovulation. Factors contributing to variation of results are; ewe breed, ram breed, time of year, period of isolation from rams and period of progestogen treatment.

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Chapter 3. Journal Article

Melengestrol Acetate And Norgestomet For The Induction of Synchronized Estrus In Seasonally Anovular Ewes

Abstract

Two commercially available progestogen products for cattle, melengestrol acetate (MGA) and norgestomet (SMB), were evaluated for their ability to induce synchronized estrus in anovulatory ewes. Seasonally anestrous ewes (n=232; determined by blood serum progesterone concentration) of mixed breeding were randomly assigned within broad age groups to one of seven treatments: 1) control (C); 2) MGA only (OMGA); 3) MGA + zeranol (RMGA); 4) MGA + PG-600 (PMGA; 400 IU pregnant mare's serum gonadotropin + 200 IU human chorionic gonadotropin in a 5 mL dose); 5) SMB only (OSMB); 6) SMB + zeranol (RSMB); and 7) SMB + PG-600 (PSMB). Beginning 10 d before breeding, OMGA, RMGA, and PMGA ewes were fed .3 mg MGA/d provided through a mixture of shelled corn and a commercially prepared pelleted supplement containing MGA. Concomitantly, OSMB, RSMB, and PSMB ewes were given a 3 mg norgestomet implant inserted subcutaneously on the back of the ear. Immediately preceding initiation of the MGA and SMB treatments, RMGA and RSMB ewes were given a single i.m. injection of 2.5 mg zeranol. At the end of the 10-d treatment period, MGA feeding was discontinued and the norgestomet implants were removed. Concomitantly, PMGA and PSMB ewes were given a single i.m. injection of PG-600 (5 mL). All treatment groups were combined into one breeding group on May 4, 1992, with a ram to ewe ratio of 1:17 for a 30-d breeding period. Mating to synchronized estrus was greater ($P < .0001$) for progestogen-treated ewes. Within progestogen treatments, more ($P < .0001$) SMB ewes were marked within the first 5 d of breeding than MGA ewes. Overall, there were no

treatment differences in estrus response for the 30-d breeding period. Blood serum samples collected during the first 14 d of breeding were analyzed for progesterone as an indicator of corpora lutea formation. Even though a large proportion of C ewes displayed luteal activity, only 12 % exhibited behavioral estrus within the first 17 d of breeding. Progestogen treated ewes exhibited a shorter mean interval ($P < .0001$) from ram introduction to lambing. Fertility and prolificacy were not different for C, MGA, or SMB ewes. Of the two progestogen treatments used alone, lambing rate was 85 and 59 % ($P < .03$) for OMGA and OSMB ewes, respectively. Ewes primed with zeranol before MGA or SMB treatment exhibited similar levels of fertility and intervals from ram introduction to lambing compared with ewes receiving an injection of PG-600 after progestogen treatment. These data indicate that progestogen products commercially available for cattle may be useful in enhancing out-of-season breeding performance in sheep.

Introduction

Progestogen treatment for improved breeding and lambing efficiency (Gordon, 1977) can enhance profitability of production through strategically managed year-round lambing programs. Lack of commercially available products in the United States, specifically approved for use in sheep, has inhibited the utilization of hormonal manipulation techniques for estrous synchronization and to improve out-of-season breeding practices (Parker and Pope, 1983). Two products approved for use in beef cattle, melengestrol acetate (MGA; Upjohn, Kalamazoo, MI) and Syncro-Mate-B (SMB; Sanofi Animal Health, Inc., Overland Park, KS), have been demonstrated to have application for controlled reproduction in sheep. Melengestrol acetate, administered orally for the suppression of estrus and ovulation in feedlot heifers , has been used to synchronize and induce estrus in seasonally anovular ewes (Burke and

Keisler, 1988; Safranski et al., 1992; Umberger and Lewis, 1992). Syncro-Mate-B, administered by subcutaneous ear implant for estrous synchronization in beef cattle, has been used to synchronize estrus in cyclic ewes (Ainsworth and Wolynetz, 1982) and for the control of estrus and ovulation in anovulatory ewes (Carpenter and Spitzer, 1981; Hudgens et al., 1987; Tritschler et al., 1991).

