

## STUDIES ON THE NORMS AND CORRELATIONS OF INITIAL AND POST-THAW SEMINAL ATTRIBUTES OF TRIPLE CROSSBRED BULLS

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### ABSTRACT

One hundred and sixty split-ejaculates from five triple crossbred bulls (1/2 HF x 1/4 J x 1/4 H) were studied for the initial, post-thaw and post-refrigeration sperm characteristics and their interrelationships. The mean values for the various attributes were: sperm concentration  $83.54 \pm 3.12 \times 10^7$ /ml; progressive motility motility  $71.03 \pm 1.37\%$  initial,  $60.69 \pm 1.38\%$  prefreeze,  $34.89 \pm 1.42\%$  post-thaw,  $16.38 \pm 0.93\%$  after 1 hr of post-thaw incubation at  $37^\circ\text{C}$ , and  $58.63 \pm 1.43$ ,  $50.47 \pm 1.55$ ,  $41.75 \pm 1.59$  and  $30.13 \pm 1.60$  percent after 24, 48, 72 and 96 h of storage in Tris diluent at  $5^\circ\text{C}$ , respectively. The percentages of live and abnormal spermatozoa were  $79.86 \pm 0.98$  and  $9.58 \pm 0.86$  initially, and  $43.51 \pm 1.46$  and  $14.07 \pm 0.87$  after thawing. The abnormalities of sperm head, mid-piece, and tail were also increased significantly in post-thawed semen. Most of the characteristics studied were significantly ( $P < 0.01$ ) interrelated. Initial motility and live sperm percent were the good indicators of semen quality, freezability and liveability of crossbred bulls semen. Similarly, refrigeration motility was a good indicator of freezability and post-thaw survivability of spermatozoa, and hence could serve as a simple test in screening the bulls for freezability of their semen under the field/farm conditions, where the test freezing facilities are not available.

### INTRODUCTION

The information available on the normal seminal attributes, freezability, fertility and their interdependence which could help in predicting the worth of crossbred bulls as potential sires is meagre (Rao and Kotayya, 1977; Rao and Rao, 1978; Saxena and Tripathi, 1981; Tomar *et al.*, 1985; Belorkar *et al.*, 1990). Hence, the present study was undertaken to know the physiological norms and correlations of seminal attributes and freezability in triple crossbred bulls.

### MATERIALS AND METHODS

Five triple crossbred bulls (1/2 HF x 1/4 J x 1/4 H), aged 4-1/2 years, maintained under identical nutritional and managerial conditions at the Germ Plasm Centre of IVRI, Izatnagar, were taken up for this study. Semen was collected at weekly interval using artificial vagina. A total of 160 split-ejaculates were studied for the initial motility, sperm concentration, live sperm percent and abnormal sperm percent as per the standard procedures (Tomar, 1970). The semen samples were then diluted in Tris citric acid fructose egg yolk glycerol diluent keeping 25 million spermatozoa per 0.5 ml Medium French straw. The straws were frozen in

liquid nitrogen vapour for 10 minutes after 3 hr of equilibration at  $5^\circ\text{C}$  (Sahni and Mohan, 1988). A part of the diluted semen was also preserved in refrigerator at  $4-6^\circ\text{C}$  and was assessed for progressive motility at 24 hourly intervals upto 96 hr. The frozen straws were thawed in water bath at  $38^\circ\text{C}$  for 30 seconds, after 16 hr of storage in liquid nitrogen. The pre-and post-freeze motility was assessed under a phase contrast microscope (40 X) fitted with a biotherm stage. Post-thaw live sperm and the abnormalities of sperm head, mid-piece, tail and total abnormalities were studied in eosin-nigrosin stained smears (Sharma, 1988). The same thawed straws were then immediately transferred to an incubator at  $37^\circ\text{C}$  and motility was reassessed after 1 hr. The data were analysed statistically for the mean  $\pm$  SE values of each parameter and the correlation coefficients were worked out on a computer as per the standard procedure (Snedecor and Cochran, 1967).

