Ram mating behavior after long-term selection for reproductive rate in Rambouillet ewes
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ABSTRACT: Mating behavior is known to be heritable in several species, but it is not known if selection schemes for ewe reproductive traits affect mating behavior of rams. Therefore, our objectives were to determine if divergent (high vs low index) selection of a female reproductive trait (lambs born divided by age of ewe minus one) affects mating behavior patterns of male offspring during training to service an artificial vagina (AV) in a less than natural setting (Exp. 1) or during serving capacity tests (SCT) in simulated natural mating conditions (Exp. 2). The method for evaluating male mating behavior was a series of serving capacity tests to estimate sexual performance. For Exp. 1, five, 30-min observations were conducted to assess the mating behavior of 29 rams (22 mo old) being trained to service an AV. In Exp. 1, percentages of rams mounting (73 vs 36%) and ejaculating (67 vs 29%) differed ($P < 0.05$) between rams from high and low Rambouillet ewe selection lines, respectively. In Exp. 2, ram classification consisted of exposing each 22- to 24-mo-old ram ($n = 48$) to three unrestrained ewes in estrus for 18, 30-min tests. Any ram that had not mounted or ejaculated during SCT was evaluated for sexual orientation in a 30-min preference test in which each ram had access to both restrained estrual ewes and restrained rams. In Exp. 2, there was no difference ($P > 0.2$) in percentages of rams mounting (92 vs 78%) and ejaculating (88 vs 74%) between rams from high and low Rambouillet ewe selection lines, respectively. There was no difference ($P > 0.33$) between high and low ewe selection line rams for mounts or ejaculations for 18 SCT. Only one male-oriented ram was identified, which was from the high line. In Exp. 2, six SCT were necessary to obtain 95% reliability in sexual performance scores. Results of these studies indicate that long-term selection of ewes for litter size did not alter ram sexual performance after SCT under simulated natural mating conditions or affect incidence of male-oriented rams. Mating behavior observed during training to service an AV was lower for rams from ewes of the low selection line than for rams from ewes of the high selection line. We concluded that long-term selection for reproductive rate in ewes did not alter mating behavior patterns of male offspring; however, test conditions, such as restrained and unrestrained ewes and number of serving capacity tests, may affect conclusions in studies that evaluate sexual performance of rams.

Key Words: Libido, Reproduction, Selection, Sheep

Introduction

Studies reviewed by Kilgour et al. (1985) indicated that mating behavior of both males and females is heritable in several species. In bulls, Blockey et al. (1978) reported heritability estimate of 0.59 for serving capacity. In sheep, data on serving capacity are limited with heritabilities ranging from $0.002 \pm 0.10$ (Purvis, 1985) to $0.33 \pm 0.62$ (Kilgour, 1985). The most reliable heritability estimate for ram serving capacity is $0.22 \pm 0.04$ (Snowder et al., 2002b).

Studies relating high sexual performance of rams to fertility of ewes indicate a high degree of variability. Positive associations between high sexual performing rams and ewe fertility have been reported (Mattner et al., 1971; Perkins et al., 1992; Kilgour, 1993). In contrast, others found little relationship between ram sexual performance and flock fertility (Kelly et al., 1975; Kilgour and Wilkins, 1980; Mickelsen et al., 1982). Disparity among studies appears to be primarily related to whether rams were challenged sufficiently with numerous estrous ewes (i.e., high sexual performance will only manifest itself under such conditions). Divergent
selection for female reproductive traits (i.e., litter size) has resulted in differences in male sexual behaviors of lambs (Burfenning and Rossi, 1992) and mature offspring (Tulley and Burfenning, 1983). However, little information is available on how offspring from divergent ewe lines respond to training for semen collection or effects of divergent ewe selection on sexual behavior and orientation of male offspring. Two experiments were conducted to determine 1) if divergent selection of ewes for litter size affects sexual behavior of offspring during training for semen collection using an artificial vagina in an unnatural setting, 2) if selection of ewes affects sexual behavior and orientation of male offspring under simulated natural mating conditions, and 3) number of serving capacity tests (SCT) required to obtain accurate scores for ram sexual performance.

Materials and Methods

Experiment 1

All animals in this study were born, reared, and maintained at Montana State University, Bozeman, MT, located at 45° 40’ N and 111° 3’ W. After weaning, rams were fed a diet of mixed grass hay (timothy, fescue, crested wheat, and brome), and given ad libitum access to a trace mineral supplement and water. During the performance testing, rams were fed an additional 0.09 kg alfalfa pellets·animal−1·d−1.

