The influence of breeding intensity on above- and below-average sexual performance rams in single- and multiple-sire breeding environments

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Abstract

Two studies were conducted to evaluate the relationship between serving capacity scores and breeding performance of rams. The first study was conducted to determine whether rams with above or below mean serving capacity scores could perform equally in greater and lesser breeding intensity, single-sire mating schemes. The second study was conducted to determine whether rams with above and below mean serving capacity scores could perform equally well when only one or two ewes were in estrus daily in a multiple-sire breeding scheme (two rams/pen). Rams (n = 68) were ranked according to average number of ejaculations recorded in serving capacity tests. Sixteen rams with the greatest scores (above-average) and 16 rams with least scores (below-average) were identified for breeding. Half of above-average and half of below-average rams were used in the two studies. For study 1, each ram was individually introduced to 23 estrus-synchronized ewes for 9 d to simulate high breeding intensity. Rams were given a 5-d rest before they were individually introduced to 23–24 naturally cyclic ewes for 17 d (low breeding intensity). For study 2, 16 rams were paired across ram types, and each pair competed for 20 ewes for 18 d (8 pens). For study 1, ewe fertility (ewes lambing/ewes present at lambing) and number of lambs born were greater (P<0.001) for above-average (0.67 ± 0.03 and 27.6 ± 1.2, respectively) than for below-average rams (0.39 ± 0.07 and 15.3 ± 2.7) with greater breeding intensity. Ewe fertility and lambs born did not differ for above-average (0.91 ± 0.03 and 37.8 ± 1.9, respectively) and below-average rams (0.86 ± 0.03 and 39.0 ± 1.9) with less breeding intensity. For study 2, number of ewes lambing (99 ± 8.0 compared with 72 ± 13.6; P = 0.12) and number of lambs sired (149 ± 18.5 compared with 101 ± 22.8; P = 0.14) did not differ between above- and below-average rams, respectively, in direct competition. Sexual classifications based on serving capacity

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tests are related to breeding performance of rams in certain breeding environments. When breeding intensity is greater, above-average rams impregnate more ewes and sire more lambs than below-average rams. When only a small number of ewes are in estrus daily, below-average rams for serving capacity scores perform as well as above-average rams in multiple-sire and single-sire breeding environments. We suggest that above-average rams should be used to reduce number of rams required when breeding intensity is greater.

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1. Introduction

Reproductive performance of rams is highly variable (Terrill, 1937; Price, 1987) and can impact flock productivity. One way to improve flock productivity is to conduct serving capacity tests (Kilgour and Whale, 1980) and eliminate the rams with poor sexual performance. However, the relationship of serving capacity test performance to breeding performance has been controversial. Greater breeding performance rams increased ewe fertility in some studies (Mattner et al., 1971; Perkins et al., 1992; Kilgour, 1993), but others found little relationship between ram test breeding performance and ewe fertility (Kelly et al., 1975; Kilgour and Wilkins, 1980). We hypothesize that these differences may be due, in part, to breeding intensity in each study.

Continuous exposure of ram lambs, when they were 4.5–9.5 months of age, to estrual ewes reduced the incidence of poor performance in serving capacity tests (Katz et al., 1988), as did repeated, intermittent exposure to estrual ewes (Price et al., 1994). A continuous 17-d exposure of ram lambs to estrual ewes improved ram performance in serving capacity tests (Stellflug and Lewis, 2007).

In the present research, two studies were conducted to evaluate the relationship between serving capacity scores and breeding performance of rams. The objective of the first study was to determine whether rams ranked above or below mean serving capacity scores could perform equally well in greater and lesser breeding intensity, single-sire mating schemes. The objective of the second study was to determine whether rams ranked above and below mean serving capacity scores could perform equally well when only one or two ewes were in estrus daily in a competitive multiple-sire breeding scheme with two rams in each pen.

2. Materials and methods

2.1. General

Before breeding, all rams and ewes were maintained on native rangelands at the U.S. Sheep Experiment Station near Dubois, ID. In confinement, rams and ewes were fed daily to meet their nutrient requirements (NRC, 1985). Protocols were approved by the Agricultural Research Service institutional animal care and use committee.

2.2. Ram preparation and classification

Half of 68, 7–8-month-old ram lambs received a constant early exposure to ewes for 17 d and a constant later exposure to estrual ewes for 3 d when they were 16–19 months of age before the serving capacity tests. The other half of the rams had no early or late exposure to ewes before
the tests. After the early exposure, all rams were maintained as a single group isolated from any contact with ewes until they were 16–19 months of age.

