IJAR 18(1), 1997; 54-56

Ultrastructural Changes In Ram Sperm Head During In-Vitro Acrosome Reaction

K. AHMED*

Department of Veterinary Basic Sciences
The Royal Veterinary College
University of London
London NWI OTU

ABSTRACT

Ram Spermatozoa were incubated for 6 hrs in Hepes diluent at 39°C. The ultrastructural changes of sperm head during acrosome reaction were studied using electron microscope. Fusion of outer acrosomal membrane with plasma membrane forming a vesicle were observed in 58.17±0.79, 70.33±1.07 and 71.0±0.94 per cent spermatozoa containing 100 x 10^6, 250 x 10^6 and 500 x 10^6sperm per ml Hepes diluent respectively. The non specific degeneration and absence of acrosome was 31.67±0.04 and 8.33±0.61 in 100 x 10^6 sperm / ml, 19.5±0.46 and 6.67±0.84 in 250 x 10^6 sperm /. ml and 17.0±0.88 and 8.67±1.05 in 500 x 10^6 sperm / ml respectively.

Acrosome reaction, the progressive breakdown and fusion of the plasma membrane and outer acrosomal membrane of the male gamet (Barros *et al.*, 1967), is essential to make sperm capable of penetrating certain investments surrounding the oocyte during fertilization. This phenemenon is achieved during a period of residence in the female reproductive tract and has been successfully mimicked by incubation in Hepes medium (Robertson and Watson, 1987). The study was undertaken to record the ultrastructural changes in sperm head during in-vitro acrosome reaction in Hepes diluent.

MATERIALS AND METHODS

Semen was collected using A.V. from Finnish Landrace rams maintained at Royal Veterinary College, London. Sperm motility was verified using a Hamilton thorn motility analyser and the sperm concentration was determined with a previously calibrated colorimeter. Six ejaculates having more than 70 per cent sperm motility and 2000 x 10^6 sperm / ml were used in this study. The Hopes diluent (Nacl 139 mM, KCL 25 mM, Glucose 10 mM, Hepes 20 mM and $Cacl_2$ 3 mM) of osmolality 306 mosm and pH 7.4 was taken as incubation medium. Each ejaculate was diluted using Hepes diluents to give a concentration of 100 x 10^6, 250 x 10^6 and 500 x ^{6} sperm per ml. One ml of diluted semen of each concentration was taken in 5 ml plastic vial and incubated for inducing acrosome reaction in waterbath at 39°C for 6 hrs. Thereafter the semen samples were fixed for transmission electron microscopy maintaining 500 x ^{6} sperm / ml as per the method described by Plummer and Watson (1988). The embedded material was sectioned (50-80 nm thick) with glass knife on a Reichart ultramicrotome, mounted on 200 mesh hexagonal grids and lightly

* Present address: Assoc Professor, Deptt. of Gynaecology, Obst. and A.I. College of Veterinary Science, Khanapara, Guwahati - 781 022

stained (6 min uranyl accetate, 4 min lead citrate). The sections were examined in a JEOL 1200 Ex electron microscope for the ultrastructural changes.

RESULTS AND DISCUSSION

The percentage of different ultrastructural changes of sperm head during in-vitro incubation in Hepes diluent at 39°C is presented in Table 1. Acrosome reacted sperm (71.0±0.94%) were significantly higher and nonspecific degeneration of acrosome (17.0±0.88%) was significantly lower in samples containing higher concentration of sperm. The swelling of the acrosome and loss of homogeneity of the acrosome was observed in the present study. These changes were also considered to be the initial features of the mammalian sperm acrosome reaction (Bedford, 1983). The bulging of outer acrosomal membrane towards the wavy plasma membrane and the fusion of the outer acrosomal membrane with plasma membrane (Fig. 1) forming a fenestration of hybrid vesicles were observed in large number of spermatozoa. It was further observed that the vesiculated membrane remained in close apposition to the head to form an acrosomal "ghost" or shroud. Such an arrangement would allow gradual dispersion and release of acrosomal content. Non specific degeneration of plasma membrane and acrosome observed in the present study was also observed by Lenz *et al.,* (1983). The loss of acrosome recorded in the present investigation might be due to early acrosome reaction or non specific degeneration of the acrosome. The variation in time required for acrosome reaction was reported by Rogers (1981) and Overstreet and Cooper (1979).

Acknowledgements: The auther is grateful to Govt. of Assam for awarding state everseas fellowship to carryout the research programme at Royal Veterinary College, London.

Fig.1 Acrosome reacted sperm showing the fusion of the outer acrosomal membrane with plasma membrane

Table 1. Percentage (Mean±S.E.) of different ultrastructure of sperm head during incubation in Hepes diluent at 39°C

	Number of cells in incubation medium per ml		
	100x10^6	250x10^6	500x10^6
Intact acrosome with intact plasma membrane	1.83±0.55	3.5±0.39	3.33±0.38
Fusion of plasma membrane and outer acrosomal membrane	58.17±0.79	70.33±1.07	71.0±0.94
Non specific degeneration of acrosome	31.67±0.84	19.5±0.46	17.0±0.88
Absence of acrosome	8.33±0.61	6.67±0.84	8.67±1.05

REFERENCES

Barres C., Bedford, J.M., Franklin, L.E. and Austin, C.R. (1967). Membrane vesiculation as a feature of the mammalian acrosome reaction J.Cell Bio 1.*34:* CI-C5

Bedford, J.M. (1983). Significance of the need for sperm capacitation before fertilization in eutherian mammals. Biol. Reprod. *28:* 108-120

Lenz, R.W. Ball, G.D., Lohse, J.K., First, N.L. and Ax, R.L. (1983). Chondroitin sulfate facilitates an acrosome reaction in bovine spermatozoa as evidenced by light microscopy, electron microscop and in-vitro fertilization. Biol. Reprod. *28:* 683-690.

Overstreet, J.W. and Cooper, G.W. (1979). The time and location of the acrosome reaction during sperm transpert in the female rabbit. J. Exp. Zool. *209:* 97-104.

Plummer, J.M. and Watson, P.F. (1988). The quantitative ultrastructural assessment of head membrane damage in boar spermatozoa subjected to varying degree of cold shock. Anim. Reprod. Sci. *16:* 265-275.

Robertson, L and Watson, P.F. (1987). The effect of egg yolk on the control of intracellular calcium in ram spermatozoa cooled and stered at 5°C. Anim. Reprod. Sci. *15:* 177-187.

Rogers, B.J. (1981). Facters affecting mammalian in-vitro fertilization. In: Bioregulators of reproduction. Academic PRess, New York, pp 459-486.