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Blood Testosterone Level in Iranian Buffalo Bulls and its Relation with Semen Freezability

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Abstract: The objective of this study, was to evaluate testosterone profiles of serum in buffalo bulls and to examine its correlation with semen characteristics. Semen of 10 buffalo bulls were collected by a bovine artificial vagina. Semen characteristics such as (motility, morphology, viability and concentration) were recorded. A part of the semen sample (1 mL) was diluted by tris-egg yolk-glycerol extender, packed in French straws and was frozen in liquid nitrogen. The straws were later thawed and semen characteristics were compared with those of the fresh semen. The blood samples were collected in vacutainers without anticoagulant. After 1 h at room temperature, the samples were centrifuged at 2000 rpm for 20 min and then the sera were collected, immediately frozen and thereafter kept at -20°C until the hormone assay, performed by RIA. The results showed the overall Mean±S.E. blood testosterone concentration was 1.3±0.9 ng mL^{-1}. Blood serum testosterone was correlated with progressive motility and viability of the fresh and frozen-thawed semen. Progressive motility, viability and abnormal sperm morphology of frozen-thawed semen were highly correlated with mentioned above parameters in the fresh semen. Testosterone serum concentrations in buffalo bulls are similar to those reported in other animal species and have some correlations with semen characteristics before and after freezing.

Key words: Testosterone, semen freezability, buffalo bulls

INTRODUCTION

Testosterone secretion in most mammalian species is episodic which reflected the pulsatile release of GnRH from the hypothalamus and corresponding pulsatile release of LH from the anterior pituitary. Also, testosterone is the hormone responsible for spermatogenesis and sexual behavior, thus, the seasonal pattern of testosterone secretion could limit the male reproductive efficiency during some periods of the year. The reproductive activity of buffalo cows in Iran is considered season linked and under photoperiodic control, as can be inferred from the unequal distribution of the spontaneous sexual activity and of the births throughout the year. In extensive, traditional breeding the cyclic ovarian activity is mainly high in autumn, while during spring and early summer it is very low (Zicarelli, 1994; Alavi-Shoushtari and Babazadeh-Habashi, 2006). The available data on the endocrine activity in buffalo bulls are notably scarce but, as in other species, a reproductive seasonality seems to exist in the buffalo males: seasonal variations of libido, sexual performance, testis weight, semen characteristics, testosterone levels were repeatedly reported, but the presented data often offer marked discordances, probably due to the different breeds and environment (Sengupta *et al*., 1963; Singh and Singh, 2000). The ability to determine the testosterone producing capacity in the testis has important implications for the assessment of male fertility (Malfatti *et al*., 2006).

However, there is a little information available regarding to buffalo testosterone levels in blood serum. This study was carried out to assess the testosterone profile of the buffalo by using RIA and to investigate a possible relationship between testosterone of serum and the freezability of the spermatozoa in buffalo bulls.

MATERIALS AND METHODS

Animals: Semen samples were collected by a bovine artificial vagina from 10 sexually mature buffalo bulls (4-5 years old) in Urmia from The Buffalo Breeding Center northwest of Iran. Four semen samples obtained from each buffalo bull at different seasons during a period of 2005-2006.

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Semen evaluation: Immediately after collection, the ejaculate was placed in a 37°C water bath and the volume was recorded. The percentage of progressively motile spermatozoa was estimated by microscopic examination at 400× magnification on a pre-warmed slide (37°C) and a subjective assessment of the progressive status was recorded according to procedure of Ax *et al.* (2000). Sperm concentration was measured using standard hemocytometer methods (Hausser Scientific, Horsham, PA. USA), the percentage of viable spermatozoa was estimated by viewing 200 spermatozoa under 400× magnification using eosin-aniline blue staining method of Ax *et al.* (2000). One mL of the ejaculate of each bull was diluted in tris-egg yolk-glycerol extender, kept refrigerated for 18 h, packed in French straws and the straws were frozen in liquid nitrogen (-196°C) according to the routine methods in the center. The straws were later thawed in 37°C water bath for 30 sec. Semen characteristics were evaluated as mentioned before and the results compared with those of the frozen semen.

Hormone measurement: The blood samples were collected of jugular venipuncture without anticoagulant. After 1 h at room temperature, the samples were centrifuged at 2000 rpm for 20 min, the sera were collected, immediately frozen and thereafter kept at -20°C until the hormone assay, performed by RIA. After double diethyl ether extraction (500 mL sample and 3 mL diethyl ether) and addition of assay buffer (1 mL), concentration of testosterone was determined by an assay kit (TRK 600, Amersham Pharmacia Biotech UK Limited) for human or animal species plasma or serum, combining a tritiated hormone and a specific antibody for testosterone and 5α-dihydrotestosterone (respective cross reactivity 100 and 45-50%). All determinations were done in duplicate.

