Acta vet. scand. 1994, *35,* 17-26.

Membrane Alterations in Bull Spermatozoa after Freezing and Thawing and after In Vitro Fertilization

By *A. Krogenæs, K. Andersen Berg, A. L. Hafne* and *E. Engeland*

Department of Reproduction and Forensic Medicine, and Department of Anatomy, Norwegian College of Veterinary Medicine, Oslo, Norway.

Krogenæs, A., K. Andersen Berg, A.L. Hafne and E. Engeland: Membrane alterations in bull spermatozoa after freezing and thawing and after in vitro fertilization. Acta vet. scand. 1994, 35, 17-26. – Membrane alterations in bull spermatozoa after freezing and thawing and after the process of in vitro capacitation and fertilization were studied by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Even if the majority of the spermatozoa exhibited intact membranes after freezing and thawing (90%), one could distinguish between 3 types of membrane defects depending of the different structures involved. The first type showed loss of plasmalemma over the entire acrosome. In the second category the anterior part of the outer acrosomal membrane exhibited a pronounced extension, but was covered by a partly intact plasmalemma. The last category consisted of spermatozoa with extensive vesiculation and disruption of plasmalemma and the outer acrosomal membrane. This type of defect could not easily be distinguished from a true acrosome reaction. The cumulus cells showed an active phagocytosis of both intact and acrosome reacted spermatozoa.

acrosome reaction; bull spermatozoa; in vitro fertilization; cumulus oophorus; electron microscopy.

Introduction

Mammalian spermatozoa must undergo capacitation and a subsequent acrosome reaction (AR) prior to penetration of zona pellucida and fertilization of the ovum *(Bavister* 1973, *Austin* 1975, *Gwatkin* 1977, *Richardson et al.* 1991). The oviductal fluid contains glycosaminoglycans of which heparin is probably the most important capacitating factor. Heparin has also been shown to an effective capacitating factor in in vitro fertilization systems *(Parrish et al.* 1985). Capacitation is considered to be a prerequisite for the acrosome reaction which results in release of enzymes necessary for penetration of zona pellucida and, in some species, probably also the cellular investment outside this structure. *(Austin & Bishop* 1958, *Austin* 1975, *Austin & Bavister* 1975). For different species several authors have shown that the acrosome reaction of penetrating spermatozoa occurs at the zona pellucida *(Gwatkin et al.* 1976, *Szollösi & Hunter* 1978, *Florman & Storey* 1982, *Crozet* 1984). *Gwatkin et al.* (1976) showed that hamster sperm became infertile if they underwent an acrosome reaction apart from the egg, and that to penetrate zona pellucida and subsequently get in contact with the egg, the sperm had to undergo the acrosome reaction on the surface of the zona.

Two types of AR have been described for mammalian spermatozoa, a true AR (TAR) consisting of progressive vesiculation of the outer acrosomal membrane and the overlying

plasmamembrane, and a false AR (FAR) in which the acrosomal changes occur in association with sperm death and/or irreversible injury *(Bedford* 1970, *Meizel* 1978).

This investigation was carried out to study the membrane alterations in bull spermatozoa after freezing and thawing and during the process of in vitro fertilization. The aim was also to see whether EM studies enables differentiation of spermatozoa which have undergone a TAR from those which have undergone a FAR.

Materials and methods

Semen from bulls from Norwegian Cattle was used in these experiments. After collection the semen was first diluted to a sperm concentration of 200×10^6 / ml with an extender containing 11 % skim milk, 2% fructose and 5 vol% egg yolk. After cooling to 4°C within 30 min, the semen was further diluted to a concentration of 100×10^6 spermatozoa / ml by adding the semen extender containing 14 vol% glycerol, and equilibrated for 4 h before freezing in minipayettes (0.27 ml) with liquid nitrogen (LN_2) vapour in a Digitcool 5300 freezer and stored in LN_2. The semen was thawed at 35°C. Some of the semen was fixed immediately after thawing for examination of membrane alterations after freezing/thawing. The rest was used in an in vitro fertilization experiment to study the membrane alterations during this process.

