Evaluation of three-ram cohort serving capacity tests as a substitute for individual serving capacity tests\textsuperscript{1,2}

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ABSTRACT: Alternatives to time-consuming, laborious individual serving capacity tests (ISCT) are needed to classify ram sexual behavior. The objective of study 1 was to evaluate the relationship between the first 3-ram cohort test (COSCT) scores and the mean of 5 ISCT scores. The objective of study 2 was to determine whether 1 or 2 additional COSCT improved the ability to predict ISCT scores. For study 1, rams (n = 69) were assigned to either a COSCT given before or after 9 ISCT. For study 2, rams (n = 127) were given 3 COSCT before or after 6 ISCT. For repeated COSCT, rams were initially grouped at random and subsequently rerandomized so that each ram was grouped with at least 1 different ram for each test. For both studies, the number of ejaculations from COSCT was compared with the mean number of ejaculations across the second through sixth ISCT. A threshold between high- and low-performing rams was defined in each analysis as the mean ISCT scores of sexually active rams. Rams with a mean number of ejaculations in ISCT greater than the threshold were classified as having high sexual activity, whereas rams below the threshold were classified as having low sexual activity. Rams with no ejaculations in ISCT were classified as sexually inactive. Data from studies 1 and 2 were used to evaluate the relationship between the first COSCT and the mean of 5 ISCT scores. Data from multiple COSCT were fit to various models to determine whether the ability to predict ISCT scores was improved with 1 or 2 additional COSCT. The best model for ISCT and COSCT was a piecewise linear regression model. The first COSCT correctly identified all sexually inactive rams in both studies. The first COSCT, however, also classified 56\% of low sexually active rams and 18\% of high sexually active rams as inactive. Rams had a 71\% probability of high sexual activity in ISCT if they were classified as sexually active in the first COSCT. We conclude that a single COSCT is a reliable, albeit more conservative, and efficient alternative to a series of ISCT for characterizing sexual activity of rams. Multiple COSCT can provide some protection against culling rams with high sexual activity (i.e., approximately 50\% less with 2 additional COSCT) and still retain most of the efficiency compared with ISCT. It is important to use high-performance rams for breeding because they will approximately double the number of ewes bred and lambs sired compared with low-performance rams if a large number of ewes need to be serviced daily.

Key words: ram, sexual behavior, serving capacity test

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INTRODUCTION

Reproductive performance of rams is greatly variable (Terrill, 1937; Price, 1987), and high-performance rams identified with individual-ram serving capacity tests (ISCT) can improve flock fertility when breeding intensity is great (Perkins et al., 1992; Stellflug et al., 2006). A series of serving capacity tests is usually required for a reliable estimate of ram reproductive performance because of the multitude of factors that can influence ISCT, such as unfamiliarity with test pens and estrual ewes (Zenchak and Anderson, 1980; Price et al., 1991, 1996). Six 30-min individual-ram performance tests were required to obtain 95\% reliability for serving capacity test scores (Stellflug and Berardinelli, 2002). A quick and reliable alternative is needed to estimate ram reproductive performance because ISCT, as developed by Kilgour and Whale (1980), are time-consuming and laborious.

An alternative may be a multiple-ram (cohort) serving capacity test (COSCT), which is similar (i.e., identical observation data and ewe:ram ratio) to the ISCT.
Multiple males compete for estrual ewes and can be observed simultaneously in the COSCT, resulting in a more efficient use of time. Separation anxiety should be minimal because rams remain with other cohorts, and competition among rams may help separate high sexually performing rams from low sexually performing rams. However, it is unknown whether the cohort test provides a reliable evaluation of a ram’s sexual behavior.

Our primary objective was to evaluate the relationship between single COSCT scores and the mean of 5 ISCT scores (i.e., how well a single COSCT score would predict the ISCT scores). The second objective was to determine whether 1 or 2 additional cohort tests would improve our ability to predict ISCT scores.

**MATERIALS AND METHODS**

The ARS Institutional Animal Care and Use Committee approved the protocols.

