COMPARISON OF SPERM ATTRIBUTES IN FROZEN THAWED SEMEN OF PURE AND CROSSBRED JERSEY BULLS

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ABSTRACT

Thirty-six ejaculates (3 ejaculates / bull) of pure and crossbred Jersey bulls were used to compare sperm attributes of frozen thawed semen. The seminal attributes viz. semen volume, sperm concentration, post thaw motility and acrosome integrity were similar ($p>0.05$) between the breeds, whereas, the percentage of live sperm during post thaw period were higher ($p<0.05$) in pure compared to crossbred Jersey bulls. The overall sperm abnormalities as well as the majority of head, mid piece and tail abnormalities were similar ($p>0.05$) between pure and crossbred Jersey bulls, however, the proportion of detached / free head, bent mid piece, free tail and highly coiled tail was higher ($p<0.05$) in crossbred and the proportion of asymmetric head and terminally coiled tail was higher ($p<0.05$) in pure Jersey bull. In both breeds, the total sperm head abnormalities showed negative correlation with live sperm ($p>0.05$), HOST positive sperm ($p>0.05$) and acrosomal integrity ($p<0.05$). In brief, based upon the sperm attributes in frozen thawed semen, it can be concluded that semen quality of pure and crossbred Jersey bulls was almost similar.

Keywords: Crossbred, Jersey bull, Semen, Sperm abnormality, Sperm attributes

INTRODUCTION

About one half of the young crossbred bulls are rejected because of poor semen quality, libido and freezability (Vijetha *et al*., 2014). Also, a positive association exists between increased morphological abnormalities of spermatozoa and reproductive efficiency of bulls (Walters *et al*., 2005). Therefore, the evaluation of sperm morphology in frozen thawed semen is an important aspect to assess the freezability and fertility of bulls. Hence, the present study was carried out to evaluate the differences in various sperm attributes in frozen thawed semen of pure and crossbred Jersey bulls.

MATERIALS AND METHODS

The present research was conducted on 12 (Pure = 6, Crossbred = 6) mature and healthy Jersey bulls maintained at semen station, Nagpur. Total 36 ejaculates (3 ejaculate / bull) were collected and evaluated for various seminal attributes. The volume of semen was recorded directly from the graduated semen collection tube immediately after collection. The concentration of spermatozoa (million/ml) in the neat semen was determined by the bovine photometer. The semen was diluted with tris egg yolk-glycerol and freezing of semen sample was done by programmable Bio freezer for 7 min and finally cryopreserved in LN_2 container. The French mini straws were removed and thawed in water bath at 37°C for 30 seconds. In frozen thawed semen, the live sperm count and abnormalities were determined by differential staining technique using Eosin-Nigrosin stain (Blom, 1950). Total live and abnormal sperm count was determined by observing 200 spermatozoa in each smear. Hypo osmotic swelling test (HOST) was conducted by using 2.7% aqueous solution of fructose (1.351 gm/50 ml distilled water) and 1.47% aqueous solution of sodium citrate (0.735 gm/50 ml distilled water). Equal volumes of both solutions (0.5 ml each) were mixed and kept in a incubator at 37°C for 10 min. About 50µl

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Table 1: Sperm attributes in fresh and frozen thawed semen of pure and crossbred Jersey bulls (Mean± SE, No. of ejaculates = 18 each)

Seminal attributes	Pure	Crossbred
Fresh semen		
Volume (ml)	6.50±0.39	5.98±0.34
Sperm concentration (x10^6/ml)	1171.01±65.09	1093.48±48.25
Frozen thawed semen		
Post thaw motility (%)	46.11±1.43	47.22±1.08
Live sperm (%)	58.67±1.02[a]	51.63±0.97[b]
HOST +ve (%)	47.27±1.05	45.94±1.33
Acrosome integrity (%)	71.94±0.86	69.38±0.53

[a] *vs.* [b] $p<0.05$

of semen was added in hypo-osmotic solution and incubated at 37°C for 30 minutes. After incubation, the contents were mixed gently followed by addition of 0.1 ml buffered formal saline. About 10µl of this mixture was taken on glass slide, covered with glass cover and observed under microscope (10x) for 100 spermatozoa, to determine the percentage of HOS positive spermatozoa. Acrosomal integrity of semen was assessed by Giemsa stain (Watson, 1975) and acrosomal intact was determined in 100 spermatozoa. The data obtained was analysed using standard statistical procedures.

RESULTS AND DISCUSSION

The sperm attributes in fresh and frozen thawed semen viz. the fresh semen volume, sperm concentration, post thaw motility, HOS reacted spermatozoa and acrosome integrity was similar ($p>0.05$) between pure and crossbred Jersey bulls, except the live sperm percentage in frozen thawed semen, which was higher ($p<0.05$) in pure Jersey bulls (Table 1).