Independent of the breeding studies, a study to evaluate multiple sire serving capacity tests (cohort tests) was conducted, with rams from each of the two exposure treatments being randomly assigned to a single cohort test given either before or after a series of nine individual serving capacity tests (sequence of tests). All of the 30-min serving capacity tests were conducted over a 2-month period. For a cohort test, three rams were penned with nine unrestrained, estrual ewes, and ram breeding performance data were recorded. For the individual tests, individual rams were penned with three unrestrained estrual ewes and breeding data were recorded. Chronically ovariectomized ewes were estrus-synchronized using pessaries containing 60 mg of 6α-methyl-17α-hydroxyprogesterone acetate (Veramix, Pfizer, Orangeville, ON). 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-17β 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 1-week 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; tests were postponed until a sufficient number of ewes (n = 24) were in estrus. 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 ewes. The rams were considered sexually active when at least one ejaculation was observed in one of the nine individual tests.

An electroejaculator (Ideal Instruments Inc., Chicago, IL) was used to collect semen 11–12 d before onset of breeding. Sperm was evaluated for morphology and motility with a phase contrast microscope (Nikon Instruments Company Inc., Melville, NY). Normal and motile sperm were estimated to the nearest 10%, followed with a forward progressive movement score (1–4; 1 < 25%, 2 = 25–50%, 3 > 50–75%, 4 > 75% motility) using a modification of Terrill’s (1937) system, which was based on a 1–6 motility rating scheme. Metabolic activity of spermatozoa was evaluated with a resazurin reduction assay (Wang et al., 1998) using a Spectramax Plus 384 spectrophotometer (Molecular Devices Corporation, Sunnyvale, CA) to measure the relative absorbance at 600 nm wavelength.

The 68 rams were ranked according to average number of ejaculations across the nine individual serving capacity tests within each of the four prebreeding treatment combinations (exposure to estrual ewes with cohort before or after individual tests or no exposure to ewes with cohort before or after individual tests). Thirty-two rams, representing four rams from each treatment combination with the greatest scores (above-average test scores) and four sexually active rams with the least scores (below-average test scores) and satisfactory semen, were identified for the breeding studies. Above- and below-average rams were divided across the two breeding studies maintaining the four prebreeding treatment combinations.

2.3. Study 1: breeding management

Rams with individual sexual performance scores above (n = 8) or below (n = 8) the average serving capacity test score of the group were used in a single-sire breeding scheme. Rams were individually introduced to 23 estrus-synchronized ewes on November 17 for 9 d to simulate a high intensity breeding environment. Estrus was synchronized with Veramix pessaries for 12 d and ewes were placed in breeding 24 h after pessary removal. It was anticipated that each ram was exposed to 8–12 ewes in estrus daily for a 2- to 3-d period. After the greater intensity mating, rams were given a 5-d rest period before they were individually introduced to 23–24 ewes that
were expressing estrus at typical intervals for 17 d to provide a minimal mating demand (low intensity breeding environment) with only one to two ewes expected in estrus daily. Rambouillet ewes (739 total; stratified for age and prolificacy) were assigned to 32 breeding pens. Fertility and prolificacy data were based on the number of ewes present at lambing because 33 ewes died from predation primarily by stray dogs or unknown causes before lambing time.

2.4. Study 2: breeding management

Sixteen rams (different from rams in study 1) with their individual sexual performance scores above \((n = 8)\) the group mean serving capacity test score or with their individual sexual performance scores below \((n = 8)\) the group mean serving capacity test score were used in a multiple-sire breeding arrangement. The 16 rams were paired across ram types, and each pair was placed with 20 ewes on November 17 for 18 d \(8\) pens; 2 rams/pen). Rams with known similar genetic relationships were assigned to different pens. The 160 Rambouillet ewes were stratified for age and parity. Results were based on the number of ewes present at lambing because 16 ewes died from predation primarily by stray dogs or unknown causes before lambing.

2.5. Blood collection and paternal typing

To determine parentage of lambs in study 2, blood from the 16 rams and 282 white-faced lambs and their dams was collected with 10-mL Vacutainers containing EDTA (Becton Dickinson, Franklin Lakes, NJ) and stored at \(4\) °C. Genomic DNA was extracted from blood within 7–10 d after blood collection using a standard salt-precipitation method \((Miller et al., 1988)\). Five microsatellite markers were used in parentage testing of lambs: \(McM111\) \((Smith et al., 1995a)\) for pens 1, 3, 4, and 7; \(BMS460\) \((Stone et al., 1995)\) for pen 2; \(McM63\) \((Smith et al., 1995b)\) for pen 5; \(McM137\) \((Smith et al., 1995b)\) for pens 6 and 8; \(OarFCB304\) \((Buchanan and Crawford, 1993)\) for pen 8. Microsatellite genotyping was performed as described by Cockett et al. \((1996)\). Sire of each lamb was determined as the ram that could exclusively provide the remaining microsatellite allele after accounting for the dam’s microsatellite allele. For most lambs, only one microsatellite was required to determine the sire, whereas two microsatellites were required for differentiation for the two sires in pen 8. If a sire could not be unequivocally assigned after examination of genotypes for up to two microsatellites, sire was designated as ambiguous for that lamb.