**General**

Rams were weaned at approximately 120 d of age and maintained as an all-male group at times in confinement and at times on native rangelands at the US Sheep Experiment Station near Dubois, Idaho. Animals were fed daily to meet their nutrient requirements (NRC, 1985) in confinement.

**Experimental Protocol**

For study 1, 16- to 17-mo-old Rambouillet rams (n = 69) were tested for sexual performance in 30-min ISCT and 30-min COSCT. The number of ejaculations was recorded as the test score during all serving capacity tests. A total of 34 of the rams were previously exposed to estrual ewes for 17 d at 7 to 8 mo of age and again for 3 d immediately before onset of the ram tests at 16 to 17 mo of age, whereas the other 35 rams were not exposed to ewes before the tests. Seventeen rams from each previous exposure group were evaluated in a single COSCT before a series of 9 ISCT. The remaining rams (n = 35) were evaluated in a single COSCT after 9 ISCT to allow evaluation of the order of COSCT and ISCT (test order). The COSCT was conducted in a 7.2- × 4.0-m pen with 9 unrestrained, estrual ewes and 3 rams to maintain a 3:1 ewe:ram ratio, with the exception of 1 COSCT with 2 rams and 6 ewes because 1 ram with fly strike was killed. The series of 9 ISCT was conducted in 2.4- × 2.4-m pens in which individual rams were observed with 3 unrestrained, estrual ewes, also a 3:1 ewe:ram ratio. Rams were subjected to 1 ISCT per day for 3 consecutive days, for a total of 3 test periods with an approximate 12-d interval between test periods. The COSCT was conducted either 1 d before or 1 d after the series of ISCT. All serving capacity tests were conducted in October. Ewes used for serving capacity tests were ovariectomized and estrus was induced by using pessaries containing 60 mg of 6α-methyl-17α-hydroxyprogesterone acetate (Veramix, Pfizer, Orangeville, Ontario) and injection of 50 μg of estradiol-17β (Stellflug and Berardinelli, 2002). Ewes were considered to be in estrus when they would stand to be mounted.

In study 2, 16- to 17-mo-old purebred Columbia, Rambouillet, Targhee, and Polypay rams (n = 127; 27 to 37 rams per breed), which were not previously exposed to estrual ewes, were tested in ISCT and COSCT as described for study 1. Seventy rams were given 6 ISCT followed by 3 COSCT, and 57 rams were given 3 COSCT followed by 6 ISCT. Pens and conditions were the same as for study 1. Rams were subjected to 1 ISCT per day for 3 consecutive days for a total of 2 test periods, with an approximate 6-d interval between test periods. Rams were subjected to 1 COSCT per day for 3 consecutive days beginning either 12 d before or after the series of ISCT. Cohort rams were grouped at random for the first COSCT and were rerandomized for subsequent COSCT to ensure that at least 1 ram in the cohort group was different. Two of the COSCT were conducted with 2 rams and 6 ewes. All serving capacity tests were conducted over a 37-d period beginning in late August.

The ISCT score used in the analyses was the mean of ISCT 2 through 6 to provide similar data sets for both studies. Data from the first ISCT were excluded from the analyses because the unfamiliarity of rams with ewes and the testing environment made them less reliable than data from subsequent tests (Price et al., 1991). Rams that had a mean number of ejaculations from the 5 ISCT >0 were classified as sexually active. The mean number of ejaculations from sexually active rams in the ISCT was considered the threshold between high and low sexual activity levels (Stellflug et al., 2008). Rams with a mean number of ejaculations in ISCT greater than the threshold were classified as having high sexual activity, whereas sexually active rams with a mean number of ejaculations in ISCT below the threshold were classified as having low sexual activity. Rams with no ejaculations in ISCT were classified as sexually inactive.

