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Effect of follicular fluid proteins on post-thaw characteristics of buffalo spermatozoa

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ABSTRACT

Follicular fluid collected from buffalo ovaries obtained from slaughter house was exposed to SDS-PAGE to study the electrophoretic profiles of follicular proteins (FP). A total number of 28 protein bands were observed, of which, 10, 14 and 4 were of >97, 29-97 and <29 kDa, respectively. No follicular fluid specific protein could be observed. Follicular proteins isolated by ammonium sulphate precipitation and dialysis were added to frozen thawed buffalo semen at the rate of 1, 2, 4, 8 and 16 mg/ml and incubated for 4 h at 37°C. Samples were evaluated for motility, viability and acrosomal integrity measurements immediately after thawing and at hourly interval. A net beneficial effect of FP on spermatozoan motility, viability and acrosomal integrity was observed in samples added with 1 mg/ml of FP. Samples containing higher FP (>4 mg/ml) showed inferior spermatozoan characteristics than that of control. From this study it is inferred that FP have a beneficial effect on spermatozoan function in a dose dependent pattern.

Key words : Follicular fluid, spermatozoa, buffalo, follicular protein

Follicular fluid is a complex fluid composed of secretions derived from the ovarian follicle as well as from blood plasma. Recently, based on the fact that follicular fluid maintains sperm motility and viability, the use of follicular fluid to improve the quality of ejaculated spermatozoa in different mammalian species has been the subject of study. Different workers claim that fractions of follicular fluid are responsible for maintenance of sperm functions. The sperm motility stimulatory factor has been purified in human (Kao *et al.*, 1993) and in porcine (Lee *et al.*, 1992) follicular fluid. However, the information about the follicular protein (FP) profile and their effect on spermatozoan characteristics in buffaloes appears to be scanty. Hence, the present study was undertaken to study (a) the electrophoretic profile of FP by SDS-PAGE and (b) the effect of FP on post-thaw spermatozoan characteristics.

MATERIALS AND METHODS

The study was conducted at Artificial Insemination Laboratory, Division of Animal Reproduction, IVRI. Buffalo ovaries were procured from local abattoir immediately after slaughter and brought to the laboratory in ice. The ovaries were kept at refrigeration temperature till aspiration of follicular fluid. The ovaries were washed with sterile normal saline and wiped with filter paper. Follicular fluid was aspirated with a sterile syringe and needle (20 G) from the medium and large sized follicles, pooled and centrifuged at 3000 rpm for 30 min. The protein content was estimated (Lowry *et al.*, 1951) in clear supernatentand stored at -20°C until further use.

Sodium dodecyle sulphate polyacrylamide-gel electrophoresis (SDS-PAGE)

To study the elctrophoretic profile of FP the follicular fluid along with serum and molecular marker (Genei, Bangalore) were exposed to 10% SDS-PAGE as per the standard procedure of Laemmelli (1970) with slight modifications. The gel was stained with coomassie brilliant blue and analysed for band pattern in Gel Doc. system.

Preparation of follicular proteins : FP were precipitated by slowly adding ammonium sulphate to the pooled follicular fluid and stirred until 80% saturation was reached. The solution was kept at refrigeration temperature overnight. The precipitated proteins were pelleted by centrifuging at 6000 rpm in a refrigerated centrifuge for 30 min. Pellets were resuspended in two pellet volumes of deionized water and dialyzed overnight. Retentates (>12 kDa) was filtered through a non-pyrogenic filter (0.2 µm). Protein concentration was determined (Lowry et al., 1951) and aliquots of FP were lyophilized to dryness and stored frozen at -20°C.