A single i.m. injection of pregnant mare's serum gonadotropin (PMSG) administered at the end of progestogen treatment advances the onset of behavioral estrus, increases ovulation rate, and induces a tighter synchrony of ovulation (Cognie, 1990). Until recently, sources of PMSG were limited. The introduction of PG-600 (Intervet, Millsboro, DE), used to induce estrus in prepuberal gilts, provides a commercially available supply of PMSG. One 5 mL dose of PG-600 contains 400 IU PMSG and 200 IU human chorionic gonadotropin.

Foote (1967) suggested that pretreatment with estrogen might further enhance the induction of estrus and ovulation in anestrous ewes treated with progestogen and PMSG. Beck and Reeves (1973) reported peak LH concentrations similar to preovulatory serum LH levels in cyclic ewes when anestrous ewes were administered a single i.m. injection of 12.5 μg or higher estradiol-17β. Elsasser et al., (1983) reported that i.m. injections of zeranol (Ralgro; Pitman-Moore, Terre Haute, IN) or estradiol-17β had similar effects on LH, FSH, and prolactin concentrations in ovariectominzed ewes.

Availability of product, price, method of delivery, and efficacy affect the extent to which progestogens can be used to improve breeding performance in seasonally anestrous ewes. This study was conducted to evaluate further the potential use of MGA and SMB used with and without Ralgro or PG-600 to induce ovulation and control estrus in spring-breeding of seasonally anovular ewes.

Materials and Methods

Dry, multiparous ewes (n=244), characterized genotypically to consist of approximately 50 % Suffolk and varying percentages of Rambouillet, Dorset, and Finnsheep breeding, were bled on April 13 and 17 by jugular venipuncture to determine estrual status via blood serum progesterone analysis. Ewes (n=8) with blood serum progesterone concentrations of .5 ng/mL or higher were considered cyclic and were not included in the study. The remaining ewes were characterized as being seasonally anovular and were randomly assigned within broad age groups to one of seven treatments (Table 1): 1) control (C); 2) 10 d MGA only (OMGA; fed at .30 mg·head^{-1}·d^{-1}); 3) 10 d MGA + an i.m. injection of 2.5 mg zeranol (Ralgro) suspended in corn oil (2 mL) that contained 10% ethanol (RMGA); 4) 10 d MGA + an i.m. injection of PG-600 (5 mL; PMGA); 5) 3 mg norgestomet (Syncro-Mate-B) implanted for 10 d (OSMB); 6) 3 mg norgestomet + an i.m. injection of 2.5 mg zeranol (RSMB); and 7) 3 mg norgestomet + an i.m. injection of PG-600 (5 mL; PSMB). Thus, this study was designed to include a negative control accompanied by a 2 X 3 factorial arrangement of treatments involving two progestogens administered with and without zeranol or PG-600.

More than 90 % of the ewes in this study had lambed the previous fall. All ewes had been isolated from rams for ≥ 60 d. Beginning 1 wk before treatment and continuing 3 wk into the breeding period, ewes were fed .45 kg whole shelled corn·head^{-1}·d^{-1}. Trough space was in excess of 25 cm/ewe. Two weeks before turn-in, a breeding soundness examination was performed on the Suffolk, Dorset, and Hampshire rams used for breeding.

Beginning 10 d before breeding, OMGA, RMGA and PMGA ewes were fed, at the same time each morning, .45 kg of a 70:30 mixture of whole shelled corn and a

commercially prepared pelleted supplement (2.2 mg/kg MGA). Concomitantly, OSMB, RSMB, and PSMB ewes were given a 3 mg norgestomet implant (one-half cattle implant, ≈1 cm long) inserted subcutaneously on the back of the ear. Immediately preceding initiation of the MGA and SMB treatments, RMGA and RSMB ewes were given a single i.m. injection of 2.5 mg zeranol. To prepare zeranol suspensions, Ralgro pellets were transferred to injection vials, and ethanol (10 % of the final volume) was added to the vials, which were allowed to stand until the pellets dispersed. As the final step, an appropriate amount of corn oil was added to the vials to provide a 2.5 mg dose of zeranol delivered in a 2 mL injection.

At the end of the 10-d treatment period, MGA feeding was discontinued and the norgestomet implants were removed through a small incision made in the skin at the distal end of the implant. Concomitantly, PMGA and PSMB ewes were given a single i.m. injection of PG-600 (5 mL). All treatment groups were combined into one breeding group on May 4, 1992, with a ram to ewe ratio of 1:17 for a 30-d breeding period. All rams were equipped with marking harnesses. Marker colors were changed on d 10 and d 20 of the breeding period. Ewes were checked daily at 0900, and marking information was recorded. To monitor luteal activity, blood samples for serum progesterone analysis were collected by jugular venipuncture from C ewes 11 and 4 d before ram introduction, and from C, OMGA and OSMB ewes at 4, 7, 9, and 14 d after ram introduction. After collection, blood samples were stored out of direct sunlight and allowed to clot. Serum samples were collected and frozen until all samples could be assayed for progesterone concentration.