Data analysis: Data analysis was performed using SPSS software (SPSS version 11.5 for Windows; SPSS Inc., Chicago, IL, USA) computer program. Results are quoted as arithmetic Mean±standard error (S.E.) and significance was attributed at $p<0.05$ and $p<0.01$. Pearson's correlation coefficient (2-tailed) test was used to examine the correlation between all the parameters of the semen and the correlations of testosterone with all the parameters of the semen were tested by multiple linear regression test.

RESULTS

The results of the semen evaluation of 10 buffalo bulls are summarized in Table 1 and depicted as

Table 1: Semen characteristics of the buffalo bulls

Number of bulls (n)	10
Ejaculate volume (mL)	3.7±1.400
Sperm concentration ($\times 10^6$ cells mL^{-1})	1121.0±167.0
Progressive motility	
Fresh (%)	70.0±8.5[a]
Frozen-thawed	59.6±7.1[b]
Abnormal morphology	
Fresh (%)	15.9±4.7[a]
Frozen-thawed (%)	23.6±5.8[b]
Viability	
Fresh (%)	75.8±9.8[a]
Frozen-thawed (%)	59.8±6.2[b]

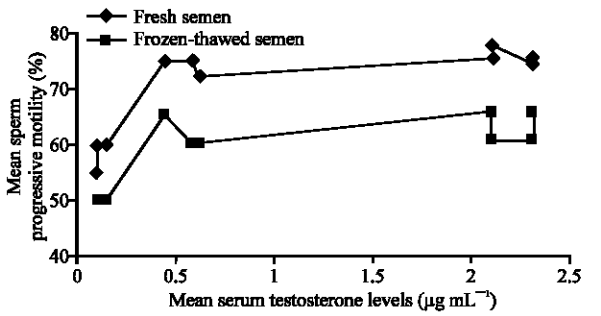

Fig. 1: Changes of the sperm progressive motility in relation to serum testosterone level

Mean±S.E. The mean values obtained for progressive motility, abnormal morphology and viability of fresh semen was highly significantly different with those of the frozen-thawed semen ($p<0.001$). Progressive motility ($r = 0.504$, $p<0.01$) and viability ($r = 0.435$, $p<0.01$) of frozen thawed semen were highly correlated with the motility and viability of the fresh semen and its abnormal morphology was correlated ($r = 0.611$, $p<0.01$) with those of the fresh semen.

Mean values denoted by letters (a and b) are different in paired groups ($p<0.05$). All values are Mean±S.E.

The overall mean±S.E.M. concentration of blood testosterone was 1.3±0.9 ng mL^{-1}. Blood serum testosterone were correlated with progressive motility and viability of the fresh and frozen-thawed semen $r = 0.475$, $p<0.01$ and $r = 0.414$, $p<0.01$, respectively (Fig. 1).

DISCUSSION

The relationship between testosterone blood levels and libido or semen quality in buffalo bulls is not obvious (Dixit *et al.*, 1985; Gupta *et al.*, 1984) but low testosterone levels have been a suggested cause of scarce libido in buffalo males (Sharma *et al.*, 1986; Bugalia *et al.*, 1998; Afiefy *et al.*, 1984) managed to stimulate the bulls' libido by exogenous testosterone injection. Furthermore, a high

positive correlation between serum testosterone levels and libido score was found by Taha *et al.* (1984), Tuli *et al.* (1991) and Javed *et al.* (2000) reported a positive correlation between seminal plasma testosterone concentration and higher quality semen characteristics in this species. Our data seem to suggest that testosterone secretion in buffalo bulls throughout the year is season related and probably linked to environmental influences other than that of photoperiodism (such as nutrition, temperature, air humidity) but it is clear that a major stimulus on the sexual endocrine axis is exerted by the contact with females. However, a little information is available regarding the buffalo testosterone serum. This study was designed to get some information in this field. In this study, the semen characteristics and the records of freezability of the spermatozoa obtained from 4 samplings in different seasons were available (Table 1), the seasonal variations in buffalo bull semen characteristics had been studied previously (Alavi-Shoushtari and Babazadeh-Habashi, 2006) so, were not mentioned here. In our study, statistical analyses showed that blood serum testosterone was correlated with progressive motility and viability of the fresh and frozen-thawed semen ($r = 0.475$, $p<0.01$ and $r = 0.414$, $p<0.01$, respectively).

