The Indian Journal of Animal Reproduction; 28(2): 52-58; Dec. 2007 **Research Article**

Studies on micro and macro mineral profiles of seminal plasma of Tarai buffalo bulls[1]

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ABSTRACT

A total of 60 semen ejaculates were collected from six Tarai buffalo bulls using sterilized artificial vagina twice weekly and were evaluated as per standard procedures for the routine tests. Seminal plasma was separated by centrifugation. The various macro and micro mineral constituents of the seminal plasma were analyzed. Mmanganese, iron, copper, cobalt and zinc averaged 0.029±0.002, 2.85±0.16, 0.031±0.002, 0.177±0.019 and 2.54±0.05 mg/dl, respectively. The macro minerals, *viz.* sodium, potassium, calcium and phosphorus averaged 272.85±9.84, 75.78±3.23, 42.01±0.01and 7.55±0.16 mg/dl, respectively. On the basis of present investigation, it may be concluded that micro and macro mineral constituents in Tarai buffalo bulls were in the normal rang.

Key words: Tarai buffalo, Seminal Plasma, Macro and Micro Mineral.

INTRODUCTION

The buffaloes are the backbone of Indian farmer's economy and Indian dairy Industry. In spite of having world's largest buffalo population, India has only nine well recognized and documented breeds of buffalo. Most of the nondescript buffalo populations are unique in their adaptation to the local agro climatic conditions. The Tarai buffalo is one of such buffalo breeds that has adopted and is thriving under stressful climatic conditions of the Tarai region. For multiplication of superior germ plasm of Tarai buffaloes, good quality frozen semen would be need of the hour. The knowledge on micro and macro mineral constituents of seminal plasma of the Tarai buffalo bull would help in formulation of suitable dilutor for production of good quality deep frozen semen. In the light of above facts, the present study was undertaken to study various micro and macro mineral profiles of semen of Tarai buffalo bull.

MATERIALS AND METHODS

The experimental animals comprised of six Tarai buffalo bulls aged 4 to 6 years. They were maintained under standard managemental conditions. The semen samples were collected using sterilized artificial vagina from the bulls twice weekly. Soon after collection, the semen samples were evaluated for routine tests and only good quality semen samples were used for the study. Seminal plasma was separated by centrifugation at 3000 rpm for 10 minutes. The separated plasma samples were collected in micro centrifuge tubes and were stored in deep freezer at -20°C for further biochemical analysis.

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Estimation of sodium and potassium:

The sodium and potassium concentration in the seminal plasma were estimated using flame photometer. The stock standard solutions (NaCl-11.68 gm and KC1-0.746 gm in 1000 ml triple glass distilled water) were prepared containing sodium and potassium concentration of 200 meq/lit and 10 meq/lit, respectively. The stock standard solutions were further diluted 1:100 with triple glass distilled water. The diluted stock standard solutions were further diluted to obtain a series of working standard solutions of Na and K, respectively, as given below:

Diluted stock standard solution (ml)	Triple glass distilled water (ml)	Effective concentration in mEq/Lit. equivalent to diluted seminal plasma	
		Na^+	K^+
200	-	200	10
160	40	160	8
120	80	120	6
80	120	80	4
40	160	40	2

The seminal plasma samples were diluted with triple glass distilled water (1:100) as that of standard and were aspirated in flame photometer. The concentrations of Na and K were expressed in meq /lit of seminal plasma and finally converted in to mg/dl.

