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Short Communication

Relation of seminal plasma hormonal levels with quality of frozen semen in cattle

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ABSTRACT

Levels of seminal plasma Testosterone, Tri-iodo-thyronine (T_3) and Thyroxin (T_4) in the fresh ejaculates were estimated and its correlation with post thaw seminal quality was found out. The levels of these hormones were significantly ($p<0.01$) higher in HF semen than in crossbred and Murrah semen. These hormones were positively correlated with sperm motility, volume, total sperm concentration and percent live sperm. A significant positive correlation with sperm penetration distance (SPD), percent sperm responding to hypo-osmotic swelling test (HOS) in fresh and post-thawed semen with these hormones was also recorded. A positive correlation of SPD and HOS positive sperm with testosterone, T3 and T4; both in fresh and post-thawed semen proves their important role in maintaining initial quality of semen, indicating estimation of these hormones as a valuable tool for initial selection of bulls and also for the selection of ejaculates for freezing.

Key Words: Testosterone, Thyroid hormones, Semen, Freezing, Sperm penetration distance, Hypo-osmotic swelling test.

Supply of quality frozen semen with good predictable potential fertility has remained a challenge till date to meet its ever-increasing demand for artificial insemination in bovines. The fertility results with frozen semen are not comparable to that with the fresh semen (Vishwanath *et. al.* 1996). The poor freezability and fertility of frozen semen of buffalo and crossbred bulls further augment this problem. Semen has been frozen successfully with acceptable post-thaw recovery rate in these species too, however, not to the extent to that of the *taurine* breeds. Attempts are being made in order to improve freezability and predict the fertilizing potential of frozen semen. Many *in vitro* fertility tests have been developed for predicting the quality of frozen semen and among them cervical mucus penetration test (CMPT) and hypo-osmotic swelling test (HOST) are two simple and reliable tests.

Thyroid hormones are the important metabolic hormones exerting profound effect on the metabolic rate of body and thus on the quantity and quality of the semen. The values for testosterone, T_3 and T_4 in blood plasma are known to fluctuate depending on the age, time and frequency of semen collection. In mature semen donating bulls, these hormones are also detectable in seminal plasma. Circulating levels of Testosterone affects the quality of freshly ejaculated semen in bovines. A close relationship of Testosterone in blood plasma to total number of sperm and motility of the semen is reported (Sharma et al, 1986). Testosterone level is, reportedly, positively correlated with post-thaw motility of frozen semen (Wolf, 1996). However, no report seems to be available to state how these hormones affect the fertilization potential of the spermatozoa.

Perusal of literature reveals that the effect of these seminal plasma hormones on the quality, especially on the fertilization potential

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of sperm, measured in terms of *in vitro* fertility tests seems to be lacking. Therefore, we hypothesized that concentrations of these hormones in the seminal plasma are likely to reflect more reliable fertility potential than that in the blood plasma. The present study was therefore, undertaken to record the concentrations of these hormones (Testosterone, T_3 and T_4) in the seminal plasma of sexually mature semen donating HF, Murrah and crossbred bulls and their relationship with initial quality of semen especially with respect to freezability and fertilization potential of spermatozoa.

Thirty-eight semen ejaculates were collected from sexually mature semen donating (4 -6 yrs age) bulls (8 ejaculates each from 2 HF and 2 crossbred (HFxH), and 6 ejaculates from one Murrah bull), maintained under uniform feeding and management regime at the institute. Semen was collected in artificial vagina twice a week in the morning hours before feeding. The semen sample was split, in three parts – part one for routine semen evaluation, part two for estimation of hormones and part three for freezing and subsequent investigations.

Semen evaluation:

One part of semen was evaluated for general physico-morphological seminal attributes (Salisbury, *et al* 1978). The acrosomal integrity was assessed using Giemsa stained smear (Watson *et. al.* 1975). The fresh semen was also subjected to cervical mucus penetration test (Prasad *et. al.* 1997) and Hypo-osmotic swelling test (Jayendran *et. al.* 1984).