The overall Mean±S.E. blood testosterone concentrations in this study, were 1.3±0.9 ng mL^{-1}. The lower testosterone level observed in this study, although, not significantly different from the other reported and it suggest that seasonal factors may have affected the control of the gonadal activity in buffalo males. It is well known that environmental factors (such as climate, food intake an quality) exert substantial effects on male gonadal activity in other seasonal species, such as sheep (Gupta *et al*., 1984). Malfatti *et al.* (2006), reported the profiles of the blood testosterone during the year appear to be linked with season, with lower level recorded in autumn-winter compared to values in spring-summer. Moreover various authors observed better semen characteristics in spring than in autumn or winter months (Manikn *et al*., 1989) even though, Sengupta *et al.* (1963) had partially contrasting results, with better quality of semen in autumn than in summer. In data seem to suggest that testosterone secretion in buffalo bulls throughout the year is season related and probably linked to environmental influences other than that of photoperiodism (such as nutrition, temperature, air humidity).

CONCLUSION

When serum testosterone concentrations were higher in buffalo bulls semen volume, sperm concentration and frozen-thawed sperm viability and progressive motility were higher.

REFERENCES

Alavi-Shoushtari, S.M. and B. Babazadeh-Habashi, 2006. Seasonal variations in the characteristics of the Azarbaijani buffalo (*Bubalus bubalis*) semen. Iran J. Vet. Res., 7: 49-54.

Afiefy, M.M., A.A. Idris and K. Zaki, 1984. Seasonal variations in the volume of the ejaculate in buffalo and Friesian bulls with special reference to the role of Vitamin A and testosterone. In: 10th international congress on animal reproduction and artificial insemination, Urbana, Illinois (USA), 2 (1-2): 49.

Ax, R.L., M.A. Dally, R.W. Lenz, C.C. Love, D.D. Varner, B. Hafez and M.E. Bellin, 2000. Semen Evaluation. In: Hafez, B. and E.S.E. Hafez (Eds.). Reproduction in Farm Animals. 7th Edn. Lippincott Williams and Wilkins, Philadelphia, pp: 365-373.

Bugalia, N.S., B.R. Inderjeet, S.L. Gulati Garg and S.K. Verma, 1998. Thyroid functions, testosterone profiles and preputial microbes in cow and buffalo bulls. Indian. J. Anim. Sci., 68: 341-343.

Dixit, N.K., S.P. Agarwal V.K. Agarwal and P.H. Dwarkanhath, 1985. Seasonal variation in serum levels of steroid hormones and their relation with seminal quality and libido in buffalo bulls. Theriogenology, 24: 293-303.

Gupta, R.C., A.K. Sharma and N.K. Khurana, 1984. Testosterone levels and libido in buffalo-bulls. In: 10th International Congress on Animal Reproduction and Artificial Insemination, Urbana, Illinois (USA), 2 (1-3): 271.

Javed, M.T., Ahrar-Khan and Mumtaz-Ali, 2000. Influence of season on seminal plasma testosterone and oestrogen in healthy and abnormal buffalo bulls and their relationship with other semen parameters. Veterinarski. Arkiv., 70 (3): 141-149.

Malfatti, A., O. Barbato, L. Todini and G.M. Terzano, 2006. Blood testosterone levels in Italian buffalo bulls managed in 2 different breeding conditions. Theriogenology, 65: 1137-1141.

Manikn, R.S., A. Srivastava and W.D. Mudgal, 1989. Seasonal changes in the digestibility and seminal attributes in buffalo bulls fed different levels of dietary protein. Indian J. Dairy Sci., 59: 1177-1180.

Sharma, I.J., S.P. Agarwal, S.P. Shukla and P.K. Dwaraknath, 1986. Comparative study of hormonal and seminal characteristic of cross-bred bulls and buffalo bulls. Indian Vet. J., 63: 636-638.

Sengupta, B.P., M.S. Misra and A. Roy, 1963. Climatic environment and reproductive behavior of buffaloes. I. Effect of different seasons on various seminal attributes. Indian J. Dairy Sci., 16: 150-165.

Singh, P. and N. Singh, 2000. Effect of multiple showering and artificial aeration on sexual behaviour, quality and freezability of buffalo bull semen during hot-humid season. Indian J. Anim. Sci., 70: 914-916.

Taha, A.H., A. Heshmat and S.M. Sharawy, 1984. Peripheral serum luteinizing and testosterone concentration in buffalo bulls and its influence on libido. In: 10th International Congress on Animal Reproduction and Artificial Insemination, Urbana, Illinois (USA), 3 (1-3): 294.

Tuli, R.H., I.S.R.L. Lohan and S.P. Goyal, 1991. Single Testosterone and progesterone hormones in buffalo bull seminal plasma and their correlations with seminal characteristics. Indian J. Dairy Sci., 44: 587-589.

Zicarelli, L., 1994. Management in different environmental conditions. Buffalo. J. Sup., 2: 17-38.