POOR REPRODUCTIVE RESPONSE OF ANESTROUS SUFFOLK EWES TO RAM EXPOSURE IS NOT DUE TO FAILURE TO SECRETE LUTEINIZING HORMONE ACUTELY

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ABSTRACT
Twenty Polypay-sired ewes (Group P) and 14 Suffolk ewes (Group S) were bled at 48-h intervals for 10 d beginning on April 6, 1989, and the serum was assayed for progesterone to determine which ewes were anestrous; 9/20 Group P ewes were anestrous, compared with 14/14 Group S ewes ($P < .001$). Catheters were placed into the jugular vein of anestrous ewes from both breed groups (eight of Group P, seven of Group S), and samples of serum were collected at 12-min intervals for 4 h. Then, the ewes were exposed to mature, intact rams, and additional samples were taken at 12-min intervals for 4 h after ram exposure. The serum was later analyzed to determine the secretion of LH in response to ram introduction. After the acute bleeding period, all Group P and Group S ewes were commingled and exposed to a ram continuously for 42 d. Samples of serum were collected thrice weekly and analyzed for progesterone to monitor ovulatory response to ram introduction through the 42-d period. In addition, breeding activity and lambing data were recorded. When all Group P ewes were compared to Group S ewes, a greater proportion ($P < .001$) of Group P ewes were mated (20/20 vs 3/13; one Group S ewe died during the 42-d mating period) by the end of the 42-d period and more ($P < .001$) ewes lambed in the fall (17/20 Group P vs 2/13 Group S). Furthermore, when the reproductive responses of only anestrous ewes at ram introduction were compared, a greater proportion ($P < .001$) of anestrous Group P ewes were mated and lambed (9/9 and 8/9, respectively) than Group S ewes (3/13 and 2/13, respectively). The average concentration of LH was increased ($P = .07$), and the frequency of pulses of LH tended to be increased ($P < .13$) in both breeds of ewes after introduction of the ram, and these increases did not differ between the two breed groups. Finally, the profiles of progesterone during the period of ram exposure indicated that a greater proportion ($P < .001$) of anestrous Group P ewes had consecutive ovulations (more than one full-length luteal phase) after ram introduction (9/9 anestrous Group P) than Group S ewes (4/13). Thus, most Group S ewes either ovulated and had only one full-length luteal phase (6/13) or failed to have a full-length luteal phase (3/13) after ram introduction. Results of the current study do not support the hypothesis that ewes of highly seasonal genotypes respond more poorly to ram introduction than ewes of less seasonal genotypes because they fail to release LH acutely upon ram introduction. Rather, anestrous ewes of more highly seasonal genotypes seem to be less likely to have more than one full-length luteal phase after ram introduction; therefore, they are less likely to be mated in spring for fall lambing.

Key Words: Rams, Breeds, Ewes, LH, Reproductive Performance

Introduction

The ability of rams to stimulate ovulation in anestrous ewes is well-established (see Knight, 1983 for review). This effect is due largely to the immediate and impressive release of LH in ewes previously isolated from contact with rams (Martin et al., 1980, 1983). However, some breeds or crosses seem to be more easily stimulated by ram introduction than others. Fewer Hampshire ewes than Dorset ewes ovulated and mated when exposed to rams in May or June (Nugent et al., 1988). In addition, nulliparous Suffolk ewes completely failed to ovulate in response to introduction of rams in April (Minton, 1989).

With the foregoing in mind, an experiment was designed to evaluate between-breed variation in the ability of anestrous ewes to respond reproductively to ram introduction. We hypothesized that anestrous ewes of some genotypes failed to respond to introduction of rams because they failed to release LH acutely upon ram introduction. In this study, the ewes compared were Suffolk and (Polypay × [Rambouillet × Dorset]); these genotypes of ewes were chosen because of their relative failure (Minton, 1989) and success (J. E. Minton, unpublished data), respectively, in spring-mating regimens.