Post-thaw spermatozoan characteristics : Frozen semen straws of buffalo bulls were thawed at 37oC for 30 sec. They were cut and semen was taken in microcentrifuge tubes. The FP were added at the rate of 1, 2, 4, 8 and 16 mg/ml of frozen thawed semen while semen with no addition of FP acted as control. The sperm motility, viability (Campbell *et al.*, 1953) and acrosomal integrity measurements (Watson, 1975) were assessed immediately after thawing and at 1,2, 3 and 4 h of

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incubation at 37°C. All the experiments were conducted in triplicate. Data were statistically analyzed as per the standard methods of Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Electrophoretic profile of follicular proteins : The result of SDS-PAGE of FP is shown in Fig. 1. Twenty eight protein bands were observed, of which, 10 bands were of molecular weight higher than 97 kDa. Fourteen bands were observed between 29 and 97 kDa where as only four bands were detected below 29 kDa. It was found that almost all these bands had their counter part in blood serum and it suggests that almost all the

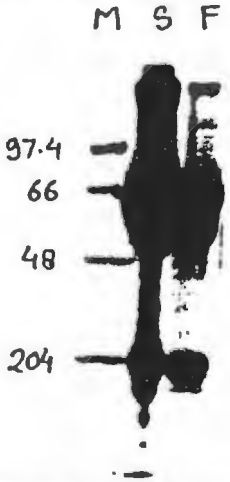

Fig. 1 SDS-PAGE profile of buffalo follicular proteins (M-molecular marker, S-serum, F-Follicular fluid

FP of the buffalo is originated from blood serum. Our findings are in agreement with Kulkarni (1988) in buffaloes and Gerena and Killian (1990) in cattle. The major protein in follicular fluid had a relative molecular mass of 66 kDa and apparently was bovine serum albumin (BSA) since it behave similar to the BSA molecular weight marker.

Our study could not demonstrate any buffalo follicular fluid specific protein and this might be due to the extremely small amounts of these proteins in follicular fluid which might have been masked by higher concentrations of proteins of blood serum (Kulkarni, 1988). The importance of follicular proteins in development and function of ovarian follicles and spermatozoan functions has recently been recognized. Factors such as bovine serum albumin in follicular fluid favours capacitation and acrosome reaction in bovine (Parrish *et al.*, 1988). A 45 kDa protein in human (Suraez *et al.*, 1986) and 52 kDa protein in porcine (Lee *et al.*, 1992) follicular fluid has been shown to stimulate the motility and induce acrosome reaction of spermatozoa. However, no such reports are available on buffaloes, hence, identification and characterization of follicular proteins would help in better understanding of their role in sperm matabolism.

Follicular proteins and spermatozoan characteristics : A net beneficial effect of FP in terms of maintaining the spermatozoan motility (Fig. 2), viability (Fig. 3) and acrosomal integrity (Fig. 4) was observed at low concentrations. Significantly ($P<0.05$) higher motility and viability were observed in samples added with 1 mg/ml of FP upto 4 h incubation when compared with all other groups. Samples containing >4mg/ml of FP had a significantly ($P<0.05$) lower motility and viability. There was no significant difference in acrosomal integrity percentage between control and samples with upto 4 mg/ml of FP, however, over all maintenance of acrosomal integrity was higher in samples with 1mg/ml of FP. Acrosomal integrity percentage decreased in a linear pattern along with increased dose of FP as well as with incubation period. Our observations are in agreement with Kumar (1995). McNutt and Killian (1991) observed a non-significant difference

