PLASMA MEMBRANE INTEGRITY OF BULL SPERMATOZOA AT DIFFERENT STAGES OF PROCESSING OF SEMEN

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ABSTRACT

Present investigation was carried out on 51 semen ejaculates (26 from Holstein Friesian and 25 from crossbred bulls) collected from ten bulls maintained at Animal Breeding Centre, Salon, Rae Bareli (UP). Plasma membrane integrity of sperms at various stages of cryo-preservation was studied with the help of Hypo Osmotic Swelling Test (HOST). The results obtained revealed that the process of cryopreservation adversely affected the plasma membrane integrity. Percentage of HOST reacted sperms was reduced by more than 20 percent after freezing. There was a highly significant positive correlation (r=0.43) between number of HOST reacted sperms after dilution and after freezing. Too early (<15 minutes) or any delay in processing time (> 30 minutes) was found to have adverse effect on sperm plasma membrane integrity. A non significant but negative correlation was observed between the processing time and % of HOST reacted sperms after equilibration (r = -0.19) and freezing (r = -0.22).

Key words: Bull, Cryo-preservation of semen, HOST, Plasma membrane integrity

The Artificial Insemination (AI) technology is considered to be the most powerful tool in the hands of animal scientists for the genetic improvement of the livestock and for the control of sexually transmitted diseases. The genetic gains through AI are achieved by maximum utilization of an elite sire to produce thousands of superior progenies, which otherwise is not be possible through natural breeding. Cryo-preservation of semen plays an important role in adoption of this technology. However, for obtaining maximum progress it is very much important to control numerous environmental and management factors affecting the fertility of the females and the quality of the cryo-preserved semen.

In the process of semen freezing and thawing, a large number of sperms apparently become damaged (Nishizono *et al.*, 2004; Peris *et al.*, 2004; Bollwein *et al.*, 2008). It has been reported that compared to fresh semen, eight times more frozen bovine sperms were required to achieve the equivalent fertilization rates in vivo (Shannon and Vishwanath, 1995).

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Spermatozoal motility and integrity of plasma membrane as well as acrosome are considered to be the important quality parameters effecting fertility of frozen semen. However, these viability parameters are extremely sensitive to various stages of cryo-preservation, which can alter the fertilizing capacity of the sperms.

Keeping this in mind, the present study is designed to investigate the extent of damage to plasma membrane at various stages of cryo-preservation through HOST.

The study was based on 51 ejaculates (26 from Holstein Friesian and 25 from Crossbred bulls) collected from ten breeding bulls maintained at Semen station, Animal Breeding Centre, Salon, Rae Bareli (UP). Semen samples with 70 percent or above initial motility were included in this study. The sampling of semen was done at three different stages, namely after dilution, after completion of four hours equilibration and 24 hours post-freezing. The freezing of semen samples was done by programmable Bio-freezer (IMV make). HOST for the collected samples was conducted following the procedure described by Jayendran *et al.* (1984).

The effect of different stages of cryo-preservation on the integrity of plasma membrane of spermatozoa has been studied by using Least-squares technique as suggested by Harvey (1975).

Least-squares analysis of variance revealed that the various stages of cryo-preservation had highly significant (P<0.01) effect on sperm membrane integrity. The percentage of HOST reacted spermatozoa had gone down from 87.8 percent at dilution to 79.8 percent after equilibration and finally to 65.3 percent after freezing. For both Holstein Friesian and Crossbred bulls, more than 20 per cent of spermatozoa were observed to have been damaged in the process of cryo-preservation. Hammerstedt *et al.,* (1990), Parks and Graham (1992) and Watson (1995) reported that the plasma membrane of the sperms got damaged by cryo-preservation. Bull sperms, due to higher ratio of unsaturated to saturated fatty acid are found to be more susceptible to peroxidation (Halliwell and Gutterridge,1984) which results in membrane damage, inhibition of respiration and leakage of intercellular enzyme on freezing (White, 1993).

In the present study, statistically highly significant positive correlation was observed between percentages of sperms reacted following dilution and post-freezing (r=0.43). This revealed that good quality semen has the better probability to survive the freezing process. Similar observation was reported by Cerolini *et al.,* (2001) in boar semen. Shanon and Curson (1972) reported that the presence of dead sperms result in activation of aromatic amino acid oxidase, which is a major source of ROS (Reactive Oxygen Species) production in semen. The release of oxidase from dead sperms reduces the motility and viability of the remaining living sperms in bovine semen

Status of sperm plasma membrane integrity (as evident through % of HOST reacted sperms) after equilibration period and freezing was studied for different processing time. Figures presented in the table revealed that maximum percentage of HOST reacted sperms after equilibration (81.32±1.83) and freezing (66.12±2.21) was observed when the processing time is between 15-30 minutes. While, in case of too early processing (<15 minutes) or delay in processing (>30 minutes), figures for % of HOST reacted sperms were relatively less. In the present study, a non significant but negative correlation was also observed between the processing time and % of HOST reacted sperms after equilibration (r = -0.19) and freezing (r = -0.22).

From the present study, it can be concluded that processing of semen ejaculates should be done without any delay at different stages namely filling-sealing, printing and racking to avoid deterioration in semen quality.

Table: Percentage of HOST reacted sperms after equilibration and after freezing for different processing time

Processing time (Minutes)	Percentage of HOST reacted sperm	
	After Equilibration	After Freezing
Less than 15	76.42±2.93	69.92±2.95
15-30	81.32±1.83	66.12±2.21
31-60	80.12±2.80	63.92±2.89
More than 60	78.72±2.61	64.52±3.18

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