Intracellular Vacuoles or Vesicles and Invagination in Boar Spermatozoa

Yoshio KOJIMA

*Laboratory of Reproductive Physiology, Faculty of Agriculture,
Shizuoka University, 836 Ooya, Shizuoka 422*

(Received for publication July 2, 1980)

Abstract. By means of a transmission electron microscope, various types of intracellular vacuoles or vesicles were observed in ejaculated boar spermatozoa. These membrane bounded structures were frequently noticed in the head (acrosome and sperm nucleus) and the principal piece, but were hardly ever seen in the middle piece except in the cytoplasmic droplet, or in the end piece. In two cases of sterility (asthenozoospermia) and poor semen quality (hairpin curved deformity), the vacuoles appeared in the neck area, even in the center of proximal centriole, and some of them represented an invagination of the plasma membrane.

It is well known that the head of mature mammalian spermatozoa commonly show vacuoles or vesicles of various size at the ultrastructural level [11]. Especially in the human spermatozoon, vacuoles frequently appear in the nucleus and their nature has been discussed [16]. Various types of acrosome deformities have been observed during morphological examinations of boar semen in our laboratory. The present study reports intracellular vacuoles or vesicles in boar spermatozoa not only in the sperm head but also in the flagellum.

Materials and Methods

Semen samples were collected from 8 Landrace and 4 Hampshire boars, which belonged to a breeding stock. One Landrace boar showed a poor semen quality (the appearance rate of the hairpin curved sperm was more than 13%) [15] and another boar, a Hampshire, was sterile (asthenozoospermia). The ejaculates were obtained on a phantom by use of artificial vagina.

The methods used in this investigation are similar to those previously described [15]. Briefly, about 1 ml of the fresh semen was double fixed with cacodylate-buffered (pH 7.2) 3% glutaraldehyde and

2% osmium tetroxide. After dehydration, the samples were embedded in Epon 812. Sections were cut on a Porter-Blum MT-1 ultramicrotome, double-stained with uranyl acetate and lead citrate, and examined with an electron microscope (JEOL, MT-7).

Results

In some cases the spermatozoa showed a bulky vacuole in the acrosome, and sometimes this vacuole indented the nucleus. Generally, the nucleus of mature boar spermatozoa does not have the prominent vacuole seen in human spermatozoa. When present, it is small without a limiting membrane. However, in the sterile boar, a prominent vacuole could often be seen in more than 10% level of the sperm number (Fig. 1).

In the neck (connecting piece), various types of vacuoles or vesicles occasionally appeared (Figs. 2-4) even within the proximal centriole (Fig. 2). Most of the cases appeared in the subcentriolar, central part of the neck. Some of them showed a multi-vesicular structure inside the vacuole (Fig.

3). In the sterile boar, some of the vacuoles appeared to represent deep invaginations at the plasma membrane (Fig. 4). Vesicles and/or vacuoles were commonly observed in the cytoplasmic droplet of immature spermatozoa. However, the spermatozoa seldom had vacuoles in the middle piece or the end piece.

Usually, most of the vacuoles in the principal piece appeared in the distal part and were located inside the fibrous sheath, dislocating axial filament complex over to the other side (Fig. 5). Regardless of the location of the displaced tail fibers, most of the vacuoles were usually elongated in a plane parallel with the central pair of axonemal microtubules (Fig. 5).

Discussion

Various kinds of acrosome defects and nuclear deformities with vacuoles or vesiculations have been described in human spermatids [13], in boar spermatozoa [1, 3, 5], and in bull spermatozoa [10]. Coulter et al. [9] reported a narrow-mouthed invagination of the nuclear membrane called "crater defect" in bovine spermatozoa. Such cases have been recognized as a cause of total sterility or subfertility in farm animals. Systematic registrations of different acrosomal deformations have been started in order to find out if there is a genetical background (Hancock, 1972). For example, some cases of this type of abnormalities were described as an inbreeding defect, called "SME—defect", in the boar [8]. Sometimes this deformity is so severe that it influences the contour of the nucleus [2, 7]. The cases of pouch formations by invaginations of the nuclear envelope have been noticed in bovine and porcine testicular sperm [4]. Similar defects appeared in experimental cryptorchidism in the rabbit [17] and in a quaking mutant mouse [6].

