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Ultrastructural Observations of the Acrosome Reaction of Goat Spermatozoa Preincubated in the Hamster Uterus

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햄스터子宮에서 前培養한 山羊精子의 微細構造的 觀察

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摘 要

本 試驗은 山羊精子의 前培養환경으로서 摘出햄스터子宮의 이용가능성을 검토하기 위해 摘出햄스터子宮에서 6시간 前培養한 山羊精子의 微細構造的 변화를 透過型電子顯微鏡으로 관찰하였다.

山羊精子를 採取 직후 固定하였을 때는 精子頭部의 微細構造에 변화가 없었으나, 摘出햄스터子宮에서 6시간 前培養한 후 固定하였을 때는 53%의 精子에서 尖體에 小胞形成이 관찰되었으며, 32%는 尖體가 消失되었다. 따라서 精子頭帽의 小胞形成은 尖體反應에 의한 것이고, 精子의 頭帽가 消失된 것은 죽은 精子에서 관찰되었을 가능성을 示唆했다.

Introduction

Before the mammalian spermatozooon can fertilize the ovum it must reside for a time in the female genital tracts. This phenomenon was first described independently by Chang (1951) and by Austin (1951), who termed the process: "capacitation" (Austin, 1952).

The importance of the acrosome reaction in mammalian fertilization was first recognized by Austin and Bishop (1958). The observation that the acrosome reaction or multiple fusions between the plasma and underlying outer acrosomal membranes was first made by Piko and Tyler (1964) for the rat, and followed by Barros et al. (1967) for the hamster and rabbit. These and subsequent studies using an electron microscope have demonstrated that the morphological pattern of the acrosome reaction is fundamentally the same in all mammalian species (Yanagimachi and Noda, 1970; Bedford and Cooper, 1978). In the domestic animals, however, there have been few reports on the morphological pattern of the acrosome reaction of spermatozoa preincubated *in vitro*, except for the boar and goat spermatozoa (Imai et al., 1983).

The present study was performed to observe the ultrastructural changes of goat spermatozoa preincubated in the uterus isolated from an estrous hamster.

Materials and Methods

The medium used for the manipulation of spermatozoa was a modified Krebs-Ringer bicarbonate solution (m-KRB containing 94.6 mM-NaCl, 4.78 mM-KCl, 1.71 mM-$CaCl_2$, 1.19 mM-KH_2PO_4, 1.19 mM-$MgSO_4$, 25.07 mM-$NaHCO_3$, 21.58 mM-sodium lactate, 0.5 mM-sodium pyru-

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vate, 5.56 mM-glucose, 1 mg crystalline bovine serum albumin (Sigma Chemical Co.)/ml, 50g streptomycin sulphate/ml and 75 g potassium penicillin/ml) (Toyoda and Chang, 1974).

Semen (0.8ml) was collected from a goat of the Saanen breed using an artificial vagina and percentage of motile spermatozoa after collection was 85%. A part of the semen was used as a control immediately after semen collection and the another part was diluted to 10 ml m-KRB solution, and washed once by centrifugation at 500 g for 10 min. The sedimented sperm-pack was resuspended in about 2 ml m-KRB solution. About 0.05 ml of the sperm suspension at the concentration of 15×10^8 spermatozoa/ml was introduced into each uterine horn isolated from hamster superovulated by PMSG and hCG, and then the uterotubal junction and the cervical end of the uterus were ligated. The isolated uterus was dipped in 0.9% NaCl solution and kept in a CO_2 incubator (5% CO_2 in air at 37°C) for 6h.

The spermatozoa were recovered by flushing the reproductive tracts after incubation. Contaminating blood cells and cellular debris were removed by centrifugation at 150 g for 10 min. All sperm samples were washed again by centrifugation at 500 g for 10 min. Small drop (0.01 ml) of sperm mass were fixed for 1 h with cold 2.5% glutaraldehyde in a 0.1 M phosphate buffered solution (pH 7.4). After having been rinsed by centrifugation (1,500 g, 10M phosphate buffered solution, the pieces of sperm mass were postfixed for 1 h with 1% osmium tetroxide maintained at pH 7.4 with a phosphate buffered solution. Dehydration of the fixed specimens was always performed with a series of acetone at room temperature (Yanagimachi and Noda, 1970). The sperm samples were then embedded in a mixture of Epon 812 and 815. Ultrathin sections of sperm samples cut with ultramicrotome (Poter-Blum I Type) were stained with uranyl acetate and lead citrate, and examined with a Hitachi H-300 electron microscope.

