The Indian Journal of Animal Reproduction; 25(2): 120-122; December 2004

Ultrastructural studies on fresh-extended and frozen-thawed goat spermatozoa

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Received : March 13, 2003
Accepted : November 10, 2003

ABSTRACT

Use of routine *in vitro* maturation, fertilization and culture (IVMFC) protocol in this laboratory with some modifications did not produce satisfactory results in goats due to low cleavage rates. Post-fertilization evaluation of *in vitro* inseminated oocytes using an inverted microscope indicated absence of sperm penetration into the zona pellucida. It was, therefore, considered necessary to examine the ultrastructure of frozen-thawed sperm in order to determine its integrity and viability. Ultrastructure of freshly ejaculated extended goat sperm was taken as control. Electron micrographs of frozen-thawed sperm indicated that inner and outer acrosomal membranes were undergoing signs of degeneration such as lysis of the membranes specifically at the tip region. Since acrosome plays an important role during fertilization, any changes in its membrane are likely to affect the fertilization rates.

Key words: Goat sperm, acrosome, ultrastructure, frozen-thawed sperm, fresh sperm

Sperm and oocyte both play vital roles in the fertilization process. Reproductive biologists have been trying to mimic *in vivo* environment existing in the reproductive tract of domestic animals to produce large number of embryos *in vitro*. Earlier studies have indicated that artefactual environment in which the oocytes and embryos are cultured induce ultrastructural changes in them (Kanwal, 1999). Since freshly ejaculated sperm are subjected to processes such as dilution, freezing and thawing before they are used for *in vitro* insemination, these processes may induce changes in them that probably affect the success rates following IVF. Cryopreservation of sperm has failed to establish itself commercially in other species of domestic livestock than cattle and buffaloes. It results in a more homogeneous population of cell population; accelerate capacitation-like process and lowers post-thaw sperm survival (Curry, 2000). There is not enough data available on the effects of sperm processing on its ultrastructure. The objective of the current investigation was to examine ultrastructural changes in goat spermatozoa that were induced following freezing and thawing processes.

MATERIALS AND METHODS

Fresh semen from a selected crossbred buck Jermasia (Katjang x German Fawn) reared and maintained at the farm of the Institute of Biological Sciences, University of Malaya was collected using an artificial vagina. Following collection, the semen was extended with tris egg yolk citrate in the ratio of 1:24. Sperm samples from freshly ejaculated extended and from frozen-thawed specimen from the same buck were used. A total of eight sperm samples (fresh-extended: 4, frozen-thawed: 4) were processed for transmission electron microscopy according to a given protocol. Sperm were fixed in 2.5 percent glutaraldehyde in 0.2 M sodium cacodylate, post-fixed in 1 percent osmium tetra oxide, dehydrated with a graded series of acetone, infiltrated with resin and embedded in epoxy resin. Thin sections were stained with uranyl acetate and lead citrate and viewed with a Philips CM 12 transmission electron microscope at an accelerating voltage of 60 kW. The exposed film was developed at the electron microscope lab and the micrographs were printed at a local photographic shop.

RESULTS AND DISCUSSION

Electron micrographs of goat sperm obtained from freshly ejaculated extended and frozen-thawed sperm specimens are shown in Figs. 1-5. The heads of the sperm were elongated in shape with a tapering and bulbous tip. At the tip subacrosomal membrane was found comparatively wider and a bulb-like structure with higher electron density surrounded it (Fig. 1). The sub-acrosomal substance corresponded to the structure commonly referred to as the

