The Behavioral Component of the Ram Effect: 
The Influence of Ram Sexual Behavior on the 
Induction of Estrus in Anovulatory Ewes

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ABSTRACT: The objective of this study was to test whether the sexual behavior of the ram affects the ram effect. Rams exhibiting either high (HP) or low (LP) levels of sexual performance (on the basis of serving capacity tests) were exposed to 89 anestrous ewes for 28 d. Thirty-two anestrous ewes were not exposed to rams. The objective of this study was to compare the efficacy of estrus induction by HP (n = 4) vs LP (n = 4) rams. Plasma progesterone concentration was used as an index of ovarian activity. Groups of ewes were exposed to either an HP or an LP ram in a .32-ha pasture. Courtship behaviors of rams were recorded for 6 h on the initial day of exposure and for 30-min periods on alternate days thereafter. A greater percentage of ewes exposed to HP rams ovulated (95%) compared with ewes exposed to LP rams (78%) (P < .02). On the 1st d of exposure, the HP rams exhibited more courtship behavior and spent more time near the ewes (P < .04). The HP rams spent more time within 1 m of ewes during the 28-d exposure. There were no differences in the amount of contact with rams (LP or HP) between ewes that did and did not ovulate (P > .05). The first rise in progesterone indicative of ovulation tended to occur earlier (P = .06) in ewes penned with HP rams. A greater percentage of ewes exposed to LP rams (P = .03) had early elevations of progesterone with no concurrent sexual behavior. These data imply that in addition to a pheromone the sexual behavior of the ram may be important in initiating ovarian cycle activity.

Key Words: Rams, Sexual Behavior, Estrus Ovulation

Introduction

Toward the end of seasonal anestrus in sheep, introduction of the ram results in an earlier and synchronized appearance of estrus. This phenomenon was first reported by Underwood et al. (1944) and has since been referred to as the “ram effect” (reviewed by Martin et al., 1986). When ewes are isolated from rams for ≥ 1 mo, they react to the introduction of a ram with a rapid increase in their plasma LH concentration and a preovulatory LH surge. Ovulation occurs 35 to 40 h later. Early researchers hypothesized that an olfactory stimulus (pheromone) from the ram released the ewe from its inhibitory feedback system during anestrus. Morgan et al. (1972) observed that noncycling ewes with an impaired sense of smell were not as stimulated to exhibit estrus after exposure to rams as were intact ewes or those ewes with impaired senses of touch or hearing. More recently, Cohen-Tannoudji et al. (1986) have demonstrated that although chemical stimulation alone can elicit ovulation, other sensory cues may be involved. Anosmic ewes did not cycle in response to a rams fleece but did display an LH release similar to that of controls when exposed to intact rams. These authors suggested that sensory cues other than olfactory stimuli could not only act synergistically with chemical signals but also replace the pheromone in triggering the same physiological responses. Lindsay and Signoret (1980) casually observed that small numbers of rams of poor libido were less effective than rams of high libido in stimulating an ovulatory response in anestrous ewes. Given that ewes may integrate several sensory messages to perceive the presence of a ram, the objective of this experiment was to determine whether the sexual behavior (performance) of the ram contributes to the efficacy of the “ram effect.”
Materials and Methods

Rams

Rams that had been previously identified as sexually inactive or exhibiting low levels of sexual performance (LP) and rams exhibiting high levels of sexual performance (HP) (Perkins et al., 1992) were restested during the breeding season (September 15 through October 31). Rams were individually exposed to three estrus-synchronized ewes for 30 min on three occasions. Two LP and two HP rams were selected for this experiment based on their average number of mounts and ejaculations achieved during the three tests. The HP rams averaged six ejaculations (range 4 to 10). The LP rams had a maximum of two ejaculations during any single 30-min test. Rams were balanced for this experiment by breed (Rambouillet and Targhee). The sexual experience of these rams was limited to serving capacity tests. Rams were maintained in drylot and were fed alfalfa hay (each ram received 2.5 kg/d) daily with water and salt available ad libitum until they were released into breeding pastures for the experiment. At that time they grazed freely and had access to creek water, and salt was provided.