**Statistical Analyses**

Our objective was to determine the ability of COSCT scores to predict ISCT scores. These tests are a measure of ram behavior, and previous experiences or the lack thereof may affect subsequent tests (Price et al., 1991, 1996). Because the ISCT is the mean of multiple tests, with the first test score considered as “training” and thus discarded, the ISCT score is relatively resistant to previous testing experiences. A single COSCT score is presumably less resistant to previous testing experiences because no scores are discarded and there is no averaging effect. Thus, it was hypothesized that the relationship between the test scores would differ depending on the order in which the rams were subjected.
to the tests, and initial plots of the data supported this. The relationship between ISCT and COSCT scores was also modeled separately for each test order because rams in routine practice would not be exposed to a battery of both tests. All analyses were conducted with the R statistical software package (R Development Core Team, 2006). Significance was accepted at $P < 0.05$.

Inferences about the effects of breed of ram or previous exposure were not objectives of this study, and in preliminary linear models that used data from each study separately, neither of these effects interacted with COSCT scores (data not shown), so these effects were not included in subsequent models. In preliminary linear models that included data from both studies, the effect of study did not interact with COSCT scores when the COSCT was given first, but the interaction was significant when the ISCT was given first. However, it was thought justified to combine data, within test order, from both studies into a single analysis and to partition the effects of study to the error term because 1) the effects of study cannot be exactly replicated in the future; and 2) partitioning the effects of study to the error term yielded more conservative estimates of prediction intervals.

The ability of COSCT scores to predict ISCT scores was modeled in 4 scenarios: 1) using only the first COSCT score, when the COSCT was given first (n = 91); 2) using the mean of multiple COSCT scores, when the COSCT was given first (n = 57); 3) using only the first COSCT score, when the ISCT was given first (n = 104); and 4) using the mean of multiple COSCT scores, when the ISCT was given first (n = 70). The ISCT scores were modeled as linear and quadratic functions of COSCT scores in each scenario, and piecewise linear regression (i.e., broken stick) models were tested at all COSCT breakpoints. The model within each scenario that yielded the smallest Akaike’s information criterion was considered best. The COSCT scores used as independent variables for scenarios 2 and 4 included the first COSCT score, the second COSCT score, the mean of the first 2 COSCT scores, the mean of the last 2 COSCT scores, or the mean of all 3 COSCT scores.

The best model for every scenario was a piecewise regression with a breakpoint between 0 and >0 ejaculations, and the distribution of residuals for each piece was tested for normality by using a Shapiro-Wilk test ($\alpha = 0.05$). The assumption of normality was accepted for each scenario at a COSCT score >0, and a 95% prediction interval was calculated for each level of COSCT score. The prediction interval was used to estimate the probabilities that future COSCT scores would classify rams, according to the ISCT, as having either high sexual activity or low sexual activity, or as being sexually inactive. The assumption of normality was rejected for each scenario at a COSCT score = 0; thus, the probabilities associated with sexual classification were calculated from an empirical distribution function.

RESULTS

First COSCT Given Before a Series of ISCT

Rams (n = 91) with a COSCT score >0 had a high probability (0.71; n = 31) of high sexual activity, a low probability (0.29; n = 15) of low sexual activity, and no probability of sexual inactivity as classified with ISCT. Rams with a COSCT score = 0 from the same group of 91 rams had a low probability (0.16; n = 7) of high sexual activity and approximately an equal probability of low sexual activity (0.38; n = 17) or of being sexually inactive (0.47; n = 21). These probabilities (given only in the text) are based on an ISCT high-low threshold value of 2.92 ejaculations. A plot of these data with the 95% prediction interval is displayed in Figure 1. The best prediction model for ISCT scores in this scenario was a piecewise linear regression with the breakpoint between COSCT scores 0 and 1. No slope was detected with this model when COSCT scores were >0; thus, the COSCT score was effectively modeled as a class variable with separate means for a COSCT score = 0 and a COSCT score >0. The fitted ISCT scores ($\pm$SE) were $3.27 \pm 0.15$ ejaculations for a cohort test score >0 and $1.20 \pm 0.21$ ejaculations for a COSCT score = 0.

