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Short Communication

Trace minerals and freezability of cross-bred bull semen*

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ABSTRACT

In the present study the effect of trace elements of semen on freezing from four good and four poor freezable crossbred bulls were investigated. The mean concentration of copper (μg/ml), cobalt (μg/ml), zinc (μg/ml) and iron (μg/ml) in the neat seminal plasma of good freezable semen was 0.874±0.092, 0.481±0.038, 10.519±0.648 and 48.98±3.09, whereas the corresponding concentration in the post-thaw semen samples was 0.084±0.011, 0.128±0.039, 2.282±0.241 and 15.73±1.06, respectively. Similarly, the concentration of the above trace minerals in the poor freezable bull semen was estimated to be 0.629±0.071, 0.341±0.037, 5.065±0.446 and 45.45±2.60 in neat and 0.066±0.009, 0.103±0.023, 1.993±0.251 and 19.00±1.01 in post-thaw semen samples. Highly significant difference ($P < 0.01$) was observed between the semen samples of good and poor freezable bulls with respect to zinc, whereas the difference with respect to copper and cobalt was significant ($P < 0.05$) in the neat state. The post-thaw concentration of only iron registered significant difference between the good and poor freezable semen samples.

Key words : Freezability, crossbred, trace minerals

The deficiency of various minerals including the trace elements can lead to reproductive failures in males. The elements are known to function as co-factors, as activators of enzymes or stabilizer of secondary molecular structure. Yet very little is known about the level and importance of these elements in bovine semen. In the present investigation the level of certain microelements in crossbred bull semen have been studied to correlate with freezability and conception rate.

The present study was carried out on twenty crossbred bulls (Red Sindhi x Jersey) of 50 per cent exotic inheritance maintained at Frozen Semen Bank, Khapuria, Cuttack under standard manage mental and nutritional status. Semen collected from 20 bulls was subjected to cold shock resistance test to identify the good and poor freezable semen and the percentage of spermatozoa surviving after cold shock was studied according to Lasely *et al.* (1942) in 80 semen samples. All the 80 samples from 20 bulls were subjected to freezing in 0.5 ml French straws in Tris-Fructose-Yolk-Glycerol (Davis *et al.*, 1963) following the standard protocol.

After 24-hr storage in liquid nitrogen, the straws were thawed at 37°C for 30 seconds. The post thaw sperm motility, motility of sperm after 1-hr incubation at 37°C and post thaw livability was studied to identify the good and poor freezable bull semen. This criterion was employed for selecting four bulls of poor freezability (PTM < 50%) and four bulls of good freezability (PTM >50%) for subsequent investigation.

Semen was collected once in a week, from each of the four poor and four good freezable bulls with at least six ejaculates from each of the eight bulls were taken under the present course of investigation. After routine semen evaluation (Zemjanis, 1970) the semen was splitted into two parts. One part was centrifuged at +4°C (2200 g) to remove the seminal plasma and stored at -20°C for estimation of trace minerals. The other part was immediately processed for freezing in French straws and stored in liquid nitrogen. After storage in liquid nitrogen for a period of 1 month, the straws were thawed at 37°C for 30 sec and assessment of seminal characteristics were done (Bhavsar *et al.*, 1989).

During the determination of trace minerals, at least 10 straws from each batch after thawing were taken in a centrifuge tube and post thaw seminal plasma was collected by centrifugation at 2000 rpm for 30 min (2200 g) at +4°C. The seminal plasma of each bull, neat and post thaw was wet digested as per the procedure prescribed by Kolmer *et al.* (1951). The digested samples were then diluted to 5 ml with triple distilled water. A total of 96 samples were digested for estimation of trace minerals. The concentration of copper

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(Cu), cobalt (Co) and zinc (Zn) were estimated in the digested samples by using atomic absorption spectrophotometer (ECIL) at the wave length of 324.8, 240.7 and 213.9 nm and at lamp currect of 6, 7 and 7 ma, respectively after suitable dilution. The concentration of iron was estimated by spectrophotometer method as per the procedure laid down by Sandell (1959).

The mean values of trace minerals viz., copper, cobalt, zinc and iron in the seminal plasma of good and poor freezable bulls in neat and after freezing are presented in Table 1. The level of trace elements in good freezable bull semen was significantly higher for copper ($P < 0.05$), cobalt ($P < 0.05$) and zinc ($P < 0.01$) in neat state whereas the iron level was significantly higher ($P < 0.05$) with respect to post thaw concentration in poor freezable bull semen.

The overall mean copper content in the seminal plasma of good freezable and poor freezable semen were 0.874±0.092 and 0.629±0.071 μg/ml, respectively which concurs with the findings of Misra *et al.* (1986) but contradicts with Bhavsar *et al.* (1989) and Misra *et al.* (1989). This might be due to the effect of seasons or breed types as observed by Goswami *et al.* (1993). The concentration of copper in good freezable semen had significant negative correlation with mass activity (-0.70), initial motility (-0.87), initial live sperm (-0.75) and post thaw abnormal sperm (-0.92) which might be responsible for maintaining functiopnal moieties of the sperm membrane through its action on sperm structural integrity. As copper is an important constituent of enzymes tyrosinase, Uricase etc. and acts as a catalyst in oxidation of sulph-hydral group (Wiesner, 1968), so this might have contributed for maintaining the sperm viability and motility during the process of freezing and preservation in the good freezable bull semen.

