Slovak J. Anim. Sci., 45, 2012 (3): 76-82

ISSN 1337-9984

EFFECTS OF VISUAL STIMULI OR CHANGE OF THE STIMULUS EWE ON LIBIDO AND SEMEN CHARACTERISTICS OF CROSSBRED RAMS DURING BREEDING SEASON

A. OLFATI, G. H. MOGHADDAM*, S. ALIJANI, S. A. RAFAT

Department of Animal Science, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

ABSTRACT

The main purpose of this study was to determine the effects of visual stimuli or change of the stimulus ewe on libido and semen characteristics of crossbred rams during breeding season. In experiment 1: four 3-year-old Arkharmerino×Ghezel rams and eight 3-year-old ewes were exposed to different sexual stimulation procedures. In the first procedure, the same ewe was used as the female stimulus for three consecutive presentations, but in the second procedure the stimulus ewe was replaced after the second presentation by a new female. In experiment 2: twenty mature crossbred rams consisting of Baluchi×Moghani, Ghezel×Baluchi, Arkharmerino×Ghezel and Arkharmerino×Moghani were used in this study. Animals were randomly allocated into two equal groups. In the first group, the rams were affected by visual stimuli (VS), while in the second group, rams were depriving of visual stimuli (NVS). Reaction time, semen volume, sperm concentration, forward progressive motility, live sperm and sperm abnormality rates were scored. In rams treated with change of the stimulus ewe 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 (1.09 vs. 0.81 and 0.85 mL, and 2.91 vs. 2.14 and 2.15 x 10^{12}/mL to the first and third ejaculation respectively; $P<0.05$). The most significant differences were observed in either forward progressive motility (70.73 vs. 69.31 and 82.3 %), live sperm (70.84 vs. 72.37 and 82.5 %) and sperm abnormalities rates (10.73 vs. 9.91 and 6.7 %) in the first and third ejaculation, respectively ($P<0.05$). Libido and mounting behavior variables were substantially decreased in reaction time in the third service when the ewe was changed. However, for rams treated with visual stimuli, only the semen volume increased (0.84 vs.1.03 mL, $P<0.05$). No significant differences were observed either in reaction time, mating behavior, sperm concentration; forward progressive motility, live sperm and sperm abnormality rates. These findings suggest that visual stimulus or change of the stimulus ewe increased sexual potency in breeding and semen output of crossbred rams.

Key words: libido; rams; reaction time; semen; visual stimuli

INTRODUCTION

Genetic improvement of livestock animals relies on the intensive use of a few superior males either for natural mating or in artificial insemination (AI) schedules. As a consequence the effects of management and handling methods on the ram's level of sex drive could be of importance obtaining the best use of the sire. Intensive management also necessitates short breeding periods and hence a high ejaculation frequency by the rams to breed a large number of ewes, particularly when estrus is synchronized or induced. A knowledge of how different livestock species respond to sexual stimulation is useful to persons involved in semen collection, „hand" (controlled) mating, and the administration of sexual performance evaluations (i.e., serving capacity tests), all of which require a significant outlay of time and labor (Price *et al*., 1991a). The reduction of the stimulus value of the female after several, or even after one mating, appears to be the

***Correspondence:** E-mail: ghmoghaddam@tabrizu.ac.ir
Gholamali Moghaddam, Department of Animal Science,
Faculty of Agriculture, University of Tabriz, 5166614766 Tabriz, Iran
Tel.: +98 411 3392041 Fax: +98 411 3356004