For the in vitro fertilization, the following procedure was: Ovaries were collected at an abattoir immediately after slaughter and transported to the laboratory in saline at 35°C. Follicular fluid from small antral follicles of 2-10 mm in diameter was aspirated, and the cumulus-oocyte complexes were picked up by use of a light microscope. Only oocytes with a compact cumulus cell layer were selected for maturation. These oocytes underwent 3 washes in modified Tyrode's medium (TL-Hepes) supplemented with 3 mg/ml bovine serum albumin (BSA) (Sigma chemical Co., St. Louis, MO), 0.2 mM pyruvate (Sigma) and 50 µg/ml gentamycin (Gibco, Life Technologies, Inc., Gaithersburg, MD) *(Bavister et al.* 1983). The maturation medium consisted of TCM 199 (Gibco) with the addition of 10% fetal calf serum (FCS) (Gibco), 2 µg/ml FSH (NIADDK -oFSH-17), 10 µg/ml LH (NIADDK-oLH), 1 µg/ml oestradiol-17-β (Sigma), 0.2 mM pyruvate and 25 µg/ml gentamycin *(Sirard et al.* 1988). The maturation took place in an incubator at a temperature of 39°C and a humidified atmosphere of 5% CO_2 for 24 h.

The semen preparation for the fertilization experiment consisted of the swim-up technique and 2 centrifugations at 130 G for 10 min conducted in sperm-Talp medium containing 6 mg/ml BSA, 1 Mm pyruvate and 25 µg/ml gentamycin *(Parrish et al.* 1984, 1986). The semen was diluted to 25 million spermatozoa/ml and added to the fertilization drops at a concentration of 50.000 sperm per 10 oocytes and 50 µl drop.

The fertilization medium consisted of modified TL-Hepes with 6 mg/ml fatty acid free BSA (Sigma), 0.2 mM pyruvate (Sigma), 25 µg/ml gentamycin and for capacitation 10 µg/ml heparin (Sigma), 2 mM penicillamine (Sigma), 1mM hypotaurine (Sigma) and 250 µM epinephrine (Sigma) *(Bavister & Yanagimachi* 1977). Following culture for 24 hours at 39°C and in an atmosphere of 5% CO_2, the complexes were prepared for EM.

Preparation for Scanning Electron Microscopy (SEM)

Semen samples of 0.5 ml were transferred to 10 ml 3% glutaraldehyde in 0.1 M cacodylate buffer. After sedimentation, the sediment was washed in 0.1 M cacodylate buffer (pH 7.4)

for 2×10 min. The sediment was subsequently fixed in 2% OsO_4 in the same buffer for 2 h before being washed 2×10 min in 0.1 M cacodylate buffer. The samples were centrifuged between each wash. One drop of the buffer suspension was placed on cover slips treated with polylysin and placed in a vapour chamber in the refrigerator for sedimentation overnight. After dehydration through an ascending series of ethanol solutions, the material was critical point dried in a Balzer critical point dryer, sputter coated with gold (Au) and examined by a Cambridge Stereoscan 90.

Preparation for Transmission Electron Microscopy (TEM)

The semen samples were prefixed in 3% glutaraldehyde in 0.1 M cacodylate buffer. Afterwards, the semen was washed in cacodylate buffer for 2×10 min, postfixed in 2% OsO_4 in 0.1 cacodylate buffer for 2 h and then washed again in cacodylate buffer for 2×10 min. The samples were dehydrated in ascending series of ethanol and propylene oxide before infiltration of epon-propylene oxide (1:1 for 1 h and 3:1 overnight). The material was embedded in Epon LX 112. Ultrathin sections were cut on an LKB ultrotome. Electron microscopy was performed by use of a Jeol JEM 100 S Elmiscope.

Results

After freezing and thawing

The majority of the spermatozoa seemed to have intact membranes after freezing and thawing (Figs .1a, 1b). However, a proportion of the cells (usually less than 10%) exhibited either minor disruptions of the plasmalemma, most frequently at the anterior level of the equatorial segment (Fig. 2), or more extensive alterations, which could be classified into 3 main categories depending of the structures involved. Defect type A represented loss of the plasmalemma frequently over the entire acrosome, while the outer acrosomal membrane (i.e. the outer part of the acrosomal membrane) was intact (Fig. 3). Typical for defect type B, which was the most frequent one, was a pronounced extension of the anterior part of the outer acrosomal membrane covered by a partly intact plasmalemma (Fig. 4). The third category (type C) consisted of spermatozoa with sperm heads showing extensive vesiculation and disruption of plasmalemma and the outer acrosomal membrane (Fig. 5).

After in vitro fertilization

The majority of spermatozoa were acrosome reacted and therefore devoid of the plasmalemma and the outer acrosomal membrane proximal to the equatorial segment (Figs. 8a, 8b). However, a proportion (about ⅓) of the sperm cells displayed extensive loss of plasmalemma, marked swelling of the anterior part of the acrosome and vesiculation of the acrosomal matrix (Fig. 7). Sperm heads with intact acrosomes under a partly fractioned plasma membrane were also observed (Fig. 6).

