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Research Article

Effect of Additives on Interrelationships Among Various Spermatozoal Attributes of Fresh, Frozen-Thawed and Refrigerated Semen of Triple Crossbred (Hf X J X K) Bulls

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ABSTRACT

Thirty six ejaculates from 4 mature triple crossbred (25% HF ×25% J ×50% Kankrej) bulls were split into 3 aliquots each and were processed for freezing and refrigeration storage in TFYG diluent without and with EDTA (0.1%) or Cysteine HCl (0.1%). The percentages of motile and live sperm and intact acrosome were significantly (P < 0.01) higher in the presence of EDTA and/or cysteine as compared to control Tris diluent at all steps of semen processing/preservation. Furthermore, the motility at initial, prefreeze, post-thaw and post-refrigeration stages were highly significantly (P<0.01) interrelated (r=0.35–0.88), and it was the same with live sperm (0.26–0.76), abnormal sperm (0.20–0.62) and intact acrosome (0.30–0.76). Moreover, the sperm motility at different steps of semen processing/preservation had significant (P 0.05) positive correlations with post-thaw and post-refrigeration live sperm (0.21–0.30), negative correlations with prefreeze and post-refrigeration abnormal sperm (–0.19 to –0.32) and highly significant positive correlations with intact acrosome at all steps of semen processing/preservation (0.19–0.39). Similarly, live sperm percent at different steps of semen processing/preservation had significant negative correlations with abnormal sperm (0.19–0.43) and positive correlations with intact acrosome (–0.20 to –0.39), except that the initial and post-refrigerated live sperm did not reveal significant relation with intact acrosome at initial, prefreeze and post-thaw stages, whereas abnormal sperm at different steps of semen processing/preservation had significant negative correlations with intact acrosome particularly at initial and prefreeze stages (–0.20 to –0.34). These correlations suggested that the assessment of motile, live abnormal sperm and intact acrosome could be of practical utility in routine semen evaluation to predict its keeping quality and freezability in triple crossbred bulls, and that only the motility, which is a very simple rapid and subjective way of assessment, could serve the purpose of more tedious and time consuming staining procedures in routine semen analysis to predict its quality.

KEY WORDS: Semen additives, crossbred bulls, freezability, storage ability, interrelationships

INTRODUCTION

The literature on the beneficial effect of extender-additives on the keeping quality and freezability of bovine semen is quite large (Misra et al., 1988; Virani 1992; Dhami et al., 1993; Kumar et al., 2001). Moreover, Vyas et al. (1992) and Rana and Dhami (2003) have reported the interrelationships of various spermatozoal attributes at the initial, post-thaw and post-refrigeration stages after sephadex filtration of crossbred and Gir bulls' semen. However, the reports on such interrelationships for spermatozoal attributes at different steps of semen processing and preservation with the use of additives were meagre. Hence, an attempt was made to study and report the same for semen of triple crossbred bulls.

MATERIALS AND METHODS

This study was undertaken during autumn at Livestock Research Station of the University at Anand, on 36 ejaculates (9/bull) of 4 mature triple crossbred (25% HF ×25% J ×50% Kankrej) bulls. The study covered evaluation, using standard procedures, of sperm motility, viability, morphology and acrosome integrity of fresh, frozen–thawed and refrigerated (5oC) aliquots of semen split-diluted (1:10 dilution) in standard Tris fructose yolk

glycerol diluent without and with EDTA disodium salt (0.1%) or cysteine hydrochloride (0.1%), with a view to evaluate their relative efficacies for improving the freezability and storage ability of semen. The parts of split-diluted ejaculates were preserved at refrigeration temperature (5°C) in glass tubes placed in half-filled beakers and the remaining parts were frozen in liquid nitrogen vapour. The samples were examined for all the above traits at initial, prefreeze and post-thaw stage as well as after 24 and 48 h of refrigeration storage as detailed earlier (Rana and Dhami, 2003). The means and standard errors of all the traits were calculated using 108 observations each (36 ejaculates ×3 split-samples) for tabulation and were subjected to test of significance using factorial CRD, and their correlation coefficients were worked out (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