Received: January 30, 2012
Accepted: September 10, 2012

major factor accounting for the temporary interruption of the sexual activity of the male. Changing the stimulus female has been reported as one of the most efficient methods of stimulating the male's sexual reaction in the ram (Pepelko and Clegg, 1965) and the goat (Silvestre *et al*., 2004). In sheep, the ejaculation rate of sexually satiated males exposed to a new unmated estrous female was restored in up to 95 % of animals (Pepelko and Clegg, 1965). In general, the volume of the ejaculate and sperm number decrease (Amir *et al*., 1986) and reaction time (RT) increases as the number of successive collections rises (Pepelko and Clegg, 1965). Visual stimulation provided a definite, clearly recognizable end point for establishing that a ram was sexually stimulated. McGrath *et al.* (1979) reported that a visual stimulus has positive effects on semen characteristics in Suffolk rams. Sheep producers in the northwest of Iran use natural mating, and the libido of the ram is an important factor to be considered. The breeds of rams used in the present study were developed for use in a cold region, as well as the ability to exhibit sexual behaviors, including interest in induced estrous ewes and ejaculations. But there are no published reports on the effect of sexual stimulation procedure on the libido and semen characteristics of the crossbred rams. Hence, in the present study, the main objective was to study the effect of visual stimuli and change for new female on libido and semen characteristics in crossbred rams during breeding season.

MATERIAL AND METHODS

Experiment 1

This experiment was carried out over 5 weeks during the summer (July, 2011), at the agricultural research station, University of Tabriz, East Azerbaijan province, Iran (38° 07′ N and 46º 29′ E). Four 3-year-old Arkharmerino×Ghezel rams and eight 3-year-old ewes were used. All rams were housed in a common covered pen and separated from females.

One month before beginning the experiment, rams were trained for semen collection by artificial vagina (AV) using induced-estrus ewes as teaser. Training was finished when males mounted and ejaculated regularly. After the male training period, the 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 prior to introduction of the first ram to the test arena. Rams had 5 min to attempt to ejaculate. After ejaculation or 5 min, whichever occurred first, the male was moved to the pen. After 15 to 20 min, males were again placed into the test arena.

Animals were submitted to two different sexual stimulation procedures, which were balanced over two days during per weekly sessions (Tuesday and Friday) and throughout the experiment. In each session, the males were introduced to the test arena three times and the same females were used for all four rams. Sequence of semen collection was established by male hierarchy; in this way, approximately the same sequence was used during all sessions. In the first procedure (A), the same ewe was used as female stimulus for the three presentations. In the second procedure (B), the ewe was replaced after the second presentation by a new female.

Experiment 2

This experiment was carried out over 5 weeks during the summer (August, 2011) to determine the role of visual stimuli in the development of libido in the rams receiving initial attention. Subsequent attention was focused upon the relation between visual stimuli and semen output of rams. Before beginning the main experiment and collecting the original data, the rams did not appear to give their full attention to the sexual behaviors of the stimulator female prior to sexual performance tests. In order to test the visual stimuli of the males, the test arena was adjacent to the male pen and rams were able to see the other males mounting behavior towards the attached ewe. Twenty mature crossbred rams were randomly allocated into two equal groups (from each genetic group n = 5, Baluchi×Moghani (BL-MG), Ghezel×Baluchi (GH-BL), Arkharmerino×Ghezel (MR-GH) and Arkharmerino×Moghani (MR-MG). The rams in two groups were similar in age and body weight at the start of the study (Table 1). Two successive ejaculates were collected by AV twice weekly (Tuesday and Friday) throughout the experiment. In the first group, the rams were affected by visual stimuli (VS), while in the second group the rams were deprived of visual stimuli (NVS). The period from introduction of the ram to the test arena for his first ejaculation was defined as RT. All tests were conducted in the morning between 8.00 and 12.00 hours. The data were collected by a single observer. The number of mounts per each ejaculate was also recorded.