Rams (n = 29) used in Exp. 1 were 22-mo-old offspring from Rambouillet lines of ewes that had been selected for high (H) and low (L) reproductive rates since 1968 at Montana State University. Details of the selection criteria used for these lines were reported by Schoenian and Burfenning (1990). Briefly, this entailed selecting ewes at approximately 16 mo of age based upon the following index (I): I = total lifetime lambs born/age of ewe − 1. Selection using this I has resulted in an ovulation differentiation of 0.61 between ewes of these lines. Each fall, four rams from ewes with the highest indices and four rams from ewes with the lowest indices within the H line and four rams from ewes with the lowest indices within the L line, that were not paternal half-sibs, were selected for breeding the following year. Fifteen H and 14 L line rams from H and L ewe lines that had been selected for 22 yr were used in Exp. 1. These rams had not been exposed to ewes after they were weaned at 90 to 130 d of age. Rams were acclimated to the test arena and husbandman for 21 d before the first performance evaluation test. These tests were conducted over a 3-wk period from late December to mid-January.

Training to Service an Artificial Vagina (AV). Five 30-min tests with estrual ewes restrained in a breeding crate were used to evaluate sexual performance during training for collection of semen by AV (Nasco International, Atkinson, WI) at Montana State University. Ewes were identified in estrus by epididectomized rams. A mount was recorded when the forelegs of the ram crossed the hip joints of the ewe without ejaculatory. An ejaculation was recorded if the ram mounted, achieved intromission into the AV, exhibited pelvic thrusts, threw back his head, and ejaculated, followed by a period of no interest in the ewe. The number of mounts with and without ejaculations, and number of rams mounting and ejaculating were recorded. Each time a ram mounted, an attempt was made to place the AV on the penis by the husbandman who the rams had been acclimated to. If the warm AV (36.5°C) cooled to less than 34.5°C, then it was replaced with a warm AV. Each AV was lubricated with a small amount of lubricating jelly.

Experiment 2

All rams in this study were obtained from Montana State University where they had been reared under the same conditions and management used for rams in Exp. 1. These rams were maintained at the U.S. Sheep Experiment Station (USSES), Dubois, ID, located at 44° 14’ N and 112° 11’ W on a diet of 1.6 kg long-stem alfalfa hay·animal−1·d−1 and had free access to trace mineral salt and water after they were transported to the USSES from Montana State University. Rams were managed in all male groups at the USSES without access to females other than during testing.

Rambouillet rams (n = 48) for Exp. 2 were offspring from the same selection lines of ewes as described in Exp. 1, except that selection had continued for six more years. Rams were 18 to 24 mo of age when they underwent serving capacity and sexual preference tests from December to February.

Estrus Induction Procedure. Estrus was induced in ovarioectomized ewes by using pessaries containing 60 mg of 6α-methyl-17α-hydroxyprogesterone acetate (Tuco Products Limited, Orangeville, Ontario, Canada). One pessary per ewe was inserted into the vagina for a period of approximately 12 d. At time of pessary removal each ewe received a 0.5 mL injection i.m. of estradiol (100 μg estradiol benzoate per milliliter of corn oil). Each ewe was given additional estradiol injections at 24, 48, and 72 h after the first injection to maintain estrus for three test days over a 1-wk interval that began 48 h after first injection. Ewes that were not in estrus during the tests were replaced with ewes that were in estrus, and tests were postponed until sufficient number of ewes (n = 24) were in estrus.