Multiple COSCT Given Before a Series of ISCT

The best model (i.e., lowest Akaike’s information criterion) for the multiple COSCT on rams (n = 57) used the mean of the second and third COSCT scores as the independent variable. The best prediction model for ISCT scores in this scenario was a piecewise linear regression, with the breakpoint between COSCT scores 0 and 0.5. No slope was detected for COSCT scores >0; thus, COSCT score was effectively modeled as a class variable with separate means for COSCT score = 0 and COSCT score >0. Consequently, multiple COSCT did not help differentiate between high and low sexual activity levels of sexually active rams. Compared with the first COSCT score from the same rams, however, multiple COSCT increased the probability that rams with a COSCT score = 0 were sexually inactive and decreased the probability that they had high sexual activity (Table 1). These probabilities are based on an ISCT high-low threshold value of 2.92 ejaculations. Figure 2 shows the plot of data and prediction interval for the first COSCT score (panel a) and for the mean of the second and third COSCT scores (panel b). The fitted ISCT scores ($\pm$SE) were $3.28 \pm 0.22$ and $3.22 \pm 0.17$ ejaculations for a COSCT score >0 and $1.07 \pm 0.29$ and $0.47 \pm 0.24$ ejaculations for a COSCT score = 0 for the first COSCT score and the mean of the second and third COSCT scores, respectively.

First COSCT Given After a Series of ISCT

Rams (n = 104) with the first COSCT score >0 had increasing probabilities of high sexual activity (21% to
92%) and decreasing probabilities of low sexual activity (76% to 8%) and sexual inactivity (3% to <1%) as the COSCT score increased from 1 to 7 (Table 2; Figure 3). Rams with the first COSCT score = 0 had a very low probability (5%) of high sexual activity and a high probability (70%) of being sexually inactive (Table 2). These probabilities were based on an ISCT high-low threshold of 2.46 ejaculations. The fitted ISCT scores were ISCT score = 1.40 + (0.34 × COSCT score) for COSCT score >0 and ISCT score = 0.42 when COSCT score = 0.

### Multiple COSCT Given After a Series of ISCT

Rams (n = 70) with a COSCT score >0 had an increased probability of high sexual activity and a decreased probability of low sexual activity and sexual inactivity as the score increased from 1 to 6 (Table 3), which was similar for the first and mean of the first and second COSCT. Rams with a COSCT = 0 had a greater probability of being sexually inactive (80% for the mean of the first and second COSCT vs. 55% for the first COSCT) and a lower probability of having low sexual activity (20% for the mean of the first and second COSCT vs. 36% for the first COSCT). Rams with a COSCT = 0 had no chance of having high sexual activity when using the mean of the first and second COSCT, compared with a 9% probability when using only the first COSCT. The probabilities are based on an ISCT threshold of 2.19 ejaculations. In Figure 4, data for the first COSCT (panel a) are given for comparison on the same rams represented in the first and second COSCT score mean (panel b), which explained the most variation in ISCT scores. The fitted ISCT scores = 1.53 + (0.23 × COSCT score) and 1.21 + (0.33 × COSCT score) for COSCT score >0 and 0.60 and 0.04 when the COSCT score = 0 for the first COSCT and for the mean of the first and second COSCT scores, respectively.

### DISCUSSION

Our data indicate that a single 3-ram, 30-min COSCT is a reliable and efficient alternative to a series of ISCT for characterizing sexual activity in rams. However, the COSCT seems somewhat more conservative than the ISCT. Rams with a cohort test score of 0 have an almost equal probability of being sexually inactive and having low sexual activity (47 and 38%, respectively), whereas they have a low probability (16%) of having

### Table 1. Probability of sexually inactive (SI = 0) or sexually active (SA >0) rams from the first or from the mean score of the second and third cohort serving capacity tests (COSCT), given before the 30-min individual-ram serving capacity tests (ISCT), being SI, low SA, or high SA as classified in ISCT

| Item                  | COSCT score\(^2\) | ISCT classification |
|-----------------------|-------------------|---------------------|
| 1st COSCT             | 0                 | SI (n)  Low SA (n)   High SA (n) |
|                       | >0                | 0.55 (18) 0.30 (10) 0.15 (5) |
| Mean of 2nd and 3rd COSCT | 0                | 0.64 (18) 0.29 (8) 0.07 (2) |
|                       | >0                | 0.34 (10) 0.66 (19) |

\(^1\)ISCT classifications are based on the threshold value (2.92) of mean ejaculations of two through six 30-min ISCT for 57 rams.