Materials and Methods

Multiparous Suffolk (n = 14; Group S) and (Polypay × [Rambouillet × Dorset]) (n = 20; Group P) ewes, which had lambed in January and had weaned their lambs (March 31, 1989) were used in the experiment. Ewes had been isolated from rams since the breeding season of the previous fall. All ewes were bred by jugular venipuncture every 48 h for 10 d (beginning on April 6, 1989), and concentrations of progesterone in serum were used to identify cyclic and anestrous ewes. Ewes were judged to be anestrous if progesterone never exceeded 1 ng/ml during the 10-d period.

On April 19, catheters were placed into the jugular vein of seven Group S and eight Group P ewes that had been determined to be anestrous. Samples of serum were collected at 12-min intervals for 4 h. Then, Suffolk rams were introduced into the rooms housing the ewes (a single ram per room), and samples of serum were collected for an additional 4 h, at 12-min intervals. Two rams were used because simultaneous introduction of the rams into the groups of ewes was desired and because the ewes were housed during bleeding in separate rooms. Approximately equal numbers of Group S and Group P ewes were exposed to each ram. After the period of blood collection, all ewes were maintained together as a single group. One Suffolk ram, whose brisket was smeared with colored grease daily, remained with the ewes for 42 d.

Samples of serum were collected thrice weekly during the 42-d breeding period; the concentration of progesterone in these samples was used to monitor ovulatory responses. In addition, breeding marks were recorded daily, and lambing data were recorded in the fall.

Progesterone in unextracted serum was quantified by a RIA in which the antibody (lot TPG-1305) had been coated to polypropylene assay tubes. The antibody did not cross-react significantly (<2.4%) with any of 16 other steroid hormones tested (data from the manufacturer). Radiolabeled tracer for the assay was [125I]progesterone. When .05, .25, 1, 5, 10, or 20 ng progesterone/ml was added to ovine serum, .06, .29, 1.07, 5.07, 10.03, or 20.34 ng/ml was recovered, respectively (average recovery of 108%). All samples were quantified in the same volume of serum. The between- and within-assay CV averaged 10.2 and 19.6%, respectively. We found this assay to be as effective in determining reproductive status of ewes (Hanson et al., 1990) as the extracted assay for progesterone validated previously in our laboratory (Minton, 1990).

The RIA used to quantify LH in serum was that reported previously (Minton, 1990). The between- and within-assay CV averaged 13.3 and 11.4%, respectively. Pulsatile secretion of LH was determined with the aid of an algorithm (Merriam and Wachter, 1982) adapted for a personal computer (Gitzen and Ramirez, 1987).

The proportion of ewes within each breed that was mated by the ram and the proportion lambing was analyzed by chi-square tests. Based on profiles of progesterone, the proportion of anestrous ewes within each group that: 1) ovulated (once or twice) and had more than one full-length luteal phase, 2) ovulated and had only a single full-length luteal phase, or 3) did not develop a full-length luteal phase in

3Diagnostic Products Corp., Los Angeles, CA.
response to the ram also were analyzed by chi-square tests. The average concentration of LH, the number of pulses of LH, and the amplitude of pulses of LH before and after ram exposure were analyzed with a split-plot ANOVA with breed of ewe as a source of variation in the main plot (tested by the ewe within-breed mean square) and time (before and after ram exposure) and the breed x time interaction as sources of variation in the subplot. The number of lambs from each ewe given an opportunity to be mated was analyzed by one-way ANOVA. One Group S ewe died a few days into the ram exposure period, leaving only 13 ewes for analysis of data collected after ram introduction (profiles of progesterone, mating, and lambing).