### RESULTS AND DISCUSSION

The mean values with their standard errors for various seminal attributes studied at the initial, prefreeze, post-thaw and post preservation at  $4-6^\circ\text{C}$ , and their correlation coefficients have been presented in Tables 1 and 2. The present

Restricted	Level of significance	Avik - Breed Feed Exer-	cise	BxF	BxE
Alin					
6	6.98-XX	XX	—	—	X
237	$\pm 0.195$				
69	10.72-—	—	X	—	X
494	$\pm 0.472$				
4	2.61-XX	—	X	—	—
064	$\pm 0.070$				
4.59	142.70XX	X	XX	—	—
29	$\pm 2.17$				
54	5.85—	XX	X	—	X
12	$\pm 0.12$				

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findings with respect to the normal seminal profiles are comparable with the earlier reports on crossbred bulls semen (Biswas *et al.*, 1976; Rao and Rao, 1978; Garg and Pandit, 1983; Chauhan *et al.*, 1983; Sharma, 1988; Belorkar *et al.*, 1990), and agree to the general views for triple crossbred bulls.

### Initial motility and sperm concentration

The initial motility ( $71.03 \pm 1.27\%$ ) had significant ( $P < 0.01$ ) positive correlations with the live sperm percent at the initial and post-thaw stage, and motility at prefreezing, post-thaw (0 h & 1 h incubation) and at different hours of preservation at  $4-6^\circ\text{C}$ . It had negative correlations with sperm abnormalities of head, mid-piece, tail and total in the fresh and post-thawed semen. Whereas, sperm concentration showed significant ( $P < 0.01$ ) positive correlations only with sperm tail abnormalities in fresh and post-thawed semen (Table 2). These were the expected relationships as better the initial motility of semen better would be the freezability/preservability with higher live sperm and lower dead/abnormal spermatozoa, and are in agreement with the reports of Saxena and Tripathi (1981), Rao and Rao (1978), Biswas *et al.* (1976), Patel *et al.* (1989), Sharma (1988) and Belorkar *et al.* (1990) in crossbred bulls.

### Live sperm count (pre-and post-freezing)

As with initial motility, the live sperm in fresh semen ( $79.88 \pm 0.98\%$ ) was significantly ( $P < 0.01$ ) and positively correlated with the motility at pre-and post-freezing, refrigeration motility and post-thaw live sperm percent. It was negatively correlated with sperm head, mid-piece, tail and total abnormalities in the fresh and frozen-thawed semen. Similarly, the post-thaw live sperm ( $43.51 \pm 1.46\%$ ) had significantly ( $P < 0.01$ ) positive correlations with motility at pre-and post-freezing and at different hours of refrigeration, and negative correlations with sperm abnormalities pre- and post-freezing (Table 2). Correlation coefficients of similar magnitude were reported by earlier

workers (Saxena and Tripathi, 1981; Sharma, 1988; Bhavsar *et al.*, 1988 & 1990; Belorkar *et al.*, 1990) in crossbred buffalo bulls. Thus, greater the liveability of spermatozoa, better would be the quality and motility pre- and post-freezing.

### Sperm abnormalities (fresh semen)

The abnormalities of sperm head, mid-piece, tail and total in the fresh semen (Table 1) were significantly ( $P < 0.01$ ) and positively interrelated with one another, and were further correlated positively with the sperm abnormalities of different segments in post-thawed semen, and negatively with pre- and post-freezing motility (Table 2). These were the expected correlations, since the abnormalities of spermatozoa, particularly the tail and mid-piece, hinder the sperm forward motility or kinetics, and also such spermatozoa are non-viable, hence negatively correlated with motility and liveability. Similarly, the post-thaw sperm quality is dependent on the initial quality. The present findings support observations of Biswas *et al.* (1976), Rao and Rao (1978), Bhavsar *et al.* (1990) and Belorkar *et al.* (1990) in bovines.

### Sperm abnormalities (Post-thaw)

In the post-thawed semen also, the abnormalities of sperm head, mid-piece, tail and total were significantly ( $P < 0.01$ ) and positively interrelated, and all were negatively correlated with the motility at different hours of refrigeration, as well as with motility and live sperm percent at pre- and post-freezing (Table 2). Although the mid-piece abnormalities had poor correlations with tail abnormalities and refrigeration motility. Since the post-thaw motility, sperm and refrigeration motility were positively correlated, their negative correlations with sperm abnormalities were anticipated. Belorkar *et al.* (1990) have made similar observations in crossbred bulls.