\(^2\)COSCT score = number of ejaculations during a 30-min COSCT.

![Graph](https://example.com/graph)
**Table 2.** Probability of sexual classification as sexually inactive (SI), low sexually active (low SA), or high sexually active (high SA) rams, as classified with 30-min individual-ram serving capacity test (ISCT) scores, for each 30-min cohort serving capacity test (COSCT) score (0 to 7 ejaculations) when the COSCT was given after the ISCT.

| COSCT score | ISCT classification |
|-------------|---------------------|
|             | SI  | Low SA | High SA |
| 0           | 0.70| 0.25   | 0.05    |
| 1           | 0.03| 0.76   | 0.21    |
| 2           | 0.01| 0.65   | 0.34    |
| 3           | <0.01| 0.52  | 0.48    |
| 4           | <0.01| 0.37  | 0.63    |
| 5           | <0.01| 0.24  | 0.76    |
| 6           | <0.01| 0.14  | 0.86    |
| 7           | <0.01| 0.08  | 0.92    |

1ISCT classifications are based on the threshold value (2.46) of mean ejaculations of two through six 30-min ISCT for 104 rams.
2COSCT score = number of ejaculations during a 30-min COSCT.

**Figure 2.** Cohort serving capacity test (COSCT) score data are plotted against the mean of individual serving capacity test (ISCT) scores from ISCT 2 through 6 for rams (n = 57) that were given the COSCT before the series of ISCT. Panel a displays the data for the number of ejaculations during the first 30-min COSCT, and panel b displays the data for the mean of the second and third 30-min COSCT. Data are plotted with a small random offset on the x-axis for display purposes. Open circles (○) represent data for COSCT scores = 0. Solid circles (●) represent data for COSCT scores >0. In each respective panel, the dotted lines indicate the threshold values from the ISCT for either high or low sexual activity, or sexually inactive rams. The solid line represents the mean ISCT scores, with 95% prediction intervals (dashed lines) for the sexually active rams from the piecewise linear model. The diamond (◊) indicates the mean ISCT score for rams sexually inactive in the COSCT.

**Figure 3.** Data for the first cohort serving capacity test (COSCT) score (number of ejaculations during the first 30-min tests; n = 104) are plotted against the mean of individual serving capacity test (ISCT) scores from ISCT 2 through 6 for rams that were given the COSCT after the series of ISCT. Data are plotted with a small random offset on the x-axis for display purposes. Open circles (○) represent data for COSCT scores = 0. Solid circles (●) represent data for COSCT scores >0. The horizontal dotted lines indicate the threshold values from the ISCT for either high or low sexual activity, or sexually inactive rams. The solid line represents the expected ISCT scores, with corresponding 95% prediction intervals (dashed lines) for the sexually active rams from the piecewise linear model. The diamond (◊) indicates the mean ISCT score for rams sexually inactive in the COSCT.
high sexual activity. Therefore, eliminating all rams with a cohort score of 0 could dramatically reduce the chance of selecting low-performance rams for breeding and could have only a low probability of eliminating high-performance rams. Rams with a cohort test score greater than 0 have a high probability (70%) of having high sexual activity. These results were generated in less than 6% of the observation time that was required for the ISCT (i.e., 10 vs. 180 min per ram). The first cohort test could not distinguish between high and low sexual activity levels, as characterized with the series of 6 ISCT. Thus, no new information about a ram was gained after the first ejaculation was observed during the cohort test.