Hormone estimation:

A part of semen was centrifuged, seminal plasma separated and stored at –20° C till estimation of hormones. The concentration of T_3, T_4 and testosterone was assayed by RIA method using Immuchem coated tubes (supplied from ICN Pharmaceutical, Inc. Diagnostics Division Coasta Mesa, CA 92626) as described according to the literature supplied with the kit.

Freezing of semen:

Another part of semen was diluted in tris-fructose-eggyolk-glycerol extender (Davis, *et. al.* 1963) using 5% yolk for buffalo and 10% for cattle semen. The extended semen was frozen in liquid nitrogen vapour (Kumar, 1989). The data were analyzed statistically and correlation coefficients between hormone concentrations and seminal parameters were found out according to Snedecor and Cochran (1989). The correlation coefficients were worked out by pooling the data irrespective of species and breed.

The mean (± SE) values of various seminal attributes in fresh and post-thawed semen are given in table1. A significant ($P < 0.01$) difference in testosterone concentrations was found between two cattle breeds. The Testosterone Concentration in the seminal plasma of HF bull was significantly higher (12.23±0.49 ng/ml) than crossbred (7.06±0.77) and Murrah (7.02±0.07). A great variation in testosterone concentration between two crossbred bulls was recorded. The T_3 concentration was significantly lower in Murrah bull seminal plasma than that of HF and crossbred. However, the concentration of thyroxin (T_4) did not differ. As such no literature could be traced about the concentration and role of T_3 and T_4 in the seminal plasma of crossbred and HF bull. Tuli *et. al.* (1991) reported seminal plasma testosterone concentration in Murrah bull to be 1.41 ± 0.14 ng / ml. The values of these circulating hormones in blood plasma can not be compared because of many varying factors associated with this parameter like, age and health of bulls, climate, nutritional status, time and frequency of semen collection and many other (Gupta *et al* 1984; Sharma *et al*, 1986; Wolf, 1996)

Relationship of Hormones with Fresh Seminal Attributes:

In the fresh ejaculate of crossbred seminal plasma T_4 showed a negative correlation with individual motility ($r = - 0.33$; $P< 0.05$) and seminal plasma T_4 level ($r = - 0.47$; $P < 0.05$). However, T_3 level revealed a weak positive correlation with the individual motility ($r = 0.37$;

P<0.05). T3 was found to be negatively associated with total abnormal sperms (r = -0.49; p < 0.01) and testosterone levels (r = 0.51; p<0.01) suggesting its indirect role in cellular metabolism during spermatogenesis and epididymal maturation. The study also revealed a negative association of testosterone with individual motility (r = -0.44; P<0.05) and sperm concentration (r = 0.56; P<0.01). This negative relationship of testosterone with motility and sperm concentration lacks sufficient explanation, as this study was limited to two bulls, therefore it requires an elaborate study for further verification. No significant relationship of testosterone with other seminal attributes was recorded. In the fresh ejaculate of HF bulls T4 concentration was positively correlated with testosterone (r = 0.51; p< 0.01), intact acrosome (r = 0.38; P < 0.05), ejaculate volume (r = 0.38; P< 0.05), but negatively with GPT (r = - 0.39; P< 0.05). Similarly T_3 level was positively correlated with sperm concentration (r = 0.58; P<0.01), sperm penetration distance (r = 0.44; P< 0.05) and HOS positive sperms (r = 0.65; P< 0.01) but negatively with mass activity (r = - 0.41; P< 0.05).Seminal plasma testosterone level showed a weak negative correlation (r = -0.39; P<0.05) with sperm concentration. No other parameters measured were found to be significantly associated with testosterone.