Concentrations of progesterone were determined in 100 µl of serum, in duplicate, with the Coat-A-Count (Diagnostic Products Corporation, Los Angeles, CA) RIA. Samples were re-evaluated if duplicate values differed by more than 25 % and .1

ng/mL. The intra- and inter-assay coefficients of variation were 3.3 % and 9.6 %, respectively.

After breeding, ewes were maintained under pasture conditions until approximately 4 wk before lambing when grain supplementation was initiated. Individual ewe lambing information was collected. Four ewes that died between breeding and lambing were not included in the analysis.

Chi-square analyses were conducted using the frequency procedure in SAS (1990) to determine significance of treatment on the categorical variables mating, luteal activity, fertility, and prolificacy. Analysis of variance was conducted, using the GLM procedures in SAS, to evaluate the fixed effect of treatment on lambing interval. Orthogonal contrasts for control vs treated ewes, and the factorial effects of progestogen, injection of zeranol or PG-600, and their interactions were tested for all dependent variables using the CATMOD and GLM procedures in SAS.

Results and Discussion

Estrus and Luteal Activity

A greater proportion ($P < .0001$) of progestogen-treated ewes mated to synchronized estrus (Table 2). More ($P < .0001$) SMB ewes were marked by rams during the first 5 d of breeding than MGA ewes. Within MGA treatments, RMGA ewes tended to exhibit ($P < .08$) a more synchronized estrus than OMGA or PMGA ewes. Except for the C and PMGA treatments, repeat matings to synchronized estrus exceeded 20 %. Overall, there were no treatment differences in estrus response for the 30-d breeding period.

Progestogen priming of seasonally anovular ewes, previously isolated from rams, dramatically increases estrous response upon ram exposure (Oldham and Fisher,

1992). Cognie et al. (1982) treated anovular Merino ewes for 12 d with intravaginal sponges impregnated with 30 mg flurogestone acetate (FGA) before ram exposure. Response to synchronized estrus was 97 % for FGA-treated ewes compared to only 20 % for non-treated control ewes. Ewe response to synchronized estrus obtained by Hunter et al. (1971) using a 14-d priming of anovular ewes with FGA intravaginal sponges were similar to those found in this study, with repeat matings to synchronized estrus of 25 and 16.2 %, respectively for the 2 yr study.

Safranski et al. (1992) reported a 55.2 % and 69.8 % response to synchronized estrus in seasonally anestrous ewes treated for 9 d with MGA or MGA/PG-600, respectively, compared to 9.4 % for non-treated control ewes. Five groups of anestrous ewes treated at different times from February through June with a 3-mg norgestomet implant for 10 d followed by an injection of 750 IU PMSG at implant removal exhibited an average response to synchronized estrus of 72 % compared to 10 % for non-treated control ewes (Carpenter and Spitzer, 1981). Neither of these studies monitored repeat matings at subsequent estrous periods.

All blood serum samples obtained from C ewes at 11 and 4 d before breeding, to monitor overall luteal activity of experimental ewes, measured less than .5 ng/mL progesterone. Blood serum samples obtained from C, OMGA, and OSMB ewes during the first 14 d of breeding were used as an indicator of corpora lutea (CL) formation. Blood serum progesterone levels greater than .5 ng/mL were found in 96, 100, and 93 % of OMGA, OSMB and C ewes, respectively (Table 2). Even though a large proportion of C ewes displayed luteal activity, only 12 % exhibited behavioral estrus within the first 17 d of breeding. Similar results were obtained by Safranski et al. (1992). Acute introduction of rams to seasonally anovular ewes, after an appropriate period of isolation from rams, causes spontaneous ovulation within 50 h after ram

exposure commonly referred to as the "ram effect" (Martin et al., 1986). Typically, this ovulatory response is not accompanied by estrus and frequently results in premature regression of CL (≈ 50 %) within 6 d after ram introduction followed by a second estrus-free ovulation and CL with normal life spans (Oldham and Martin, 1979). Subsequent ovulations to the initial ram-induced ovulation are accompanied by behavioral estrus, thus giving two peaks of breeding activity at 18 and 24 d after ram introduction (Signoret,1990). A single i.m. injection of 20 mg of progesterone administered immediately before ram exposure prevented premature regression of CL in 100 % of ewes (9 of 9) ovulating from the ram effect (Pearce et al., 1985).