Estimation of Copper, Zinc, Iron, Manganese, Cobalt and Calcium:

The seminal plasma samples were digested first by nitric acid and then by triple acid mixture for the estimation of copper, zinc, iron, manganese, cobalt and calcium. For digestion, 5 ml of concentrated nitric acid was added to test tube containing one ml of seminal plasma. The tube was kept over hot plate for 30 min. or till the sample volume reduced to one ml. After cooling, 5 ml of triple acid mixture (nitric acid, perchloric acid and sulphuric acid in the ratio of 10:4:1) was added into it. It was again kept on hot plate till the content reduced to one ml. After cooling, triple glass distilled water was added to make final

Element	Max. Lamp Current (mA)	Wave length (nM)	Slit setting (nM)	Flame type
Copper	3.0	324.7	0.5	Air- C_2H_2 (Oxidizing)
Zinc	5.0	213.9	0.5	Air- C_2H_2 (Oxidizing)
Iron	7.0	248.30	0.2	Air- C_2H_2 (Oxidizing)
Manganese	5.0	279.50	0.2	Air- C_2H_2 (Stoichiometric)
Cobalt	6.0	240.70	0.2	Air- C_2H_2 (Oxidizing)
Calcium	10.0	422.7	0.5	Nitrous Oxide- C_2H_2 (Oxidizing)

volume as 10 ml. The concentration of copper, zinc, iron, manganese, cobalt and calcium were estimated by atomic absorption spectrophotometer (GBC-Avanta Sigma) using following setting.

Statistical analysis:

Data obtained during the study were statically analyzed by standard statistical procedures (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

The biochemical composition of seminal plasma was studied in a total of 60 ejaculates, 10 from each of six Tarai buffalo bulls. The semen was of good quality as evidenced by progressive sperm motility (79±1.79 to 87.00±1.52), livability (90.10±1.26 to 94.00±0.85) and abnormality (5.50±0.34 to 8.00± 0.57). The results are summarized in Table.

Sodium concentration in the seminal plasma of Tarai buffalo bull in the present study was 272.85±9.84 mg/dl, which is fairly comparable to the finding of Dhami and Sahni (1993; 295.11 mg/dl) in Murrah bulls. However, higher concentration of 326.09 mg/dl (Gupta *et al.,* 1983) in Murrah bulls and lower concentration of 186.89 mg/dl (Singh *et al.,* 1970) and 196.07mg/dl (Dhami *et al.,* 2002) in Jafarabadi bulls is also reported. Potassium concentration in the seminal plasma of Tarai buffalo was 75.78±3.23 mg/dl. It is relatively comparable to the findings of Dhami *et al.* (1990; 94.56 mg/dl) in Surti buffalo and Dhami and Sahni (1993; 96.22 mg/dl) in Murrah bulls. However, other workers reported higher concentration of 202.28 mg/dl (Reddy and Raja, 1979), 175.52 mg/dl (Gupta *et al.,* 1983) in Murrah bulls and 132.44 mg/dl (Dhami *et al.,* 2002) in Jafarabadi bulls.

Sodium and potassium together with the anions are chiefly responsible for the maintenance of the proper osmotic conditions in seminal plasma, which provides an external milieu for maintaining the functional integrity of the spermatozoa (Nath, 1988). The increased ratio of Na:K and lower potassium content in bovine semen was found to be associated with better quality and freezability of the semen (Mohan *et al.,* 1992). It was found that the narrow Na:K ratio and higher potassium content in buffalo semen were associated with the lower semen quality, freezability and fertility as compared to cow bull semen (Dhami *et al.,* 1990). Dhami *et al.* (1987) in Surti buffalo and Dhami and Kodagali (1988) in HF bulls recorded that the increased ratio of Na:K and low potassium content in the semen was associated with increased freezability and fertility of frozen semen. The high levels of potassium inhibits sperm metabolism due to change in the Na:K ratio rather than potassium alone. The variations from earlier reports may be due to different agro-climatic conditions (Sinha *et al.,* 1966), season of study (Reddy and Raja, 1979) and breed. The significant variation among the bulls in the present study might be due to age (Saxena and Tripathi, 1983) and genetic makeup of the bulls (Nath, 1988).