Servicing Capacity and Preference Tests. Sexual performance tests employed at the USSES, Dubois, ID, consisted of three parts. Eighteen, 30-min serving capacity tests were conducted in 2.4 × 2.4-m pens. Each ram was exposed to three unrestrained, estrual ewes only once on any day and only three times during a week. To accomplish this, 24 estrual ewes were assigned randomly in groups of three to one of eight observation pens. On the first day of testing, rams were assigned randomly to a sequence of tests for each of the three weekly observation periods. Depending upon whether a ram was tested first, second, third, or fourth
on the first of three observation days, there were either zero, one, two, or three rams exposed to ewes in an observation pen before their test, respectively. Then, on the second observation day, there were either 12, 13, 14, or 15 rams exposed to the ewes before their tests, respectively, because another group of rams was tested with the same ewes after these study rams each day. Similarly, on the third observation day of each week, there was a maximum of 23, 24, 25, or 26 rams exposed to the ewes before their tests, respectively. After each week for the first 3 wk of testing, estrual ewes were replaced with a different set of 24 ewes. On the fourth, fifth, and sixth week of testing, the first, second, and third group of ewes, respectively, were again used for 3 d. After the seventh to the eleventh serving capacity test, an overnight test was conducted on each ram that had not exhibited any sexual activity. The overnight test of 16 to 20 h in duration was used only to identify female-oriented rams. The overnight test consisted of placing each ram into a pen with three ewes that had been identified in estrus in the previous serving capacity test. At the start of the overnight test, ewes were painted with a mineral oil/cement pigment mixture just above the tailhead. Mineral oil was mixed with dry cement pigment to a consistency that will stick to wool on the brisket. At the end of the overnight test, the brisket of rams was checked for pigment. Rams that had not shown any sexual activity in the serving capacity or overnight tests were observed in a sexual preference test similar to that used by Price et al. (1988). Rams were isolated for 9 d to prevent mounting and courtship behavior with male penmates prior to the preference test. Then each ram was observed for 30-min in a testing pen that contained two restrained rams and two restrained estrual ewes. The method of restraint was described by Perkins and Fitzgerald (1992). A mount was recorded if the front legs left the ground and the brisket of the ram made contact with the rump of the restrained teaser ram or ewe, but there was no ejaculation. An ejaculation was recorded if the ram mounted, achieved intromission, exhibited pelvic thrusts, threw back his head, and ejaculated, followed by a latent period of interest in the restrained animals. In the preference test, a ram was classified as male oriented if and only if the ram mounted or ejaculated on a restrained ram.

**Statistical Analyses**

**Experiment 1.** Chi-square analyses were used to evaluate differences between selection lines for proportions of number of rams mounting and ejaculating over the five AV training periods.

**Experiment 2.** Chi-square analyses were used to evaluate differences between selection lines for proportions of number of rams mounting and ejaculating for the first five serving capacity tests and for the entire evaluation period.

Mixed model analyses of SAS (SAS Inst. Inc., Cary, NC) were used to analyze mounts and ejaculations for the 18 serving capacity tests. Line (H or L) of rams was in the main plot, and serving capacity test number and the two-way interaction were in the subplot. Line was tested by rams nested within line as the error term, and the residual was used as the error term to test the subplot. The First-Order Autoregressive Moving-Average command was used to structure the covariance among the repeated measures on the rams. Degrees of freedom were calculated using the Satterthwaite procedure of SAS (SAS Inst. Inc., Cary, NC). Data for number of mounts showed heterogeneous variance using Bartlett’s Box F-test. Data were transformed to the square root of X + 1 to stabilize the variances among rams for the fixed effect. Least squares means and confidence intervals (CI) for mounts were changed back to original units after analysis. A standard error for the original units was estimated using the 95% CI; this is an approximation and not appropriate for estimating CI for means, that would asymmetrically match the data distribution. The Tukey-Kramer method was used for mean separation.

Proc GLM was used to calculate $r^2$ values to estimate how many serving capacity tests were necessary to obtain reliable sexual performance scores. The dependent variable was the average of ejaculations of the 18 serving capacity tests (SCT). The independent variable was combinations of SCT, starting with SCT3; thereafter, combinations were the average of consecutive additions of SCT (i.e., mean of SCT3 + SCT4, then mean of SCT3 + SCT4 + SCT5, and so on). Each serially derived combination was analyzed separately.

**Results**

**Experiment 1**

During the five training periods to service an AV, percentages of H line rams mounting and ejaculating were higher ($P < 0.05$) than those for L line rams (Table 1).
Experiment 2

During serving capacity tests in Exp. 2, there was no difference (P > 0.2) for percentages of H and L line rams that mounted or ejaculated after the first five tests or for all 18 tests (Table 2).

Number of mounts over the 18 SCT did not differ (P > 0.67) between H line rams (9.4 ± approximate SEM 1.63 with a range of 0 to 59) and L line rams (8.5 ± approximate SEM 1.61 with a range of 0 to 72; Figure 1). There was no (P > 0.10) line by SCT interaction for mounts, but number of mounts differed (P < 0.01) among the consecutive 18 SCT (Figure 1).

There was a difference (P < 0.01) in the number of ejaculations among the serving capacity tests (Figure 1), but there was no difference (P > 0.33) between all H (2.2 ± 0.26 with a range of 0 to 8) and L line rams (1.8 ± 0.25 with a range of 0 to 7). There was no (P > 0.66) line by SCT interaction for ejaculations (Figure 1).

