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Effect of exposing rams to a female stimulus before semen collection on ram libido and semen quality[1]

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ABSTRACT: Rams with strong libido and desirable semen characteristics can provide more insemination doses per ejaculate and produce more progeny, improving population genetic linkage to improve the accuracy of EBV. The objective of this study was to determine if teasing rams, either by sight and smell alone (Exp. 1), or physical contact (Exp. 2), could improve libido and semen quality of rams. In Exp. 1, there were 3 treatments in which rams were exposed to the sight and smell of the ewe for 1 h: control treatment (n = 5) in which rams were exposed to a ewe not in estrus; non-novel treatment (n = 6) in which rams were exposed to a ewe in estrus and the same ewe was used for semen collection; and novel treatment (n = 6) in which rams were exposed to a ewe in estrus and a different ewe in estrus was used for semen collection. In Exp. 2, rams were individually given full access to a ewe, which had a cotton apron fitted to cover her vulva, for 15 min. The 3 treatments in Exp. 2 were: control treatment (n = 5) in which rams were placed in a pen with a ewe not in estrus; a non-novel treatment (n = 5) in which rams were placed in a pen with a ewe in estrus and the same ewe was used for semen collection; novel treatment (n = 6) in which rams were placed in a pen with a ewe in estrus and a different ewe in estrus was used for semen collection. Experiment 1 was repeated for 5 consecutive days and Exp. 2 was repeated for 4 consecutive days. Data on reaction time, number of mounts, semen volume, semen concentration, sperm wave motion, and progressive linear motion (Exp. 1 only) were collected and analyzed as a randomized complete block design, where rams were initially blocked for breed and age. In Exp. 1, there was an effect of day ($P < 0.05$) and a treatment × day interaction ($P < 0.05$) on semen volume, whereas there was also an effect of treatment ($P < 0.05$) and day ($P < 0.01$) on semen concentration, which was most evident on d 1. In Exp. 2, there was an effect of treatment on reaction time ($P < 0.05$) and semen volume ($P = 0.08$), which was most evident on d 1. This study demonstrates an acute effect on d 1 on semen concentration when rams were exposed to the sight and smell of a ewe in estrus. Alternatively, when rams were stimulated with physical contact of a ewe in estrus, an acute increase in semen volume was evident on d 1. These effects were not evident on subsequent days and thus the overall benefits on ram libido and semen quality of exposing rams to ewes in estrus are minimal.

Key words: estrus, female effect, libido, ram, semen quality

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INTRODUCTION

Artificial insemination is essential for the implementation of a sheep genetic improvement program to maximize progeny per ram and conduct evaluations of rams without compromising biosecurity (Hanrahan, 2003; Fair et al., 2005). The number of progeny is determined by total sperm output of the ram, number of sperm used per insemination, number of ewes lambing per insemination, and litter size. Acceptable pregnancy rates can be achieved after cervical AI, using fresh semen inseminated on the day of collection with 200 million sperm per dose (O'Hara et al., 2010), which limits the number of doses to 10 to 15 per ejaculate. Lapa-

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roscopic AI with fresh semen enables sperm number to be reduced to <50 million sperm per dose (Ehling et al., 2003), which limits the number of doses per ejaculate to <100 ewes to achieve pregnancy rates >70% (Sayre and Lewis, 1997). This coupled with the seasonality of sheep production requires a large number of rams to enter AI to facilitate a genetic improvement program.

Semen collection using an artificial vagina (AV) requires each ram to be trained; this can be an expensive task that takes up to 2 wk (Wulster-Radcliffe et al., 2001). Changing the female on which the male mounts when collecting semen using an AV has been used as an aide to train sheep and goats. In goats, changing the female stimulus stimulates sexual activity and increases sperm output in bucks (Silvestre et al., 2004). Thiery and Signoret (1978) observed a shorter sexual reaction time in rams by changing the female stimulus. There is limited published work on investigating the effect of teasing the ram before semen collection using a female stimulus in estrus on ram libido and semen characteristics. Our objective was to investigate if ram libido and semen quality could be increased by teasing rams before semen collection, using a ewe in estrus, either by sight and smell only (Exp. 1), or tactile contact without ejaculation (Exp. 2).

