EFFECT OF BREED ON FUNCTIONAL CHARACTERISTICS OF FROZEN BULL SPERMATOZOA*

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ABSTRACT

Semen samples collected from Jersey cross (n=6) and Friesian cross bulls (n=6) were extended in Tris-egg yolk-citric acid diluent. The samples were split and cryopreserved in static vapor freezing and programmed freezing. A significant influence of breed was evident on the progressive motility, percentage of intact acrosome and loss of enzyme Aspartate transaminase while a non significant influence was noticed on the hypo-osmotic swelling response and percentage of live spermatozoa with intact acrosome. It was evident from the present investigation that the there is selective influence of breed on the functional characteristics of frozen thawed semen processed by routine freezing protocols and by programmed freezing.

Key words: Bull spermatozoa, Cryopreservation, Friesian cross, Jersey cross, Programmed freezing.

INTRODUCTION

Cross breeding programs have resulted in rapid genetic improvement and the same has been achieved through Artificial Insemination (AI) by using semen from progeny tested sires. At present a large network of AI facilities are available throughout the country like India. However, the overall conception rate with AI is poor. To sustain the effects of existing crossbreeding programmes there is a need to improve the conception rate in bovines. The problem still, is that with the currently available cryopreservation technique to date, post-thaw survival is restricted to about 50 per cent of the sperm population. The reason assigned to the poor post-thaw survival is that there is no control over the freezing rate apart from setting the initial vapor temperature in the current cryopreservation procedures. Many studies over the past 50 years have attempted to optimize the freezing conditions for bovine spermatozoa. With the advent of programmable biological freezers, it is possible to have control over freezing rates leading to higher post thaw survivability of spermatozoa. No comprehensive study has been reported on the effect of programmed freezing on the crossbred bull semen. Further, the quality of semen after cryopreservation has not been elucidated in detail. The present investigation was undertaken in crossbred bulls to compare the conventional method of cryopreservation using static vapor phase freezing and a programmed freezing using a biological freezer on bull spermatozoa, to assess the breed effect on the functional characteristics of frozen thawed bull spermatozoa.

MATERIALS AND METHODS

Semen from sexually mature and healthy cross bred bulls [Jersey x Sindhi cross bulls (n=6) and Holstein-Friesian crosses (HF x Sahiwal; n=2, HF x Sindhi; n=2, HF x Gir; n=1 and HF x Tharparkar; n=1)] stationed at Nucleus Jersey and Stud Farm, Udhagamandalam in Tamil Nadu was collected by artificial vagina (AV) after proper pre-collection stimulus by way of one false mount before each collection. All the bulls were housed in well-ventilated houses with sufficient running area adjoining the houses, under uniform and standard management condition. The animals were regularly exercised when no collection is done. They were provided with adequate concentrate feed twice a day, adlibitum green and dry roughage and water. Five semen samples (two ejaculates per collection) were collected from each bull in the early hours of the morning and semen samples that showed more than 60 per cent gross motility was used for

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processing. A total of 120 semen samples were analyzed.

The ejaculates were examined for volume (ml), initial motility (% of motile spermatozoa) and concentration (million per ml). The semen samples were extended using Tris-egg yolk-citric acid diluent (Mathew, 1984) and was further diluted with one step addition of 5 per cent glycerol at 5°C. The two ejaculates from each bull were pooled after the addition of glycerol and equilibrated for 4 hours. The extended semen samples were filled into 0.25ml French mini straws (IMV, France) containing the name, identification number and the breed of the bull and automatically sealed. Dilution rate was fixed so that each dose had at least 25 million sperms. At the end of the equilibration period, the influence of type of freezing i.e. the freezing rate was studied using two types of freezing techniques.

One set of straws from each bull were arranged in racks and placed inside a LR-440 container (MVE, Ohio, USA), by fixing the freezing rack at 4 cm from the level of the liquid nitrogen for 4 minutes. Immediately after freezing the semen straws were collected in goblets with liquid nitrogen and stored at -196°C. The second set of the straws were frozen using a programmable bio-freezer DIGIT COOL 5300 (IMV, France). The program for the freezing rate was set as 4 hours of equilibration at +25°C to +4°C, followed by a freezing rate of -5°C / min to reach -12°C from +4°C and - 60°C / min to reach -140°C. At the end of the freezing cycle the straws were collected into goblets and directly plunged into Liquid Nitrogen.

The pre and post freeze evaluation of spermatozoa for post thaw motility, membrane integrity and acrosome status was performed during equilibration and after 20 days. The straws were thawed in a water bath at 37°C for 30 seconds for post-thaw evaluation of spermatozoa. The progressive motility was assessed based on the percentage of progressively motile spermatozoa in the field. Acrosome staining technique of Hancock (1952) using Giemsa stain was adopted to determine the percentage of intact acrosome before and after freezing. The stained slides were examined under oil immersion objective and a minimum of 100 spermatozoa was examined for the changes in the acrosome. The concentration of Aspartate Transaminase (Aspartate transaminase) enzyme released into the seminal plasma was estimated by colorimetric method as per Reitman and Frankel (1957) using Systronics® Colorimeter. The concentration of enzyme Aspartate transaminase (µmol/L) was estimated just before freezing (pre-freeze) and after freezing and thawing (post-thaw) to assess the leakage of the enzyme as a result of freezing damage. Supra vital triple staining was performed as described by Vazquez *et al.* (1992). The spermatozoa were examined under a Light microscope at oil immersion objective. The spermatozoa were classified as live spermatozoa with intact acrosome, live spermatozoa without acrosome, dead spermatozoa with intact acrosome and dead spermatozoa without acrosome based on the staining patterns. The plasma membrane integrity was assessed using Hypo-osmotic Swelling Test (Jeyendran *et al.*, 1984) to determine the percentage of spermatozoa showing the hypo-osmotic swelling reaction before and after freezing. The curling or swelling percentage was recorded after counting around 200 sperms. The data collected were statistically analyzed using a computer programme Microstat®.