2.6. Statistical analyses

The prebreeding exposure and sequence of serving capacity tests were maintained in the statistical models as part of the experimental design, but the sequence of serving capacity tests was not included in the interpretation because all of the rams had received equal exposure time with ewes during the serving capacity tests before the breeding studies. The Glimmix procedures of SAS \((Ver. 9.1.3, SAS Institute Inc., 2004, Cary, NC)\) with the Poisson link were used to analyze the motility, progressive forward movement, and resazurin score as semen characteristics estimated before breeding for rams used in breeding for studies 1 and 2. For study 1, the model consisted of amount of exposure (exposure or no exposure), sequence of tests (cohort test before or after individual serving capacity tests), amount of ram type (above or below average), and interactions, with the residual as the random effect. For study 2, the model was the same as for study 1, except that breeding pen within exposure and sequence of tests was included as an additional random effect. Degrees of freedom were calculated using the Kenward–Roger procedure \((Kenward and Roger, 1997)\).
In study 1, mixed model procedures of SAS for a split plot were used to analyze fertility (ewes lambing/ewes present at lambing), total number of lambs born, and prolificacy (lambs/ewes present at lambing). Rams were the experimental unit. The main plot consisted of amounts of exposure, sequence of tests, ram type, and interactions. The subplot consisted of breeding intensity (high or low) and interactions. The fixed effects and interactions in the main plot were tested with the random effect of exposure, sequence of tests, and ram type nested within ram as error term. The subplot was tested with the residual error term. Fertility, number of lambs born, and prolificacy data showed homogeneous variance using Bartlett’s Box $F$-test. The slice test option of mixed model procedures of SAS was used for mean separation for interactions.

In study 2, mixed model procedures of SAS were used to analyze the number of ewes lambing and number of lambs sired with rams as the experimental unit. Fixed effects included amounts of exposure, sequence of tests, ram type, and interactions, with breeding pen within exposure and sequence of tests as a random effect. Bartlett’s Box $F$-test was used to test for homogeneity of variance. Variances for ewes lambing and lambs sired were homogeneous. The slice test option was used for mean separation for interactions.

3. Results

3.1. Study 1

Incidence of sperm abnormalities before breeding was less than 10% for heads, midpieces, and tails for above- and below-average rams that were used in breeding. The motility (62.0 ± 7.3% versus 60.8 ± 7.3%) and progressive forward movement scores (3.4 ± 0.3 versus 3.2 ± 0.3) before breeding did not differ between the above- and below-average rams, respectively. The relative absorbance at 600 nm wavelength with a resazurin reduction assay did not differ between the above- and below-average rams (0.65 ± 0.13 versus 0.69 ± 0.13, respectively).

The serving capacity test scores for the above-average and below-average rams were 3.5 ± 0.3 and 1.9 ± 0.3 ($\bar{X} \pm \text{S.E.}$) ejaculations, respectively. Rams with a serving capacity score above average had increased ($P < 0.001$) fertility (0.67 ± 0.03) compared with the below-average rams (0.39 ± 0.07) when the rams were exposed to a greater number of ewes to breed daily. However, differences for the above-average compared with below-average rams were not detected ($P = 0.45$) when rams had less breeding pressure with ewes that were expressing estrus at typical intervals (0.91 ± 0.03 compared with 0.86 ± 0.03, respectively).

The above-average rams increased ($P < 0.001$) the number of lambs born (27.6 ± 1.2) from approximately 22 ewes in each of eight breeding pens compared with below-average rams (15.3 ± 2.7) when the rams were required to breed a high number of ewes daily. Differences were not detected ($P = 0.55$) when rams had less breeding pressure (23 estrous cycling ewes/breeding pen in a 17-d period) for the above-average compared with below-average rams (37.8 ± 1.9 versus 39.0 ± 1.9, respectively). The exposure of rams to estrual ewes before the serving capacity tests increased ($P < 0.03$) the number of lambs born (32.1 ± 3.1) compared with rams that were not exposed to estrual ewes before the serving capacity tests (27.8 ± 2.4). There were no significant interactions with the prebreeding exposure treatments.