All of the remaining rams are expected to be sexually active if rams with a first cohort test of 0 are culled. This practice may be of benefit to producers who want an efficient means to “guarantee” rams that are to be sold, or producers who wish to guard against nonpregnant ewes after planned matings. This culling practice, however, would remove approximately 50% of the rams from the flock, including approximately 16% of the highly sexually active rams, whereas culling for sexual inactivity, when assessed by using the ISCT, would result in removal of approximately 23% of the rams.

Our data indicate that the use of multiple cohort tests can provide some protection against unnecessary culling of highly sexually active rams. When using the mean of the second and third cohort test scores, less than 10% of the highly sexually active rams are expected to be culled unnecessarily. In addition, the percentage of all rams expected to be culled would be reduced by nearly 9 percentage points (33 out of 57 vs. 28 out of 57). These results were generated in less than 17% of the observation time that was required for the ISCT (i.e., 30 vs. 180 min per ram). Nevertheless, the mean cohort test scores could not distinguish between high and low sexual activity levels.

It is important to identify high sexual performance in rams for breeding because high-performance rams approximately doubled the number of ewes bred and lambs sired compared with low-performance rams in multiple-sire and single-sire breeding environments, in which 7 to 10 ewes need to be serviced daily (Stellflug et al., 2006, 2008). When breeding intensity was low (1 to 2 ewes daily), the low-performance rams achieved reproductive rates comparable to those of the high-performance rams (Stellflug et al., 2008).

Some of the differences between the COSCT and the ISCT may be related to competition between rams in the cohort test, which may enhance breeding behavior for high-performing rams and intimidate rams that are reluctant breeders (Shreffler and Hohenboken, 1974). In addition, Mania and Katz (1997) determined that exposure of rams to recently mated rams enhanced sexual activity. Furthermore, rams may adapt more quickly to the unfamiliar testing environment in 3-ram cohort tests, in which rams have been reared with their cohorts. These opportunities are at least provided in a 3-ram cohort test, unlike in individual-ram tests, and may have a positive influence on cohort test results to help separate sexually inactive rams from sexually active rams.

The first cohort test score was a better predictor of the ISCT score when the cohort test was given after the individual tests. This was indicated with the significant linear relationship between test scores, and the decrease in root mean square error (0.87 vs. 1.02 ejaculations). For this order of testing, use of the mean of multiple cohort test scores (i.e., the first and second cohort test scores) also improved the reliability of the cohort test. This was evidenced by an increase in the regression coefficient (i.e., 0.33 vs. 0.23) and a slight decrease in the root mean square error (0.86 vs. 0.87). In addition, no highly sexually active rams, according to the individual tests, are expected to be lost if all rams with a mean cohort test score = 0 are culled. These testing scenarios have no practical value for producers, because in routine practice, rams would not be subjected to a battery of both tests. These data suggest,
however, that previous exposure of rams to the testing environment, to estrual ewes, or both may increase the reliability of the first cohort test score. Results of ISCT are affected by previous exposure to both the testing environment and estrual ewes (Zenchak and Anderson, 1980; Price et al., 1991, 1996). The design of our study does not allow us to determine the type of exposure, or the extent that might increase the reliability of the first cohort test score. The COSCT needs to be validated by using conception data from single- and multiple-sire breeding scenarios with varying breeding intensities.

This research indicates that a single 3-ram, 30-min cohort serving capacity test is a reliable, albeit more conservative, and efficient alternative to a series of ISCT for characterizing sexual activity in rams. Eliminating rams with a cohort score of 0 will eliminate sexually inactive rams and dramatically reduce the chance of selecting low-performance rams, but approximately 18% of rams with high sexual activity would also be lost for breeding. Use of multiple cohort tests can provide some protection against culling rams with high sexual activity (i.e., less than 10% of highly sexually active rams are expected to be culled), and the multiple cohort test score data can be generated in less than 17% of the observation time required for the ISCT.

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