EFFECT OF CRYOPRESERVATION ON THE POST THAW SURVIVEABILITY OF BUFFALO BULL SPERMATOZOA WITHOUT SEMINAL PLASMA

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ABSTRACT

Semen samples from Nili Ravi buffalo bulls were collected and divided for three treatments i.e. A (whole semen), B (spermatozoa free of seminal plasma) and C (spermatozoa resuspended in seminal plasma). Each fraction was extended in two different extenders viz. E1 (milk-egg yolk glycerol), E2 (lactose-fructose-egg yolk-glycerol) by the ratios of 1:10. After equilibration of 5 hours, all samples were frozen at -196°C. The average (Mean ± SD) post thaw motility (47.75 ± 3.87, 57.25 ± 4.38 and 34.50 ± 5.30 per-cent) and 5.30 ± 0.81, 8.00 ± 0.82 and 3.05 ± 0.699 hours for liveability at 37°C while 102.62 ± 15.16, 217.25 ± 19.08 and 46.87 ± 9.09 for absolute index of liveability for three treatments respectively. There was highly significant difference ($P < 0.001$) among the three treatment. Treatment B proved the best and followed by treatment A and C. Extender lactose-fructose-egg yolk-glycerol proved superior than milk-eggyolk-glycerol.

INTRODUCTION

It is well known that ejaculated semen is composed of spermatozoa and seminal plasma. There is no evidence that any component of seminal plasma or any of the accessory sex glands are absolutely indispensible to fertility (Price and Williams, 1961). The presence of seminal plasma causes an apparent reduction of glucose uptake by spermatozoa (Flipse, 1954). A heat labile toxic protein has been insolated from bovine seminal plasma (Shannon, 1965). The presence of seminal plasma causes increase in the release of the amino acid oxidase, an enzyme responsible for reduction in motility (Martinus *et al.*,1991). Many research workers have cryopreserved buffalo bull whole semen (Anand *et al.*, 1978; Ala-ut-Din *et al.*, 1981; Boserekar *et al.*, 1991; Singh *et al.*, 1992) but the results indicate lower viability of spermatozoa. Therefore, this project was carried out to cryopreserve buffalo bull spermatozoa at -196°C after removing the toxic components through the drainage of seminal plasma with the objective to improve the viability of buffalo bull spermatozoa after freezing.

MATERIALS AND METHODS

Semen collection

Semen from two Nili Ravi buffalo bulls was collected twice weekly by artificial vagina. Two successive ejaculates per collection were obtained. Ten ejaculates from each bull were selected (a total of five from each bull). Immediately after collection the samples were transferred to water bath at 37°C for evaluation. The color, volume, concentration, pH, mass activity and rate of motility were observed. Ejaculates having at least 60% motility were selected for further processing.

Each pooled semen sample was divided into six equal aliquots. Two of them were maintained as control (A). While the other four aliquots were centrifuged at 1000g for 15 minutes for removal of seminal plasma. The seminal plasma from two of these centrifuged aliquots was removed (B), while the seminal plasma of other two centrifuged aliquots was allowed to remix (C). Three out of six aliquots (1A + 1B + 1C) were extended wit extender E1 (Table 1) and similarly others with the extender E2 (Table 1) by a ratio of 1:10.

Cryopreservation

French straws were filled with extended semen. After sealing of the open end, the straws were stored at 4°C for an equilibration time of 5 hours. Motility rate after the equilibration time was recorded. Freezing was done by holding straws in liquid nitrogen vapours 5 cm above the level of liquid nitrogen in a freezing chamber for 8 minutes. Then they were immensed in liquid nitrogen and stored at least for 24 hours (Ahmad and Chaudhry, 1980).

Table 1: Composition of experimental extenders

Ingredients	Extenders	
	E1	E2
11% Lactose (mL)	-	55
6% Fructose (mL)	-	18
Whole milk (mL)	73	-
Glycerol (mL)	7	7
Egg yolk (mL)	20	20
Penicillin (1U/mL)	1000	1000
Streptomycin (mg/mL)	1	1

E1 = Whole milk-egg yolk-glycerol (WMEYG)
E2 = Lactose-fructose-egg yolk-glycerol (LFEYG)

Post thaw motility, surviveability and absolute index of surviveability

Thawing was carried out in a water bath at 37°C for 15 minutes. Immediately after thawing each sample was checked for initial post thaw motility. Then each sample was incubated at 37°C and the rate of motility was checked after every hour till the death of all spermatozoa to compute the surviveability of spermatozoa. Initial post thaw motility and liveability of spermatozoa at 37°C were used to compute absolute index of liveability (Ahmad *et al.*, 1994a).

Data analysis

Data thus collected was analysed using CRD with factorial experiment design (Snedecor and Cichron, 1967). DMR test was applied for multiple mean comparison (Duncan, 1955).