Prolificacy was greater ($P < 0.002$) for ewes bred in the lesser intensity breeding environment compared with ewes bred in the greater intensity breeding environment with exposure rams that had below-average serving capacity test scores (Table 1), as indicated with the exposure $\times$ sequence of tests $\times$ breeding intensity interaction ($P < 0.02$). Prolificacy did not differ for
Table 1
Means ± S.E.M. for prolificacy (lambs/ewes present at lambing) of ewes exposed to above-average or below-average serving capacity score rams in a greater or lesser breeding intensity single-sire mating environment.

<table>
<thead>
<tr>
<th>Treatment and ram type</th>
<th>High breeding intensity</th>
<th>Low breeding intensity</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EX, above-average rams (n = 4)</td>
<td>1.93 ± 0.05</td>
<td>1.91 ± 0.06</td>
<td>0.77</td>
</tr>
<tr>
<td>EX, below-average rams (n = 4)</td>
<td>1.74 ± 0.09</td>
<td>2.10 ± 0.04</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>NEX, above-average rams (n = 4)</td>
<td>1.80 ± 0.12</td>
<td>1.86 ± 0.04</td>
<td>0.46</td>
</tr>
<tr>
<td>NEX, below-average rams (n = 4)</td>
<td>1.94 ± 0.14</td>
<td>1.95 ± 0.02</td>
<td>0.93</td>
</tr>
</tbody>
</table>

The greater breeding intensity environment exposed each ram to 8–12 ewes in estrus daily, whereas the lesser breeding intensity environment exposed each ram to one or two ewes in estrus daily.

Rams were exposed to ewes for 17 d at 7–8 months of age and later exposed to estrual ewes (EX) for 3 d at 16–19 months of age just before serving capacity tests or not exposed to ewes (NEX) before serving capacity tests. Ram type was based on rams ranking the greatest (above average) or least (below average) from the average number of ejaculations across the nine, 30-min individual serving capacity tests within each of the four prebreeding treatment combinations (EX and cohort test before individual serving capacity tests; EX and cohort test after individual tests; NEX and cohort test before individual tests; NEX and cohort test after individual tests).

3.2. Study 2

Incidence of sperm abnormalities before breeding was less than 10% for heads, midpieces, and tails for both above- and below-average performance rams used in breeding. The motility (65.0 ± 3.3% versus 65.0 ± 4.2%) and progressive forward movement score (3.5 ± 0.2 versus 3.5 ± 0.2) before breeding did not differ between the above- and below-average rams, respectively. The relative absorbance at 600 nm wavelength with a resazurin reduction assay did not differ between the above- and below-average rams (0.80 ± 0.08 versus 0.81 ± 0.09, respectively).

Of the 160 ewes used in study 2, 144 lambed and 16 died before lambing. Data for ewes (n = 13) that had no identified lambs were deleted from the data set. Data for ewes that had partially identified litters were included in the data set with their lambs that had identifiable sires. For ewes that had all of their lambs with identifiable sires (n = 117), 25 ewes had single lambs, 58 ewes had multiple lambs sired by 1 ram, and 34 ewes had lambs sired by more than 1 ram. The total count for ewes lambing was 171 because the same ewe was included twice if she had identifiable lambs from two sires. A total of 250 lambs were assigned unambiguously to 1 of the 2 rams in their dam’s breeding pen and 32 lambs were unidentified (Table 2). Unidentified lambs were excluded from the data set. Their sires could not be identified because of (a) missing samples (either lamb, or dam, or both; n = 2), (b) failure to extract DNA from the blood sample (n = 1), (c) dam or ram misparentage (n = 4), or (d) failure to unambiguously distinguish between rams within the breeding pen (n = 26).

Test scores for the above- and below-average rams were 3.6 ± 0.4 and 2.1 ± 0.4 ejaculations, respectively. There were no differences in the current multiple-sire study for number of ewes lambing (99 ± 8.0 versus 72 ± 13.6; P = 0.12) or number of lambs sired (149 ± 18.5 versus 101 ± 22.8; P = 0.14) between the above- and below-average rams, respectively. In addition, there were no differences for number of ewes lambing (96 ± 12 versus 75 ± 11.2; P = 0.20) or number of lambs sired (138 ± 23.2 versus 112 ± 20.9; P = 0.37) regardless of whether rams had exposure or no exposure to estrual ewes as ram lambs and again later before the serving capacity tests, respectively.
Table 2
Number of lambs sired by above-average or below-average serving capacity rams and unidentified lambs in each of eight multiple-sire breeding pens with 20 ewes for 18 d when only one or two ewes were in estrus daily