Seminal plasma T_4 level in Murrah bulls showed a positive correlation with sperm concentration (r = 0.76; P< 0.01), sperm penetration distance (SPD) during cervical mucus penetration test (CMPT) (r = 0.87; P<0.01) and total abnormal sperm (r = 0.54; P<0.05), however negative with live sperm (r = - 0.63; P< 0.01) and HOS positive sperms (r = - 0.64; P< 0.01). T3 level had a very strong positive correlation with live sperm percent (r = 0.9; P< 0.01) and ACP level (r = 0.86; P< 0.01). It also revealed a positive correlation with mass activity (r = 0.59; P< 0.01) and AKP (r = 0.38; P< 0.05). Seminal plasma testosterone concentration had a positive correlation with GPT level (r = 0.45; P<0.05) and sperm concentration (r = 0.43; P< 0.05) but negative with GOT (r = 0.69; P< 0.01), ejaculate volume (r = 0.37; P< 0.05) and total abnormal sperms (r = 0.38; P< 0.05).

Relationship of hormonal levels with post-thawed seminal attributes:

The correlation coefficient values 'r' value) of seminal plasma testosterone, T_3 and T_4 with other seminal parameters are given in table 3. Perusal of data revealed that testosterone was significantly positively correlated with almost all the post-thawed seminal characteristics including SPD value and percent HOS positive sperm. A significant positive correlation of seminal plasma testosterone concentration with sperm motility in Murrah bulls (r = 0.43; P<0.01) has been reported (Tuli *et. al.* 1991). Significant positive correlation of blood plasma testosterone concentration with libido, ejaculate volume, sperm concentration, sexual activity, number of sperm and motility has been reported. Tuli *et. al.* (1991) reported significant positive correlation of testosterone with semen volume, concentration and sperm motility, but Sharma *et. al.* (1986) reported that higher concentration of testosterone decreases the semen quality. Similarly, the T_3 and T_4 concentration were also significantly positively correlated with post-thaw motility, livability, per cent intact acrosome, per cent HOS positive sperm and SPD travelled by spermatozoa in the cervical mucus. Mohanty (1999) reported a positive correlation of thyroid hormone with initial motility, live sperm post-thaw motility and post-thaw livability of crossbred sperm. El-Anwar *et. al.* (1991) reported higher concentration of seminal plasma T_3 and T_4 in Friesian bulls donating good quality semen as compared to those donating poor. However, Sekasiddhi and Buaban (1997) did not find any difference in circulating T_3 and T_4 levels in the fertile and low libido bulls.

Guyton (1991) suggested that elevated thyroid hormone increases the rate of secretion of most other endocrine organs. A positive correlation of T_3 and T_4 with testosterone in the present study is justifiable on these lines. However, Mohanty (1999) did not find any definite trend in

Table 1.
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Table2
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Seminal Attribute
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(C8)
(C9)
(C10)
(C11)
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C2=Initi
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C4=Cerv
C5= Per
C6= Post
C7=Post-
C8=Post-
C9= Spe
C10= Pe
C11= Co
C12= Co
C13= Co
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**- Signi

Table 1. Seminal characteristics in frozen cattle and buffalo semen and hormonal concentration in fresh seminal plasma (Mean and SE).

Sl. No.	Seminal Characteristics	Holstein Friesian	Crossbred	Murrah
1.	Mass motility (0-5 scale)	4.00± 0.36	3.06±0.25	4.00+0.36
2.	Volume (ml.)	4.90+1.86	5.60+1.33	3.98+0.37
3.	Sperm concentration (Million /ml.)	917.50+127.98	968.12+111.36	1158.34+88.48
4.	Initial motility (%)	82.19+3.64	68.13+6.29	81.67+1.05
5.	Live sperm (%)	87.38+1.46	84.13+2.31	88.33+0.88
6.	Intact acrosome (%)	89.94+11.57	87.38+2.34	90.67+0.49
7.	CMPT (distance travelled in mm.)	45.06+3.32	39.94+2.98	43.17+0.40
8.	Per cent hypo-osmotic swollen sperm	49.38+2.80	42.06+1.93	46.50+0.96
9.	Post-thaw sperm motility (%)	30.31+1.25	28.13+1.36	27.50+1.71
10.	Post-thaw sperm livability(%)	55.50+1.01	54.94+1.32	55.33+1.43
11.	Post-thaw per cent intact acrosome	70.50+0.79	67.75+1.25	70.67+1.09
12.	Post-thaw distance of cervical mucus penetrated (mm)	19.69+0.47	18.38+0.59	19.00+0.86
13.	Post-thaw HOS swollen sperm (%)	21.88+0.44	21.00+0.57	22.17+1.01
14.	Testosterone (ng/ml.)	12.23+0.49	7.06+0.77	7.02+0.70
15.	T_3 (ng/ml.)	1.13+0.05	0.91+0.04	0.80+0.03
16.	T_4 (ng/ml.)	36.56+2.39	26.87+1.15	24.50+0.62

