Slovak J. Anim. Sci., 41, 2008 (2): 67 - 71

INTERRELATIONSHIP BETWEEN RAM PLASMA TESTOSTERONE LEVEL AND SOME SEMEN CHARACTERISTICS

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ABSTRACT

A total number of 6 rams (breed Rahmani) were used in this experiment to determine the effect of testosterone level before and after semen collection on semen evaluation. After semen collection, semen samples were subjected to evaluation of the following criteria: initial sperm motility percentage, methylene blue reduction time test (MBR-T), sperm concentration per mililitre, semen volume and reaction time (RT). These criteria were measured for first and second semen ejaculates, respectively. All the rams were allowed to exert a false mounting before blood sample collection and second blood sample was collected after second semen ejaculate collection. Data showed that there were no significant differences between testosterone levels before first semen collection and after second semen collection. These levels were 5.46 ± 1.01 and 6 ± 1.06 ng/ml of blood serum. But there were significant differences among different rams. These levels were 4.21 ± 0.93, 9.27 ± 1.95, 5.57 ± 2.17, 5.96 ± 1.93, 2.51 ± 0.83 and 5.42 ± 1.98 ng/ml of blood serum for 6.0 different rams, respectively. In addition, there was a high correlation between testosterone levels and semen characteristics. Testosterone levels correlated significantly with sperm motility, sperm concentration and methlyene blue reduction time test. These correlations were 0.83, 0.84, 0.87 and -0.82 for the above mentioned criteria, respectively. It could be concluded that testosterone levels could be a detrimental factor in semen assessment.

Key words: rams, testosterone, semen characteristics, sperm motility, sperm concentration

INTRODUCTION

Testosterone, the primary male hormone, is responsible for male characteristics (Seideman et al., 1982). Ram blood testosterone levels vary according to breed, nutrition level, season and age (Boland et al., 1985; Zamiri and Khodaei, 2005).

Testosterone secretion is correlated to external stimulants such as behaviour of ewes, odour of ewes and ewe estrous manifestation (Walkden-Brown et al., 1999). Both sexual behaviour (D'Occhio and Brooks, 1982; Perkins and Fitzgerald, 1992 and 1994) and pheromone production (Fulkerson et al., 1981; Signoret et al., 1982) are dependent on the action of androgens.

Ewes did not migrate to tethered rams when free rams or free 'aproned' rams were available. The number of ewes migrating was also sharply reduced when ewes were distracted by penned rams even when these rams were out of visual contact (Lindsay, 1965).

The purpose of this study was to monitor the levels of testosterone at the time of semen collection and after second semen ejaculate collection. Another aim of this investigation was to study the relationship between testosterone levels at the time of semen collection and ram semen characteristics for first and second semen ejaculates.

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Received: February 21, 2008; Accepted: May 15, 2008

MATERIAL AND METHODS

A number of 6 Rahmani rams (having 2 years of average age and 72.5 kg of average weight) were subjected to semen collection with artificial vagina (AV). The frequency of semen collection was twice per week for a period of 35 days. Rams were reared and fed in Experimental Farm of Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. Blood samples were collected two times for each ram for measuring testosterone levels by ELIZA technique (Bio Check, Inc, CA 94404, USA). First blood sample was collected after first false mounting and second blood sample was collected after second semen collection. Semen samples were subjected to the following measurements: semen volume, sperm motility %, methylene blue reduction time-test (MBR-T), sperm concentration and reaction time. Required materials for MBR-T test were as follows (after El-Mekawy, 2000):

Ram semen
Sodium Citrate 3.6 %
Egg - yolk
Methylene blue stain 1 %
Mineral oil
Water bath
Test tubes

The experimental procedure for MBR-T test was as follows:

1. A quantity of 0.8 ml of sodium citrate – egg yolk extender (88 % sodium citrate solution 3.6% + 12 % egg yolk) was added to 0.2 ml of semen sample in a test tube.
2. One drop (100 µl) of methylene blue stain (1% in water) was added and mixed carefully with sodium citrate extender.
3. Mineral oil layer (1.25 ml) was added to previous mixture and then incubated in a water bath at 43 - 46 °C.
4. Required time to change stain colour was adjusted.

Statistical analysis was carried out using SPSS 8.0 (1997) software to study the effect of time of blood sample collection on testosterone level. ANOVA test was performed to examine differences among 6 rams according to testosterone level and semen characteristics. Duncan multiple range test was used to compare different means of studied parameters. Correlations between testosterone levels and semen characteristics were examined for studied rams by using Winks SDA-Statistical Data Analysis Version 6.0.4 (Copyright 1991-2007).