Lambing

Progestogen-treated ewes exhibited a shorter mean interval from ram introduction to lambing ($P < .003$; Table 3). Within progestogen treatments, OMGA ewes had a longer ($P < .05$) mean interval from ram introduction to lambing than RMGA, OSMB, RSMB, and PSMB ewes. Fertility for MGA, SMB, and C ewes was not different. However, within SMB treatments, only 59 % of OSMB ewes lambed compared ($P < .05$) to 90 % of RSMB and PSMB ewes, respectively. Poor synchronization of ovulation may have occurred in OSMB ewes due to the lack of gonadotropin stimulation after implant removal. Cognie (1990) indicated that a single i.m. injection of PMSG at the end of progestogen treatment induced a tighter synchrony of ovulation. Gordon (1975) reported that many spring-mated ewes treated with progestogen/PMSG return to anestrus upon failure to conceive at first estrus. Of the OSMB ewes not conceiving to synchronized estrus, only 5 of 8 were remarked in the second period compared to 9 of 10, and 7 of 9 for the RSMB and PSMB ewes, respectively.

A greater proportion of progestogen-treated ewes lambed within the first 10 d of lambing as a result of more fertile matings to the ram-induced first ovulation (Figure 1). There was no difference in prolificacy due to treatment. Ewes primed with a single injection of zeranol before MGA or SMB treatment exhibited similar levels of fertility and intervals from ram introduction to lambing compared to ewes receiving an injection of PG-600 after progestogen treatment. Because the cost was less than 10 % of PG-600, zeranol may provide a more economical means of assuring adequate fertility levels when progestogen treatments are used. More work should be done to determine the physiological responses to zeranol in anovular ewes, especially at the level of the ovary. Zeranol has estrogenic activity (Peck and Chesworth, 1977) and elicits an increase in LH production after a single injection in ovariectomized ewes (Elsasser, 1983). Furthermore, zeranol stimulates estrus at the end of progestogen treatment (Burke and Keisler, 1988; Lewis and Goebel, 1993). Foote (1967) reported that the incidence of both estrus and ovulation and the subsequent proportion of pregnant ewes was consistently higher in anestrous ewes receiving estrogen pretreatment before progestogen/PMSG treatment.

Results from this study are in general agreement with those reported in other studies for anovular ewes treated with MGA (Keisler, 1992; Umberger and Lewis, 1992; Safranski et al., 1992) or SMB (Carpenter and Spitzer, 1981; Alifakiotis et al., 1982; Tritschler et al., 1991). In general, out-of-season lambing response tended to be higher for all treatments in this study compared to those reported elsewhere. This may have occurred as a result of the population of ewes used in this trial having a history of fall lambing. Higher fertility rates for OMGA vs OSMB ewes merits further evaluation. Other studies have demonstrated no benefit to gonadotropin treatment (Safranski et al., 1992) or treatment with estrogen-like compounds (Umberger and

Lewis, 1992) upon discontinuation of MGA feeding. This is the only study where direct comparisons have been made between these two progestogens.

Implications

Results obtained in this study for the occurrence of estrus, luteal activity, and lambing are similar to generally accepted arguments presented in other studies for progestogen-treated anovulatory ewes responding to acute introduction of a ram. Syncro-Mate-B and MGA used in combination with zeranol or PG-600 are effective in shortening the interval from ram introduction to lambing. Priming with zeranol before progestogen treatment is as effective and more economical than gonadotropin treatment at progestogen removal.

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Table 1. Treatment schedule for anestrous ewes

	Day of treatment		
	-10	-9 to -1	0[a]
Control (C)	Corn	Corn	Ram
MGA only (OMGA)[b]	MGA	MGA	Ram
MGA/Ralgro (RMGA)	(Inject zeranol)[c]/MGA	MGA	Ram
MGA/PG-600 (PMGA)	MGA	MGA	(Inject PG-600)[d]/Ram
SMB only (OSMB)[e]	SMB	SMB	Ram
SMB/Ralgro (RSMB)	(Inject zeranol)[c]/SMB	SMB	Ram
SMB/PG-600 (PSMB)	SMB	SMB	(Inject PG-600)[d]/Ram

[a]30-d breeding period with 1:17 ram to ewe ratio.
[b].3 mg MGA/d.
[c]2.5 mg zeranol in 2 mL corn oil containing 10% ethanol.
[d]5 mL PG-600 (400 IU PMSG + 200 IU hCG).
[e]3 mg norgestomet implant.