Small vacuoles without a limiting membrane in the nucleus of the sperm head have been commonly observed in many mammalian species [11], as well as a comparatively big vacuole in the human sperm head. They are generally accepted to be within physiological limits, produced by an occasional lack of chromatin condensation, and are not considered to impair the function in human sperm [16]. This interpretation seems acceptable for the small vacuoles in the sperm head also seen in the farm animals (Fig. 1), but the large, membrane limited vacuoles or vesiculations are probably more serious changes. The correlation between these vacuolations or vesiculations in mammalian spermatozoa and infertility was discussed by Jones [14]. He suggested the possibility that these changes are artifacts or caused by cytolysis, and also compared them with the morphological changes taking place in the sperm head during capacitation and fertilization.

The appearance of vacuoles in the sperm neck was noticed in the rat testis [20] and in ejaculated spermatozoa from a low fertility group of bulls [19]. The former cases were seen in connection with the disappearance of the proximal centriole during rat spermiogenesis. However, invaginations of the plasma membrane (Fig. 4) did not occur in their cases. Except for the present observations, we have not seen vacuole formations in the neck piece of the spermatozoa from farm animals. Vesicles and sometimes myelin figures were observed in the neck of rat spermatids [20]. A structure similar to the vesicle in the proximal centriole (Fig. 2)—a central vesicle in the centriole—was already reported in somatic cells of the rodent [18], but the significance and function of this structure are entirely unknown.

Except for the appearance of mitochondrial "vacuolizations" as artifacts, vacuoles

were very rare in the middle piece in this study.

As in bull spermatozoa [19], membrane limited vacuoles in the principal piece were rather frequently observed in the boar spermatozoa. It is interesting that most of these vacuoles were parallel to the central pair of axonemal microtubules (Fig. 5). This may be related to the position of the longitudinal columns of the fibrous sheath. Concerning the origin of the vacuole in the principal piece, it may be a remnant of some cytoplasmic organelle (smooth endoplasmic reticulum) present during spermateliosis.

Further details about the morphogenesis of these defects will be available at a later date.

Acknowledgment. The author wishes to express his deep appreciation to Prof. L. Nicander, The Veterinary College of Norway, for his encouragement throughout this work and revising the manuscript.

References

- [1] Andersen, K. (1974). Morphological abnormalities in the acrosome and nucleus of boar spermatozoa. *Nord. Vet.-Med.* **26**, 215-218.
- [2] Andersen, K., and O. Filseth (1976). The occurrence of a "SME"-defect-like abnormality in the head of spermatozoa from a Norwegian Landrace boar. *Nord. Vet.-Med.* **28**, 511-514.
- [3] Bane, A. (1961). Acrosomal abnormality associated with sterility in boar. In *Proc. IVth Int. Congr. Anim. Reprod.* Haque, Vol. IV, 810-817.
- [4] Bane, A., and L. Nicander (1965). Pouch formations by invaginations of the nuclear envelope of bovine and porcine sperm as a sign of disturbed spermiogenesis. *Nord. Vet.-Med.* **17**, 628-632.
- [5] Bane, A., and L. Nicander (1966). Electron and light microscopical studies on spermateliosis in a boar with acrosome abnormalities. *J. Reprod. Fertil.* **11**, 133-138.
- [6] Bennett, W. I., A. M. Gall, J. L. Southard, and R. L. Sidman (1971). Abnormal spermiogenesis in quaking, a myelin-deficient mutant mouse. *Biol. Reprod.* **5**, 30-58.
- [7] Blom, E., and A. Birch-Andersen (1975). The ultrastructure of a characteristic sperm head-defect in the boar: The SME-defect. *Andrologia*. **7**, 199-209.
- [8] Blom, E., and P. Jensen (1977). Study of the inheritance of the SME seminal defect in the boar. *Nord. Vet.-Med.* **29**, 194-198.
- [9] Coulter, G. H., R. J. Oko, and J. W. Costerton (1978). Incidence and ultrastructure of "crater" defect of bovine spermatozoa. *Theriogenology* **9**, 165-173.
- [10] Cran, D. C., and H. M. Dott (1976). The ultrastructure of knobbed bull spermatozoa. *J. Reprod. Fertil.* **47**, 407-408.
- [11] Hancock, J. L. (1966). The ultra-structure of mammalian spermatozoa. In *Advances in Reproductive Physiology*, A. McLaren editor, Logos Press/Academic Press, London, New York, 125-154.
- [12] Hancock, J. L. (1972). Spermatogenesis and sperm defects. In *Genetics of the Spermatozoon*, Beatty, R. A., and S. Gluecksohn-Waelsch, editors, Univ. Edinburgh, Edinburgh and New York, 121-130.
- [13] Holstein, A.-F. (1975). Morphologische Studien an abnormen Spermatiden und Spermatozoen des Menschen. *Virchows. Arch. Pathol. Anat. Histol.* **367**, 93-112.
- [14] Jones, R. C. (1975). Fertility and infertility in mammals in relation to sperm structure. In *The Biology of the Male Gamete*, Duckett, J. G., and P. A. Racey, editors, Academic Press, London, 343-365.
- [15] Kojima, Y. (1977). Light and electron microscopic studies of "Hairpin curved deformity" on boar spermatozoa. *Jpn. J. Vet. Sci.* **39**, 265-272.
- [16] Pedersen, H., and D. W. Fawcett (1976). Functional anatomy of the human spermatozoon. In *Human Semen and Fertility Regulation in Men*, Hafez, E. S. S., editor, Mosby Comp., Saint Louis, 65-75.
- [17] Plöen, L. (1973). Observations on rabbit spermatogenesis: Electron and light microscope studies with special reference to normal spermateliosis and to the immediate and delayed effects on spermateliosis of a short-time experimental cryptorchidism. Thesis, Stockholm, p. 27.
- [18] Stubblefield, E., and B. R. Brinkley (1967). Architecture and function of the mammalian centriole. In *Formation and Fate of Cell Organelles*. Vol. 6, Warren, K. B., editor, Academic Press, New York and London, 175-218.
- [19] Uzu, G., J. L. Courtens, and M. Courrot (1976).