The cumulus cells exhibited an active phagocytosis of spermatozoa with both intact and defect acrosomes (Figs. 9, 10).

Some intact spermatozoa could be observed in contact with zona pellucida (Fig. 11), and acrosome reacted spermatozoa were also seen penetrating this structure at different levels (Figs. 12, 13). After penetration such spermatozoa were further observed in the perivitelline space in contact with the microvilli of the oocyte as a manifestation of the first step of gamete conjugation (Fig. 14).

Discussion

Semen frozen from bulls of Norwegian Cattle generally displays a high post-thaw quality, and this experiment showed that the majority

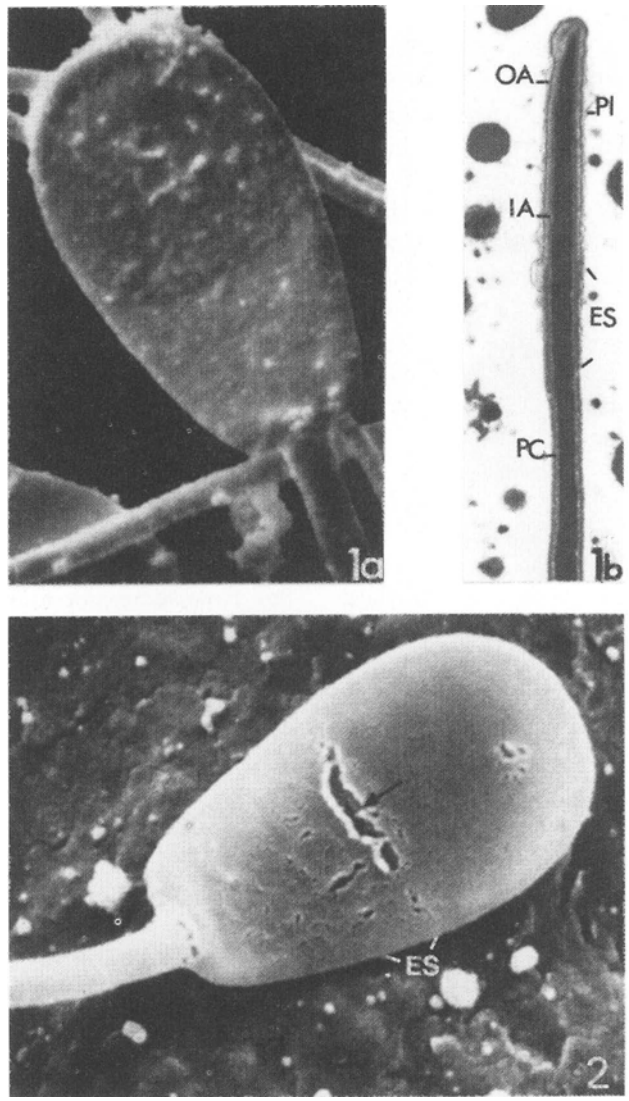

Figure 1a. Figures 1-14 Electron micrographs, scanning (SEM) and transmission (TEM) of frozen bull spermatozoa immediately after thawing, during the process of penetration of the cumulus layer and zona pellucida and in the perivitelline space. Pl = plasmalemma, OA = outer acrosomal membrane, IA = inner acrosomal membrane, ES = equatorial segment, PC = postacrosomal cap, CC = cumulus cell, Zp = zona pellucida, Pvs = perivitelline space, Oo = Oocyte and Mv = microvilli. Fig. 1a. Head of a spermatozoon after thawing with an intact plasmalemma. SEM×10.700.

Figure 1b. Sperm head after thawing with intact plasmalemma, acrosome and postacrosomal cap. TEM×16.000 (corresponding to figure 1a).

Figure 2. Head of a spermatozoon after thawing with partial disruption (arrow) of the cell membrane at the anterior level of the equatorial segment. The outer acrosomal membrane is intact. SEM×11.200.