The pooled means (±SE) of most of the spermatozoal attributes studied in the freshly diluted, prefreeze, post-thawed or post-refrigerated (24 and 48 h) semen were significantly better in the presence of EDTA and/or cysteine than the control Tris diluent, motile, live sperm and intact acrosome being higher and abnormal sperm and damaged acrosome being lower (Table 1). Furthermore, the values of motile and live sperm and intact acrosome were quite higher than the minimum acceptable standards reported in the literature for the fresh, refrigerated or frozen–thawed semen of bovines for use in AI. Some earlier workers have documented similar beneficial effects of cysteine and/or EDTA on the semen of cow–bulls and buffalo–bulls either at refrigeration temperature (Adbou and El-guindi, 1977; Tomar et al., 1979; Dhami et al., 1993) or at subzero temperature preservation (Singh et al., 1989; Kumar et al., 2001) or both (Virani, 1992; Dhami et al., 1993, 1995). However, Misra et al. (1988) and Saxena et al. (1988) could not find a beneficial effect of cysteine or EDTA on freezability and storage ability of bovine semen, respectively.

Cysteine HCl is a sulfhydrile group containing amino acid, which acts as a reducing substance and chelates heavy elements and stimulates aerobic fructolysis by the spermatozoa. EDTA also chelates calcium and other heavy metals, thereby protects sperm motility and maintains phosphorylation (Sengupta et al., 1969; Saxena et al., 1988; Dhami et al., 1993). The present findings suggest that EDTA (preferably) and/or cysteine (each 0.1%) can be supplemented as a routine in the semen extender to improve keeping quality and freezability of bovine semen so as to enhance the conception rate in inseminated females.

The interrelationships established through correlation matrix analysis between various spermatozoal traits studied at initial, prefreeze and post-thaw stage, and after 24 h and 48 h of refrigeration preservation of triple-bred bulls' semen (irrespective of diluent-additives) (Table 2) revealed highly significant ($P<0.01$) and positive interrelationships for the percentages of motile spermatozoa in fresh, post–thawed and refrigerated semen of triple-bred bulls (r=0.35–0.88). It was the same case for the percentages of live sperms (r=0.24–0.76), abnormal sperms (r=0.20–0.62), intact acrosome (r=0.30–0.76) and even damaged acrosome (r=0.29–0.73). Furthermore, only post-thaw live sperm percent revealed significant positive correlations with sperm motility at different steps of cryo-freezing/refrigeration preservation (r=0.21–0.30). The percentages of abnormal sperms in fresh, frozen-thawed and refrigerated semen had significant negative correlations with the percentages of live sperms and intact acrosome at various steps of freezing/preservation (r=0.19–0.43). Intact acrosome in fresh, post-thawed and refrigerated semen had highly significant ($P<0.01$) positive correlations with motile and live spermatozoa and negative correlations with damaged acrosome, while the latter (damaged acrosome) had negative correlations with the percentages of motile and live spermatozoa. In general, the correlations of motile sperms with live and

Table 1. Spermatozoal attributes of fresh, post-thawed and refrigerated semen of triple-bred bulls in TFYG diluent without and with additives (Mean ± SE)

Semen Processing/ Preservation Steps	Diluent-additive	Motile sperm (%)	Live sperm (%)	Abnormal sperm (%)	Intact acrosome (%)	Damaged acrosome (%)
Initial (Fresh)	-	82.08 ± 0.85	88.89 ± 0.57	7.44 ± 0.28	92.33 ± 0.33	7.67 ± 0.33
Prefreeze	Control Tris	76.11 ± 0.75	83.53 ± 0.73	9.56 ± 0.27	88.44 ± 0.35	11.65 ± 0.45
	Tris + EDTA	81.39 ± 0.84	85.72 ± 0.73	8.58 ± 0.27	91.22 ± 0.38	8.78 ± 0.38
	Tris + Cysteine	79.86 ± 1.04	84.17 ± 0.81	9.08 ± 0.32	90.17 ± 0.39	9.97 ± 0.39
Post-thaw	Control Tris	46.39 ± 1.27	57.22 ± 1.09	13.92 ± 0.26	81.42 ± 0.44	18.58 ± 0.44
	Tris + EDTA	53.19 ± 1.26	61.50 ± 0.76	13.03 ± 0.32	85.08 ± 0.39	14.92 ± 0.39
	Tris + Cysteine	52.22 ± 1.20	57.17 ± 1.25	13.78 ± 0.41	84.44 ± 0.35	15.56 ± 0.35
24-h Refrigeration	Control Tris	73.47 ± 0.77	78.72 ± 0.70	10.11 ± 0.25	87.36 ± 0.38	12.75 ± 0.35
	Tris + EDTA	78.06 ± 0.94	80.08 ± 0.65	9.31 ± 0.26	90.22 ± 0.36	9.94 ± 0.42
	Tris + Cysteine	77.36 ± 1.14	78.11 ± 0.77	9.81 ± 0.27	88.69 ± 0.37	11.31 ± 0.37
48-h Refrigeration	Control Tris	70.00 ± 0.91	72.28 ± 0.62	11.42 ± 0.23	85.31 ± 0.26	14.69 ± 0.26
	Tris + EDTA	74.31 ± 0.96	74.50 ± 0.68	10.53 ± 0.28	88.08 ± 0.27	11.92 ± 0.27
	Tris + Cysteine	72.92 ± 1.17	72.89 ± 0.57	11.11 ± 0.23	87.08 ± 0.39	12.92 ± 0.39