The concentration of cobalt in the semen of good and poor freezable bull was 0.481±0.038 and 0.341±0.037 μg/ml with the difference between the groups of bulls, being significant ($P < 0.01$) in the good freezable bulls, the cobalt concentration had significant positive correlation with semen volume (0.87), whereas negative correlation was obtained with mass activity (-0.82), initial live sperm (-0.90), post thaw motility (-0.82) and post thaw live sperm (-0.86). The lower cobalt concentration in bull semen might have a beneficial effect as regards to pre and post thaw saminal characteristics.

The level of zinc in the good freezable and poor freezable bull neat seminal plasma was estimated to be 10.519±0.648 and 6.065±0.446 μg/ml which is higher than those reported by Singh and Gangwar (1977) and Pangawkar

Table 1. Trace elements in semen (Mean±SE) of good and poor freezable crossbred bulls before freezing and in post thaw samples

Bull No./ Trait	Copper (mcg/ml)		Cobalt (mcg/ml)		Zinc (mcg/ml)		Iron (mcg/ml)	
	Neat semen	Post thaw	Neat semen	Post thaw	Neat semen	Post thaw	Neat semen	Post thaw
Good freezable bull semen								
X-145	0.484±0.070	0.065±0.008	0.482±0.059	0.075±0.013	7.365±0.528	2.102±0.359	48.75±4.47	11.22±1.64
C-73	0.681±0.135	0.049±0.006	0.458±0.027	0.062±0.011	9.920±1.250	3.123±0.758	50.10±9.18	14.15±1.59
IX-59	0.910±0.124	0.147±0.034	0.508±0.118	0.244±0.152	12.705±1.148	1.987±0.576	42.17±3.47	15.67±1.42
C-3627	1.422±0.133	0.073±0.006	0.477±0.088	0.131±0.029	12.087±1.032	1.925±1.032	54.87±6.59	21.91±1.13
Overall	0.874±0.092^{Ca}	0.084±0.011^{b}	0.481±0.038^{Ca}	0.128±0.039^{b}	10.519±0.648^{Aa}	2.282±0.241^{b}	48.98±3.09^{a}	15.73±1.06^{Cb}
Poor freezable bull semen								
X-208	0.496±0.139	0.099±0.025	0.519±0.089	0.151±0.088	5.672±0.862	1.660±0.275	36.40±7.06	20.45±1.75
X-180	0.916±0.145	0.083±0.08	0.383±0.034	0.053±0.004	3.940±0.493	2.815±0.523	59.91±4.04	21.46±2.70
X-185	0.682±0.129	0.033±0.004	0.228±0.053	0.073±0.019	6.062±1.009	1.865±0.779	41.70±3.95	14.49±0.90
C-193	0.419±0.086	0.047±0.009	0.235±0.042	0.135±0.013	4.585±1.043	1.568±0.162	43.80±2.77	20.32±0.54
Overall	0.629±0.071^{Da}	0.066±0.009^{b}	0.341±0.037^{Da}	0.103±0.023^{b}	5.065±0.446^{Ba}	1.993±0.251^{b}	45.45±2.6^{a}	19.00±1.01^{Db}

et al. (1988). Significantly higher zinc content in good freezable semen compared to poor freezable bullsemen is in consonance with the observation made by Pangawkar *et al.* (1988) indicating the role of zinc in seminal plasma influencing the structural integrity, motility and the survivability of spermatozoa. The high positive significant correlation of zinc was marked in good freezable semen with mass activity (0.86), initial live sperm (0.96), initial motility (0.81) and post thaw motility (0.87). This is suggestive of the fact that zinc acts on the membrane maintaining chromatin stability and possibly for the mechanical properties of the accessory fibers, tails morphology and sperm motility as suggested by Baccetti *et al.* (1973). Recent revelation by Gavella and Lipovac (1998) has demonstrated that this metal ion participates in the oxidative changes occurring after ejaculation and thus may modulate the properties of germ cells.

The level of iron in the seminal plasma of good and poor freezable bull was 48.98±3.09 and 45.45±2.86 μg/ml, respectively, which was higher than the values reported by Bhavsar *et al.* (1989) and Misra *et al.* (1989). Iron content of seminal plasma in good freezable bull had significant negative correlation with semen volume (-0.97), mass activity (-0.81), initial live sperm (-0.71), sperm concentration (-0.96), post thaw motility (-0.82) and post thaw livability (-0.96) which led to conclude that higher iron content in cross bred bull semen is not favourable for achieving good freezability. This is in agreement with Bhavsar *et al.* (1989), whereas Singh and Gangwar (1977) observed significantly positive correlation between iron content of semen and initial motility but no correlation with any other seminal traits. The iron concentration in good freezable bull seminal plasma registered a significant correlation with copper (0.82) and zinc (0.84) indicating the essential role played by zinc in conjunction with copper and iron by inhibiting DNAase and thus conserving the energy during transport (Byer, 1974), exerting an inhibitory effect on super oxide anion regardless of the initial O_2 radical level and maintaining the functional and structural integrity of the sperm cells (Gavella and Lipovac, 1998).

The post thaw concentration of all the trace minerals studied in good and poor freezable semen samples did not register any significant difference except iron, which was significantly higher in poor freezable semen. Therefore, the initial concentration of all the trace minerals studied is more important in its determination prior to freezing of semen and could be inferred that the initial concentration of the aforesaid trace minetrals may be evaluated in crossbred bull semen samples for optimum fertility rate or before incorporating a breeding bull into the A.I. station.

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