Table 1. Norms of various seminal attributes in fresh, frozen and refrigerated semen of triple crossbred bulls

Sl.No.	Attribute	Means $\pm$ SE*	Sl. No.	Attribute	Mean $\pm$ SE*
1.	Initial motility (%)	71.03 $\pm$ 1.27	11.	Post-thaw live sperm (%)	43.51 $\pm$ 1.45
2.	Sperm conc ( $\times 10^7$ /ml)	83.54 $\pm$ 3.12	—	Post-thaw sperm abnormalities:	
3.	Live sperm (%)	79.86 $\pm$ 0.98	12.	% Head abnormalities	4.63 $\pm$ 0.48
—	Initial sperm abnormalities		13.	% Mid-piece abnormal	2.03 $\pm$ 0.36
4.	% Head abnormal	2.81 $\pm$ 0.52	14.	% Tail abnormalities	7.31 $\pm$ 0.50
5.	% Mid-piece abn.	0.99 $\pm$ 0.37	15.	% Total abnormal	14.07 $\pm$ 0.87
6.	% Tail abnormal	5.70 $\pm$ 0.49	—	Refrigeration preservation :	
7.	% Total abnormal	9.58 $\pm$ 0.86	16.	Motility at 24 hr (%)	58.63 $\pm$ 1.43
8.	Prefreeze motility	60.69 $\pm$ 1.38	17.	Motility at 48 hr (%)	50.47 $\pm$ 1.55
9.	Post-thaw motility	34.89 $\pm$ 1.42	18.	Motility at 72 hr (%)	41.75 $\pm$ 1.59
10.	Post-thaw incubation (1 hr) motility	16.38 $\pm$ 0.93	19.	Motility at 96 hr (%)	30.13 $\pm$ 1.60

\* Values are the means of 160 observations each.

### Pre- and Post-freezing motility

Prefreeze motility of semen ( $60.69 \pm 1.34\%$ ) had significant positive correlations ( $P < 0.01$ ) with post-thaw (0 h & 1 h incubation) motility, post-thaw live sperm motility at different intervals of fridge preservation, and negative correlations with post-thaw sperm abnormalities. Similar correlations were also observed for post-thaw motility at 0 hr ( $34.89 \pm 1.42\%$ ) and 1 hr ( $16.38 \pm 0.93\%$ ) incubation with the post-thaw live and abnormal sperm percent and motility after refrigeration (Table 2). These correlations revealed that better the preservability of semen at  $5^\circ\text{C}$ , better would be its freezability. Thus, one can indirectly depict the another one, and hence, either can be used in evaluating the semen/bull under field conditions before selecting as potential sire for semen bank. The findings are well in accordance with the correlations reported by Bhavsar *et al.* (1988 & 1990), Verma and Sharma (1989) and Belorkar *et al.* (1990) in the bovines.

### Refrigeration motility

Highly significant positive interrelationships were observed between sperm motility values at different intervals of storage in the refrigerator and these were also correlated with the initial and post-thaw semen characters. These findings were in accordance with the findings of Saxena and Tripathi (1981) and Verma and Sharma (1989) in crossbred bull semen.

### ACKNOWLEDGEMENT

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Table II: Correlation coefficients between various seminal attributes of crossbred bulls.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1.		.01	.84**	-.34**	-.26**	-.31**	-.45**	.79**											
2.				-.12	-.01	-.12	.25**	.13	-.11										
3.				-.42**	-.31**	-.40**	-.55**	.73**											
4.					.39**	.17	.69**	-.31**											
5.						.11	.58**	-.12											
6.							.75**	-.40**											
7.								-.44**											
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17.																			
18.																			
19.																			

M = Initial motility; SC = Sperm concentration; \* = Significant; 1. M, 2. SC, 3. Live sperm (%), 4. Head abnormalities, 5. Mid-piece abnormalities, 6. Tail abnormalities, 7. Total sperm abnormalities, 8. Prefreeze motility, 9. Post-thaw motility, 10. Post-thaw incubation (1 hr) motility, 11. Post-thaw live sperm, 12. Head, 13. Mid-piece, 14. Tail, 15. Total sperm abnormalities, 16. Motility at 24 hr, 17. Motility at 48 hr, 18. Motility at 72 hr, 19. Motility at 96 hr.