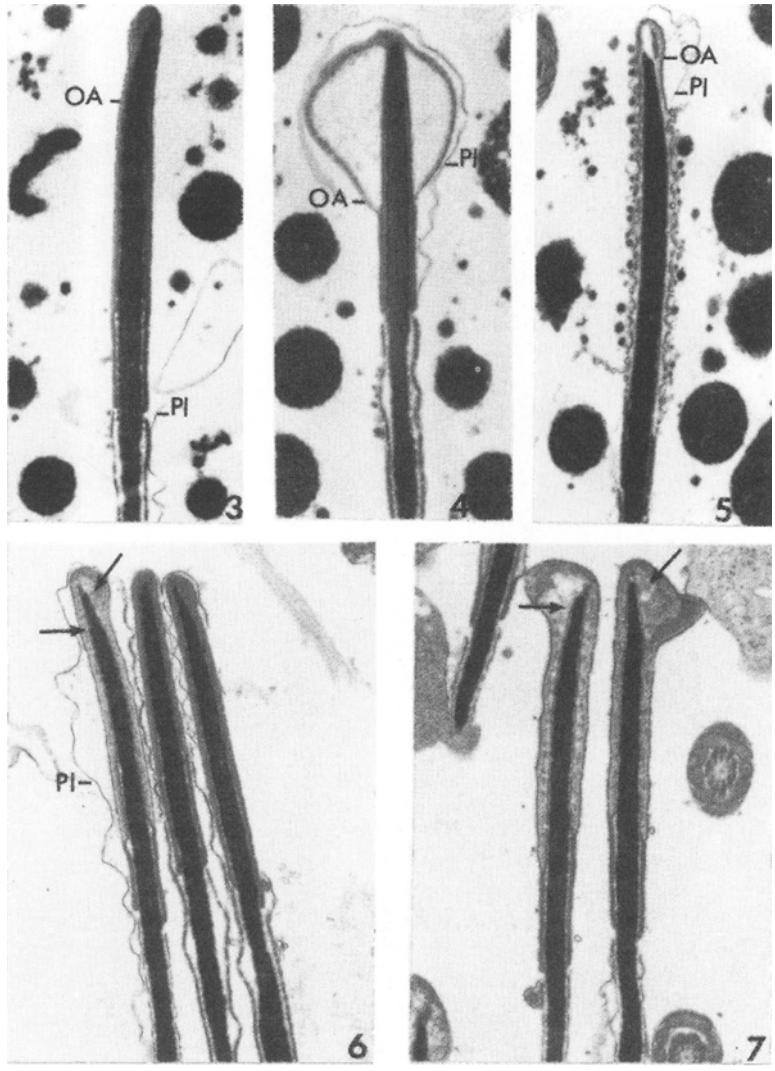

Figure 3. Sperm head after thawing with loss of plasmalemma over the entire acrosome (Defect type A) TEM×16.000

Figure 4. Sperm head after thawing, exhibiting a pronounced extension of the anterior part of the outer acrosomal membrane covered by a partly intact plasmalemma (Defect type B). TEM x 16.000.

Figure 5. Sperm head after thawing showing extensive vesiculation and disruption of plasmalemma and outer acrosomal membrane (Defect type C). TEM×16.000.

Figure 6. Sperm heads during penetration of the cumulus cell layer, with intact acrosomes under a partly fractioned plasmalemma The sperm head to the left shows incipient vesiculation and granulation (arrows) of the acrosomal matrix. TEM×16.000.

Figure 7. Sperm heads between cumulus cells exibiting extensive loss of plasmalemma, marked swelling of the anterior part of the acrosome and vesiculation of the acrosomal matrix (arrows). TEM×16.000.