abnormal sperms in fresh, frozen–thawed and refrigerated semen were poor and of very low magnitude, and it was similar for the initial live sperm with the abnormal sperm, intact acrosome and damaged acrosome in fresh, frozen–thawed and refrigerated semen (Table 2). These findings on correlations corroborated well with some of the earlier reports (Saxena and Tripathi, 1978; Vyas et al., 1992; Belorkar et al., 1993; Shelke and Dhami, 2001 and Rana and Dhami, 2003).

The sperm motility, morphology and acrosome integrity after freezing and post-thaw incubation are shown to be good indicators of fertility of frozen–thawed bovine semen (Brown et al., 1982; Anzar et al., 1997). Post-thaw motility and intact acrosome are reported to have significant positive correlations with those in fresh semen of crossbred (Sharma et al., 1992) and buffalo bulls (Kumar et al., 1993). Saxena and Tripathi (1978) reported significant positive correlations of initial sperm motility of Jersey × Sahiwal crossbred bulls with sperm motility at different hours of refrigeration storage (+ 0.486 to 0.773). Dhami et al. (1993) recorded highly significant positive correlations (0.68–0.98) for the sperm motility traits of fresh, refrigerated and frozen–thawed semen of HF bulls at various storage intervals/processing steps, and concluded that freezability of semen could be predicted based on its keeping quality at 5°C. Belorkar et al. (1993) obtained good correlations for initial motility and sperm concentration with post-thaw motility and fertility of frozen semen of crossbred bulls. Vyas et al. (1992) reported significant positive correlations between the initial quality of sephadex/glasswool filtered semen and its freezability, post-thaw incubation survival and keeping quality at 5°C mainly for the traits of motile, live and abnormal sperm in crossbred bulls. Shelke and Dhami (2001) found highly significant positive interrelationships (0.277–0.925) among initial, prefreeze and post-thaw motility, sperm concentration and live sperm percent, and all these were negatively correlated with abnormal sperm percent in both Gir and Jafari bulls. Rana and Dhami (2003) found highly significant ($P<0.01$) positive interrelationships among the percentages of motile and live sperms, and intact acrosome in fresh, post-thawed and post-refrigerated semen (r=0.17–0.90). All these traits had significant negative correlations with the sperm/ acrosome abnormalities in Gir bull semen.

The present findings of significant positive correlations of motile and live sperm and intact acrosome of fresh semen with the same traits at all other processing/

preservation steps and their negative correlations with abnormal sperm and acrosome in both post-thawed and post-refrigerated semen, suggest that these tests can be of practical utility in routine semen evaluation initially to predict its keeping quality and freezability in triple-bred bulls. Moreover, The findings suggest that motility estimation in fresh, post-thawed and post-refrigerated semen is a fairly good indicator of live and abnormal sperm and acrosome integrity of sperms at various steps of semen processing/freezing/preservation and hence, this trait alone can be adopted in routine assessment of semen quality, instead of going into the tedious and time consuming staining procedures for assessment of viability, morphology and acrosomal integrity, which in fact are not always correlated with in vivo fertility.