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Rao, A.V.N. and Kotayya, K. (1977). Studies on ejaculate charac-

Freeze motility of semen ( $60.69 \pm 1.34\%$ ) showed significant positive correlations ( $P < 0.01$ ) with post-thaw (0 and 1 h incubation) motility, post-thaw live sperm and motility at different intervals of fridge preservation, and negative correlations with post-thaw sperm abnormalities. Similar correlations were also observed for post-thaw motility at 0 hr ( $34.89 \pm 1.42\%$ ) and 1 hr ( $16.38 \pm 0.93\%$ ) of incubation with the post-thaw live and abnormal sperm percent and motility after refrigeration (Table 2). These correlations revealed that better the preservability of semen at  $-15^\circ\text{C}$  better would be its freezability. Thus, one character correctly depicted the another one, and hence, either one can be used in evaluating the semen/bull under field conditions before selecting as potential sire for semen bank. The present findings are well in accordance with the correlations reported by Bhavsar *et al.* (1988 & 1990), Verma and Sharma (1989) and Belorkar *et al.* (1990) in the bovines.

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Table II: Correlation coefficients between various seminal attributes in fresh, post-thawed (PT) and preserved (at 5°C) semen of crossbred bulls.

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**Abnormalities (fresh semen)**  
The abnormalities of sperm head, mid-piece, tail and fresh semen (Table 1) were significantly ( $P < 0.05$ ) interrelated with one another, and were found positively with the sperm abnormalities of all segments in post-thawed semen, and negatively with pre- and post-freezing motility (Table 2). These results suggest correlations, since the abnormalities of sperm head, tail and mid-piece, hinder the sperm forward movement or kinetics, and also such spermatozoa are found to be negatively correlated with motility. Similarly, the post-thaw sperm quality is dependent on the initial quality. The present findings support the findings of Biswas *et al.* (1976), Rao and Rao (1980), Kaur *et al.* (1990) and Belorkar *et al.* (1990) in

**Abnormalities (Post-thaw)**  
The post-thawed semen also, the abnormalities of head, mid-piece, tail and total were significantly and positively interrelated, and all were negatively correlated with the motility at different hours of refrigeration as well as with motility and live sperm percent after freezing (Table 2). Although the mid-piece abnormalities had poor correlations with tail abnormalities and total motility. Since the post-thaw motility, and refrigeration motility were positively correlated with the mid-piece abnormalities, the positive correlations with sperm abnormalities were not significant. Belorkar *et al.* (1990) have made similar observations in crossbred bulls.

frigerated semen of triple crossbred bulls

Attribute	Mean $\pm$ SE*
live sperm (%)	43.51 $\pm$ 1.45
low sperm abnormalities:	
normalities	4.63 $\pm$ 0.48
se abnormal	2.03 $\pm$ 0.36
normalities	7.31 $\pm$ 0.50
normal	14.07 $\pm$ 0.87
ation preservation :	
24 hr (%)	58.63 $\pm$ 1.43
48 hr (%)	50.47 $\pm$ 1.55
72 hr (%)	41.75 $\pm$ 1.59
96 hr(%)	30.13 $\pm$ 1.60



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## EFFECT OF FEEDING UREA ON THE YIELD FROM

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Ten cross-bred milch animals on the G-1 and G-2. The G-1 group of animal concentrate mixture to meet the protein requirements of the animals were fed with urea ammoniated straw for maintenance and milk production.

In the group fed with urea treated straw, at the same time a reduction in concentrate treatment of straw has the potential for increase milk yield.

### INTRODUCTION

Urea upgraded straw supported better growth as compared to urea supplemented straw (Wieringa, 1988). *et al.*, (Dahiya 1990) while increased dry matter intake and better nutrient utilization of urea treated wheat straw in milking buffaloes, 2% urea in concentrate requirement has been reported.

Several studies have shown that urea treated (*Oryza sativa*) straws (Doyle *et al.*, 1986) can increase intake and digestibility in adult large ruminants has indicated that urea treatment of the straw in diets given to dairy animals can increase milk production, reduce live weight losses in lactating animals (Davis, 1981, Perdok *et al.*, 1982, 1984; Davis, 1985).

The experiment described here was carried out to test the straw component in concentrate/straw diet for lactating crossbred cows.

### MATERIALS AND METHODS

Ten 75% Holstein Friesian crossbred cows on a diet comprising concentrates (30% crushed wheat bran, 20% groundnut cake, 2% salt and 2% vitamin A), 5 kg (about 1 kg DM) green grass (*Cynodon dactylon*) and minerals for the first 57-59 days of lactation concentrates were given according to NRC (1980).