Morphological description of spermatozoa was made according to the criteria shown in Plate 1-2.

Results and Discussion

As shown in Table 1, when goat spermatozoa were fixed immediately after collection and examined under an electron microscope, no detectable change was found in the majority (81.0%) of spermatozoa (Plate 1, a-b), but some of the spermatozoa were classified as "vesiculated" (3.0%), "acrosome lost" (14.5%) or "degenerated" (1.5%). In contrast, when spermatozoa were preincubated for 6 h in the uterus isolated from an estrous hamster, morphological changes were observed in most of the spermatozoa. These morphological changes were classified into four categories depending upon their general appearance and the morphology of the acrosome; "intact" (10.5%) in which the acrosome remained attached to the nucleus, but the plasma membrane became rarely disjoined from the surface of the sperm, "vesiculated" (53.0%) in which extensive vesiculation occurred between plasma membrane and outer acrosomal membreane at the acrosomal cap region, but no morphological change was observed at the portion of the equatorial segment, "acrosome lost" (32.0%) in which the outer acrosomal membrane was completely separated from the inner acrosomal membrane and totally lacked the acrosomal cap region, and "degenerated" (4.5%) in which the acrosome remained attached to the nucleus, but the cell membrane of the postacrosomal region was abnormally separated (Plate,

1, c-d, Plate 2, a-d).

Since the percentages of motile spermatozoa were more than 80% in spermatozoa immediately after collection and 40% in spermatozoa preincubated for 6 h in the isolated hamster uterus (Song and Iritani, 1985), the present results suggested that the "acrosome lost" spermatozoa might have been dead and the membrane vesiculation might be the true acrosome reaction rather than a random degenerative process associated with sperm death (Esbenshade and Clegg, 1980). This suggestion has also been supported by several reports, in which the acrosome reaction consisted of an ordered vesiculation of the plasma membrane and outer acrosomal membrane that was similar to the pattern described in other mammals (Piko and Tyler, 1964; Barros et al., 1967; Bedford, 1968; Soupart and Strong, 1974; Yanagimachi and Usui, 1974; Szollosi and Hunter, 1978: Imai et al., 1983; Crozet, 1984).

In the present experiment, the membrane vesiculation was observed in 53.0% of goat spermatozoa preincubated for 6 h in the isolated hamster uterus. These results also suggested that the isolated hamster uterus may have an ability to induce the acrosome reaction of goat spermatozoa. Mechanisms of the occurrence of the acrosome reaction under the present conditions are not known, but Imai et al. (1983) reported that the acrosome reaction was observed in 42.5% and 20% of boar and goat spermatozoa preincubated for 6 h in the isolated gilt uterus.

Since it has been reported that goat spermatozoa prepared by the same procedures as in the present experiment were able to penetrate zona-free hamster eggs (Song and Iritani, 1985) but were unable to penetrate zonaintact goat oocytes matured in culture (Song, 1985), it is suggested that "vesiculated" spermatozoa induced acrosome reaction before fusion with eggs were able to fuse with the plasma membrane of the zona-free hamster egg, but were unable to penetrate into zona pellucida of the goat eggs. This suggestion agreed with the results for the hamster (Barros et al., 1973) and pig (Imagi, 1983).

Table 1. Morphological examination of goat spermatozoa preincubated for 6 h in the uterus isolated from an estrous hamster.*

	Morphological change in acrosome			
	Intact	Vesiculated	Acrosome lost	Degenerated
Control	162	6	29	3
(%)	(81.0)	(3.0)	(14.5)	(1.5)
Hamster uterus	21	106	64	9
(%)	(10.5)	(53.0)	(32.0)	(4.5)

*Total number of spermatozoa examined was 200.

Summary

Goat spermatozoa were fixed immediately after collection and after 6 h of preincubation in the isolated hamster uterus, and the ultrastructure of the acrosome reaction of the goat spermatozoa was exmined under an electron microscope.