Table 2. Interrelationships between various spermatozoal traits at initial, prefreeze and post-thaw stage and after 24-h & 48-h of refrigeration preservation in triplebred bulls' semen

Spermatozoal traits	Motile sperm					Live sperm					Abnormal sperm					Intact acrosome			
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4
Motile Sperm																			
Initial	-																		
Prefreeze	0.88**	-																	
Post-thaw	0.41**	0.43**	-																
24-h Refrigeration	0.73**	0.86**	0.35**	-															
48-h Refrigeration	0.66**	0.73**	0.40**	0.81**	-														
Live Sperm																			
Initial	0.01	0.05	0.15	0.13	0.11	-													
Prefreeze	0.11	0.14	0.15	0.04	–0.05	0.50**	-												
Post-thaw	0.25*	0.30**	0.26*	0.25*	0.21*	0.07	0.40**	-											
24-h Refrigeration	0.17	0.15	0.11	0.01	–0.10	0.42**	0.76**	0.28**	-										
48-h Refrigeration	0.28**	0.26*	0.13	0.10	–0.05	0.24*	0.57**	0.26**	0.73**	-									
Abnormal Sperm																			
Initial	0.14	0.18	0.31**	0.12	0.08	–0.26*	–0.28**	–0.33**	–0.20*	–0.19*	-								
Prefreeze	–0.05	–0.22*	–0.22*	–0.16	0.02	–0.11	–0.40	–0.43**	-.22*	-.29*	.45**	-							
Post-thaw	–0.02	–0.07	–0.06	–0.02	–0.03	0.01	–0.10	–0.24*	–0.34**	–0.05	0.17	0.29**	-						
24-h Refrigeration	0.02	–0.12	–0.14	–0.09	0.06	–0.06	–0.32**	–0.30**	–0.14	–0.23*	0.24*	0.67**	0.30**	-					
48-h Refrigeration	–0.19*	–0.32**	–0.13	–0.25*	–0.01	0.04	–0.19*	–0.26*	0.07	–0.22*	0.20*	0.58**	0.20*	0.62**	-				
Intact Acrosome																			
Initial	0.26*	0.20*	0.23*	0.17	0.19*	0.07	0.20*	0.41**	0.13	0.06	–0.24*	–0.20*	–0.03	–0.24*	–.20*0	-			
Prefreeze	0.34**	0.27*	0.13	0.25*	0.22*	0.07	0.34**	0.34**	0.23*	0.13	–0.11	–0.20*	–0.11	–0.21*	–0.24*	0.48**	-		
Post-thaw	0.30**	0.23*	0.29**	0.19*	0.20*	0.17	0.32**	0.30**	0.18	0.11	–0.10	–0.25*	–0.07	–0.17	–0.14	0.30**	0.60**	-	
24-h Refrigeration	0.39**	0.36**	0.12	0.27*	0.22*	0.21*	0.37**	0.34**	0.24*	0.20*	–0.34**	–0.34**	–0.27*	–0.26*	–0.30**	0.36**	0.75**	0.57**	-
48-h Refrigeration	0.38**	0.35**	0.25*	0.27*	0.28**	0.18	0.34**	0.39**	0.24*	0.22*	–0.33**	–0.25*	–0.18	–0.25*	–0.33**	0.32**	0.65**	0.63**	0.76**

No. of observations =108, 1. Initial stage, 2. Prefreeze, 3. Post-thaw, 4. 24-h Refrigeration, 5. 48-h Refrigeration, * P<0.05; ** P<0.01.

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anoestrous cows. However, the present findings in terms of values of serum trace minerals in cows are in close conformity with the observations of Desai et al. (1979). Reddy and Reddy (1988) recorded blood serum levels of iron in fertile and infertile cows to range from 96 to 296 and 80 to 220 µg/100 ml, respectively. They also stated that the low level of iron could possibly result in improper tissue oxygenation to the uterus resulting in impaired nutrition to the uterus. Vadnere and Singh (1989) also reported the comparable values of copper and iron, in postpartum anoestrus crossbred cows, which were significantly lower than the levels in normal cyclic cows. Sharma et al. (1988) opined that excess zinc concentration could be a cause of infertility in Kankrej heifers they investigated.

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