RESULTS AND DISCUSSION

The data on the characteristics of neat semen has been presented in Table. There was no significant (>0.05) difference on the mean volume of the ejaculate, mass activity and concentration of spermatozoa between the breeds. The Jersey cross bulls showed an increased post-thaw motility than the Friesian cross bulls in both the freezing methods. However, the increase was statistically non-significant ($P>0.05$). In the preset study, there was no significant ($P>0.05$) difference in the percentage of progressive motility ($P>0.05$) between breeds in both the freezing methods. This concurs with Roy *et al.*, (1975) who observed no significant variation in the post freeze motility of spermatozoa between the genetic groups while comparing the freezability of spermatozoa in three genetic groups of crossbred bulls. The Friesian cross bulls showed a significant increase ($P<0.01$) in percentage of intact acrosome than the Jersey cross bulls in both the freezing methods. The results of the present study might be due to the variation between the two genetic groups to freezing and the variability in response in the cell membrane to freezing. Further investigation is warranted since there is paucity of literature in this aspect.

The Jersey cross bulls showed an increased hypo-osmotic swelling response than the Friesian cross bulls in both the freezing methods. However, the increase was statistically not significant ($P\leq0.05$). The non significant increase in the percentage of hypo-osmotic

swelling response in Friesian bulls observed in this study shows that the Friesian cross bulls withstood the freezing effect well than the Jersey cross bulls. The non-significant difference between the breeds may be explained based on non-significant difference observed in the post-thaw motility. This finding has to be studied in detail with different freezing rates with the programmable freezer as there is want of sufficient literature to compare the results obtained in the present study.

The Jersey cross bulls possessed highly significant percentage ($P \leq 0.01$) of live spermatozoa with an intact acrosome than the Friesian cross bulls in the prefreeze semen. Nevertheless, the difference was not statistically significant in the post-thaw semen in both the freezing methods though there was a highly significant difference between the prefreeze and post-thaw semen. Critical analysis sowed that the effect of freezing was more on Jersey cross bulls than on the Friesian cross bulls though the interaction between the freezing effect and the breeds was not significant ($P>0.05$). The Jersey cross bulls possessed a higher percentage of live spermatozoa with intact acrosome than the Friesian bulls in the prefreeze semen. However, due to freezing the percentage of live spermatozoa with intact acrosome decreased in the Jersey cross bulls more than in the Friesian cross bulls though the decrease was statistically not significant. The difference in loss of enzyme Aspartate transaminase was significantly lower ($P \leq 0.01$) in the Jersey cross semen than in the Friesian cross semen in both the freezing methods. In the present study, the Jersey cross bulls lost lesser amount of Aspartate transaminase than the Friesian cross bulls in both the freezing methods. The same effect is noted in the percentage of intact acrosome as well. The result therefore shows that the Jersey cross bulls had a high percent of intact acrosome post freeze and hence the lower loss of enzyme into the seminal plasma. This was in difference with the findings of Belorkar *et al.*, (1993) who reported no significant variations between the breeds. However, Dhami *et al.*, (1987) reported a significant difference between bulls in the buffalo semen.

The results therefore suggest that although the Jersey cross bulls possessed higher percentage of intact acrosome and lost less enzyme they had a poor membrane integrity post freeze. Further studies on post-thaw motility, acrosome changes, membrane integrity and enzyme leakage are needed before any conclusion can be made on the influence of breed on the freezability of the bull spermatozoa between the two freezing methods used.

Table : INFLUENCE OF BREED ON FREEZING OF BULL SPERMATOZOA (Mean ± SE)

Parameters	Jersey Cross			Friesian Cross		
	Prefreeze	Static Vapor Freezing	Programmed Freezing	Prefreeze	Static Vapor Freezing	Programmed Freezing
Progressive Motility (%)	63.33± 0.53[a]	56.83± 0.74[b]	60.00± 0.59[c]	62.58± 0.53[a]	57.50± 0.79[b]	61.17± 0.62[c]
Intact Acrosome (%)	71.27± 1.68[a]	54.87± 1.12[b]	56.67± 1.34[c]	72.30± 1.47[a]	58.27± 0.99[d]	62.06± 0.89[e]
Hypo-osmotic Swelling Reaction (%)	66.45± 1.03[a]	58.20± 1.07[b]	60.02± 1.00[b]	66.50± 0.86[a]	57.81± 0.88[b]	59.14± 0.87[b]
Live Spermatozoa with Intact Acrosome (%)	57.68± 1.93[a]	50.86± 1.48[c]	51.67± 1.54[e]	55.94± 1.88[b]	49.61± 1.71[c]	50.54± 1.46[c]
Aspartate transaminase (µmole/ml)	12.92± 12.92[a]	37.64± 7.69[b]	23.92± 8.82[c]	13.28± 5.12[a]	41.00± 4.30[d]	30 .63± 4.54[e]

Means having the same superscript within rows do not differ significantly ($P>0.05$)

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