When spermatozoa were fixed immediately after collection no structural change was observ-

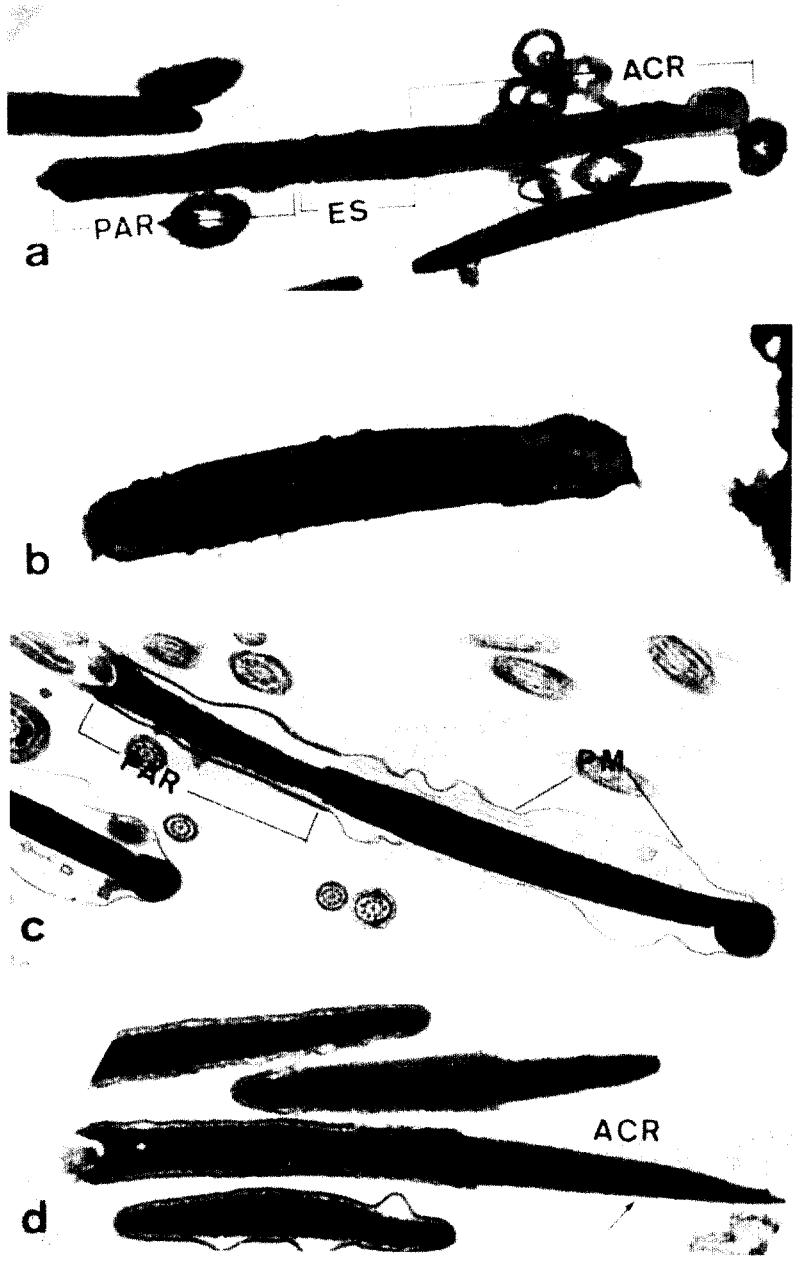

PLATE 1

Ejaculated goat spermatozoa preincubated for 6 h in the isolated hamster uterus (c – d) or just after collection (a – b), were sectioned after fixation and stained, and then photographed under an electron microscope.

a) and b). A sagittal (a) or transversal (b) section showing the acrosomal cap region (ACR), the equatorial segment (ES) and the postacrosomal region (PAR) which remains intact. x24,000 (a – b).

c). A sagittal section of degenerating spermatozoon showing the swelling of the plasma membrane (PM) around the sperm head and the cell membrane of the postacrosomal region (PAR) were abnormally separated. x24,000.

d). A sagittal section showing the outer acrosomal membrane which was completely separated from inner acrosomal membrane (arrow) and completely lacked at the acrosomal cap region (ACR). x 24,000.