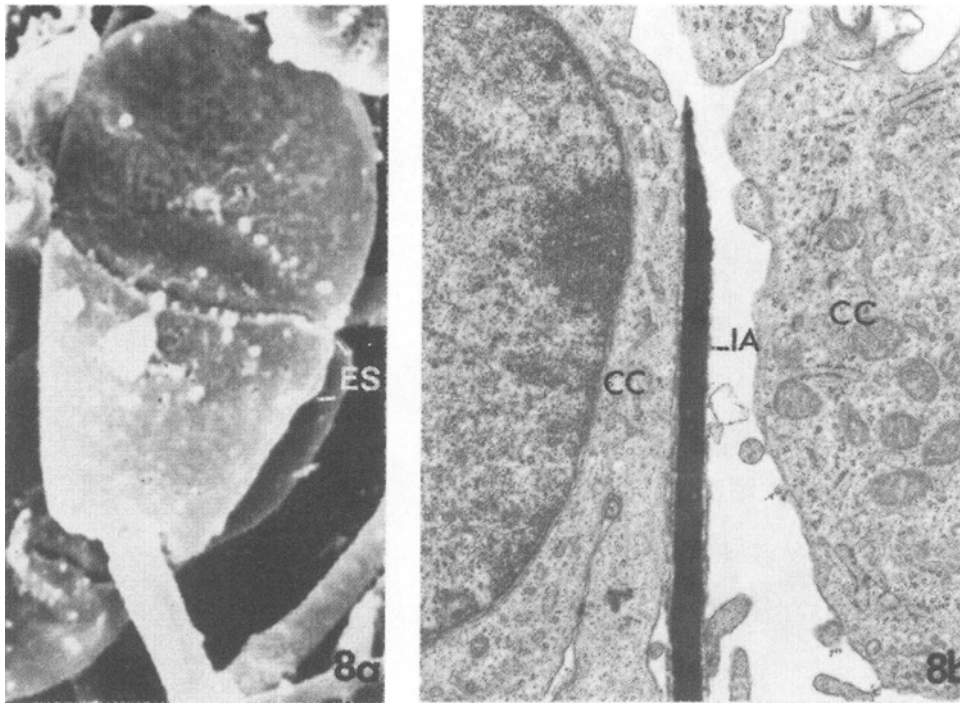

Figure 8a. Head of an acrosome reacted spermatozoon with total loss of plasmalemma and outer acrosomal membrane proximal to the equatorial segment. SEM×8.700.
Figure 8b. Sperm head of an acrosome reacted sperm cell in close apposition to a cumulus cell, devoid of plasmalemma and outer acrosome membrane proximal to the equatorial segment (corresponding to Fig. 8a). TEM×16.000.

of the spermatozoa exhibited intact membranes after freezing and thawing. However, some defects in the plasmalemma and acrosome could be observed.

The incipient disruptions of plasmalemma might represent the first step in membrane injuries leading to the formation of the defect of type A. Cell membrane damages of this type may be caused by conditions other than freezing and thawing, since corresponding defects have been found in fresh blue fox spermatozoa *(Hofmo & Andersen Berg* 1989). The lesions in the acrosome which are seen in the defects type B and C, are probably caused by the intracellular crystallization which occurs during freezing and thawing, even though mammalian spermatozoa totally contain less than 50% water in contrast to 80-90% in most other cells *(Barer et al.* 1953). Similar defects have been described in frozen spermatozoa from other species *(Healy* 1969, *Pedersen & Lebech* 1971, *Nath* 1972, *Yasuda & Tanimura* 1974, *Courtens & Paquignon* 1985). Conventional methods for sperm cryopreservation implies rapid freezing *(Weitze* 1991). A high freezing rate generally prevents major dehydration damages, but will instead cause intracellular crystallization which requires rapid thawing *(Mazur* 1985). Post-thaw viability of bull spermatozoa expressed as percentage of

