Effect of changing female stimulus on intensive semen collection in young Murciano-Granadina male goats

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ABSTRACT: The aim of this research was to study the effect of changing female stimulus on libido and semen characteristics from young Murciano-granadina male goats submitted to intensive semen collection using females not in estrus as teasers. Males were submitted to two different sexual stimulation procedures. In the first procedure, the same doe was used as the female stimulus for three consecutive presentations. In the second, the doe was replaced after the second presentation by a new female. Semen volume, concentration, forward progressive motility, and live spermatozoa were scored. To analyze reaction time (RT), three types of analysis were performed. In the first one, RT was analyzed by multifactor ANOVA, taking as a missing value 300 s when a buck did not ejaculate. In the second, RT also was analyzed by multifactor ANOVA, but data from males that did not ejaculate were removed. In the third, a Cox Survival analysis was carried out by censoring data when a buck did not ejaculate within 5 min of entering the test arena. A decrease in semen volume and sperm concentration in the successive ejaculations was observed, being highly marked in the third ejaculation independent of the stimulation procedure (0.62 vs. 0.38 and 0.43 mL, and 2,828 vs. 2,183 and 2,223 million spermatozoa/mL to the first and third ejaculation respectively; \( P < 0.05 \)). No significant differences were observed either in forward progressive motility or live sperm rate. Changing the female stimulus in the third presentation had no significant effect on any seminal characteristic. Regarding libido and mounting behavior variables, there was a substantial decrease in RT in the third service when the female was changed (with both types of ANOVA). When censored data were taken into account, the relative risk showed that the probability of a male ejaculating in the third presentation increased almost fourfold when the female was replaced than when the female was the same in all services (\( P < 0.05 \)). In conclusion, young Murciano-granadina bucks can be used as semen donors because none of the most important semen variables used to reject or accept an ejaculate before freezing process decreased after intensive semen collection. We also recommend changing the female stimulus to make the semen collection procedure more efficient and using survival analysis methodology to analyze time data, mainly when a high rate of censored data are scored.

Key Words: Goats, Libido, Mating Behavior, Reaction Time, Semen

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Introduction

In breeding programs, to shorten the generation interval, the semen must be collected from the youngest males possible (Picard-Hagen et al., 2002). To make the semen collection process more efficient, it is important to obtain the maximum number of best-quality seminal doses from each male in the shortest time possible. However, libido, sperm production, and other sexual characteristics may limit the extent of use of these males. In general, semen volume and sperm number decrease (Amir et al., 1986) and reaction time (RT; Pepelko and Clegg, 1965) increases as the number of successive collections rises.

Changing the female stimulus could improve the young male sexual response after successive ejaculations. However, little information is available about this, especially on the relationship between changing...
female stimulus and semen characteristics of young (9-mo-old) bucks. Semen volume and concentration variation have been studied between two successive ejaculates from adult male goats (Prado et al., 2002) but not in young bucks. Effects on other important semen variables, such as sperm motility and live cell rate, have not been studied.

Finally, from the statistical point of view, in the study of the male RT, when a buck does not ejaculate within a determined time and goes back into the pen without ejaculating, these data are usually excluded from the statistical analysis (Ritar et al., 1992) or, alternatively, a maximum value is used (Roca et al., 1991). To avoid this, these RT must be included in the analysis as censored data (uncompleted data), and the analysis must be conducted using survival analysis methodology.

Our objective was to study the effect of changing female stimulus in libido and semen characteristics from young male goats submitted to intensive semen collection with females not in estrus as teasers. We also compared the differences in the obtained results corresponding to the statistical analysis methodologies applied.

Materials and Methods

Semen Collection

This study was carried out over 5 wk during the summer (July), at the University Polytechnic of Valence, Spain (39°28’long and 0°22’lat), which is 13 m above sea level with average rainfall and temperature of 9 mm and 25°C, respectively, for July (Instituto Nacional de Meteorología). Four 9-mo-old Murciano-granadina bucks and eight intact 3-yr-old does were used. All bucks were housed in a common covered pen and separated from females. One month before starting the experiments, bucks were trained for semen collection in an artificial vagina using an induced-estrus doe as teaser. Training was considered finished when males mounted and ejaculated regularly when presented with any sort of female as a teaser.