Ewes

Fifty-one mature Targhee and Rambouillet ewes of known fertility were isolated from all rams on May 1. Blood samples were taken from each ewe twice a week beginning on May 3. Plasma progesterone concentration was assayed and used as an index of estrous cycle activity. Two ewes with persistently high progesterone concentrations were eliminated from the study. Based on progesterone profiles, all remaining ewes were determined to be in anestrus as of July 1. After balancing for age and breed, ewes were randomly assigned to one of three treatments: no exposure to rams, exposure to an LP ram, or exposure to an HP ram. The breeding pastures were approximately .32 ha each of mountain meadow terrain. Pastures assigned to LP rams were at a separate location from the pastures assigned to HP rams. There were eight or nine ewes per pasture. Each ewe that had been assigned to an LP or HP ram was painted on both sides with a unique color so that it could be easily identified from a distance.

On a daily basis beginning on July 5, ewes were gathered into a small catch pen and fed grain. On July 14, one experimental ram was introduced into each of four pastures (two HP and two LP). Control ewes were not exposed to rams. Rams were observed constantly for the first 6 h of exposure to ewes and for 30-min intervals on alternate days thereafter. The experiment ended on August 6. Instantaneous scan sampling was used to estimate the total amount of time that a ewe spent within 1 m of a ram. At 1-min intervals an observer recorded the identity of all ewes estimated to be within one sheep length of the experimental ram. This procedure was used for the first 6 h of exposure (0900 to 1500) and during 30-min intervals on alternate days during 24 more days (12 sample periods x 3 min). All subsequent observations were made between 0600 and 1000. The observations were discontinued when all animals were brought back to the research headquarters to prepare ewes for laparoscopy (d 26). All occurrences of courtship behaviors including foreleg kicks, tongue flicks and mount attempts were noted during observation periods. To prevent interobserver variability (and boredom) volunteers were rotated in a round-robin fashion, allowing each observer to spend time watching each test ram. Observers remained as unobtrusive as possible, using binoculars to identify ewes and to observe rams when necessary.

Serum progesterone concentrations of ewes were determined from blood samples taken after the 30-min observation period every other day for 24 d after the initial exposure to rams. On d 28 and 29 ovarian activity of ewes was evaluated through laparoscopy.

This experiment was replicated the following year with 72 ewes assigned to the three treatment groups (24 ewes in each group). Two different HP and two different LP rams, identified by procedures as previously described, were released into the same single-sire breeding pastures. The procedure was nearly identical and conducted within 1 yr and 1 wk of the first experiment. Individual ewes were not identified during the 2nd yr. The general activity of each ram was recorded at 5-min intervals during the first 6 h of exposure to ewes. The briskets of rams were painted so the identity of ewes that had been mounted could be recorded. Observations of sexual activity were made before blood samples were taken every other day.

Hormones

Serum progesterone concentration was analyzed by RIA (Fitzgerald and Butler, 1982). Interassay CV was 12% and intraassay CV was 10%. A ewe was considered to have a functioning corpus luteum when progesterone concentration was ≥.5 ng/mL. A short cycle was defined as a period of elevated progesterone concentration lasting for <7 d (Pearce et al., 1985). Both LP and HP rams had similar plasma testosterone concentrations and showed no difference in testosterone or LH concentrations in response to an injection of LHRH (Perkins et al., 1992).

Statistical Analyses

Numbers of ewes cycling in exposed vs isolated pastures and for HP vs LP rams were analyzed by categorical procedures using chi-square (PROC FREQ; SAS, 1988). Analysis of variance (PROC GLM;
Table 1. Percentage of anestrous ewes ovulating in control groups isolated from rams or exposed to rams classified as high sexual performers (HP) or low sexual performers (LP)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of ewes</th>
<th>Percentage ovulating (no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control isolated</td>
<td>40</td>
<td>17.5&lt;sup&gt;b&lt;/sup&gt; (7)</td>
</tr>
<tr>
<td>Exposed HP</td>
<td>40</td>
<td>95.0&lt;sup&gt;b&lt;/sup&gt; (38)</td>
</tr>
<tr>
<td>Exposed LP</td>
<td>41</td>
<td>78.0&lt;sup&gt;c&lt;/sup&gt; (32)</td>
</tr>
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</table>

<sup>a,b,c</sup>Percentages differ between ewes cycling from exposure vs isolation (<i>P < .01</i>) and by ram performance category (<i>P < .02</i>).

