DETRIMENTAL IMPACT OF CRYOPRESERVATION ON BUFFALO BULL SPERM MOTILITY, VIABILITY AND MEMBRANE INTEGRITY DUE TO EFFLUX OF MEMBRANE CHOLESTEROL

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ABSTRACT

Thirty ejaculates from six buffalo bulls were used in the present study. Subsequent to cryopreservation, cholesterol content of buffalo bull sperms was reduced as compared to pre-freeze stage ($p<0.05$). From pre-freeze to post-thaw stage, a marked decrease was recorded in sperm motility, viability and membrane integrity ($p<0.05$). It brief, the efflux of cholesterol from sperm membrane during cryopreservation might lead to decrease in sperm motility, viability and membrane integrity *vis-à-vis* decrease in fertility of frozen-thawed semen.

Keywords: Buffalo bull, Cholesterol, Cryopreservation, Semen, Sperm

A major reason for poor quality of buffalo bull semen and the fertility of frozen-thawed semen is cryocapacitation inflicted damage to sperm during freezing and thawing (Bailey *et al.,* 2000). Sperm possessing high cholesterol to phospholipid ratio (rabbit and human) are more resistant to freezing induced damage than the sperm having low ratios (stallion, ram and bull; Watson, 1981; Parks and Lynch, 1992 and Foote, 2002). The membrane cholesterol is involved in inflicting harmful effects as cholesterol gets depleted gradually after ejaculation, which initiates calcium ion influx, a first signal for cryocapacitation (Buhr *et al.,* 1994). The capacitation-like changes inflict considerable damage to motility apparatus, plasma membrane and acrosomal cap (Rasul *et al.,* 2001). The cholesterol efflux may represent an integral part of intrinsic regulatory property of sperm to undergo capacitation-like changes during cryopreservation. Hence, the present study was designed to evaluate the effect of cholesterol efflux on various sperm parameters like motility, viability and membrane integrity.

Five ejaculates each from six breeding Murrah buffalo bulls (age, 4 yr) were collected and semen with mass motility +++ or above and individual motility 75-80% was used for present study. The sperm concentration was assessed using Accucell bovine photometer. Semen was diluted with Tris egg yolk dilutor (TYC) to 120 million sperms/ml. The extended semen was divided into two aliquots for evaluation at pre-freeze and post-thaw stage. The aliquot for pre-freeze evaluation was cooled at 4^0C for 4 h in cold handling cabinet for equilibration. The aliquot for post-thaw stage was filled and sealed by manual filling and sealing method. The filled and sealed straws were also cooled at 4^0C for 4 h and cryopreserved in liquid nitrogen by following standard procedure. Pre-freeze and frozen-thawed semen samples were evaluated for sperm motility, viability and HOST using standard procedures. Pre-freeze and post-thaw sperms were washed thrice with PBS (pH 7.4) at 3000 rpm for 10 min. Sperm proteins were extracted by suspending 250 x 10^6 sperms in 0.5 ml of 62.5 mM Tris-HCl buffer (pH 6.8) containing 2% SDS, 10 µl cocktail protease inhibitors (Serva). The mixture was sonicated (3 bursts of 20 sec each) and centrifuged at 15000 rpm for 30 minutes (SDS-SE). Cholesterol was estimated in pre-freeze SDS-SE and post-thaw SDS-SE (Zlatkis *et al.,*1953). The data was analysed using student's t-test.

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Table 1: Impact of cryopreservation on total motility, viability and membrane integrity and cholesterol content in buffalo bull semen (30 ejaculates from six bulls)

Parameters	Pre-freeze	Post-thaw	Decrease by
Motility, %	74.6±1.5[a]	48.5±1.8[b]	26.1%
Viability, %	85.3±1.03[a]	65.8±1.2[b]	19.5%
Membrane integrity, %	74.4±3.78[a]	55.9±4.83[b]	18.5%
Cholesterol content, µg/10^9 spermatozoa	503.17±31.55[a]	424.18±52.85[b]	79.0%

[a vs. b] $p<0.05$, within a row

Subsequent to thawing of cryopreserved buffalo bull semen, the percent motility, viability and HOS decreased by 26.1%, 19.5% and 18.5%, respectively as compared to pre-freeze stage ($p<0.05$, Table 1). The decrease in cholesterol might cause loss of motility by reducing the survival rate after cryopreservation. In fact, a decrease of 79 µg cholesterol/10^9 buffalo spermatozoa was observed at post-thaw stage over pre-freeze stage ($p<0.05$, Table 1). Cholesterol aids in the stabilization of sperm membrane by regulating its fluidity during cryopreservation and the efflux of cholesterol or decrease in cholesterol phospholipid ratio in sperm membrane lead to capacitation (Muller *et al.,* 2008). Cryocapacitation is a major factor associated with reduced longevity and poor survivability of cryopreserved spermatozoa in female reproductive tract (Bailey *et al.,* 2000), resulting in reduced fertility of frozen-thawed semen. It may be concluded that efflux of cholesterol during freezing-thawing might cause decline in sperm motility, viability and membrane integrity which may further reduce fertility.

REFERENCES

Bailey, J.L., Bilodeau, J.F. and Cormier, N. (2000). Semen cryopreservation in domestic animals: a damaging and capacitating phenomenon. *J. Andro.,* **21**: 1-7.

Buhr, M.M., Curtis, E.F., and SomnapanKakuda, N. (1994). Composition and behavior of head membrane lipids of fresh and cryopreserved boar sperm. *Cryobiology*, **31**: 224-238.

Foote, R.H. (2002). The history of artificial insemination: selected notes and notables. *J. Anim. Sci.*, **80**(E 2): 1-10.

Muller, K., Muller, P., Pincemy, G., Kurz, A. and Labbe, C. (2008). Characterization of sperm plasma membrane properties of rainbow trout spermatozoa. *Bio. Reprod.,* **78**: 390-399.

Parks, J.E. and Lynch, D.V. (1992). Lipid composition and thermo tropic phase behavior of boar, bull, stallion, and rooster sperm membranes. *Cryobiology*, **29:** 255-266.

Rasul, Z., Ahmad, N. and Anzar, M. (2001). Changes in motion characteristics, plasma membrane integrity, and acrosome morphology during cryopreservation of buffalo spermatozoa. *J. Andro*., **22**: 278-283.

Watson, P.F. (1981). The effects of cold shock on sperm cell membranes. In: Morris, G.J., Clark, A. (eds), *Effects of low temperatures on biological membranes*. Academic Press, London, pp. 189-218.