After the male training period, females used in this experiment did not exhibit estrus. All females were attached and partially immobilized to a stanchion similar to that used by Price et al. (1992) 10 min before the introduction of the first buck to the test arena. In order to increase the sex drive of males, the test arena was adjacent to the male pen and bucks were able to see the other males mounting the attached doe (Price et al., 1984). Bucks had 5 min to attempt to ejaculate. After ejaculation or 5 min, whichever occurred first, the male was moved to the pen. After 10 to 15 min, males were again placed in the test arena.

During the semen collection process, when the buck mounted the doe, his penis was gently guided inside the artificial vagina, which had a temperature ranging from 43 to 46°C. Immediately following collection, the ejaculates were immersed in a warm water bath at 37°C until their assessment. Semen assessment was performed within approximately 20 min. The same technician oversaw all of the semen collection processes.

Experimental Design

Animals were submitted to two different sexual stimulation procedures, which were balanced over 2 d during each weekly session (Tuesdays and Fridays) and throughout the experiment. In each session, males were introduced to the test arena three times and the same females were used for all four bucks. Order of semen collection was established by male hierarchy; in this way, approximately the same order was respected in all sessions. In the first procedure (A), the same doe was used as female stimulus for the three presentations. In the second (B), the doe was replaced after the second presentation by a new female.

Assessment of Ejaculates

Time from introduction of the buck to the test arena to ejaculate was defined as RT, and this was measured in seconds. The number of mountings required for an ejaculation was also scored. Each ejaculate was analyzed separately after collection. Volume was measured in a conical tube graduated at 0.1-mL intervals, and concentration was determined by a spectrophotometer (Acucell, IVM Technologies, Paris, France) calibrated for goat species (1:400 dilution rate). In order to evaluate forward progressive motility (FPM), semen samples were diluted in a sodium citrate solution, pH 6.7 to 6.9, transferred to a warmed slide, and observed under a microscope. Spermatozoa FPM was classified using a scale from 1 to 5 (1 to 20%, 21 to 40%, 41 to 60%, 61 to 80%, and 81 to 100% of the motile spermatozoa showing progressive motility). Live sperm were evaluated by eosin/nigrosin stain exclusion. Briefly, a drop of stain was mixed with a drop of pure semen and extended on the slide. One hundred spermatozoa were counted, and the unstained spermatozoa were determined as viable cells. The proportion of live sperm was also classified using a scale from 1 to 5 (1 to 20%, 21 to 40%, 41 to 60%, 61 to 80%, and 81 to 100% of the viable cells, respectively).

Statistical Analyses

Results of semen volume, concentration, FPM, and live sperm were analyzed by multifactor ANOVA. Explanatory variables were days of sessions (Tuesday or Friday), male (4), presentations (1, 2, 3A, and 3B) and RT. Results of the males ejaculating or not ejaculating within 5 min were analyzed by a Fisher’s exact test.

To analyze RT, results from three types of analysis were compared. In the first one, RT was analyzed by multifactor ANOVA, taking as a missing value 300 s (5 min) when a buck did not ejaculate. In the second, RT was again analyzed by multifactor ANOVA, but
if males did not ejaculate, those data were removed. Analysis of variance and Fisher’s exact test were carried out using the Statgraphics Plus 5.1 Software (Manugistics Inc., Rockville, MD). In the third analysis, a Cox Survival analysis was done (Kalbfleisch and Prentice, 1980) using the Survival kit V3.0 program (Ducrocq and Sölkner, 1998). If a buck did not ejaculate within 5 min of entering the test arena, those data were included into the analysis as censored. Censored data give partial information because we know with absolute certainty only that these males did not ejaculate in the first 5 min. In this type of data analysis, results are expressed as relative risks defined as the probability of an ejaculation in the active group divided by the probability of an ejaculation in the first presentation (reference level). An increase in relative risk indicates a lower RT to ejaculate. Explanatory variables included in the RT models were days of sessions, male, number of mountings and presentation 1, 2, 3A, and 3B. Results of number of mountings were analyzed with a proportional hazard discrete model (Kalbfleisch and Prentice, 1980) using the Survival kit V3.0 program (Ducrocq and Sölkner, 1998). In this type of data analysis, results were expressed as relative risks defined as the probability of an ejaculation in the active group divided by the probability in the first presentation (reference level). An increase in relative risk indicates a lower number of previous mountings to ejaculate. Explanatory variables included in the model were days of sessions, male, RT, and presentation 1, 2, 3A, and 3B.