Quantitative analysis of ultrastructural abnormalities of spermatozoa from bulls of different fertility. *In Proc. VIIIth Int. Congr. Anim. Reprod.* Vol. IV, Krakow, 748-751.

[20] Woolley, D. M., and D. W. Fawcett (1973). The degeneration and disappearance of the centrioles during the development of the rat spermatozoon. *Anat. Rec.* **177**, 289-302.

要 約

豚精子に出現した空（液）胞および細胞膜の嵌入：小島義夫（静岡大学農学部家畜繁殖学教室）——種雄豚12頭（ランドレース種8，ハンプシャー種4）の射精精液中の精子の切片標本を作製し，電子顕微鏡により細胞内空（液）胞の存在，分布を調べた．限界膜を有する空（液）胞は，頭帽に最も多く，次いで頭部核質内と尾部主部に認められ，中片部と尾端部には殆んど出現しなかった．精子無力症と貧精液症（ヘアーピン形異常精子の出現率13%以上）の各1例の雄豚の精子ではこれらの空胞の出現頻度が高く，特に頸部の中心体内部にも現われ，頸部に多胞体様構造も認められた．さらに頸部における細胞膜の嵌入が見出された．一般に尾部に出現する空胞は長軸に沿って細長く，軸線維束の中心微小管対と平行に位置する傾向がみられた．これらの成績について先人の報告を対比しながら多少の検討を加えた．

Explanation of Figures

Fig. 1. Electron micrograph of an oblique section of a sperm head of a sterile boar. Under the equatorial segment of the acrosome, the nucleus has a prominent vacuole. $\times 50,000$.

Fig. 2. Longitudinal section of boar sperm neck in flat view. Note the membrane limited, small vacuoles in the proximal centriole and in the sub-centriolar space of the neck. $\times 50,000$.

Fig. 3. Tangential section of boar sperm neck in

flat view. A multivesicular structure is seen in the sub-centriolar space. $\times 45,000$.

Fig. 4. Ejaculated sperm from a sterile boar. The sperm neck shows a prominent invagination of the plasma membrane. $\times 50,000$.

Fig. 5. Cross section of two boar sperm flagella. A membrane bounded vacuole is seen between the axonemal microtubules and the fibrous sheath in one of the tails. $\times 63,000$.