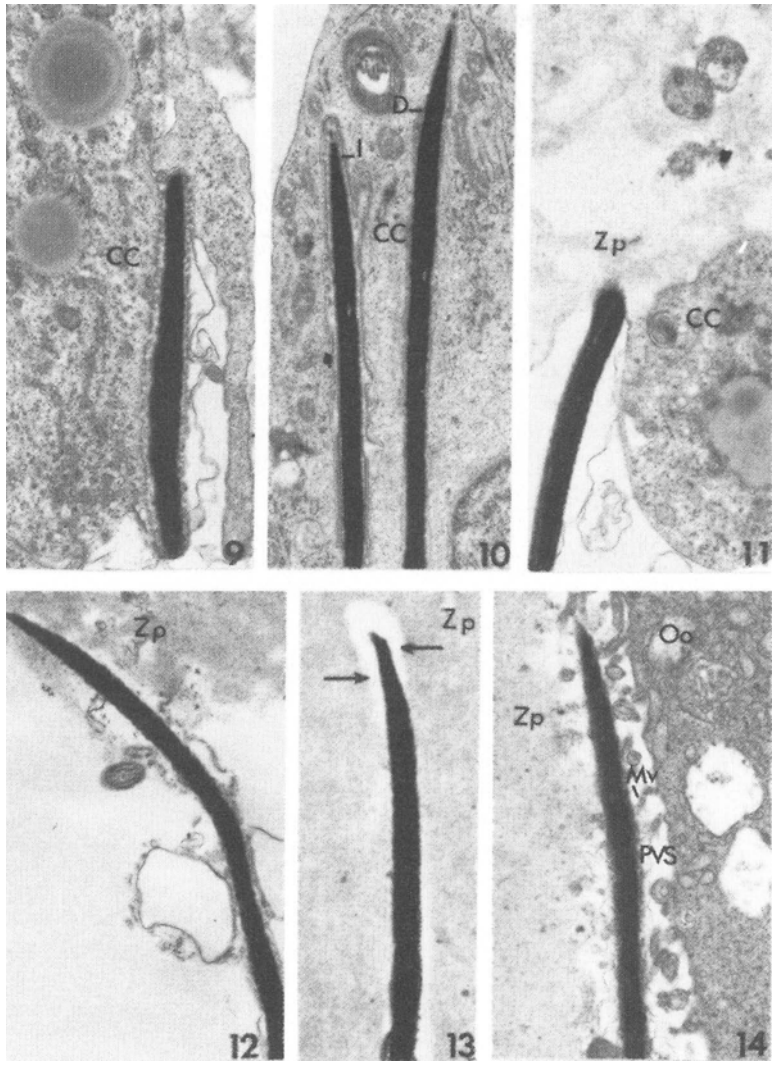

Figure 9. Head of a spermatozoon being phagocytosed by a cumulus cell close to the zona pellucida. TEM×16.000.

Figure 10. Two sperm heads, 1 with an intact (I) and 1 with a defect (D) acrosome phagocytosed by a cumulus cell. TEM×16.000.

Figure 11. Proximal part of a sperm head with intact acrosome in contact with zona pellucida. TEM×16.000.

Figure 12. Acrosome-reacted spermatozoon starting to penetrate zona pellucida. TEM×16.000.

Figure 13. Head of an acrosome reacted spermatozoon penetrating the zona pellucida by dissolution of the surrounding zonal material (arrows). TEM×16.000.

Figure 14. Head of a spermatozoon in the perivitelline space after penetration of zona pellucida. The sperm head becomes tangentially attached to the oocyte by initial contact with the microvilli of the latter. TEM×16.000.

unstained cells after eosin-nigrosin staining was markedly improved by thawing the straws at 75°C for 12 seconds *(Aamdal & Andersen* 1968). A faster thawing procedure might thus have reduced the incidence of defects of type B and C.

The acrosome has an important role in the process of fertilization. To bind to the zona pellucida, the plasmalemma must be intact, since it contains the species specific receptors which bind to the zona proteins *(Bleil & Wassarman* 1980). This binding triggers the acrosomal reaction *(O'Rand & Fisher* 1987), and the spermatozoon releases the acrosomal enzymes and penetrates the zona pellucida, whereupon it is incorporated in the ooplasm. Defect type A represents a severe cellular damage as the plasmalemma was absent over the entire acrosome. Spermatozoa with this type of defect will be totally unable to bind to the zona pellucida. Defect type B probably also leads to impaired fertilizing capacity. Small lesions of this type may be of minor functional importance as long as the spermatozoa can bind to the zona pellucida and release enzymes at the appropriate time. Defect type C will result in complete loss of fertilizing capacity, since the spermatozoa will be unable to bind to the zona pellucida and probably will release the acrosomal enzymes prematurely.

During fertilization a major proportion of the spermatozoa observed between the cumulus cells had undergone the acrosome reaction. These spermatozoa would therefore be unable to bind to the zona pellucida and to fertilize the oocyte. A major role of these acrosome-reacted cells may be the delivery of enzymes involved in dissolution of the cumulus cell layer, thus preparing the way for acrosome intact spermatozoa.

The functional implications of irregular swelling and vesiculation of the acrosome observed in about ⅓ of the spermatozoa found among the cumulus cells is somewhat unclear. It is however unlikely that these spermatozoa would be able to bind to and penetrate zona pellucida.

Defect type A and B could easily be distinguished from the true acrosome reaction which consists of fusion and subsequent vesiculation of the outer acrosomal membrane and the plasma membrane *(Barros et al.* 1967). However, defect type C could not easily be distinguished from a true acrosome reaction by electron microscopy.

In this experiment pronounced phagocytosis of spermatozoa with both intact and defect acrosomes was observed. After this phagocytosis the cumulus cells showed marked signs of degenerative changes including formation of lipid vacuoles and mitochondria exhibiting tight, parallel cristae. The effect of this phagocytosis has been discussed by several authors. It has been suggested that, through spermiophagy, the cumulus-corona (CC) – cellmass might constitute a selective barrier towards abnormal and/or supernumerary spermatozoa *(Pereda & Coppo* 1987, *Pijnenborg et al.* 1985). In human it has been shown that CC cells may favour fertilization, both *in vivo* and *in vitro,* by modifying, selecting and orienting the spermatozoa present around the oocyte *(Nottola et al.* 1989).