Assessment of ejaculates

In two experiments immediately following semen collection, the ejaculates were held in a warm water bath at 37 °C until their assessment. Semen assessment was performed within approximately 20 minutes. The semen was subjected to the following tests: (1) Volume, measured directly in milliters and with the exactness of 0.1 mL using a glass graduated tube. (2) Sperm concentration was determined using semen diluted with 3 % NaCl; the diluted semen was placed into a hemocytometer and the sperm were counted in five squares of one chamber (80 small squares with 0.2 mm volume). (3) For counting of live and dead sperm in the semen, one drop of eosin-nigrosin stain was placed onto a clean glass slide. A small

quantity of mixed semen was placed onto the slide; spread by another slide and dried rapidly by placing them on a hot plate. Spermatozoa with stained head were counted as dead. 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). (4) In order to evaluate forward progressive motility (FPM), semen samples were diluted in a sodium citrate solution (2.9 %), pH 6.7 to 6.9, placed onto a warmed slide, and observed under a light 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). (5) Abnormal morphologies: detached head, abaxial head, malformed head, damaged acrosome cap, bent and coiled tail, and the presence of cytoplasmic droplets (Evans and Maxwell, 1989).

Statistical analysis

The present experiment was based on a completely randomized design.

Table 1: Age and body weight of rams prior to visual stimulus tests

Breed	n	Age (month)	Weight (kg)
BL-MG	5	44	64 ± 1.5
GH-BL	5	51	76 ± 2.1
MR-GH	5	45	71 ± 1.2
MR-MG	5	51	72 ± 1.6

Baluchi×Moghani (BL-MG)
Ghezel×Baluchi (GH-BL)
Arkharmerino×Ghezel (MR-GH)
Arkharmerino×Moghani (MR-MG)

Experiment 1: Recorded data for RT, number of mounts per ejaculation, semen volume, sperm concentration, FMP, live sperm, and sperm abnormalities rate were analyzed by multifactor ANOVA using the GLM procedure of SAS software (SAS, 2003). Explanatory factors were days of sessions (Tuesday or Friday), rams and presentations (A, B). To compare the presentations Least Square mean was used. In other analyses, by considering RT as categorical data (with zero and one levels), a contingency table analysis was done by Fisher's exact test by use of SAS software (SAS, 2003). When each of the rams ejaculated within five minutes from introduction of the ewes, the RT was one, otherwise the RT was zeros.

Experiment 2: In this experiment the above-mentioned semen characteristics and libido were analyzed by Proc GLM procedure of SAS software (SAS, 2003). But in the statistical model the effect of visual stimuli was added to the model as a substitute for presentation. The statistical model is as follows:

Model 1: $Y_{ijk} = \mu + Animal_i + Time_j + Treat_k + e_{ijk}$

In experiment 1 and 2, Y_{ijk} equals animal performance, μ = population mean, $Animal_i$ = effect of i animal, $Time_j$ = effect of j time, $Treat_k$ = in experiment 1 represented procedure stimulus and in experiment 2 indicated visual stimulus, e_{ijk} = residual or error.

RESULTS AND DISCUSSION

Experiment 1: Semen variable of ejaculates obtained using the two established procedures of sexual stimulation are presented in Table 2. Results of the present study showed that changing female stimulus

Table 2: Effect of changing female stimulus on semen characteristics of adult rams submitted to intensive semen collection (n = 4)

Presentation[a]	Volume (mL)	Sperm concentration (x.10^9 sperm/mL)	Live sperm (%)	FPM (%)	Abnormal sperm (%)
1	1.09 ± 0.032[b]	2.9 ± 0.07[b]	3.55 ± 1.13[b]	3.54 ± 1.24[b]	10.7 ± 0.51[b]
2	0.96 ± 0.032[bc]	2.67 ± 0.07[b]	3.65 ± 1.15[b]	3.67 ± 1.26[b]	9.46 ± 0.51[b]
3A	0.81 ± 0.048[c]	2.14 ± 0.11[c]	3.62 ± 1.71[b]	3.47 ±1.87[b]	9.9 ± 0.74[b]
3B	0.85 ± 0.044[c]	2.15 ± 0.11[c]	4.13 ± 1.56[c]	4.12 ± 1.71[c]	6.7 ± 0.71[c]

[a] In presentation 3A, the same ewe was used as the stimulus female for the three presentations. In presentation 3B, the ewe was the same in the first and second presentations, but was replaced by a new ewe in the third presentation.
[b, c] Within columns, values with different superscripts differ ($P < 0.05$).