Results

Semen variables of ejaculates obtained with two established procedures of sexual stimulation are presented in Table 1. A decrease ($P < 0.05$) in semen volume was observed in the successive ejaculations, which was especially important in the third ejaculation independent of the stimulation procedure. Regarding sperm concentration, a significant decrease ($P < 0.05$) in the third ejaculate was also observed. However, no differences were observed either in forward progressive motility or in live sperm rate. Changing the female stimulus in the third presentation had no significant effect on semen volume, concentration, forward progressive motility, or live sperm rate.

With respect to mating behavior (Table 2), we observed that the risk of a lower number of mountings before ejaculating increased in the third service, reaching levels of significance ($P < 0.05$) when the female stimulus was changed in comparison with the relative risk of the first service. With respect to RT, in the third ejaculation, males required more time to ejaculate than in the two first ejaculations in the two types of ANOVA ($P < 0.05$). However, if the female was changed, RT was decreased ($P < 0.05$) in the third service in the two statistical cases (110 and 51 vs. 19 and 27 s, to 3A and 3B, respectively). If data from males not ejaculating were removed, results were altered and differences were detected ($P < 0.05$) in the RT between the first and the second ejaculate; however, this fact was not observed when survival analysis was performed. When censored data were taken into account, the relative risk showed that the probability of a male ejaculate in the third presentation increased by nearly fourfold when the female was replaced than when the female was the same in all the services ($P < 0.05$). This was also reflected in the rate of males ejaculating within 5 min, as this rate was lower in the third service with the same female than in the first service.

Discussion

In the present work, reaction time and semen volume and concentration were lower than previously recorded data in the Murciano-granadina breed (Roca et al., 1991, 1992), probably due to the lower age of our animals (9 vs. 13 mo). As is observed in our work, the volume and number of sperm per ejaculate declined significantly in successive ejaculates (rams: Jennings et al., 1976; Amir et al., 1986; goats: Prado et al., 2003; bulls: Everett et al., 1978; stallions: Squires et al., 1979;...
Table 2. Effect of stimulus female change on mating behavior of young male goats submitted to intensive semen collection

<table>
<thead>
<tr>
<th>Presentation</th>
<th>No. of previous mountings</th>
<th>Males ejaculating, %</th>
<th>Reaction time (RT)</th>
<th>Relative risks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>LS Mean ± SE, s</td>
<td>LS Mean ± SE, s</td>
</tr>
<tr>
<td>1</td>
<td>1.0f</td>
<td>97.5ef</td>
<td>33.4 ± 10.1f</td>
<td>22.6 ± 4.0f</td>
</tr>
<tr>
<td>2</td>
<td>1.2f</td>
<td>95ef</td>
<td>49.1 ± 10.6f</td>
<td>35.0 ± 4.1f</td>
</tr>
<tr>
<td>3A</td>
<td>1.3f</td>
<td>75f</td>
<td>110.1 ± 14.3f</td>
<td>51.2 ± 6.5f</td>
</tr>
<tr>
<td>3B</td>
<td>2.0f</td>
<td>100g</td>
<td>19.5 ± 14.4f</td>
<td>27.1 ± 5.8f</td>
</tr>
</tbody>
</table>

In Presentation 3A, the same doe was used as the stimulus female for the three presentations. In Presentation 3B, the doe was the same in the first and second presentations, but was replaced by a new doe in the third presentation.

ANOVA was carried out removing data when males did not ejaculate.

ANOVA was carried out, with the reaction time value being 300 s when males did not ejaculate.

Within columns, values with different superscripts differ (P < 0.05).