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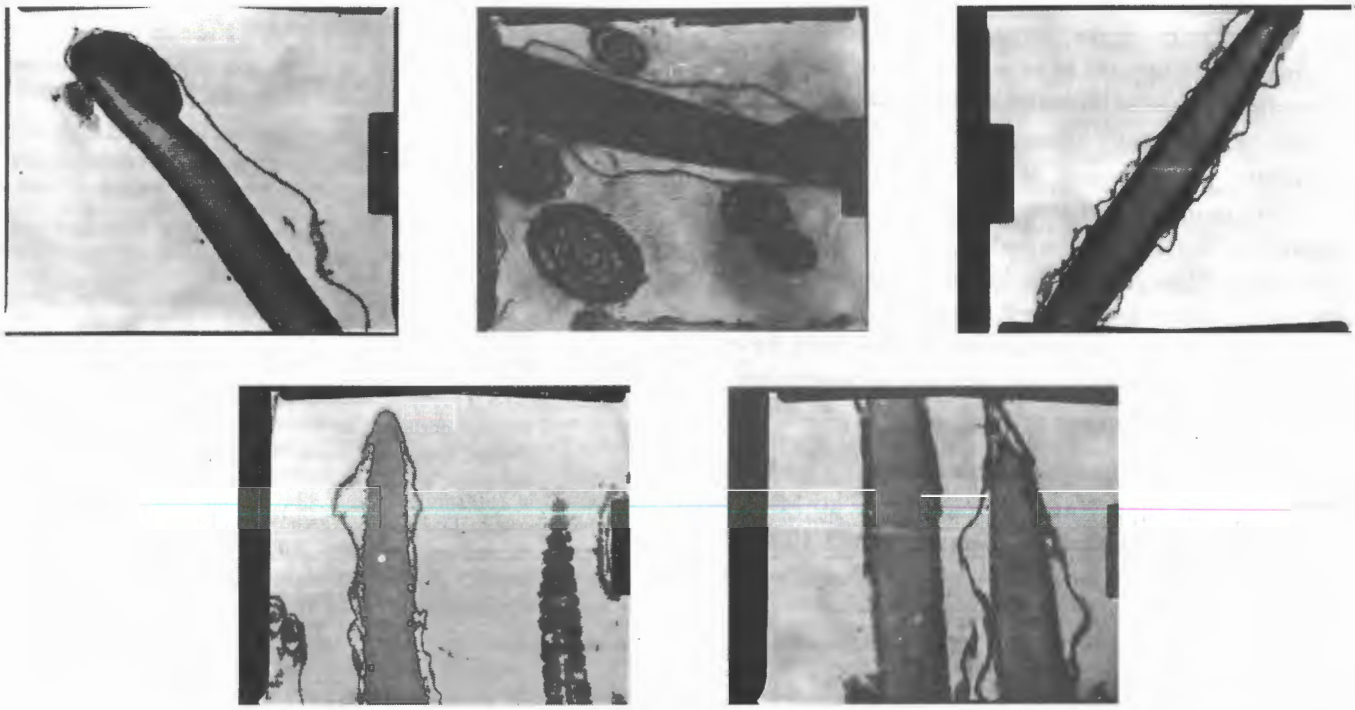

Fig. 1. Electron micrograph of freshly ejaculated goat sperm longitudinal section (LS) shows that the tip is bulbous and contains a material higher in electron density. The inner and outer acrosomal membranes are intact. X 45000.

Fig. 2. Inner, outer acrosomal, and plasma membranes are intact in freshly ejaculated and extended sperm (LS). Various cross-sections of the tail region can also be seen. X 60000.

Fig. 3. Another sperm from freshly ejaculated and extended specimen (LS) showing well-preserved inner, outer acrosomal, and plasma membranes. X 35000.

Fig. 4. Inner and outer acrosomal membranes of frozen-thawed sperm (LS) are found vesiculated. It is evident that these membranes are undergoing lysis. X 35000.

Fig. 5. Lysis of membranes is evident at higher magnification. X 45000.

perforatorium (Healey, 1969). Three membranes surrounded the head, which included inner and outer acrosomal membranes and the outer most, the plasma membrane. In the fresh-extended sperm samples, these membranes were well preserved and intact (Figs. 2, 3) while in the frozen-thawed sperm, these were seen undergoing degenerative changes such as lysis specifically at the tip region (Figs. 4, 5). Acrosome of sperm plays an important role in the fertilization process as it contains enzymes that breakdown the outer coverings of the oocyte to permit sperm penetration (Austin and Bishop, 1958). Any damage caused to the acrosomal membranes as a result of freezing and thawing protocol could interfere with the release of stored substances and thus renders the sperm incapable of penetration into the oocyte. The outer membrane of the tail in frozen-thawed sperm was also affected indicating that the sperm was undergoing degenerative changes. As tail is responsible for the motility of the sperm, any degenerative changes in it may render the sperm immotile.

Ultracentrifugation of sperm cells at a high speed is said to be another factor leading to changes in sperm motility and membrane integrity (Verberckmoes *et al.*, 2000), however, in the current investigation sperm cells were not centrifuged beyond 1200 rpm while obtaining a pellet. Changes induced in the acrosomal membrane could probably be due to specimen processing or freezing protocol. An earlier report on dog spermatozoa indicated that addition of semen extender produced no significant ultrastructural changes; however, cooling of sperm resulted in an immediate increase in the number of acrosomal abnormalities and a subsequent decrease in sperm viability (Burgess *et al.*, 2001).

Before concluding anything at this point further research will be required to ascertain whether the freeze-thawing protocol or the procedure used for processing sperm for electron microscopy induced these changes. The latter possibility is not feasible as the same procedure and chemicals were used for processing sperm from freshly ejaculated and frozen-thawed samples for electron microscopy. It may, therefore, be concluded that freezing and thawing protocol of goat sperm induced ultrastructural changes specifically in the acrosome that could have rendered the sperm immotile and incapable of penetration.

ACKNOWLEDGEMENTS

The authors are grateful to Mr. Razali Jonit for collecting and supplying the goat sperm; to Mr. T. Ragunathan, Mr. Zul and Ms. Elsie Wong Mee Hoo for providing facilities in processing samples for transmission electron microscopy.

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