MATERIALS AND METHODS

All procedures involving animals followed established standards of the UCD Lyons Research Farm for the humane care and use of animals.

Experimental Design

Experiment 1. The aim of this experiment was to examine the effect of exposing rams to a ewe for 1 h before semen collection. The exposure consisted of visual and olfactory stimuli only, and the rams did not have tactile contact with the ewe during the 1-h exposure time. The rams were individually penned in a circular arrangement around the teaser ewe. One teaser ewe was used per treatment per day and each ram had 0.75-m access to the ewe (sight and smell only). Rams were allocated to 1 of 3 treatments, according to breed and age: Treatment 1 (control); rams (n = 5) were exposed to a ewe not in estrus for 1 h and were subsequently allowed to mount another ewe in estrus, which was restrained on a ramp for semen collection. Treatment 2 (non-novel ewe); rams (n = 6) were exposed to a ewe in estrus for 1 h after which the same ewe was then restrained on a ramp for semen collection. Treatment 3 (novel ewe); rams (n = 6) were exposed to a ewe in estrus for 1 h after which the rams were allowed to mount a different ewe in estrus that was restrained on a ramp for semen collection. In all 3 treatments, the teaser ewe was removed after exactly 1 h. The experiment was repeated on each of 5 consecutive days and on each day the order of treatments and order of rams within each treatment were systematically rotated over the test days.

Experiment 2. The aim of this experiment was to assess the effect of teasing rams for 15 min before semen collection by exposing rams to tactile contact of a ewe in estrus. The rams were individually exposed to a ewe that had a cotton apron fitted to cover her vulva in a pen (3 m × 3 m). Thus, rams had full contact with the ewe and were able to mount the ewe (if she permitted them to do so) but were unable to penetrate her with his penis due to the presence of the apron. A 15-min exposure time was deemed to be sufficient so as to avoid exhaustion of the rams. Rams were allocated to 1 of 3 treatments, according to breed and age: Control; rams (n = 5) were placed in a pen with a ewe not in estrus for 15 min and after this the ram was allowed to mount another ewe in estrus, which was restrained on a ramp for semen collection. Non-novel ewe; rams (n = 5) were placed in a pen with a ewe in estrus for 15 min after which the same ewe was then restrained on a ramp for semen collection. Novel ewe; rams (n = 6) were placed in a pen with a ewe in estrus for 15 min after which the rams were allowed to mount a different ewe in estrus that was restrained on a ramp for semen collection. In all 3 treatments, the teaser ewe was removed after exactly 15 min. The experiment was repeated on each of 4 consecutive days and on each day the order of treatments and order of rams within each treatment were systematically rotated over the test days.

Animals

Experiments 1 and 2 were carried out during the breeding season at Lyons Research Farm, University College Dublin, Ireland (53°17'54" N, −6°32'8" W)). All animals were maintained outdoors on pasture with free access to water but were brought indoors daily for semen collection for the duration of the experiment. For Exp. 1, rams were sexually mature and between the ages of 18 mo and 4 yr, with a BW of 94.2 ± 1.18 kg (mean ± SEM). The rams were purebred Texel (n = 5), Suffolk (n =3), Charollais (n = 2), Belclare (n = 5), and Dorset Horn (n = 2), and had all naturally mated ewes in the previous breeding season but had not previously been trained for semen collection. Scrotum circumference and epididymal diameter were recorded daily in Exp. 1 and were 38.5 ± 0.34 cm and 32.6 ± 0.62 mm [least squares mean (**lsmean**) ± SEM], respectively. For Exp. 2, rams were sexually mature and between the ages of 18 mo and 4 yr, with a BW of 98.7 ± 2.90 kg (lsmean ± SEM). The rams were purebred Texel (n = 5), Suffolk (n =2), Charollais (n = 2), Belclare (n = 5), Dorset Horn (n = 1), and Blue Leicester (n = 1), and had all naturally mated ewes in the

previous breeding season. Except for the Blue Leicester ram, the rams used for Exp. 2 were the same rams used in Exp. 1. Scrotum circumference and epididymal diameter were recorded daily in Exp. 1, and were 36.3 ± 0.69 cm and 39.7 ± 1.49 mm (lsmean ± SEM), respectively.