After the overnight test, one ram out of six L rams that had not exhibited any sexual activity during performance tests mounted ewes and ejaculated. The remaining five L rams were not sexually active in any of the SCT or the preference test. In the H line, one ram out of four was heavily marked indicating that he was sexually active in the overnight test, and thereafter this ram continued to mount ewes and ejaculate in the remaining nine SCT. The remaining three H rams never showed sexual activity in any of the SCT with the exception of one mount by one ram during the fifth 30-min SCT that occurred before the overnight test. Only one out of four rams from the H line and no rams out of six from the L line were identified as male oriented in the sexual preference test.

The number of serving capacity tests necessary to obtain reliable sexual performance scores on rams that mounted and ejaculated was evaluated by examination of $r^2$ values. R-square values were 0.63, 0.83, 0.85, and 0.95 for SCT 3, mean of SCT3 + SCT4, mean of SCT3 + SCT4 + SCT5, mean of SCT3 + SCT4 + SCT5 + SCT6, respectively. The $r^2$ value reached 0.99 after 11 serving capacity tests.

Discussion

Percentage of rams mounting and ejaculating was not affected by selection line in serving capacity and preference tests in Exp. 2. However, sexual performance evaluation during training to service an AV in an artificial setting (Exp. 1) indicated that more H line rams mounted and ejaculated than L line rams. Although the results from these two experiments differ, it is important to note that restrained ewes were used in Exp. 1 to facilitate use of the artificial vagina, whereas unrestrained ewes were used in Exp. 2. Restraining the estrual ewe during serving capacity tests inhibits the sexual behavior of rams (Zenchak et al., 1988). Restraint of ewes in stanchions inhibited courtship and mounting activity of rams more than if ewes were unrestrained or tethered; however, the number of completed matings per unit of time was not altered by degree of restraint (Zenchak et al., 1988). Thus, these different test conditions may be important for interpreting the results in the present study and in other studies that focus upon evaluation of sexual performance in rams.

Experiment 1 was conducted under unnatural conditions (i.e., restrained ewes in crates; human intervention with AV) that were required for training rams to an AV for semen collection. We surmise that the sexual behavior of L rams may have been affected to a greater degree than that of H line rams in Exp. 1 (i.e., presence of humans may have been more inhibiting to rams with lower libido). In Exp. 2, one of six L line rams and one of four H line rams began displaying normal sexual behavior in the overnight test, which were conducted

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Table 2. Incidence of sexual behaviors of Rambouillet rams from ewe lines selected for high (H) and low (L) reproductive rate after the first 5 and all 18 serving capacity tests (SCT) in Exp. 2a

<table>
<thead>
<tr>
<th>Line</th>
<th>Mounting, %</th>
<th>Ejaculating, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First 5 SCTb</td>
<td>All 18 SCTc</td>
</tr>
<tr>
<td>H</td>
<td>25</td>
<td>88</td>
</tr>
<tr>
<td>L</td>
<td>23</td>
<td>74</td>
</tr>
</tbody>
</table>

aRams were offspring from lines of Rambouillet ewes that had been divergently selected for number of lambs born for 28 years. Each ram was given a total of 18 serving capacity tests.

bChi-square = 1.56, df = 1; P > 0.2.

bChi-square = 1.78, df = 1; P > 0.2.

bChi-square = 0.70, df = 1; P > 0.2.

bChi-square = 1.86, df = 1; P > 0.2.

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Figure 1. Least squares means for number of mounts and ejaculations (EJACS) during 18 serving capacity tests (SCT) for all rams (25 high [H] and 23 low [L] line) evaluated in Exp. 2. Rams were offspring from lines of Rambouillet ewes that had been divergently selected for number of lambs born for 28 yr. Mixed-model analyses for repeated measures indicated individual SCT differences (P < 0.01) but not between lines (P > 0.33) or for the line by SCT interaction for mounts or EJACS (P > 0.10). The approximate SEM for mounts for line by time ranged from 1.4 to 2.4. The SEM for ejaculations for line by time was 0.3.
in the absence of humans and other rams and in the presence of a limited number of ewes. Other researchers have reported that sexually inhibited rams began breeding ewes after a period of time if left in breeding pens with marking harnesses and where human activity was either absent or minimized (Hulet et al., 1964, Mattner et al., 1973). Hulet et al. (1964) reported that 35% of 23 inhibited rams began breeding within 24 h, and 70% started mating within 48 h, after they were placed into breeding pens with marking harnesses. Therefore, human presence and associated activities may be important in determining the level of sexual performance in some rams. Additional research would need to be specifically designed to determine if this type of activity differentially influences rams from the high and low selection lines as we suggested in Exp. 1.

