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Short communication

Effect of prostaglandin $F_2\alpha$, oxytocin and butylated hydroxytoluene on freezability of crossbred bull spermatozoa*

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ABSTRACT

Semen samples were collected from two crossbred bulls (HF x Hariana) and diluted in Tris with three combinations of additives i.e. prostaglandin $F_2\alpha$, oxytocin and butylated hydroxytoluene and frozen in liquid nitrogen. The sperm motility, livability and intact acrosome percentage were evaluated at pre-freeze and post-freeze stages. Except prostaglandin $F_2\alpha$, the other additives showed no significant difference in motility after dilution vs control. At pre-freeze stage, sperm motility differed significantly between treated and control groups. At post-freeze stage, sperm motility and livability differed significantly between treatment and control groups. Among the additives $PGF_2\alpha$ performed better than oxytocin and BHT in maintaining higher motility, livability and acrosomal integrity in post-thaw semen.

Key words : Bull spermatozoa, freezability, $PGF_2\alpha$, oxytocin, BHT

Prostaglandin $F_2\alpha$ found in seminal plasma is a hormone like substance reported to increase the spermatozoal motility in buffaloes (Muralinath, 1988), cattle (Lokanathan, 1993). Addition of oxytocin hormone prior to freezing was found to increase the post-thaw sperm motility and livability (Lokanathan, 1993). Butylated hydroxytoluene (BHT), an antioxidant and an organic soluble molecule which modifies the properties of lipid bilayers and membrane of sperm cell (Hammerstedt *et al.*, 1976) and serves as a scavenger of oxygen free-radicals, associated with diluent and sperm (Killian *et al.*, 1989). The present investigation was, therefore, aimed to study the effect of prostaglandin $F_2\alpha$, oxytocin and BHT on seminal characters at pre-freeze and post-freeze stages in crossbred bulls.

Twenty four ejaculates (12 each from two bulls) from crossbred bulls (HF x Hariana) maintained under identical feeding and managemental regimen were used for the study. Semen was collected in A. V twice a week. All ejaculates were evaluated for physio-morphological seminal characteristics. The ejaculates were split into 4 parts. Each part was diluted separately with Tris + additive combinations @ (1.5 ng/ml PG $F_2\alpha$, 0.1 unit/ml oxytocin (Lokanathan, 1993), Butylated hydroxytoluene (BHT), 2 mM/ml (Killian *et al.*, 1989) and 4th aliquot was kept as control. Egg yolk and glycerol were added @ of 20 and 7%, respectively. The four split samples were Frozen in medium french straw after providing combined cooling cum equilibration period of 4 hrs. The spermatozoal motility was measured immediately after dilution, at pre-freeze and post-freeze stage. The spermatozoal livability and intact acrosome percentage were measured at pre and post-freeze level. Statistical analysis of the data was done for mean and standard errors and analysis of variance.

The mean percentage of spermatozoal motility, livability and intact acrosome precentage at pre-freeze and post-freeze stage have been presented in Table 1. Except $PGF_2\alpha$ other additives showed no significant difference in motility after dilution Vs control. However, all the additive treated semen samples showed improvement in motility at the end of equilibration period at 5°C than control which was significant ($P<0.05$).

The livability and intact acrosome percentage at pre-freeze stage did not show any significant difference between the control and treatment groups. The mean percentage motility of frozen thawed spermatozoa was

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Table 1. Mean (±SE) sperm motility, livability and intact acrosome (per cent) of bull semen frozen with various semen additives in Tris diluent.

Character	Stage of processing	Bull No.	Control	$PGF_2\alpha$	Oxytocin	BHT
Motility						
	After dilution	Overall	67.63 ± 1.04^{b}	71.35 ± 0.98^{a}	69.79 ± 1.00^{ab}	70.02 ± 0.96^{ab}
	Pre-freeze	Overall	61.46 ± 1.02^{b}	66.21 ± 1.08^{a}	64.55 ± 1.09^{a}	64.44 ± 1.14^{a}
	Post-freeze	Overall	33.50 ± 0.71^{c}	44.33 ± 0.82^{a}	40.37 ± 0.84^{b}	43.41 ± 0.79^{a}
Live sperm						
	Pre-freeze	Overall	76.03±1.48	81.18±1.43	79.01±1.59	79.51±1.67
	Post-freeze	Overall	64.00 ± 1.05^{b}	73.51 ± 1.23^{a}	71.37 ± 1.21^{a}	72.46 ± 1.15^{a}
Intact acrosome						
	Pre-freeze	Overall	86.64±0.48	87.40±0.54	86.49±0.52	87.40±0.52
	Post-freeze	Overall	78.14±0.94	79.30±0.99	78.59±0.94	79.43±1.02

Means bearing different superscript in the same column differ significantly ($P<0.05$).

44.33±0.82 ($PGF_2\alpha$), 40.37±0.84 (oxytocin), 43.41±0.79 (BHT) and 33.50±0.71 (control) . Between different semen additives, comparatively higher per cent of motile sperm was recorded in prostaglandin $F_2\alpha$ treated group. The difference in post-thaw motility between the control and treatment groups was significant ($P<0.05$). The post-thaw livesperm percentage were 73.15±1.25 ($PGF_2\alpha$), 71.37±1.21 (oxytocin), 72.46±1.15 (BHT) and 64.00±1.05% (control). The results differed significantly ($P<0.05$) between treatment groups and control. However, between the treatment groups, no significant variation was obtained. The values of intact acrosome percentage were 78.14±0.94 (control), 79.30±0.99 ($PGF_2\alpha$), 78.59±0.94 (oxytocin) 79.43±1.02% (BHT) treated groups. There was no significant difference between treatment groups and control at post-thaw stage.

The $PGF_2\alpha$ treated semen revealed better results in terms of maintaining sperm motility, livability and intact acrosome percentage than oxytocin, BHT and control. This present study was well comparable with (Muralinath *et al.*, 1990) buffaloes and (Lokanathan, 1993) bull semen. This improved protection to sperm cells are possibly by the protective and stimulatory effect of PG $F_2\alpha$ during preservation. Oxytocin treated semen showed significant improvement in sperm motility and livability than control. This results is similar with (Martinek *et al.*, 1978) sows and (Lokanathan, 1993) bull semen. This protection role might be due to the pronounced stimulatory effect of oxytocin on sperm cells. BHT treated semen observed improvement in sperm motility and livability. This present study is well comparable with (Killian *et al.*, 1989) bull and (Tervit and Macmillan, 1983) ram semen. The integrity of bovine sperm membrane was destroyed by the rapid cooling during cryopreservation. BHT protect membranes of the spermatozoa from cold induced lysis thereby it increases post-thaw motility and livability, which is in agreement with (Hammerstedt *et al.*, 1978). Visualising the results of the present study on the effect of PG $F_2\alpha$, oxytocin and BHT on post-thaw semen quality of crossbred bull, it was observed that all the three additives improved the post-thaw motility, post-thaw livability and post-thaw intact acrosome percentage.

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