The overall sperm abnormalities as well as overall head, mid piece and tail abnormalities were similar ($p>0.05$) in frozen thawed semen of pure and crossbred Jersey bulls (Table 2). However, the proportion of asymmetric head and free head was different ($p<0.05$) between pure and crossbred jersey bulls (Table 2). In a previous study, the higher percentage of detached heads (6.33%) was recorded as compared to other head abnormalities in pure Jersey bulls (Sundararaman *et al*. 2014). The similar obervation was recorded in present study in pure and crossbred Jersey bulls (Table 2). The proportion of sperms with bent mid piece were higher ($p<0.05$) in crossbred Jersey bulls (Table 2). Among the various mid piece defects observed in the presnet study, the bent mid piece was most predominant, followed by highly coiled mid piece and tail piece (Table 2). Although these defects had little impact on fertility following artificial insemination, but, leads to subfertility in natural service sires (Johnson, 1997). Among the tail defects in pure and crossbred Jersey bulls, the proportion of sperms with free tail and highly coiled tail were higher ($p<0.05$) in crossbred and sperms with terminal coiled tail were higher ($p<0.05$) in pure Jersey bulls (Table 2). Compared to present observations, a higher percentage of bent and coiled sperm tail in pure Jersey bulls was recorded in a previous study (Sundararaman *et al.* 2007, 2014).

In present study, in both pure and crossbred Jersey biulls, total sperm head abnormalities revealed a negative correlation with live sperm, HOS reacted sperm and acrosomal integrity, with correlation reaching significance ($p<0.05$) for acrosomal integrity (Table 3 and 4). A similar negative correaltion was earlier reported in Sahiwal (Mandal *et al*. 2009) and

Table 2: Sperm abnormalities in frozen thawed semen of pure and crossbred Jersey bulls (Mean±SE, No. of ejaculates = 18 each)

Abnormality	Pure	Crossbred
Overall abnormalities (%)		
Total sperm abnormalities	16.75±0.42	18.11±0.64
Head	5.08±0.23	5.13±0.37
Mid piece	5.77±0.34	6.66±0.55
Tail	5.94±0.32	6.00±0.53
Sperm head abnormalities (%)		
Pear shaped	0.75±0.11	0.69±0.15
Asymmetry head	0.33±0.09[a]	0.13±0.05[b]
Double head	0.11±0.05	0.02±0.01
Knobbed defect	0.02±0.02	0.00
Detached / Free head	1.77±0.09[a]	2.61±0.23[b]
Macro head	0.13±0.05	0.27±0.10
Micro head	0.63±0.09	0.63±0.15
Narrow at base	0.69±0.27	0.55±0.09
Round head	0.36±0.13	0.25±0.08
Teratoid head	0.11±0.08	0.08±0.04
Mid piece abnormalities (%)		
Double mid piece	0.83±0.04	0.02±0.02
Dag defect	0.25±0.09	0.38±0.13
Abaxail implantation	0.11±0.050	0.05±0.038
Bent mid piece	1.91±0.24[a]	3.11±0.46[b]
Distal protoplasmic droplet	0.33±0.10	0.47±0.11
Protoplasmic droplet	0.36±0.09	0.63±0.20
Highly coiled mid piece and tail piece	1.25±0.11	1.63±0.32
Slightly bend mid piece	0.27±0.12	0.11±0.11
Swollen mid piece	0.36±0.11	0.58±0.17
Thickened mid piece	0.86±0.13	1.05±0.28
Tail abnormalities (%)		
Coiled tail	1.25±0.24	1.44±0.36
Bent tail	2.05±0.17	1.61±0.20
Free tail	1.75±0.11[a]	2.41±0.39[b]
Double tail	0.08±0.04	0.22±0.20
Terminally coiled tail	0.44±0.14[a]	0.16±0.07[b]
Slightly coiled tail	0.25±0.12	0.13±0.07
Short tail	0.22±0.10	0.11±0.06
Highly coiled tail	0.00[a]	0.16±0.09[b]

[a] *vs.* [b] $p<0.05$

Table 3: Correlation among overall abnormalities of sperm and other semen characteristics in pure Jersey bulls (No. of ejaculates = 18)

		Abnormal				Live sperm	HOST +ve	Acrosome integrity
		Head	Mid piece	Tail	Sperm			
Abnormal	Head	1						
	Mid piece	-0.13	1					
	Tail	-0.34	-0.25	1				
	Sperm	-0.22	0.49*	0.56*	1			
Live sperm		-0.27	-0.15	-0.35	-0.34	1		
HOST +ve		-0.35	0.13	-0.28	-0.19	0.18	1	
Acrosome integrity		-0.57*	0.01	-0.36	-0.35	0.41*	0.67*	1

*p<0.05

Table 4: Correlation among overall abnormalities of sperm and other semen characteristics in crossbred Jersey bulls (No. of ejaculates = 18)

		Abnormal				Live sperm	HOST +ve	Acrosome integrity
		Head	Mid piece	Tail	Sperm			
Abnormal	Head	1						
	Mid piece	-0.28	1					
	Tail	0.41	-0.03	1				
	Sperm	0.28	0.45	0.73*	1			
Live sperm		-0.27	-0.21	0.14	-0.42	-0.34	1	
HOST +ve		-0.35	-0.38	-0.03	-0.12	-0.07	0.60*	1
Acrosome integrity		-0.57*	-0.61*	0.21	-0.43	-0.23	0.58*	0.78*

*p<0.05

Jersey crossbred bulls (Sharma *et al*. 2012). These parameters give indirect assessment of bull fertility because the membrane integrity is prerequisite for the viability of spermatozoon and ability to fertilize (Rodriguez-Martinez, 2007).

In brief, in frozen thawed semen, some sperm abnormalities were higher in crossbred Jersey bulls, whereas, the live sperm proportion was higher in pure Jersey bulls. This warrants fertility trials for better assessmnet of semen quality of pure and crossbred Jersey bulls.

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