The effect of new female stimulus on semen variables or sexual response depends on degree of sexual satiation. Changing the female stimulus after the first ejaculation did not affect the semen characteristics (volume and concentration) either in adult bucks (Prado et al., 2002) or rams (Lezama et al., 2003). Nevertheless, in criollo bucks, after seven successive ejaculations, if the teaser was substituted, semen volume was notably increased (Prado et al., 2003). Concerning other semen characteristics, such as motility and live sperm rate, no references were found in the literature on caprine species and particularly on Murciano-granadina goat. Although seminal variables are not strongly related to fertility after AI, such variables are decisive to determine whether the ejaculation is rejected (Roca et al., 1997; Aboagla and Terada, 2003). In our case, changing the teaser did not affect any semen characteristics (volume, concentration, motility, live sperm rate), probably due to the fact that young bucks were not too sexually satisfied, after three ejaculations per session and 3 or 4 d between sessions. In this way, Ritar et al. (1992) suggested that in the Angora breed, for efficient collection of spermatozoa for AI, bucks should be given a rest period of 1 or 2 d between days of intensive semen collection to allow a more complete sperm replenishment.

With respect to mating behavior, the relative risk of decreasing the number of mountings before an ejaculation tended to increase slightly independent of the change of teaser, as was also observed in rams in natural mating (Pepelko and Clegg, 1965). This difference reached significant (P < 0.05) levels between the first and third service, but only when the teaser was changed. Prado et al. (2002) found similar results for the second ejaculation only when bucks were allowed to choose between two females. In addition, without a change of teaser, the rate of young males ejaculating decreased significantly.

In studies of sexual satiety in goats, after seven ejaculations, libido expression can be temporally restored with a change of teaser (Prado et al., 2003). Also, in rams, the RT was restored when a new female stimulus was presented (Pepelko and Clegg, 1965). Nevertheless, this was not observed when the female change took place after the first ejaculation. However, if the adult male was allowed to choose between two females present in the test arena at the same time, RT between ejaculations was reduced both in goat and sheep (Prado et al., 2002; Lezama et al., 2003). In the present study work, RT was increased in the third ejaculation when the female was the same (P < 0.05); however, if the female was changed, RT decreased. Conversely, in the majority of experiments studying male reaction times to ejaculate, goats in estrus are normally used as female stimulus. However, in the AI center, the use of estrus females is not necessary when males are properly trained to use an artificial vagina (Evans and Maxwell, 1987), as happens in other species. It is appropriate to point out that the results of RT obtained here with an anestrous female are similar or even lower than in other studies with an estrus-induced female (Roca et al., 1991; Prado et al., 2002).

Generally, other authors have not used the data when a buck does not ejaculate within a determined time and finally goes back into the pen without ejaculating (Ritar et al., 1992), or a maximal value is used (Roca et al., 1991), but this could lead to a bias in the results. To avoid this, these reaction times (maximum waiting time without ejaculate) must be included as censored data, taking into account their partial information and carrying out the analysis using survival methodology. The avoidance of data or the assignment of a maximal value that was observed (in this case 5 min) is not a proper method. An approach more satisfactory than transforming data is to adopt an alternative distribution model for the original data (Collet, 2003) as performed...
in the survival analysis. If the censored data are not many, the conclusions obtained could be similar to those from other types of analysis, particularly if there are great differences between treatments. In our case, when the database was analyzed with censored (8% of data) and uncensored data, we observed that when the female was replaced, the probability of the male ejaculating was increased nearly fourfold \( (P < 0.05) \) compared with when the female was the same.

**Implications**

With the Murcián-Granadina breed, the use of young male goats could be suitable for artificial insemination centers because none of the most important semen variables used to reject or accept an ejaculate before freezing process (forward progressive motility or live sperm) decreased dramatically after intensive semen collection. The efficiency of genetic selection schemes would therefore be enhanced. In future research, it will be necessary to test the cryopreservation ability of the semen from young males. We also recommend changing the female stimulus to make the semen collection procedure more efficient and the use of survival analysis methodology to analyze time data, particularly when a high rate of censored data is scored.

**Literature Cited**