Table 2. Effect of treatment on estrus and luteal activity[a]

	Treatment							Contrast[b]		
Item	C	OMGA	RMGA	PMGA	OSMB	RSMB	PSMB	1	2	3
No. of ewes exposed	60	27	28	30	29	29	29			
Ewes marked at	7^{g}	13^{hi}	20^{ij}	14^{h}	21^{ij}	27^{k}	26^{jk}			
synchronized estrus[c]	(12)	(48)	(71)	(47)	(72)	(93)	(90)	**	**	*
Ewes marked only	48^{g}	12^{h}	7^{hi}	14^{h}	4^{i}	2^{i}	3^{i}			
after d 17 of breeding	(80)	(44)	(25)	(47)	(14)	(7)	(10)	**	**	-
Repeat mating to	1 of 1^{g}	6 of 6^{hi}	5 of 6^{hi}	2 of 3^{gi}	5 of 8^{hi}	9 of 10^{h}	7 of 9^{hi}			
synchronized estrus[de]	(14)	(46)	(25)	(13)	(21)	(33)	(27)	*	-	-
Total ewes marked	54	24	26	28	28	29	29	-	-	
	(90)	(89)	(93)	(93)	(97)	(100)	(100)			-
No. of ewes with luteal	56	26	-	-	29	-	-	-	-	-
activity[f]	(93)	(96)			(100)					

[a]Percentage of ewes marked in parenthesis.

[b]Contrasts:

1 (C vs MGA and SMB).

2 (MGA vs SMB).

3 (MGA and SMB vs RMGA and PMGA and RSMB and PSMB)

[c]Synchronized estrus through d 5 for progestogen treated ewes and through d 17 for control ewes.

[d]Out of ewes not lambing to synchronized estrus.

[e]Repeat mating, % = ewes remarked/ewes marked at synchronized estrus.

[f]≥ .5 ng/mL blood serum progesterone. Blood samples collected on C, OMGA, and OSMB treatments only.

[ghijk]Values in the same row with different superscripts differ, ($P < .04$).

*$P < .02$.

**$P < .0001$.

Table 3. Effect of treatment on fertility, prolificacy and interval from ram introduction to lambing

Item	Treatment							Contrast[a]			
	C	OMGA	RMGA	PMGA	OSMB	RSMB	PSMB	1	2	3	4
No. of ewes exposed	60	27	28	30	29	29	29				
No. of ewes lambing	45	23	21	22	17	26	26				
Lambing rate, %[b]											
I	75^{de}	85^{e}	75^{de}	73^{de}	59^{d}	90^{e}	90^{e}	-	-	-	*
II	10^{d}	26^{e}	50^{ef}	36^{ef}	45^{ef}	59^{f}	59^{f}	**	*	*	-
III	2^{d}	22^{e}	11^{de}	7^{de}	7^{de}	24^{e}	21^{e}	*	-	-	*
IV	63^{d}	37^{e}	14^{f}	30^{e}	7^{f}	7^{f}	10^{f}	**	**	-	-
No. of lambs born per ewe lambing	1.62	1.65	1.67	1.68	1.71	1.58	1.88	-	-	-	-
No. of lambs born per ewe exposed	1.22^{e}	1.41^{de}	1.25^{de}	1.23^{e}	1.00^{e}	1.41^{de}	1.69^{d}	-	-	-	*
Days from ram introduction to lambing[c]	166.6 ± 1.4^{d}	159.0 ± 1.9^{e}	152.4 ± 2.0^{f}	154.8 ± 2.0^{ef}	150.9 ± 2.2^{f}	152.0 ± 1.8^{f}	152.0 ± 1.8^{f}	**	*	-	-

[a]Contrasts:
1 (C vs MGA and SMB).
2 (MGA vs SMB).
3 (MGA and SMB vs RMGA and PMGA and RSMB and PSMB).
4 (OMGA vs RMGA and PMGA; OSMB vs RSMB and PSMB).