increased semen volume and sperm concentration in the crossbred rams during natural breeding season (P<0.05). As it is observed in our study, the semen volume and sperm concentration declined significantly in successive ejaculates. These results are in line with other previous reports in rams (Jennings *et al*., 1976; Amir *et al*., 1986), goats (Ritar *et al*., 1992; Prado *et al*., 2003; Silvestre *et al.*, 2004), bulls (Everett *et al*., 1978), stallions (Squires *et al*., 1979) and rabbits (Ambriz *et al.*, 2002). Other studies concluded that semen volume and sperm concentration were not related to sexual activity (rams: Lezama *et al*., 2003; bull: Henney *et al*., 1990). FPM and live sperm rates were affected by changing female stimulus and tendency to increase FPM and live sperm rates was observed. The results obtained in this study disagree with results obtained in several studies on different species (sheep: Jennings *et al*., 1976; Amir *et al*., 1986; horse: Squires *et al.*, 1979; goats: Ritar *et al.*, 1992; Silvestre *et al*., 2004), reported that changing female stimulus did not affect FMP and live sperm rates. The effect of changing ewe's stimulus on sperm abnormalities indicated that this sexual stimulus procedure could decrease percentage of sperm abnormalities significantly (P<0.05). Decreasing sperm abnormalities was evaluated for the first time in crossbred rams, but there are no published reports for comparison. Semen volume and sperm concentration are important variables considered in most studies of AI (Ollero *et al*., 1996). In general, the data obtained provide convincing evidence that exteroceptive stimuli markedly influence semen output, especially semen volume and sperm concentration in the crossbred rams. The effect of new female stimulus on semen characteristics or sexual activity may depend 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, when the teaser was substituted, semen volume was notably increased (Prado *et al*., 2003). In young bucks, changing the new female did not affect any semen characteristics (volume, concentration, motility, live sperm rate), probably due to the fact that young bucks were not too sexually satiated, after three ejaculations per session and three or four days 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 one or two days between days of intensive semen collection to allow a more complete sperm replenishment. This stimulus remains to be classified according to sensory pathway and evaluated in terms of epididymal and accessory gland function. The speed of appearance of the semen output response after sexual preparation suggests, as it has been indicated, that perhaps the sexual preparation stimuli alter the tonicity of the musculature of the excurrent ducts of the reproductive tract of the ram, and consequently affect semen output. Results related to mating behavior are shown in Table 3. Results obtained for RT indicated that in the third ejaculation, males required more time to ejaculate than during the two first ejaculations in the two types of analysis (P<0.05, Table 3). In the present work, RT was increased in the third ejaculate when the female was the same, but, if the female was changed, RT was decreased in the third service in the two statistical cases (91 and 82 vs. 44 and 44 second for the first and third ejaculation respectively, P<0.05). The rams had greatly reduced RT when exposed to the teaser subsequent to this change in housing, which suggests that olfaction played a role. Data obtained in this experiment on RT agree with previous results obtained in rams (Pepelko and Clegg, 1965; Price *et al*., 1991a) and goats (Prado

Table 3: Effect of changing female stimulus on mating behavior of adult rams submitted to intensive semen collection (n = 4)

Presentation[a]	No. of Previous Mountings	Males Ejaculating (%)	Reaction time (Sec)	
			LS Mean ± SE*	LS Mean ± SE**
1	1.4[b]	97.5	33.8 ± 5.96[bd]	31.22 ± 6.17[b]
2	1.76[b]	95	72.53 ± 6.06[c]	69.27 ± 6.25[cd]
3A	2.35[c]	90	91.87 ± 8.95[c]	82.87 ± 8.96[c]
3B	3[d]	100	44.15 ± 8.21[d]	44.15 ± 8.72[bd]