Results

The mating and lambing responses of both groups of ewes are represented in Figure 1. First, based on the initial evaluation of progesterone in serum collected for 10 d before the onset of ram exposure, more \( P < .001 \) Group P ewes (11/20) than Group S ewes (0/14) were cycling. The mating (20/20) and lambing (17/20) responses of all Group P ewes, regardless of cycling status, were greater \( P < .001 \) than those of Group S ewes (3/13 and 2/13, respectively). Because all Group S ewes were anestrous at the onset of ram exposure, we felt that the most appropriate comparison would be between anestrous Group P and Group S ewes (Figure 1, middle and bottom panels). Even in this regard, more \( P < .001 \) anestrous Group P ewes than Group S ewes were mated (9/9 vs 3/13) and lambed (8/9 vs 2/13). Because the superior lambing rate of Group P ewes, it was not surprising that the lamb crop of these ewes (number of lambs per ewe exposed to the ram) far exceeded \( P < .005 \) that of Group S ewes (1.33 ± .22 vs .31 ± .18 lambs/ewe exposed).

The concentrations of LH and the amplitude and frequency of pulses of LH were similar between anestrous Group S and anestrous Group P ewes before introduction of the ram (Figure 2). After introduction of the ram, average concentration of LH increased \( P = .07 \), and the frequency of pulses of LH tended to be increased \( P < .13 \) in both groups of ewes. However, the amplitude of pulses of LH was not changed significantly after ram introduction. Importantly, the changes in average concentration and number of pulses of LH did not differ between Group S and Group P ewes.

The profiles of progesterone (Figure 3) in anestrous Group S and Group P ewes were classified into three categories. With only one exception, the first category (Figure 3, top panel) always was associated with mating; this group included ewes that had more than one full-length luteal phase after ram introduction. More \( P < .01 \) anestrous Group P (9/9) than Group S (4/13) ewes responded in this manner. The second type of progesterone profile (Figure 3, middle panel) was due to a single full-length luteal phase and included only ewes that were not mated (6/13 Group S ewes). The final category of progesterone profile (Figure 3, bottom panel) included only those ewes that had no evidence of a full-length luteal phase after introduction of the ram. Of course, none of these ewes (3/13 Group S ewes) was mated.

Discussion

That greater proportions of Group P than Group S ewes were mated upon ram exposure was not surprising. This was consistent with our previous observations (Thayer and Minton, 1987; Minton, 1989) and generally consistent with the study of Nugent et al. (1988), in which Dorset ewes were more responsive reproductively than Hampshire ewes to ram exposure in May and June. In both the study by Nugent et al. (1988) and the current study, ewes that were more responsive reproductively to ram introduction were generally of genotypes recognized for a lesser degree of seasonality. However, in the current study, the relative reproductive success of Group P ewes could not be accounted for solely by the fact that a greater proportion of this group was cyclic at the onset of the study. In fact, all the anestrous Group P ewes were mated and all but one eventually lambed, clearly outperforming Group S ewes (all of which were anestrous initially) in this regard.

Secretion of LH was evaluated only in ewes known to be anestrous at the time of ram introduction. The acute LH response to the ram was of interest because LH is known to increase rapidly in anestrous ewes upon ram exposure (Martin et al., 1980). Our original hypothesis was that ewes representing more highly seasonal genotypes (Group S) were less responsive reproductively to ram exposure because they did not release LH acutely in
response to the ram. This assumption was based on the observation that anestrous Suffolk ewes were extremely sensitive to the negative feedback action of estradiol on LH secretion (Legan et al., 1977) and that less seasonal genotypes were less sensitive to this steroid feedback action (Thomas et al., 1988). Therefore, we assumed that the greater feedback action of estradiol on the secretion of LH (and by implication, GnRH) in Group S ewes might be sufficiently strong so as not to be overcome by the stimulatory action of the ram. This assumption proved to be untrue, because Group S and Group P ewes responded with secretion of LH after ram introduction in an indistinguishable fashion. We cannot rule out

![Figure 1](image-url)

**Figure 1.** Mating and lambing responses of all Polypay-sired (Group P) ewes, anestrous Group P ewes, and all (anestrous) Suffolk (Group S) ewes. Ram exposure began on calendar day 109 (April 19). At the conclusion of ram exposure and lambing, a greater proportion \( P < .001 \) of anestrous Group P ewes had been mated and had lambed (9/9 and 8/9, respectively) than had Group S ewes (3/13 and 2/13, respectively).
the possibility that this increase in the secretion of LH in Group S ewes was short-lived and that the ram-induced drive for secretion of LH waned at some point after the cessation of our bleeding period (discussed subsequently).