In both Exp. 1 and Exp. 2, crossbred multiparous ewes were used as teaser ewes before semen collection and as a dummy ewe, whereby, she was restrained on a ramp for the ram to mount for semen collection using an AV. To acclimatize the rams to the semen collection ramp and operator, all rams were allowed to mount a restrained ewe in estrus once per day on 2 consecutive days before the start of Exp. 1. Ewes were artificially brought into estrus, using a 12-d intravaginal progestagen pessary (20 mg fluorogestone acetate; Chronogest, Intervet, Boxmeer, the Netherlands) and 400-IU equine chorionic gonadotropin (Intervet) was administered at pessary removal. Before use (as a teaser or dummy ewe), all ewes were checked for estrus, using a ram (which was not subsequently used in the experiment). Only ewes that stood to be mounted were deemed to be in estrus, whereas those that were known to have been in estrus 5 d earlier and did not stand to be mounted were deemed to not be in estrus and were used as a control ewe where appropriate.

Assessment of Ram Libido

Libido was measured by the reaction time and number of mounts taken before ejaculation into the AV. Reaction time was determined by the length of time (s) from when the ram put his first foot on the ramp to when ejaculation into the AV had occurred. The number of mounts was based on the number of times the 2 front feet of the ram left the ground to mount the ewe until ejaculation occurred.

Collection and Preparation of Semen

Semen collection was performed by an experienced handler using an AV. After collection, the ejaculate of each ram was assessed separately. Semen volume was determined and the semen was held in a 15-mL polypropylene tube in a water bath at 32°C. Wave motion was subjectively assessed by placing a 10-µL semen sample onto a prewarmed glass slide. The sample was then assessed using a phase-contrast microscope at 40 × magnification and a 6-point scale (0 = no currents to 5 = vigorous waves). Semen concentration was assessed in duplicate by diluting 10 µL of semen in 3,990 uL of NaCl (0.9% NaCl) and then read using a photometer calibrated for ovine semen (Accucell, IMV Technologies, L'Aigle, France). Progressive linear motion (PLM) was assessed (Exp. 1 only) by diluting 5 µL of semen in 995 µL of PBS (137 mM NaCl, 2.68 mM KCl, 10.14 mM $Na_2HP\,O_4$, 1.76 mM KH_2PO_4) containing 3% BSA. This was incubated for 1 h at 32°C and then 100 live sperm were assessed using a phase-contrast microscope at 400× magnification and the percentage of live sperm showing progressive motion were recorded. Wave motion and PLM were assessed by the same experienced individual throughout to eliminate interobserver variation.

Statistical Analyses

All analyses were carried out using the SAS software package (SAS Inst. Inc., Cary, NC). Diagnostic tests were used to determine if data had a normal distribution. Data that did not approach a normal distribution were transformed using a Box-Cox transformation (Box and Cox, 1964; Fahey et al., 2007) to meet the assumptions of ANOVA. Rams were blocked by breed and age, and then randomly assigned within blocks to their treatments. Rams were either enclosed in individual pens (Exp. 1) or exposed to a ewe individually (Exp. 2), and, therefore, ram was considered the experimental unit.

Data presented from this study show the nontransformed values. However, all *P*-values were calculated using the transformed data where required. The day before the experiments, semen was collected from all rams and analyzed and assessed as a covariate for the analysis. However, these covariates were not significant ($P < 0.05$). For the analysis, rams were blocked by breed and age. If preliminary analysis determined that the *P*-value for block or age was >0.25, they were removed from the final model. Data were analyzed using a model that included the fixed effects of breed, age, treatment, day, and treatment × day, and ram was included as a random effect. Repeated measures for day were fitted using the appropriate covariance function as determined by the Bayesian Information Criterion. Orthogonal contrasts were used to compare the control treatment vs. the novel and non-novel treatments combined. A Tukey adjustment was used to account for multiple comparisons. Total sperm number was calculated as semen concentration × semen volume. Statistical differences were reported when *P*-values were <0.05 and statistical trends were reported when *P*-values were >0.05 and <0.10. Results are reported as lsmean ± SEM, unless otherwise stated.