[b] I - Overall lambing rate per ewe exposed (Σ II, III, IV).
II - Lambing rate of ewes conceiving at synchronized estrus.
III - Lambing rate of ewes marked at synchronized estrus but conceiving to repeat mating.
IV - Lambing rate of ewes marking only in second period.

[c]Values represent least squares means ± SE.

[def]Values in the same row with different superscripts differ, ($P < .05$).

*$P < .05$.

**$P < .003$.

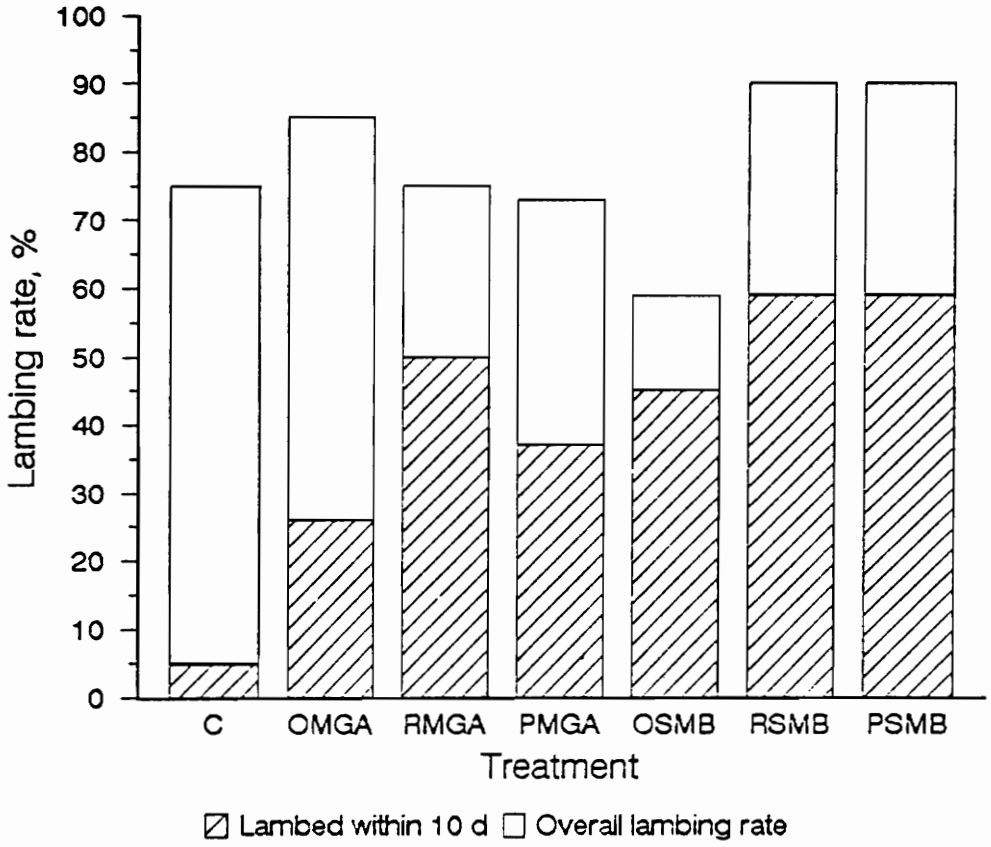

Figure 1. Effect of treatment on time of lambing.

VITA

Ghulam Jabbar was born on January 1, 1952 to Haji Bahader Khan and Zaib-u-Nisa at village Ningolai, Swat, North West Frontier Province (NWFP), Pakistan. He graduated from State High School Dherai, Swat in 1969. The same year he was admitted to the college of Veterinary Sciences Lahore, Pakistan. He received DVM degree in 1974.

He joined the Provencial Livestock and Dairy Development Department, NWFP, Pakistan as a Veterinary Officer (Health) on January 27, 1975. He worked as District Poultry Specialist in the Integrated Rural Poultry Development Program under United Nations Development Program (UNDP) from 1977 to 1980. He received M.Sc. (Hons) Animal Husbandry from NWFP Agricultural University Peshawar, Pakistan in 1988. Upon selection for the post of Deputy Program Leader (Livestock Development) in the Out-Reach Directorate, he joined the NWFP Agricultural University Peshawar, in 1989. Before coming to the USA he was actively involved in On-Farm-Research Trials in Livestock Production and Health. He will resume his present position when he is back home.

Jabar

GHULAM JABBAR