Evaluation of the acrosome reaction can be a useful tool in predicting fertility of bulls. It has been shown that the increase in the percentage of acrosome-reacted spermatozoa in heparin-treated compared to control samples was significantly correlated to the 90-day nonreturn rate of the bulls *(Whitfield & Parkinson* 1992). Our experiment indicates that it is difficult to distinguish a TAR from a FAR. However, one could use EM as a method to evaluate the increase in acrosome reacted spermatozoa in different bulls following capacita-

tion by heparin treatment, since for 1 particular bull the extent of FAR will be the same in the treatment as in the control group. Electron microscopy is a reliable method, and even if the method is rather complicated and time consuming, this will be less costly and easier to perform than assessing bull fertility by field trials and could be used as an additional method for testing bull semen.

Acknowledgements

This work was financially supported by the Norwegian Agricultural Research Council (NLVF). The authors thank the National Institute of Diabetes and Digestive and Kidney Diseases, the Center for Population Research of the National Institute of Child Health and Human Development, the Agricultural Research Service of the U S. Department of Agriculture, and University of Maryland School of Medicine for providing ovine FSH and LH. We also thank the NRF-Norwegian Cattle Association for providing bull semen.

References

Austin CR· Membrane fusion events in fertilization. J. Reprod. Fertil. 1975, *44,* 155-166

Austin CR, Bavister BD Preliminaries to the acrosome reaction in mammalian spermatozoa. In: Afzelius, B. (ed): The Functional Anatomy of the Spermatozoon Stockholm. Wenner-Gren Institute. 1975, pp 83-87.

Austin CR, Bishop MWH: Role of the rodent acrosome and perforatorium in fertilization Proc. roy. Soc B. 1958, *149,* 241-248

Aamdal J, Andersen K. Fast thawing of semen frozen in straws Zuchthygiene 1968, *3,* 22-24.

Barer R, Ross KFA, Tkaczyk S. Refractometry of living cells. Nature 1953, *171,* 720-724

Barros C, Bedford JM, Franklin LE, Austin CK Membrane vesiculation as a feature of mammalian acrosome reaction. J Cell. Biol. 1967, *34,* C1-C5.

Bavister BD· Capacitation of golden hamster spermatozoa during incubation in culture medium. J. Reprod. Fertil. 1973, *35,* 161-163.

Bavister BD, Leibfried ML, Lieberman G. Development of preimplantation embryos of the golden hamster in a defined culture medium Biol. Reprod. 1983, *28,* 235-247.

Bavister BD, Yanagimachi R The effects of sperm extracts and energy sources on the motility and acrosome reaction of hamster spermatozoa in vitro. Biol. Reprod. 1977, *16,* 228-237.

Bedford JM. Sperm capacitation and fertilization in mammals. Biol. Reprod. Suppl 1970, *2,* 128-158.

Bleil JD, Wassarman PM· Mammalian sperm-egg interaction; identification of a glycoprotein in mouse egg zona pellucida passing receptor activity for sperm. Cell 1980, *20,* 873-882.

Courtens JL, Paquignon M. Ultrastructure of fresh, frozen, and frozen-thawed spermatozoa of the boar. In: Johnson, L.A., Larsson, K. (eds): Deep Freezing of Boar Semen. Swedish University of Agricultural Sciences, Uppsala: 1985, pp. 61-87.

Crozet N: Ultrastructural aspects of in vivo fertilization in the cow Gamete Res. 1984, *10,* 241-251.

Florman HM, Storey BT· Mouse gamete interactions. The zona pellucida is the site of the acrosome reaction leading to fertilization in vitro. Dev. Biol. 1982, *91,* 121-130

Gwatkin RBL· "Fertilization Mechanisms in Man and Mammals". Plenum, New York, 1977.

Gwatkin RBL, Carter HW, Patterson H Association of mammalian sperm with cumulus cells and the zona pellucida studied by scanning electron microscopy. Scanning Electron Microscopy (part. VI) Proceeding of the Workshop on SEM in Reproductive Biology, Chicago, Illinois. 1976, pp 379-384.

Healy P: Effect of freezing on the ultrastructure of the spermatozoon of some domestic animals. J. Reprod. Fertil. 1969, *18,* 21-27.

Hofmo PO, Andersen Berg K· Electron microscopical studies of membrane injuries in Blue fox spermatozoa subjected to the process of freezing and thawing. Cryobiology 1989, *26,* 124-131.

Mazur P Basic concepts in freezing cells. Proc. 1st Int Confer. on deep freezing of boar semen. Uppsala, 1985, 91-111.