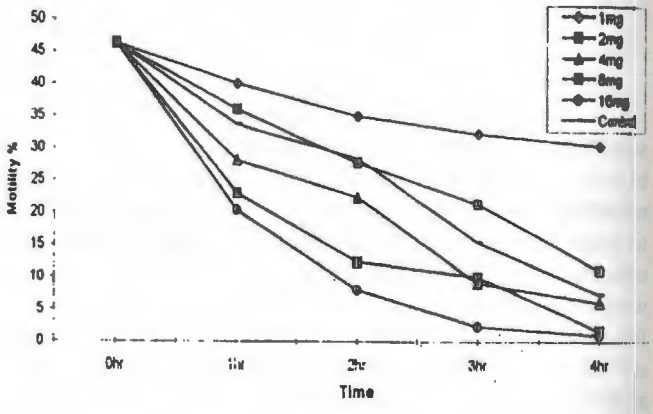

Fig. 2 Motility % after incubation with follicular proteins

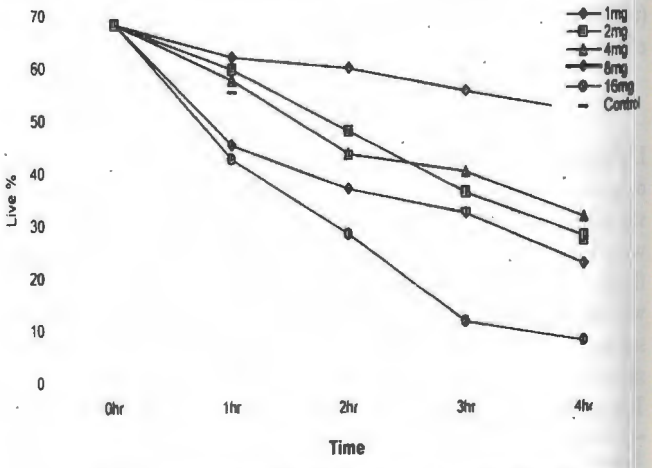

Fig.3 Live % after incubation with follicular proteins

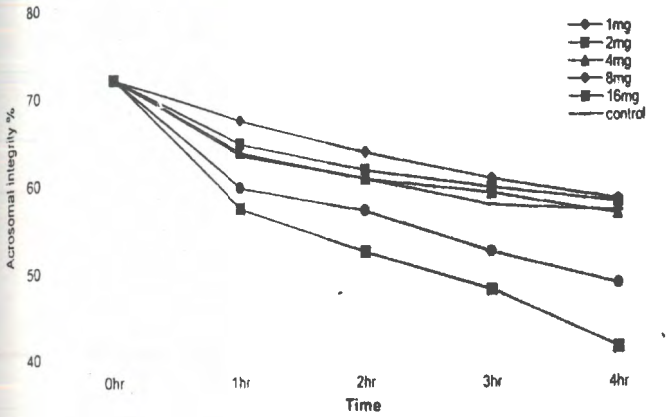

Fig. 4 Acrosomal integrity % after incubation with follicular proteins

in sperm motility between follicular fluid treated group and control even upto 6 h incubation. They also reported that the concentrations of follicular fluid were negatively correlated with sperm motility in a dose dependent pattern.

Effect of follicular fluid on various aspects of sperm function have received intense attention in recent years. Follicular fluid is known to stimulate the motility and respiration of spermatozoa to a considerable extent. The factor in follicular fluid responsible for maintenance of spermatozoan motility is an un identified fraction (Balkrishna, 1975), steroid rich fraction (Mbizvo *et al.*, 1990) or a protein (Lee *et al.*, 1992; Ramsoondar *et al.*, 1995). Most of the above studies were conducted with either heat treated or steroid free follicular fluid. Studies with follicular proteins appears to be less. Our study supports that the follicular proteins maintain the sperm motility and viability during incubation. However, at high concentrations the FP decreased the percentage of intact acrosome. It has been well documented that follicular fluid induces capacitation and acrosome reaction in spermatozoa (Yanahimachi, 1969; Suraez *et al.*, 1986; McNutt and Killian, 1991; Ramsoondar *et al.*, 1995). It was also found that acrosomal disintegration or detachment occured in high proportion of spermatozoa within 3 h of incubation with follicular fluid (Breuer and Wells, 1977). Our study supports the above findings. It has already been reported that albumin, a major protein in follicular protein, also observed in this study, apart from other factors may induce capacitation as it functions as sink for the removal of cholesterol from the sperm plasma membrane (Cross, 1998).

The results of the current study provides information about the role of FP on the spermatozoan motility, viability and acrosomal integirty. Further detailed studies on the role of follicular proteins on capacitation and acrosome reaction may help in better understanding about their dual role with opposite effect i.e. maintaining motility and inducing capacitation and acrosome reaction.

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