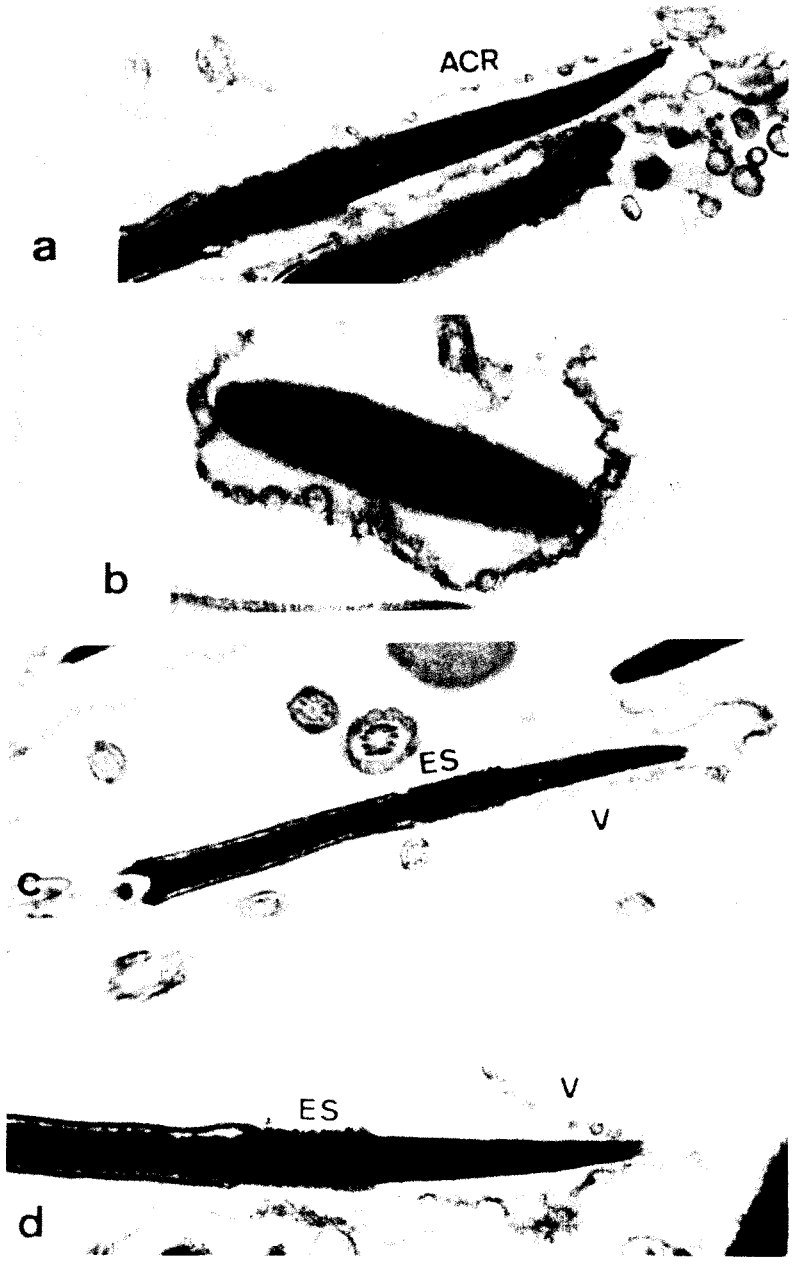

PLATE 2

Ejaculated goat spermatozoa preincubated for 6 h in the isolated hamster uterus were sectioned after fixation and stained, and then photographed under an electron microscope.

a) and b). A sagittal (a) or transversal (b) section showing the swelling of the acrosome. The acrosomal contents disperse and the microtuble like structures appear in the acrosomal cap region (ACR). x 24,000 (a - b).

c). A sagittal section showing the progressive membrane vesiculations (V) of the acrosomal cap region. The equatorial segment (ES) remains intact. x 24,000.

d). A sagittal section showing more progressive membrane vesiculation (V) of the acrosomal cap region than that was shown in (a - c). The equatorial segment (ES) remains intact. x 24,000.

ed, but when they were preincubated for 6 h in the isolated hamster uterus, 10.5, 53.0, 32.0 and 4.5% of the "intact", "vesiculated", "acrosome lost" and "degenerated" spermatozoa, respectiely, were observed.

The present results suggested that the membrane vesiculation of spermatozoa might be a true acrosome reaction and the "acrosome lost" spermatozoa might have been dead and the isolated hamster uterus may have an ability to induce the acrosome reaction of goat spermatozoa.

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