Meizel S. The mammalian sperm acrosome reaction. A biochemical approach. In: M.H.Johnson (Ed.) Development in Mammals Vol.3.North Holland Pub., Amsterdam, The Netherlands, 1978, p.164.

Nath J. Correlative biochemical and ultrastructural studies on the mechanism of freezing damage to ram semen. Cryobiology. 1972, *9,* 240-246.

Nottola SA, Familiari G, Micara G, Aragona C, Motta PM· The role of the cumulus-corona cells surrounding in vitro human oocytes and polypronuclear ova. An ultrastructural study. Prog. clin. biol. Res. 1989, *296,* 345-354.

O'Rand MG, Fisher SJ. Localization of zona binding sites on rabbit spermatozoa and induction of the acrosome reaction by solubilized zonae. Dev. Biol. 1987, *119,* 551-559.

Parrish JJ, Susko-Parrish JL, First NL. Effect of heparin and chondroitin sulfate on the acrosome reaction and fertility of bovine sperm in vitro. Theriogen 1985, *24,* 537-549

Parrish JJ, Susko-Parrish JL, Leibfried-Rutledge ML, Critser ES, Eyestone WH, First NL. Bovine in vitro fertilization with frozen-thawed semen. Theriogenology, 1986, *25,* 591-600.

Pedersen H, Lebech PE: Ultrastructural changes in the human spermatozoon after freezing for artificial insemination. Fertil Steril. 1971, *22,* 125-133.

Pereda J, Coppo M Ultrastructure of a two-cell human embryo. Anat. Embryol. 1987, *177,* 91-96.

Pinenborg R, Gordts S, Ongkowidjojo R, Brosens I· Sperm phagocytosis by corona cells in a human in vitro fertilization system Ann. N. Y. Acad Sci. 1985, *422,* 310-317.

Richardson RT, Nikolajzyk BS, Abdullah LH, Beavers JC, O'Rand MG Localization of rabbit sperm acrosin during the acrosome reaction induced by immobilized zona matrix. Biol. Reprod. 1991, *45,* 20-26.

Sirard MA, Parrish JJ, Ware CB, Leibfried-Rutledge ML, First NL. The culture of bovine oocytes to obtain developmentally competent embryos Biol. Reprod. 1988, *39,* 546-552.

Szollosi D, Hunter RHF: The nature and occurence of the acrosome reaction in the domestic pig, Sus Scrofa J. Anat (Lond.) 1978, *127,* 33-41.

Weitze KF. Kryobiologische Aspekte der Spermakonservierung (Cryobiological aspects of sperm conservation). In: Busch W, Lohle K, Peter W (eds): Kunstliche Besamung bei Nutztieren. Stuttgart. Gustav Fischer Verlag Jena: 1991, pp. 237-248.

Whitfield CH, Parkinson TJ. Relationship between fertility of bovine semen and in vitro induction of acrosome reactions by heparin. Theriogenology, 1992, *38,* 11-20.

Yasuda Y, Tanimura I Scanning electron microscopy of boar spermatozoa before and after frozen-thawed treatment. Int. J. Fertil 1974, *19,* 149-159.

Sammendrag

Membranforandringer i oksespermier etter frysing og opptining og etter in vitro fertilisering

Membranforandringer i oksespermier etter frysing og opptining og under in vitro fertilisering er undersøkt ved hjelp av scanning elektronmikroskopi (SEM) og transmisjons elektronmikroskopi (TEM). Selv om de fleste spermiene hadde intakte membraner etter frysing og opptining, kunne man skille mellom 3 typer membrandefekter avhengig av hvilke strukturer som var involvert. Den først typen manglet plasmalemma over hele akrosomet I den andre kategorien var fremre del av ytre akrosommembran sterkt utvidet, men delvis dekket av intakt plasmamembran. Den siste kategorien besto av spermier med uttalt vesikulering og desintegrering av plasmalemma og ytre akrosommembran. Denne siste defekten var ikke lett å skille fra den ekte akrosomreaksjonen som ble observert under in vitro fertilisering og besto av en sammensmelting og vesikulering av plasmalemma og ytre acrosommembran Både intakte og akrosomreagerte spermier ble aktivt fagocytert av cumulusceller

(Received March 12, 1993; accepted November 3, 1993)

Reprints may be requested from: A. Krogenæs, Department of Reproduction and Forensic Medicine, Norwegian College of Veterinary Medicine, P.O. Box 8146 Dep., N-0033 Oslo 1, Norway.