[a] In presentation 3A, the same ewe was used as the stimulus female for the three presentations. In presentation 3B, the ewe was the same in the first and second presentations, but was replaced by a new ewe in the third presentation.
[b, c, d] Within columns, values with different superscripts differ (P < 0.05).
* ANOVA was carried out, with the RT value being five minutes when males did not ejaculate.
** ANOVA was carried out removing data when males did not ejaculate.

et al., 2003; Silvestre *et al*., 2004), while at the same time, disagree with results obtained in rams (Ritar *et al*., 1992; Lezama *et al*., 2003; Godfrey *et al*., 1998) and in goats (Amir *et al*., 1986). Lezama and Orihuela (2001) reported that changing the stimulus animal in hair sheep rams after the first ejaculation resulted in little change in the behavioral response to a new estrous female. In sheep, the ejaculation rate of sexually satiated males exposed to a new unmated estrous female was restored in up to 95 % of animals (Pepelko and Clegg, 1965). 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). Results of Fisher's exact test indicated (Table 3, percentage of ejaculated males) that there is no significant difference between treatments for number of ejaculated rams in five minutes ($x^2 = 2.24$). With respect to mating behavior, it was observed that the number of mountings per ejaculation increased in the third service, reaching levels of significant difference ($P<0.05$); these results

Table 4: Correlation among different semen characteristics in treated rams with visual stimuli tests

Trait	No. of Previous mountings	Volume (mL)	Sperm concentration ($x.10^9$ sperm/mL)	Live sperm (%)	FPM (%)	Abnormal sperm (%)
Reaction time(Sec)	0.23	- 0.2	0.082	0.011	- 0.06	- 0.00003
	0.016	0.034	0.39	0.91	0.51	0.997
No. of Previous mountings		- 0.35	- 0.25	0.223	0.198	- 0.165
		0.0002	0.0064	0.018	0.36	0.079
Volume (mL)			0.26	- 0.0067	0.0096	- 0.02
			0.0064	0.94	0.92	0.83
Sperm concentration ($x.10^9$ sperm/mL)				- 0.25	- 0.147	0.177
				0.0083	0.122	0.061
Live sperm (%)					0.863	- 0.825
					< 0.0001	< 0.0001
FPM (%)						- 0.855
						< 0.0001

Notice: First and second rows indicate correlation among semen characteristic and P-value, respectively.

agree with results obtained by Silvestre *et al*. (2004) in the young Murciano-Granadina goat and are contrary to results obtained by Lezama *et al.* (2003) in the hair sheep ram. High correlation (Table 4) was seen among sperm abnormalities, live spermatozoa, and spermatozoa progressive motility ($P<0.0001$).

Experiment 2: The volume of semen per ejaculate, on the other hand, presumably represented chiefly seminal vesicular efficiency at the time of ejaculation. Further, treating rams with visual stimuli (Table 5) only increased the semen volume ($P<0.05$); the results of this study are contrary to the results obtained by McGrath *et al.* (1979) in the Suffolk rams. Concerning other semen characteristics such as sperm concentration, FPM, live sperm and sperm abnormality rates, which were not affected by visual stimulus, no references were found in the literature on sheep and particularly on crossbred rams. Although seminal variables are not strongly related to fertility after AI, such variables are decisive to determine whether the ejaculate is rejected (Roca *et al*., 1997; Aboagla and Terada, 2003). The rams were not provided with visual access to the test pens before being tested each day because it has been shown that visual stimuli are not critical for enhancing the sexual activity of rams (Price *et al*., 1991b), and these results agree with the results obtained regarding RT and number of mounts per ejaculation in this experiment. Visual stimuli are extremely important in males in order to maintain sexual activity of high quality at regular intervals, and might precede each ejaculation in an attempt to maximize semen output. Because the rams in the present study were sexually experienced, no improvements in performance were detected during the successive test days. When the rams did not ejaculate, the mating failure may be a

result of the influence of environmental condition rather than a lack of inherent libido. Another achievement of experimental visual stimuli was that the test period was shorter, and therefore may be considered economically more suitable in the AI center. The design did not allow determination of whether this visual stimulation affected second ejaculate, first refractory and refractory period. Thus the future study of the effects of visual stimulation on above- mentioned parameter in small ruminants is recommended. It is also recommended the research on the effect of ewe breed on the libido and semen characteristics in the ram. The present study indicates that under natural mating conditions in the pen, changing one female for another may keep breeding activity and sexual activity at desirable levels.