RESULTS AND DISCUSSION

The data regarding initial post thaw motility, liveability and absolute index of liveability of spermatozoa for treatment and extenders has been presented in Table 2. Analysis of the data revealed that there was highly significant ($P < 0.001$) difference among the three treatments. The extenders also differed significantly ($P < 0.005$). The highly significant results of treatment B could be due to the removal of seminal plasma which contains inhibitory factors. These results were in agreement to those reported by Flipse (1954); Shannon (1965); Chinnaiya (1982); Sahni and Mohan (1990); Sahni (1990); Martinus *et al.* (1991) and Ahmad *et al.* (1994 b).

Chinnaiay 1982 removed seminal plasma from buffalo bulls having low initial motility. The samples having motility >50 Per-cent were frozen and 25 Per-cent of frozen sample showed initial post thaw motility >40%. A heat labile toxic protein in the bovine seminal plasma to reduce the liveability from 15.8 to 9.3 hours (Shannon, 1965). Ahmad *et al.* (1994b) have observed improvement in the liveability of buffalo bull spermatozoa from 12.875 ± 1.310 (hours) for whole semen to 15.61 ± 1.91 (hours) for removal of seminal plasma at 37°C. the adverse effect of seminal plasma is more marked in buffalo semen than in cattle semen (Sahni, 1990).

The poor results of treatment C are due to extrachular release of toxic substances after injury to the sperm cell by centrifugation (Mann, 1964). These substance caused damage to the spermatozoa after resuspansion of seminal plasma to the spermatozoa. These results are in agreement to those reported by Martinus *et al.* (1991). They observed the release of amino acid oxidase enzyme activity from 0.332 ± 0.016 (spermatozoa frozen with 85% seminal plasma) to 0.125 ± 0.021 (spermatozoa frozen without seminal plasma).

Even if the separation of spermatozoa from the seminal plasma has been done with great care there is not certainity that the loss of intracellular substances has not been incurred. Because they are highly valuable to mechanical injury. These substance are drained by removal of seminal plasma after centrifugation. The seminal plasma of 'Echinus Esculentus' acquires sperm immobilizing properties after centrifugation as a result of leakage of inhibitory substances released from spermatozoa into seminal plasma (Rothschild, 1948).

Similarly, Mann (1964) reported that centrifugation may inflict an injury upon sperm cell which even not apparent, nevertheless results in leakage of certain proteins from spermatozoa into seminal plasma. Thus for instance "Cytochromes C" is easily detached from the sperm structure, as a result of cellular damage. Extra cellular detection of "Cytochrome C" provides a sensitive indicator for the "genescence" changes in spermatozoa. Probably this explains the conflicting reports concerning the effects of centrifuged seminal plasma on sperm motility.

Table 2: Average values (Mean ± S.D) of post thaw motility, Liveability and absolute index of liveability of spermatozoa at 37°C.

Extenders	Treatment A	Treatment B	Treatment C	Average
Post thaw motility (%)				
E1	46.50 ± 3.87	56.00 ± 5.30	33.00 ± 5,03	45.16 ± 4.73
E2	49.00 ± 3.87	58.50 ± 3.46	36.00 ± 5.59	47.83 ± 4.31
Average	47.75 ± 3.87 b	57.25 ± 4.38 a	34.50 ± 5.31 c	
Liveability (hours)				
E1	5.10 ± 0.77	7.30 ± 0.87	2.90 ± 0.77	5.10 ± 0.80
E2	5.50 ± 0.84	8.70 ± 0.77	3.20 ± 0.63	5.80 ± 0.75
Average	5.30 ± 0.81 b	8.00 ± 0.89 a	3.05 ± 0.70 c	
Absolute index of liveability				
E1	96.25 ± 17.13	191.75 ± 12.977	42.75 ± 8.113	110.25 ± 12.739
E2	109.00 ± 13.199	242.75 ± 25.192	51.00 ± 10.082	134.25 ± 16.157
Average	102.62 ± 15.164 b	217.25 ± 19.084 a	46.87 ± 9.097 c	

Treatment A = Whole semen. Treatment B = Removal of seminal plasma by centrifugation.
Treatment C = Centrifugation but the sediment resuspended into supernatent
E1 = Whole milk-egg yolk-glycerol (WMEYG) E2 = Lactose-fructose-egg yolk-glycerol (LFEYG)

Lactose and fructose have successfully been used to preserve buffalo bull semen (Tomar and Desai, 1961). The reason for the superiority of LFEYG extender on WMEYG is due to the reason that Monosaccharides sugars (glucose and fructose) not only provides nutritive support but also maintains the osmotic balance of extender (Singh *et al.*, 1992). Disaccharides (lactose and sucrose) provides cryoprotective benefits because of having larger molecule while acting as a dehydrating agent (Aslam *et al.*, 1992). The freezing of buffalo bull whole semen have indicated low viability rates (Ala-ud-Din *et al.*, 1981). But from this study it is evident that removal of seminal plasma before cryopreservation is beneficial in respect of post thaw motility, liveability and absolute index liveability of buffalo bull spermatozoa.

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