RESULTS AND DISCUSSION

Experimental data showed that there were no significant differences between testosterone levels determined after first false mounting and after second semen ejaculate collection. The levels were 5.46 ± 1.01 and 6.0 ± 1.06 ng/ml blood serum respectively for 6 different Rahmani rams. On the other hand, there were significant differences between 6 rams as for testosterone concentration irrespective of time of blood sample collection as shown in Figure 1.

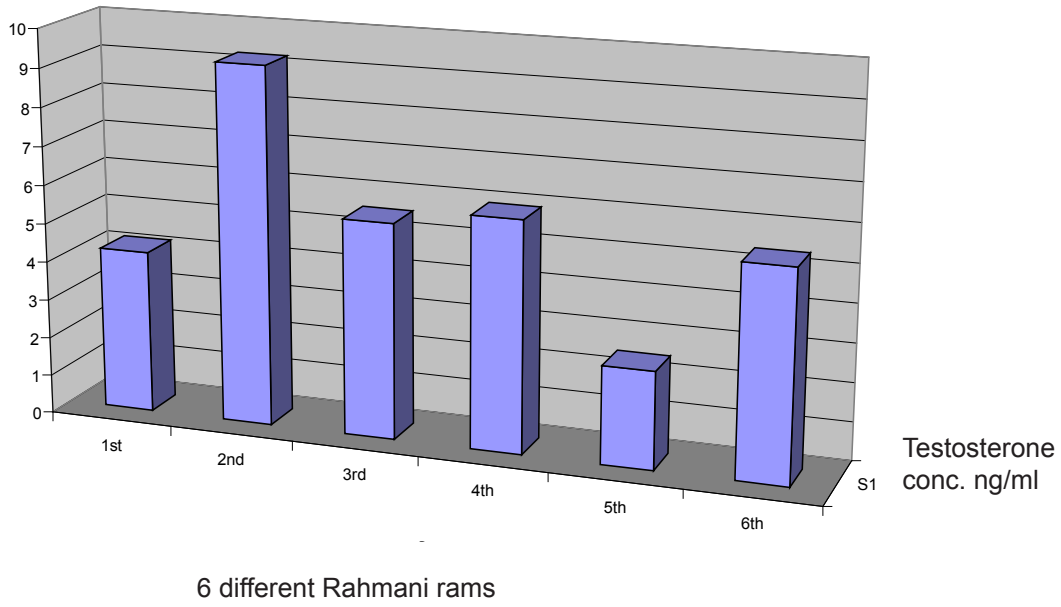

Fig. 1: Differences among 6 Rahmani rams as for testosterone levels

The second ram achieved the highest testosterone level and reached 9.2 ± 1.95 ng/ml of blood serum and differed significantly from the other rams. Testosterone (T) is involved in several male reproductive processes in rams (Johnson *et al,* 1973; Sanford *et al.,* 1974; Gomes and Joyce, 1975). Some investigators found high seasonal variation in sexual activity of rams following the exposure to an estrous female (Pepelko and Clegg, 1965; Lees, 1965). The decreased summer activity is a direct result of lowered T or a combination of environmental and physiological factors still unknown **(Schanbacher and Lunstra, 1976).** But castration to a great extent eliminates the breeding ability of rams and high levels of T or other secretory products of the testis as the main effectors of male behaviour (Clegg *et al.,* 1969). The testosterone levels in this experiment are in accordance with many authors who also studied testosterone profiles of ram serum (Whitehead and McEwan, 1973; D`Occh and Brooks, 1982; Brown *et al.*, 1984). Also, as for the effect of testosterone level semen characteristics, ANOVA test showed that there were significant differences between 6 rams in regards of sperm motility of first semen ejaculate, semen volume of first and second ejaculate, reaction time of first ejaculate and sperm concentration of first semen ejaculate but there were no significant differences in regards to MBR-T test of first and second semen ejaculate, reaction time of second semen ejaculate and sperm concentration of second semen ejaculate as shown in Table 1.

This is very recognizable from the correlations between testosterone levels and semen characteristics as shown in Table 2. These results could be explained on the basis of effects of testosterone on testes functions especially on Sertoli cells. Testosterone hormone can directly affect the process of spermatogenesis, as normal spermatozoa are directly under the influence of Sertoli cells which are responsible for sperm nourishment, division and caring as pointed out by Hafez (1993). The major action of androgens appears to be on the Sertoli cells rather than directly on the germ cells (Hafez, 1993).