RESULTS

Experiment 1

There were no effects ($P > 0.10$) of treatment, day, or their interaction on reaction time with an overall average reaction time of 69.4 ± 17.4, 67.9 ± 11.6, and 44.5 ± 5.2 s for the control, non-novel, and novel treatments, respectively. In addition, the number of mounts taken for the

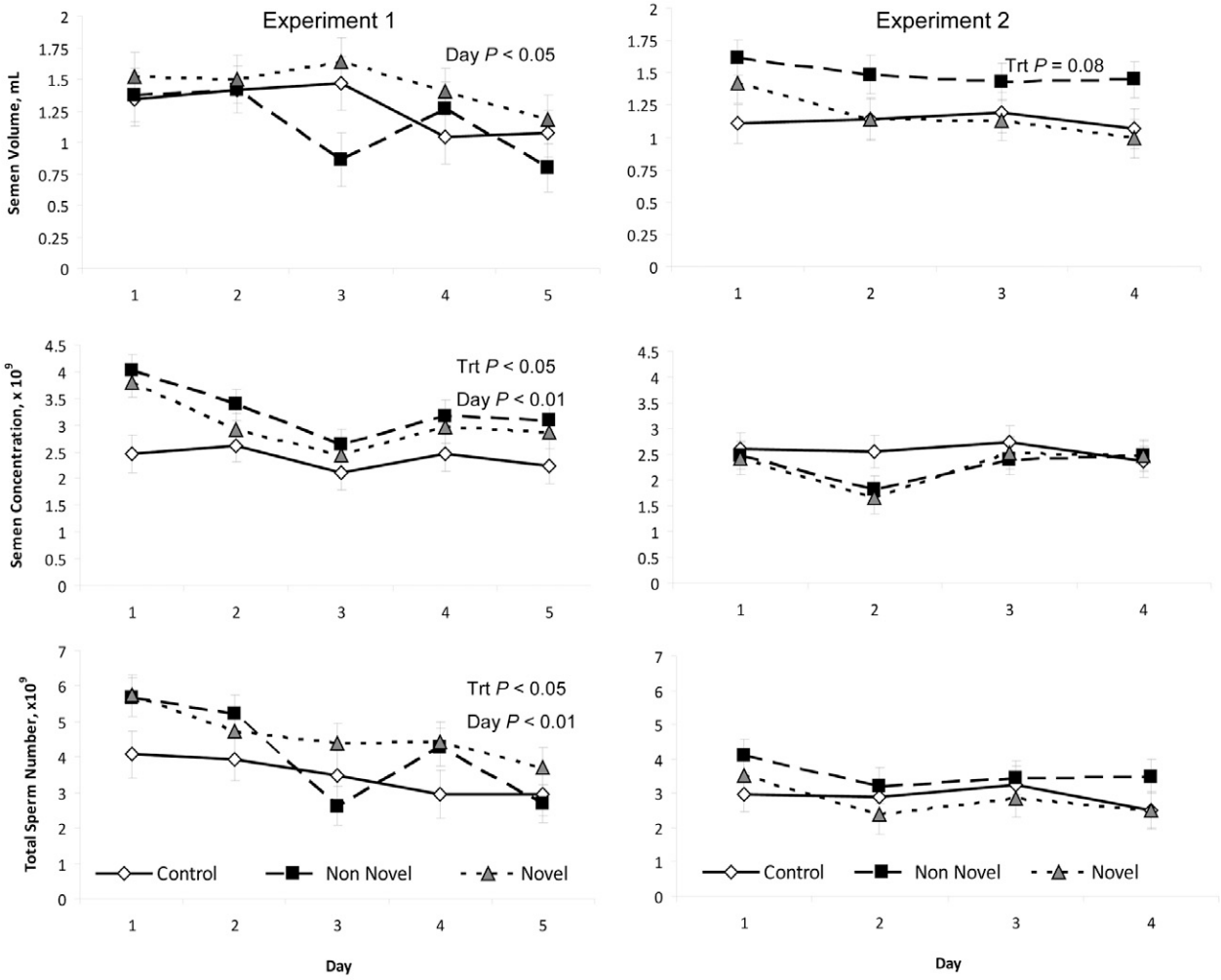

Figure 1. Least squares means (± SEM) for semen volume, sperm concentration, and total sperm number for Exp. 1 and 2.

rams to ejaculate averaged 2.0 ± 0.3, 2.7 ± 0.6, and 1.6 ± 0.2 mounts for control, non-novel, and novel treatments, respectively, and were not affected by treatment, day, or their interaction ($P > 0.05$).

There was no effect ($P > 0.10$) of treatment on semen volume; however, there was an effect of day ($P < 0.05$), with a decreased semen volume observed in all treatments on d 5 compared with d 1 (Figure 1). Both semen concentration and total sperm number were affected by treatment ($P < 0.05$) and day ($P < 0.01$; Figure 1); however, there was no treatment × day interaction ($P > 0.10$).

There was no effect ($P > 0.10$) of treatment, day, or treatment × day interaction on wave motion or PLM of sperm. The mean wave motion was 4.0 ± 0.2, 4.2 ± 0.2, and 4.0 ± 0.3, and the mean PLM was 80.0 ± 2.2, 71.0 ± 4.5, and 80.7 ± 1.9% over the 5 d for the control, non-novel, and novel treatments, respectively.

The lsmeans were combined for novel and non-novel treatments, and were compared with the control treatment. The novel and non-novel treatments (3.9 ± 0.3 × 10^9) had a greater semen concentration than the control treatment (2.5 ± 0.4 × 10^9, $P < 0.01$), and the novel and non-novel treatments (5.7 ± 0.6 × 10^9) had a greater total sperm number than the control treatment (4071.7 × 10^6 ± 666.2 × 10^6, $P < 0.05$) on d 1 of the experiment. However, these differences were not evident ($P > 0.10$) from d 2 to 5 of Exp. 1.

Experiment 2