SAS, 1988) was used to compare the number of days to first occurrence of progesterone (>&gt; .5 ng/mL) by ewes exposed to first occurrence of progesterone (> .5 ng/mL) by ewes exposed to LP vs HP or not exposed to rams each year, the amount of contact with ewes by LP vs HP rams, and the amount of contact by any ram with cycling vs noncycling ewes. The total number of contacts per ram was determined by the number of tally marks recorded for each ewe in the ram's pen during the observation periods. Where there was no interaction by year, data were combined. Contact with ewes was evaluated on the day of ram introduction for yr 1 and 2 and during 30-min observation periods every other day throughout the experiment for yr 1 only.

Results

A greater percentage of ewes exposed (n = 81) to either an LP or HP ram (87%) than of ewes isolated (n = 40) from rams (18%) ovulated (<i>P < .01</i>). The sexual behavior of the ram also influenced the percentage of ewes induced to ovulate. Ninety-five percent of ewes ovulated in response to HP rams compared with 78% in response to LP rams (Table 1). More ewes cycled (<i>P < .02</i>) when exposed to HP vs LP rams. Despite this difference, all the ewes in two out of the four LP rams' pens cycled.

The HP rams had more contacts (<i>P < .01</i>) with ewes than LP rams on the 1st d of exposure. The HP rams directed courtship activity (investigatory sniffs, foreleg licks, and mount attempts) toward 88% of the ewes penned with them during the first 6 h of exposure. All LP rams failed to court any ewes on the 1st d. After the initial exposure period, however, LP rams had more contacts than HP rams (<i>P < .04</i>) within 1 m of ewes during 30-min observation periods on alternate days over 24 d. Both LP and HP rams were observed mounting and courting ewes after the initial joining. Table 2 compares the estimated amount of LP vs HP ram contact and courtship activity with ewes on the initial day of exposure and over 24 d of exposure during yr 1. For the ewes that ovulated, the first rise in progesterone indicative of ovulation occurred somewhat earlier (<i>P = .06</i>) in ewes penned with HP rams (10.8 ± 1.5 d) than in ewes penned with LP rams (13.6 ± 1.6 d). Figure 1 shows a representative profile of the pattern of blood progesterone secreted from three individual ewes. A difference (<i>P = .03</i>) was observed in the distribution of short vs normal estrous cycles. Short cycles were characterized by early elevations of progesterone and no concurrent sexual behavior. Only 60% (19/32) of ewes that ovulated after exposure to an LP ram had cycles classified as normal, compared with 82% (31/38) of ewes exposed to HP rams. Mean number of corpora lutea, however, did not differ (1.6 ± .6 vs 1.7 ± .6). There were no differences (<i>P > .05</i>) in the total number of observed contacts with a ram between ewes that did and did not ovulate (131.2 ± 22 vs 115.4 ± 21 contacts, respectively).

Discussion

Results from this experiment suggest that overt sexual cues from a ram assist in releasing some ewes from the inhibitory feedback of their endocrine system during anestrus. In addition, the predictability of ram efficacy for the “ram effect” is more reliable when using a sexually active vs a less sexually active ram.

There is clear evidence that a pheromone produced by the wax and wool of rams induces ovulation in anovular ewes (Knight and Lynch, 1980b). The pheromone alone, however, does not account for the variation observed in ovulation response. Knight and Lynch (1980a) reported that only 48% of a group of

Table 2. Number of ram contacts within one meter of ewes and frequency of sexual behaviors of rams with high (HP) versus low (LP) sexual performance for year 1