Calcium concentration in seminal plasma of Tarai buffalo bull in the present study averaged 42.01±0.01 mg/dl, which is well comparable to the findings of Singh *et al.* (1970; 43.45 mg/dl) and Reddy and Raja (1979; 41.11 mg/dl) in Surti buffalo. However, higher level of calcium in the seminal plasma was reported by Chaudhary and Gangwar (1977; 53.0 mg/dl) and Gupta *et al.* (1983; 79.13±19.48 mg/dl) in Murrah bulls and lower concentration by Shelke and Dhami (2002; 14.40 mg/dl) in Jafarabadi bulls. Higher calcium content in semen is reported to have depressing effect on sperm metabolism (Mann and Lutwak-Mann, 1981). However, Pangawkar *et al.,* (1988) reported that calcium content in the ejaculates of different freezability groups did not differ significantly.

Phosphorus content in the seminal plasma of Tarai buffalo averaged 7.55±0.16 mg/dl, which agreed well with the finding of Singh *et al.,*(1970; 7.56 mg/dl). However, higher level of phosphorus in the

seminal plasma is reported by Chaudhary and Gangwar (1977; 12.1 mg/dl), Gupta *et al.*, (1983; 14.43 mg/dl) and lower concentration by Shelke and Dhami (2002; 5.85 mg/dl) in Jafarabadi bulls. The level of phosphorus is vital for semen preservation. It was found to be associated with higher metabolic activity and inherent capacity of spermatozoa for higher glycolytic and respiratory activity (Dabas *et al.*, 1984). These variations from earlier reports in the present study may be due to differences in breed (Shelke and Dhami, 2002), season of study (Reddy and Raja, 1979) and agro-cumatic conditions (Bhosreker, 1980; Saxena and Tripathi, 1983). The significant variation in calcium concentration among the bulls observed in the present study might be due to individual variation of bulls (Saxena and Tripathi, 1981) and age of the bulls (Tripathi and Saxena, 1983).

Micro-minerals

Concentration of manganese in seminal plasma of Tarai buffalo averaged 0.029±0.002 mg/dl. It is fairly comparable to the finding of Shelke and Dhami (2002; 0.021 mg/dl). However, higher level of 0.289 mg/dl is also reported (Dhami *et al.*, 1994). Manganese is involved in partial regulation of oxidative phosphorylation (Swenson, 1970). It is essential for spermatogenesis. Low level of manganese is associated with the poor quality semen (Goswami *et al.*, 1993). Thus, it may be concluded that manganese improves the semen quality in buffalo bulls. The variations in manganese content between present finding and earlier reports might be due to variations in breed, season and ambient temperature at the time of study (Goswami *et al.*, 1993). Gangwar and Bahga (1979) also recorded seasonal and individual variation of . manganese concentration in the seminal plasma. Iron concentration in seminal plasma of Tarai buffalo bull averaged 2.85±0.16 mg/dl, which is comparable to the finding of Dliami *et al.* (1994; 2.983 mg/dl).

However, other workers reported lower iron content of 1.94 mg/dl (Singh and Gangwar, 1977), 1.986 mg/dl (Bhavsar, *et al.*, 1989) and 0.477 mg/dl (Shelke and Dhami, 2002) in the seminal plasma. Iron in semen stimulates oxidative reduction of metabolites of spermatozoa (Mann and Lutwak-Mann, 1981). The ambient temperature significantly affects the iron concentration in the seminal plasma (Goswami *et al.*, 1993).

Mean level of copper content in the seminal plasma of Tarai buffalo was 0.031±0.002 mg/dl, which is fairly comparable to the finding of Shelke and Dhami (2002; 0.028 mg/dl) in Jafarabadi bulls. Other workers reported higher concentration of 0.061 mg/dl (Gupta *et al.*, 1983), 0.085 mg/dl (Dhami *et al.*, 1994) and 0.271 mg/dl (Bhavsar *et al.*, 1989). Copper, present in the seminal plasma had inhibitory effect on the spermatozoan activity (Singh *et al.*, 1970). It varied significantly with breed and season (Goswami *et al.*, 1993).