There was an effect of treatment on reaction time ($P < 0.05$), with an overall average reaction time of 52.0 ± 34.4, 34.7 ± 31.4, and 95.8 ± 34.4 s for the control, non-novel, and novel treatments, respectively. There were no significant effects of day or day × treatment interactions for reaction time ($P > 0.10$). Likewise, there were no significant day, treatment, or day × treatment interaction effects ($P > 0.10$) for the number of times a ram mounted a ewe before ejaculating into the AV (2.7 ± 1.1, 1.5 ± 1.0, and 3.6 ± 1.1 s for the control, non-novel, and novel treatments, respectively).

The effect of treatment on semen volume approached significance ($P = 0.08$; Figure 1), with the non-novel treatment having a greater semen volume on all days than the novel or control treatments. However, this increase in semen volume did not result in a corresponding increase in total sperm number as sperm concentration tended to be less than the control. There were no treatment or treatment × day interaction effects ($P > 0.10$) for semen concentration or total sperm number, and there were no significant day, treatment, or day × treatment interaction effects for wave motion.

When semen volume for both the novel and non-novel treatments were combined and compared with the control, it demonstrated that the novel and non-novel treatments (mean 1.5 ± 0.2 mL) had greater semen volume than the control treatment (1.1 ± 0.2 mL, $P < 0.05$) on d 1 of Exp. 2. However, this difference was not present ($P > 0.10$) for the remainder of Exp. 2.

DISCUSSION

The main findings of this study are that teasing rams before semen collection, either by tactile exposure or sight and smell of an estrous ewe, has only minimal benefits in terms of ram libido or number of sperm produced per ejaculate. The main difference was on d 1, whereby an acute increase in semen concentration and thus total sperm number was evident in Exp. 1 and in semen volume in Exp. 2.