CONCLUSION

Results obtained in the present study indicate that semen output was apparently linearly related to the logarithm of the coded sexual preparation value. Also, the changing stimulus ewe and visual stimulation before each ejaculation could prove to be practical for increasing the semen output of rams used in the AI center.

Table 5: Overall means and variation of seminal characteristics and sexual activity measurements between VS and NVS groups (n = 20)

Parameter	NVS[1]	VS[2]	S.E	Breed	Weight	Age	Treatment
Reaction time (Sec)	22.3	21.97	0.96	ns	*	*	ns
No. of Previous mountings	4.74	3.74	0.6	**	ns	ns	ns
Volume (mL)	0.84[a]	1.03[b]	0.039	**	ns	**	**
Sperm concentration ($x.10^9$ sperm/mL)	3.75	3.73	0.163	*	ns	**	ns
FPM (%)	3.44	3.55	1.069	ns	ns	ns	ns
Live sperm (%)	3.49	3.57	1.179	ns	*	ns	ns
Abnormal sperm (%)	10.05	9.09	0.51	ns	*	ns	ns

[1]NVS = Non visual stimulus
[2]VS = Visual stimulus
[a, b] Within rows, values with different superscripts differ ($P < 0.05$).
ns = not significant; *P<0.01; **P<0.001.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Dr. H. Karami Shabankareh, Dr. R. Masoomi, and Dr. A. R. Abdolmohammadi for their help and collaboration in this work.

REFERENCES

ABOAGLA, E. M. – TERADA, E. T. 2003. Trehalose-enhanced fluidity of the goat sperm membrane and its protection during freezing. *Biol. Reprod*., 2003, vol. 69, p. 1245-1250.

AMBRIZ, D. – ROSALES, A. M. – SOTELO, R. – MORA, J. A. – ROSADO, A. – GARCIA, A. R. 2002. Changes in the quality of rabbit semen in 14 consecutive ejaculates obtained every 15 minutes. *Arch. Androl. J*., 2002, vol. 48, p. 389-395.

AMIR, D. H. – GACITUA, H. – RON, M. – LEHRER, A. R. 1986. Seasonal variation in semen characteristics and the fertility of Finn cross rams subjected to frequent ejaculation. *Anim. Reprod. Sci*., 1986, vol. 10, p. 75-84.

EVANS, G. – MAXWELL, W. M. S. 1989. Salmon's Artificial Insemination of Sheep and Goat. University press, Butterworth & Co. Ltd., London, U.K.

EVERETT, R. W. – BEAN, B. – FOOTE, R. H. 1978. Sources of variation of semen output. *J. of Dairy. Sci*., 1978, vol. 61, p. 90-5.

GODFREY, R. W. – COLLINS, J. R. – GRAY, M. 1998. Evaluation of sexual behavior of hair sheep rams in a tropical environment. *J. Anim. Sci*., 1998, vol. 76, p. 714-717.

HENNEY, S. R. – KILLIAN, G. J. – DEAVER, D. R. 1990. Libido, hormone concentrations in blood

plasma and semen characteristics in Holstein bulls. *J. Anim. Sci.*, 1990, vol. 68, p. 2784-2789.

JENNINGS, J. J. – McWEENEY, J. 1976. Effect of frequent ejaculation on semen characteristics in rams. *Vet. Res.*, 1976, vol. 20, p. 230-233.

LEZAMA, V. – ORIHUELA, A. 2001. A note on the effects of libido reestablishment on the semen characteristics of hair sheep rams. In: Proceedings of 2nd International Congress Sobre pequeños ruminates y camélido. Spanish, 2001.