<table>
<thead>
<tr>
<th>Breeding pen</th>
<th>Ram type</th>
<th>Unidentified</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Above-average rams</td>
<td>Below-average rams</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9</td>
<td>28</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>23</td>
<td>9</td>
<td>5</td>
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<tr>
<td>3</td>
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<td>8</td>
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<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>149</td>
<td>101</td>
<td>32</td>
</tr>
</tbody>
</table>

4. Discussion

The breeding performance (fertility measured as ewes lambing/ewes present at lambing and number of lambs born) for rams with above-average serving capacity test scores was greater in a greater intensity breeding environment when 8–12 ewes were in estrus daily. Ewes exposed to rams with above-average test scores had approximately twice the fertility and gave birth to approximately twice as many lambs as did ewes exposed to rams with below-average serving capacity test scores in a greater intensity breeding environment. The single-sire study results with greater intensity breeding are similar to a previous study with multiple-sire breeding. In the previous study with direct competition for 10–12 ewes in estrus daily, greater performance rams impregnated approximately twice as many ewes and sired twice as many lambs as either lesser performance female-oriented or male-oriented rams (Stellflug et al., 2006). Taken together, these studies indicate that rams with above-average test scores will improve reproductive efficiency, especially when breeding intensity is greater.

In single-sire pens when breeding intensity was low (study 1), above- and below-average rams did not differ in their ability to impregnate ewes and sire lambs. This is similar to the results from the multiple-sire study 2 in which above- and below-average rams competing for only 1 or 2 ewes in estrus daily were equal in reproductive performance. The single-sire and multiple-sire studies indicate that above- and below-average rams perform equally well with or without competition in breeding when breeding requirements are less.

Reproductive performance of rams with different breeding intensities may explain the differences among reports on the relationship between sexual performance of the ram and ewe fertility (Kelly et al., 1975; Mattner et al., 1971; Kilgour and Wilkins, 1980; Mickelsen et al., 1982; Perkins et al., 1992; Kilgour, 1993). Another factor that may explain some of the conflicting reports in the literature is the degree of difference between the serving capacity test scores of above- and below-average performance rams. However, magnitude of difference between test scores for above- and below-average rams is thought to be of less importance than breeding intensity. Breeding intensity is thought to be important because similar increases in ewes lambing and number of lambs born were observed in multiple-sire (Stellflug et al., 2006) and in the current single-sire breeding environment when breeding intensity was high. However, serving capacity scores of above- and below-average rams were not as great for the current single-sire study (3.5 ± 0.3 ver-
sus 1.9 ± 0.3, respectively) as for the previous multiple-sire study (3.2 ± 0.2 versus 0.4 ± 0.2, respectively; Stellflug et al., 2006).

Number of lambs born was greater for the rams that were exposed to estrual ewes as ram lambs at 7–8 months of age and later at 16–19 months of age before the serving capacity tests than for the rams that were not exposed to estrual ewes before the tests. This supports results indicating that constant exposure of 7–8-month-old ram lambs to ewes for 17 days dramatically reduced the incidence of sexually inhibited rams (Stellflug and Lewis, 2007) and indicates an improvement in lamb production beyond improving sexual behavior in serving capacity tests. Based on the previous study (Stellflug and Lewis, 2007), the early exposure as ram lambs in the current study is thought to be more beneficial than the later exposure. The importance of exposure of ram lambs to improve reproductive behavior in serving capacity tests is supported by Katz et al. (1988) and Price et al. (1994) in which rams were either raised with ewes from 4.5 to 9 months of age or after 30-min exposure to estrual ewes at weekly intervals for 4 weeks at 8–9 months of age, respectively.

Differences in prolificacy between the greater and lesser intensity breeding environment were within the ram type (above- or below-average serving capacity test scores) rather than between ram types, as it was for fertility and number of lambs born. Prolificacy was less in the greater intensity breeding environment only for below-average rams that had been exposed to ewes before serving capacity tests, compared with such rams in the low intensity breeding environment. Thus, if reproductive performance was reduced, it occurred with the below-average rams in the greater intensity breeding environment.

5. Conclusions

Sexual classifications based on serving capacity tests are related to the breeding performance of rams in certain breeding environments. When breeding intensity is greater, rams ranked above the average serving capacity test scores impregnate more ewes and sire more lambs than rams ranked below the average test scores. When only a small number of ewes are in estrus daily, below-average rams perform as well as above-average rams in multiple-sire breeding and single-sire breeding environments. We suggest that, when breeding intensity is expected to be great, serving capacity tests should be used to select above-average serving capacity rams so that the number of rams required to impregnate a large number of ewes during a brief breeding period can be reduced.

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