Knowledge of how different livestock species respond to sexual stimulation is critical in improving the efficiency of the training period of males for semen collection. The sudden introduction of estrous females to males has previously been shown to induce certain behavioral changes in the male, such as sniffing, nudging, flehmen, mounting, and ejaculation. Endocrinological changes, such as increased plasma concentrations of LH and testosterone, have also been reported to increase in the ram when in proximity to estrous females (Gonzalez et al., 1988, 1991a; Rosa et al., 2000). The use of such social and physiological cues could provide an opportunity to increase sexual stimulation of males before semen collection, with the aim of increasing libido and semen characteristics. In the current study, when rams were stimulated, either by visual and olfactory stimuli only (Exp. 1), or full physical contact (Exp. 2), there was no difference on ram libido (as measured by number of mounts) in rams exposed to the control ewe or an estrous ewe, irrespective of whether or not the ewe used for semen collection was novel. This finding is supported by Gonzalez et al. (1991b), who assessed the endocrine response in experienced rams exposed to urine, wool, or vaginal secretions of ewes in estrus. They concluded that olfactory cues are not necessary for the stimulation of endocrine response in the sexually experienced ram. In contrast, Maina and Katz (1999) reported an increase in sexual performance of rams that were exposed to other male rams that had been in previous contact with ewes; thus, demonstrating that rams are responsive to the olfactory smell of ewes.

False mounts and physical restraint of the male before semen collection have been shown to lead to a greater volume ejaculate; thus, increasing the number of sperm in the ejaculate of dairy bulls (Almquist, 1973) and boars (Hemsworth and Galloway, 1979). Most of the work to date on sexual stimulation in small ruminants has focused on restraining the male before semen collection or changing the female stimulus during repeated semen collection. This has been reported to be successful in goats through reducing reaction time (Prado et al., 2002, 2003; Silvestre et al., 2004) and increasing sperm output (Prado et al., 2003). In sheep, Thiery and Signoret (1978) demonstrated a shorter sexual reaction time in rams by changing the female stimulus, whereas Lezama et al., (2003) found no effect of changing the female stimulus on semen characteristics or interejaculation interval. In Exp. 1, our approach of exposing the ram to stimuli of an estrous ewe for 1 h before semen collection significantly increased semen concentration. This led to an increase in the total number of sperm per ejaculate on d 1, with the non-novel treatment having a significantly greater sperm count than the control treatment, without any effect on wave motion or PLM. The pathophysiology of such an effect may be due to an effect on epididymal contractility, which is known to be influenced by interplay among complex neuronal pathways and non-neuronal factors (Vignozzi et al., 2008). We speculate that exposing the ram to a ewe in estrus may lead to an increase in the series of epididymal contractile waves; thus, propelling more sperm toward the vas deferens and leading to an increased number of sperm in the ejaculate. In Exp. 2, the novel and non-novel treatments combined resulted in a significantly greater semen volume on d 1 than the control. This is in agreement with work conducted in bulls and boars, where physical stimulation led to greater ejaculate volumes (Almquist, 1973; Hemsworth and Galloway, 1979). In both experiments, the effects observed on d 1 were not evident on subsequent days, demonstrating an acute effect. This may be due to the partial depletion of sperm reserves after repeated semen collections. Repeat collections (6 to 8 per wk) of semen from mature bulls have been shown to reduce the sperm number by up to 25% (Amann and Almquist, 1962).

In conclusion, this study demonstrated an acute effect on d 1 on semen concentration when rams were exposed to the sight and smell of a ewe in estrus. Alternatively, when the rams were stimulated with physical

contact of a ewe in estrus, an acute increase in semen volume was evident. However, neither of these effects was evident for the remainder of the collection days. Thus, the benefits of stimulating rams with ewes in estrus to increase the number of insemination doses per ejaculate are small, especially when semen collection is performed on consecutive days.

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