<table>
<thead>
<tr>
<th>Item</th>
<th>HP</th>
<th>LP</th>
</tr>
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<tr>
<td>Mean (± SE) no. of contacts during first 6 h of exposure to ewes&lt;sup&gt;a&lt;/sup&gt;</td>
<td>157 ± 12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>108 ± 8&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Percentage of ewes toward which courtship was directed during first 6 h of exposure</td>
<td>88%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0%&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total no. of courtships and/or mounts observed during 30 min on alternate days during 24 d (total of 6 h)</td>
<td>25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean (± SE) no. of contacts observed during 30-min periods</td>
<td>48 ± 12.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>71 ± 8.6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Contact means the number of times a ram was observed within 1 m of a ewe at 1-min intervals using instantaneous scan sampling.
<sup>b,c</sup>Columns with different superscript differ (<i>P < .05</i>).
Figure 1. A representative profile of the pattern of plasma progesterone secreted from three individual ewes. Ewes #14 and #15 had initial rises in progesterone characteristic of a short cycle. The estrous cycle of ewe #39 is normal. More ewes exposed to high-performing rams than ewes exposed to low-performing rams had cycles classified as normal \( P = .03 \). The arrow represents the introduction of rams.

Ewes ovulated when wool and wax collected from Dorset rams were smeared on the ewes’ noses. Cohen-Tannoudji et al. (1986) demonstrated that anosmic ewes can ovulate in response to the presence of intact rams. Many other factors, such as breed of ram, depth of anestrus, season, and length of isolation period from rams, have reportedly contributed to efficacy of the “ram effect” (reviewed by Stewart, 1985). These studies imply that ovulation in response to rams involves pheromonal as well as other information perceived from the environment. Such information may be integrated at higher brain centers rather than acting directly on hypothalamic regulation of pituitary secretion. The present study suggests that sexual behavior of rams contributes to the onset of ovarian activity in some individuals because a greater percentage of ewes cycled in response to HP than to LP rams.

The LP rams spent more time within 1 m of ewes than did the HP rams during the 24-d exposure period. Proximity to the ewes was usually in a nonsexual context (e.g., grazing or sleeping). This suggests that close proximity alone is not as effective in inducing ovulation as sexual behavior.

The differences noted in the onset of normal vs short cycles determined by blood progesterone concentration implies differences in the quality of cycles induced. Martin et al. (1986) reported the existence of short cycles in 50% of anovulatory ewes exposed to rams. They attributed variability noted in the pattern of progesterone secretion to a random occurrence and suggested that it resulted from insufficient gonadotropin secretion. It is tempting to speculate that short cycles may be attributed to the inability of LP rams to induce sufficient gonadotropin secretion by some ewes and that sexual interaction directly stimulates LH activity.

Fulkerson et al. (1981), Croker et al. (1982), and Signoret et al. (1982) have shown that wethers and ewes treated with testosterone (and estrogen) can be used as substitutes for vasectomized or intact rams in stimulating anovular ewes to ovulate and exhibit estrus. These reports indicate that the production of the pheromone is dependent on sex steroids, particularly testosterone. Testosterone concentrations were not low in LP rams (Perkins et al., 1992); hence, it is unlikely that pheromone production by LP rams is low. The LP rams spent as much time near and around the ewes as did the HP rams. If we assume that sexual behaviors are not necessary for dissemination of the pheromone, then any ewe that can ovulate in response to the pheromone alone (e.g., extract held over the nose) can probably ovulate in response to any ram (HP or LP). This may account for the high percentage (100%) of ewes that responded to half (2/4) of the LP rams.

This study supports observations by Pearce and Oldham (1988) suggesting that the ram effect is mediated by a combination of stimuli from the ram, which include visual and tactile cues. These data indicate that such cues are further processed by the ewe as perception of male sexual behavior.
Implications

This experiment provides evidence that the sexual performance of a ram is a variable that should be considered in selecting individuals for use in bringing anovulatory ewes into estrus. Many anestrous ewes can begin cycling in response to a ram’s wool, a pheromone, or any ram. Yet, a higher percentage of ewes and a better quality of estrous cycle (defined by plasma progesterone concentration) result from introducing rams that have been selected on the basis of sexual performance tests. This information is most applicable when only one ram will be used for inducing estrus.

Literature Cited