Cobalt concentration in the seminal plasma of Tarai buffalo bull in the present study averaged 0.177±0.019 mg/dl. However, Shelke and Dhami (2002) reported lower cobalt concentration of 0.037 mg/dl in the seminal plasma of Jafarabadi bulls. Information on cobalt content in buffalo semen is not sufficient and more investigation is needed. Zinc concentration in the seminal plasma of Tarai buffalo bull in the present study averaged 2.54±0.05 mg/dl, which is fairly comparable to the finding of Gupta *et al.* (1983; 2.68 mg/dl). However, other workers reported higher concentration of 3.01 mg/dl (Singh and Gangwar, 1977), 3.36 mg/dl (Bhavsar *et al.*, 1989) and 5.79 mg/dl (Dhami *et al.*, 1994) and also lower concentration of 1.094 mg/dl (Shelke and Dhami, 2002) in the seminal plasma. Zinc content in semen showed significant seasonal and breed variation (Goswami *et al.*, 1993). It is essential for normal testicular function and in its deficiency; testicular spermatogenic and endocrine functions suffer (Mann and Lutwak-Mann, 1981). On the contrary, high zinc level has detrimental influence on sperm development and morphology, which is discernible from positive correlation of zinc level with total sperm abnormalities in the present study. A poor semen quality production and a lowered testosterone level were associated

Table: Various macro and micro mineral constituents' profiles of seminal plasma of Tarai buffalo bulls

S.No.	Constituents (mg/dl)	Bulll	Bulll	Bull3	Bull 4	BullS	Bull 6	Overall
1	Na	257.85^{h}±25.27	237.86^{31}±7.45	206.67^{bcd} ±15.63	304.24^{bl} ±23.93	295. 18^{C} ±16.64	335.29^{adh} ±27.89	272.85 ±9.84
2	K	68.05^{h}±7.15	80.30 ±5.60	73.78 ±5.46	61.93^{3}±3.27	91.22^{ah}±11.04	79.40 ±10.40	75.78±3.23
3	Ca	42.10^{abc} ±0.02	41.98^{3} ±0.02	42.06^{de} ±0.04	42.06^{ts} ±0.03	41.94^{bdl} ±0.02	41.93^{ceg} ±0.04	42.01 ±0.01
4	P	7.62±0.27	8.26±0.27	7.67 ±0.25	7.19 ±0.40	7.50 ±0.75	7.10 ±0.26	7.55 ±0.16
5	Mn	0.031 ±0.007	0.037 ±0.007	0.027 ±0.006	0.025 ±0.001	0.024 ±0.001	0.031 ±0.002	0.029 ±0.002
6	Fe	2.24^{h} ±0.29	3.14 ±0.28	3.53^{h} ±0.57	2.67 ±0.26	2.85 ±0.38	2.69 ±0.44	2.85 ±0.16
7	Cu	0.026^{3} ±0.003	0.031^{b} ±0.004	$_{0048}$abcde ±0.008	0.028^{C} ±0.001	0.026^{d} ±0.001	0.027^{e} ±0.001	0.031 ±0.002
8	Co	0.037^{ah} ±0.006	0.043^{1C} ±0.007	0.079^{bhl} ±0.011	0.181^{abc} ±0.011	0.295^{abc} ±0.014	0.425^{abc} ±0.017	0.177 ±0.019
9	Zn	2.59 ±0.09	2.44 ±0.09	2.56 ±0.09	2.41 ±0.37	2.69 ±0.06	2.55 ±0.17	2.54 ±0.05

Means bearing common superscripts within a row differ significantly (a to g; P<0.01 and h to i; P<0.05)

with lower zinc concentration in blood and seminal plasma (Goswami *et al.*, 1993).

Trace elements play important role in metabolic activities by acting as co-factors in various enzyme systems. Their levels did not show any definite trend with semen quality or other biochemical constituents of seminal plasma in the present study. Shelke and Dhami (2002) also did not find any definite association between trace elements and semen quality and freezability in Jafarabadi bulls.

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