It has been noticed from Table 2 that hormone testosterone correlated with semen characteristics especially sperm motility percentage, ejaculate volume,

Table1: Semen characteristics of 6 Rahmani rams for first and second semen ejaculates

Semen* characteri-stics	1st Ram	2nd Ram	3rd Ram	4th Ram	5th Ram	6th Ram
1st Reaction Time	20.60^{A} ± 4.66	27.75^{A} ± 10.06	18.00^{A} ± 5.25	19.20^{A} ± 6.53	153.33^{B} ± 76.88	17.00^{A} ± 4.36
1st Ejaculate Volume	1.80^{A} ± 0.14	2.05^{A} ± 0.09	1.92^{A} ± 0.12	1.36^{B} ± 0.11	1.23^{B} ± 0.12	1.38^{B} ± 0.12
1st Sperm Motility%	80.00^{A} ± 1.58	86.25^{B} ± 1.25	81.00^{A} ± 2.92	79.00^{A} ± 1.00	71.67^{C} ± 3.33	80.00^{A} ± 2.24
1st Sperm Conc.	3.16×10^{9A} ± 9.36	4.05×10^{9B} ± 33.56	3.16×10^{9A} ± 23.07	2.95×10^{9A} ± 25.23	2.92×10^{9A} ± 38.01	3.17×10^{9A} ± 28.17
1st MBR-T	85.00 ± 11.40	53.25 ± 5.68	58.00 ± 15.38	67.80 ± 10.68	87.67 ± 11.35	58.00 ± 4.64
2nd Reaction Time	10.60 ± 2.87	42.25 ± 8.43	27.00 ± 13.74	7.00 ± 0.81	53.33 ± 40.10	18.20 ± 2.60
2nd Ejaculate Volume	1.38^{A} ± 0.23	1.30^{A} ± 0.19	1.36^{A} ± .08	1.04^{B} ± 0.10	0.57^{B} ± 0.06	0.90^{B} ± 0.17
2nd Sperm Motility%	83.00^{A} ± 1.22	86.25^{B} ± 1.25	85.00^{A} ± 3.15	82.00^{A} ± 2.00	68.33^{C} ± 5.77	80.00^{C} ± 6.01
2nd Sperm Conc.	2.80×10^{9} ± 38.52	3.32×10^{9} ± 57.65	3.18×10^{9} ± 20.15	2.77×10^{9} ± 54.53	2.42×10^{9} ± 53.99	2.38×10^{9} ± 33.21
2nd MBR-T	99.80 ± 30.24	70.00 ± 11.73	80.00 ± 30.66	113.40 ± 28.54	130.00 ± 18.33	70.00 ± 16.50

* Means with different letters differed significantly at $P < 0.05$

Table 2: Correlations between testosterone levels and semen characteristics for first and second semen ejaculates of 6 Rahmani rams

Correlation	stSM	stSV	stRT	stSC	stMBR-T	ndSM	ndSV	ndRT	ndSC
testos	.827*	.647	-.607	.838*	-.824*	.771	.555	-.042	.866*
stSM		.726	-.931**	.785	-.739	.975**	.834*	-.49	.986**
stSV			-.524	.493	-.455	.79	.888**	.021	.657
stRT				-.609	.63	-.921**	-.78	.754	-.919**
stSC					-.771	.64	.403	-.093	.803*
stMBR-T						-.673	-.389	.079	-.826*
ndSM							.918**	-.504	.951**
ndSV								-.431	.756
ndRT									-.464
ndSC									

tes = testosterone, stSM = sperm motility of first ejaculate
stSV = semen volume of first ejaculate stRT = reaction time of first ejaculate
stSC = sperm concentration of first ejaculate stMBR-T = methylene reduction time test of first ejaculate
ndSM = sperm motility of second ejaculate ndSV = semen volume of second ejaculate
ndRT = reaction time of second ejaculate ndSC = sperm concentration of second ejaculate
* significance at $P < 0.05$ ** significance at $P < 0.01$

sperm concentration, reaction time and methylene blue reduction time test for both first and second ejaculates. According to previous reports (Colas *et al*., 1986; Langford *et al*., 1989), the amplitude of seasonal changes in testicular size is closely related to that observed in gonadal functions for some breeds. In adult rams, the increased spermatogenesis efficiency may be related to the establishment of anatomo–histological maturity, i.e., to an increase in the number of round spermatids from which the spermatozoa are originating as well as in the capacity of the epididymal maturation (Salmon *et al*.,1984; Chevrier and Dacheux, 1988). The highest correlation was observed between testosterone level and sperm motility percentage for first and second ejaculate and sperm concentration for first ejaculate and these correlations were .827, .771, .838 and .866, respectively. In addition, high libido (as represented by reaction time) according to high testosterone levels, was found and that the reaction time negatively highly correlated to testosterone level as pointed out in Table 2. The increased serving capacity could be linked to establishment of an optimal relationship between the hypothalamo–pituitary axis and the gonadal level as reported by Mandiki and co-workers (1998). Also, correlations were high and negative between reaction time and sperm motility percentage as shown in Table 2.

It could be concluded that testosterone levels are good markers for semen quality and production. Also, reaction time is highly correlated to testosterone levels. Effects of testosterone level correlate to semen quality and quantity. This study shows that high testosterone levels continue for a period of 15 minutes between successive semen ejaculates.

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