The profiles of progesterone after introduction of the ram provide some evidence for the relative reproductive failure of anestrous Group S ewes compared with anestrous Group P ewes. It is widely recognized that the first, and usually the second, ovulation after ram exposure is not associated with expression of estrus (see Knight, 1983 for review). Estrus is usually not expressed until the third ovulation (after the first full-length luteal phase) in ram-exposed, anestrous ewes. Most Group S ewes either had only a single, normal-length luteal phase or failed to have a full-length luteal phase altogether. Therefore, these ewes would not be expected to show estrus. In contrast, all the anestrous Group P ewes had two, apparently normal-length, consecutive, luteal

Figure 2. Secretory characteristics of luteinizing hormone (LH) in anestrous ewes in response to ram exposure. Suffolk ewes (Group S) are denoted by solid bars (n = 7); Polypay-sired ewes (Group P) are denoted by open bars (n = 8), and both groups combined are denoted by shaded bars. Values represent means ± SEM. Asterisks denote an increase in average LH (*P = .07) and a tendency for an increase in number of pulses of LH (**P < .13) in both treatment groups combined after ram introduction.
phases; all were mated, and all but one eventually lambed.

Initially, most ewes seemed to have a short, low-amplitude luteal phase after ram introduction, regardless of whether or not they had subsequent full-length luteal phases. For those ewes that were eventually mated, the time from ram introduction until the average time of mating was 20.9 ± .4 d (range of 17 to 25 d). This period of time is consistent with the lag

![Figure 3. Representative profiles of progesterone in anestrous Polypay-sired (Group P) and Suffolk (Group S) ewes in response to ram exposure. The time of ram introduction is denoted by the broken arrow at calendar day 109. The top panel denotes representative profiles containing more than one full-length luteal phase; these profiles were associated with mating (solid arrows). Both ewes represented were mated at the first solid arrow, the second solid arrow denotes remating of ewe 6183 only. The type of progesterone response illustrated in the top panel included more (P < .01) Group P (9/9) than Group S (4/13) ewes. The middle panel illustrates a representative profile with only a single full-length luteal phase after ram exposure, characteristic of 6/13 Group S ewes. The lower panel illustrates a representative profile of progesterone with no full-length luteal phase after ram introduction, characteristic of 3/13 Group S ewes. None of the ewes with progesterone profiles represented in the middle and bottom panels was mated.](image-url)
time expected for ram-exposed anestrous ewes that ovulate two times before exhibiting estrus in association with the third ovulation; the first ovulation is associated with a short luteal phase and the second with a normal-length luteal phase (Oldham and Martin, 1978/1979).

The factors underlying the inability of Group S ewes to generate more than one full-length luteal phase (the only type of ovulatory response associated with mating in our study) remain unclear. One explanation may be that ovulate two times before exhibiting estrus time expected for ram-exposed anestrous ewes. One explanation may be that ovulate two times before exhibiting estrus time expected for ram-exposed anestrous ewes. However, most Suffolk ewes only had one full-length luteal phase, or, in some cases, had no full-length luteal phase after ram introduction and, therefore, failed to exhibit behavioral estrus.

Implications

The results of the current study do not support the hypothesis that ewes of some genotypes do not respond well reproductively to ram exposure because they do not release luteinizing hormone in response to the ram. At least acutely, the luteinizing hormone response of Suffolk ewes was essentially identical to that of Polypay-sired ewes. However, most Suffolk ewes only had one full-length luteal phase.

Literature Cited


