EFFECT OF BUFFALO SEMINAL PLASMA PROTEINS ON EPIDIDYMAL SPERMATOZOA MOTILITY DURING *IN VITRO* CAPACITATION

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ABSTRACT

This study was undertaken to observe the sperm motility response with the heparin binding proteins (HBPs) and gelatin binding proteins (GBPs) during *in vitro* capacitation. Washed buffalo epididymal sperms were incubated with heparin (10µg/ml) in the presence of HBPs and GBPs @ 10, 20, 30, 40, 50 and 60 µg/ml. The percentage of sperm motility was evaluated from 0 to 6 h. Both HBPs and GBPs acted in a similar way for maintaining the sperm motility during *in vitro* capacitation.

Key words: *In vitro* capacitation, Heparin, Gelatin, Proteins, Buffalo, Epididymal spermatozoa

INTRODUCTION

The physiological role of seminal plasma proteins in the development of forward progressive motility of spermatozoa and *in vitro* sperm functions, fertility, capacitation of spermatozoa, acrosome reaction, and sperm-zona interaction have been reported (Killian 1992; Bhattacharya 1992; Ronkko *et al.*, 1994; Batova *et al.*,1993; Cross 1998; Arangasamy and Singh, 2007; Arangasamy *et al.*, 2005). The addition of seminal plasma to sperm has been shown to induce hyperactivation and acrosome reaction *in vitro* (Lindholmer 1974; Agarwal and Vanha Pertulla 1987). Sperm of several domestic species and humans may be capacitated *in vitro*, and required conditions vary between species (Visconti *et al.*, 1994). Perusal of literature revealed that few attempts have been made to study buffalo seminal plasma proteins nature and their effect on *in vitro* capacitation studies. Hence the present study was undertaken to assess the effect of heparin binding proteins (HBPs) and gelatin binding proteins (GBPs) from buffalo seminal plasma on cauda epididymal spermatozoa motility *in vitro* capacitation.

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MATERIALS AND METHODS

The sperm capacitation medium used in this study was a modified Brackett and Oliphant (BO) (1975) medium. The effect of group of gelatin binding proteins (GBPs) (7 no') molecular weight ranging from 13 to 61 kDa, and heparin binding proteins (HBPs) (8 no') molecular weight ranging from 13 to 71 kDa were observed on sperm motility maintenance response during *in vitro* capacitation.

Buffalo testes with epididymes were collected from local abattoir and transported to laboratory (within 4-6 h after slaughter) in a thermosflask containing normal saline at 30-35°C. After washing, the excess tissues were trimmed and cauda epididymis was isolated from testes. Convoluted tubules were exposed by several incisions with Bard Parker blade. The gushing fluid, rich in sperms was collected in petridish and mixed with 10 ml of modified BO capacitation medium. Spermatozoa were collected on the same day from 2-3 epididymes of different bulls, mixed and used for a single test. The percentage of spermatozoa motility was assessed in fresh cauda epididymal semen and upto 6 h during *in vitro* capacitation. A total of 10 tests were conducted. The collected sperm cells were washed twice with capacitation medium (by centrifugation at 2000 rpm for 10 minutes). The sperm motility was assessed under a

phase contrast microscope. The sperm pellets were resuspended with capacitation medium containing BSA (6 mg/ml) and heparin (10µg/ml). Sperm concentrations were adjusted to 1 x 108 sperm cells/ml and thirteen 1 ml aliquots were prepared. One aliquot was kept as control, while to the remaining aliquots either GBPs or HBPs (Arangasamy 2003) were added @ 10, 20, 30, 40, 50 and 60 µg/ml. These aliquots were placed under mineral oil in eppendorf tubes in 5% CO2 at 37°C upto 6 h. Sperm motility were assessed from 0 to 6 h at 1 h intervals. The data were analysed as per the standard procedure Snedecor and Cochran, (1989). Repeated analysis of variance was carried out to compare the protein effects between hours. All values are expressed as Mean ±S.E.

RESULTS AND DISCUSSION

Higher percentage of sperm motility was observed in HBPs treated group and GBPs than the control at 5 and 6 h incubation, which was significant (P<0.05). The effect of various concentration of particular seminal plasma protein on maintenance of sperm motility was also observed in this study. HBPs treated group @ 40 µg, 30 µg and 20 µg/ml concentration performed well to maintain motility when compared to 10, 50 and 60 µg/ml. This indicated that high as well as low concentration had detrimental effect on sperm motility. Almost all concentrations (i.e. 10 to 60 µg/ml), of the GBPs in seminal plasma gave higher results compared to control.

The present study indicated that addition of seminal plasma proteins required certain duration of time to form coat over the plasma membrane of spermatozoa and bring about the protective action on sperm cell to maintain the motility. It is clearly indicated in this study that protein added samples showed more motile sperm cells than control group and probably it might be due to the seminal plasma proteins components involved in the capacitation media which might have played favourable role to maintain motility. Overall 29-31% motility loss was observed from 0 to 6 hrs incubation during capacitation. This finding is agreement with Chauhan et al. (1997) who reported 30 % reduction in motility after 8 h incubation in medium in fresh buffalo spermatozoa. In the present study, the hyperactivated motility and whiplash motion of buffalo cauda epididymal sperm were also observed and was similar to those reported by Sidhu et al. (1984) in buffalo and Parrish et al. (1988) in cattle. In the early stages, head to head agglutination was noticed which was followed at a later stage by onset of hyperactivation. Motility of spermatozoa was important for fertilization but was not an index to evaluate the ability of spermaotozoa for acrosome reaction. Seminal plasma proteins might be involved in modification of the composition of the sperm membrane lipids that occured during capacitation and acrosome reaction by the way it helped for maintaining the sperm motility in this present study.

ACKNOWLEDGEMENT

The authors thank the Director and Head, Animal Reproduction Division, Indian Veterinary Research Institute, Izatnagar, U.P, India for providing the necessary facilities.

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