Table2.Correlation coefficient (r value) among different seminal attributes and various hormones in the seminal plasma (data pooled irrespective of species and breed)

Seminal Attributes	Seminal Attributes												
	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13
(C1)	1.00												
(C2)	.73**	1.00											
(C3)	.77**	.82*	1.00										
(C4)	.85**	.66**	.72**	1.00									
(C5)	.88**	.66**	.69**	.92**	1.00								
(C6)	.35**	.26	.33*	.53**	.42**	1.00							
(C7)	.33*	.37*	.43**	.45**	.34*	.79**	1.00						
(C8)	.60**	.39*	.49*	.63**	.62**	.73**	.65**	1.00					
(C9)	.37*	.42**	.46**	.48**	.42**	.74**	.67**	.68**	1.00				
(C10)	.37**	.48**	.49**	.44**	.39*	.71**	.69**	.67**	.91**	1.00			
(C11)	.70**	.52**	.53**	.77**	.73**	.46**	.38*	.44**	.39*	.32*	1.00		
(C12)	.50**	.32*	.36*	.63**	.53**	.45**	.28	.41**	.39*	.33*	.80**	1.00	
(C13)	.56**	.45*	.47**	.71**	.59**	.55**	.42**	.42**	.40*	.40**	.81**	.84**	1.00

C1=Initial motility,
C2=Initial Livability,
C3=Initial per cent Acrosome,
C4=Cervical mucus penetration distance in fresh semen,
C5= Per cent swollen sperm in hypo-osmotic solution for fresh semen,
C6= Post-thaw motility,
C7=Post-thaw percent live sperm,
C8=Post-thaw intact acrosome percent,
C9= Sperm penetration distance traveled by post-thawed sperm,
C10= Per cent swollen sperm in post thawed semen,
C11= Concentration of Testosterone in seminal plasma,
C12= Concentration of T3 in seminal plasma,
C13= Concentration of T_4 in seminal plasma.
*- Significant at 5% level
**- Significant at 1% level

testosterone, T_3 and T_4 blood plasma values of crossbred bulls with good and poor freezability. He however, reported that conception rate was significantly correlated with the semen samples of bulls having higher hormonal concentrations.

A positive correlation of these hormones with certain tests of present study, measuring *in vitro* fertilization potential of frozen spermatozoa (CMPT and HOS) is thus a very valuable information by which the predictability of such frozen ejaculates can be made. Thyroid hormones reveal the general metabolic status of any individual. The positive correlation of thyroid hormones with initial seminal attributes reveals further the importance of balanced feeding and good management practices during rearing of bulls for production of semen.

A significant positive correlation of seminal plasma testosterone at the time of ejaculation with almost all the semen quality parameters elaborates their role on the initial quality of semen and its subsequent fertilization potential. A positive correlation of SPD and HOS positive sperm with seminal plasma testosterone further indicates its importance. It can therefore, be used as one of the tests at the time of selection of bulls.

It can be concluded that estimation of testosterone, T_3 and T_4 hormones in the seminal plasma along with some simple *in vitro* fertility tests may provide fairly reliable information on the fertilizing potential of the semen.

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