Another possible explanation for the differences in sexual performance between Exp. 1 and 2 may be related to the number of serving capacity tests given in each experiment. In Exp. 1, five tests were performed, while in Exp. 2, 18 tests were conducted; one would expect that 18 tests may be more accurate and precise than five tests and dismiss the results of Exp. 1 as limited in these reliability parameters. However, there was no difference in the percentage of rams mounting and ejaculating after the first five tests in Exp. 2. Results from Exp. 2 indicated that in order to achieve a high reliability (95%) for determining an accurate sexual performance score requires a minimum of six serving capacity tests of 30-min duration each at a rate of three tests per week. Recently, we reported a reliability of 77% with a battery of screening serving capacity tests of not more than three tests (Snowder et al., 2002b). Therefore, sexual performance scores obtained after five tests when training to service the AV in Exp. 1 may not have been as accurate or reliable as those from Exp. 2.

Experiment 2 was conducted to more closely simulate natural breeding conditions (i.e., unrestrained ewes and little to no human intervention). Under these conditions there was no difference in male sexual behavior between the lines. Rams that did not show any sexual activity in the serving capacity tests only responded in the overnight test at a low rate (2 out of 10 rams). Again, the identification of these additional sexually active female-oriented rams was attributed to the more natural conditions of the overnight test without any interference. Only one male-oriented ram was identified in either line, indicating that sexual preference or orientation did not appear to be altered by divergently selecting ewe lines for lambs born. The low incidence of male-oriented rams supports earlier reports of Katz et al. (1988) and Perkins and Fitzgerald (1992) in which two out of 24 and eight out of 94 rams were male oriented, respectively. We believe that the lack of differences in male sexual behavior between rams of these selection lines in Exp. 2 was related to the large percentage of rams mounting and ejaculating. Burfening and Rossi (1992) found no difference in serving capacity scores between these lines of rams when they evaluated only rams that had exhibited mounting and ejaculatory behavior during their testing periods. Also, the increased sexual activity by both the H and L line rams in Exp. 2 was probably stimulated by access and availability to unrestrained ewes in estrus (Zenchak et al., 1988). Differences between sexual performance evaluations of high and low line rams from these selection lines have been reported previously by Tulley and Burfening (1983). However, serving capacity tests in their study encompassed both the anestrous and breeding seasons, and it is possible that one line may have been influenced more than the other line by seasonal effects.

The present study indicates that long-term selection of Rambouillet ewes for number of lambs born has had little impact on sexual behavior or sexual orientation of their male offspring. Based upon these results and the fact that a single ram can breed many ewes, it is speculated that selection criteria involving sexual performance of rams may be more successful for increasing sexual performance of their male offspring, and this type of selection may alter ewe reproductive characteristics. Bench et al. (2001) found that direct selection for high sexual performance in rams improved sexual performance of male offspring after one generation. Also, Snowder et al. (2002a) reported small or nil estimates of genetic correlations of ram sexual performance score with number of lambs born or weaned and suggested that little or no correlated genetic improvement of lambs born or weaned will occur. Thus, direct selection for ram sexual performance appears to be less efficient than direct selection on the ewe for improving female traits, whereas combining direct selection for female sexual performance and for female traits may provide the more efficient method of improving overall reproductive efficiency in sheep.

In conclusion, selection for reproductive rate (lambs born) in Rambouillet ewes does not affect the sexual behavior or sexual orientation of male offspring. However, we recognize that test conditions under which serving capacity testing is conducted, such as restrained and unrestrained ewes and number of serving capacity tests, may affect the accuracy and reliability of sexual performance scores.

**Implications**

This study implies that test conditions for monitoring sexual behavior of rams need to be considered carefully to make sound decisions on the sexual performance of rams. Also, at least six serving capacity tests of 30-min duration, and not more frequently than at a rate of three tests per week, are required to achieve 95% reliability in sexual performance scores. This study also indicates that selecting ewes for lambs born is unlikely to have an impact on sexual behavior or orientation of male offspring.
Ram mating behavior after ewe selection