LEZAMA, V. – ORIHUELA, A. – ANGULO, R. 2003. Effects of restraining rams or change of the stimulus ewe on the libido and semen quality of rams. *Small. Rumin. Res.*, 2003, vol. 49, p. 219-222.

McGRATH, P. E. – BOLAND, M. P. – GORDON, I. 1979. Effect of sexual preparation procedures on semen characteristics in the ram. *J. agric. Sci*., 1997, vol. 93, p. 761-763.

OLLERO, M. – MUIN˜ O-BLANCO, T. – LOPEZ-PEREZ, M. J. – CEBRIAN-PEREZ, J. A. 1996. Viability of ram spermatozoa in relation to the abstinence period and successive ejaculations. *Inter. J. Androl.*, 1996, vol. 19, p. 287-292.

PEPELKO, W. E. – CLEGG, M. T. 1965. Studies of mating behavior and some factors influencing the sexual response in the male sheep (Ovis Aries). *Anim. Behav*., 1965, vol. 13, p. 249-258.

PRADO, V. – ORIHUELA, A. – LOZANO, S. – PE´REZ-LEO´N, I. 2002. Management of the female stimulus during semen collection and its association with libido re-establishment and semen characteristics of goats. *J. Anim. Sci*., 2002, vol. 80, p. 1520-1523.

PRADO, V. – ORIHUELA, A. – LOZANO, S. – PE´REZ-LEO´N, I. 2003. Effect on ejaculatory performance and semen parameters of sexually satiated male goats (*Capra hircus*) after changing the stimulus female. *Theriogenology*, 2003, vol. 60, p. 261-267.

PRICE, E. O. – BLACKSHAW, J. K. – BLACKSHAW, A. – BORGWARDT, R. – DALLY, M. R. – BonDURANT, R. H. 1992. Sexual responses of rams to ovariectomized and intact estrous ewes. *Appl. Anim. Behav. Sci.*, 1992, vol. 42, p. 67-71.

PRICE, E. O. – BORGWALDT, R. – BLACKSHAW, J. K. – BLACKSHAW, A. – DALLY, M. R. – ERHARD, H. 1991b. Effect of early experience on the sexual performance of yearling rams. *Appl. Anim. Behav. Sci.*, 1991, vol. 42, p. 41-48.

PRICE, E. O. – WALLACH, S. J. R. – DALLY, M. R. 1991a. Effects of sexual stimulation on the sexual performance of rams. *Appl. Anim. Behav. Sci.*, 1991, vol. 30, p. 333-340.

RITAR, A. J. – MENDOZA, G. – SALAMON, S. – WHITE, I. G. 1992. Frequent semen collection and sperm reserves of the male Angora goat (*Capra hircus*). *J. Reprod. Fertil*., 1992, vol. 95, p. 97-102.

ROCA, J. – CARRIZOSA, J. A. – CAMPOS, I. – LAFUENTE, A. – VA´ZQUEZ, J. M. – MARTI´NEZ, E. 1997. Viability and fertility of unwashed Murciano-Granadina goat spermatozoa diluted in Tris-egg yolk extender and stored at 5 °C. *Small. Rumin. Res.*, 1997, vol. 25, p. 147-153.

SAS Institute Inc. 2003: SAS Users Guide. SAS Institute Inc. Cary, NC, 2003.

SILVESTRE, M. A. – SALVADOR, I. – SANCHEZ, J. P. – GOMEZ, E. A. 2004. Effect of changing female stimulus on intensive semen collection in young Murciano-Granadina male goats. *J. Anim. Sci*., 2004, vol. 82, p. 1641-1645.

SQUIRES, E. L. – PICKETT, B. W. – AMANN, R. P. 1979. Effect of successive ejaculation on stallion seminal characteristics. *J. Reprod. Fertil.*, 1979, vol. 27, p. 7-12.