ELECTRON MICROSCOPIC STUDY OF THE BULL SPERMATOZOON III

Yoshio KOJIMA*

Department of Veterinary Obstetrics
Faculty of Veterinary Medicine
Hokkaido University, Sapporo, Japan

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III STRUCTURE OF THE NECK

RESULTS

The neck of the bull sperm is a slender wedge-shaped structure which is wide and flattened anteriorly but rather thick posteriorly, The internal fine structure of the neck differs extremely from that of the middle piece.

In suspension materials, the outline of the neck is generally indistinct because of its weakness to electron permeability, having little resemblance with that of the middle piece (fig. 14). The replica method is also inconvenient in clarifying the surface texture of the neck (fig. 16). Even if the sperm is treated by repeated washing, such that the connection between the head and the neck becomes loose and the covering membrane is broken, 3 or several tortuous fibrils are only shown in the neck. At the anterior end of the neck there are three protrusions corresponding to the fossula-like depressions of the head, thus these protrusions and depressions are connected with each other like an articulation (fig. 15).

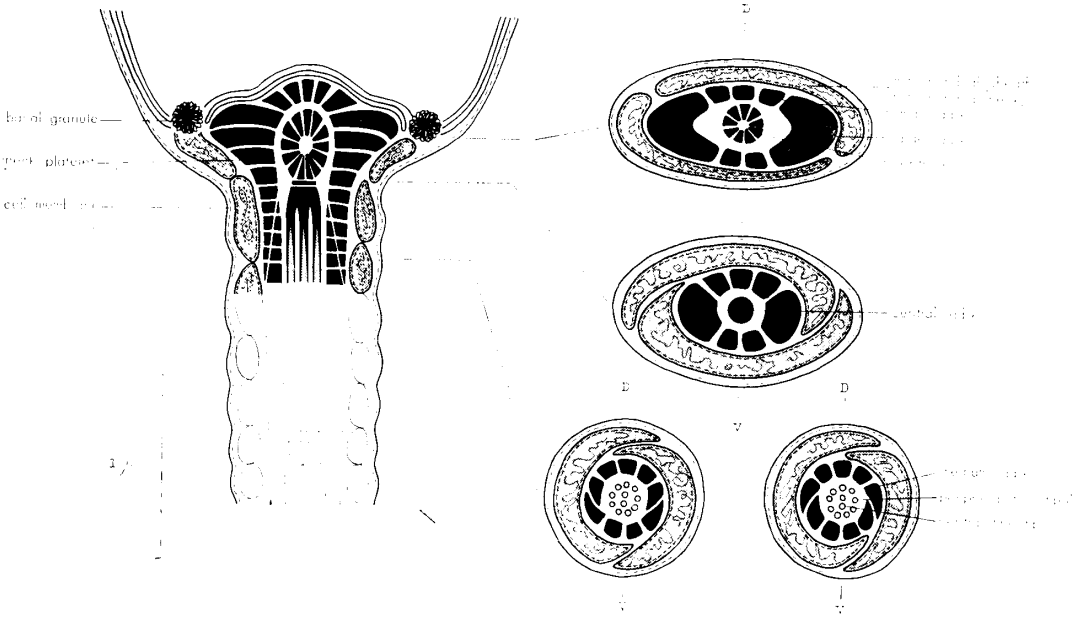
In sectioned materials, inside of the cell membrane, the mitochondrial sheath probably originating in the basal granules, forms two spiral structures, the mitochondrial helixes, irregularly surrounding the fibrillar bundle of this portion as a collar (figs. 13 & 23). The number of appearance of the mitochondrial sheath in transverse sections in 50 cases varies from 1 to 4 on each lateral side.

The neck fibrillar bundle, the original part of the axial fibril bundle, is composed of a special arrangement of the neck platelets. This complex of the neck platelets forms the 3 protrusions of the neck. In a flattened section the protrusion appears as 3 (figs. 17 & 18), but in a sagittal section as only one (figs. 8 & 9). From the many microphotographs of the neck, it can be said that the part connecting to the neck is composed of a complex of many neck platelets with high electron density (fig. 13), and the complex separates at once into 7 radixes which run downwards the middle piece; i. e., in a cross section of the anterior portion, the neck shows a composition of 2 large radixes and 5 small ones (fig. 25). This arrangement of the radixes is very characteristic; 2 large radixes run along both lateral sides inside of the mitochondrial spirals, while at the dorsal side 3 small radixes and at the ventral 2 small radixes run, respectively. In the middle portion of the neck, each of the large radixes branches off 2 medium radixes, respectively (fig. 20) and at the point of about 1 μ downward,

* Present address: Laboratory of Reproductive Physiology, Faculty of Agriculture, Shizuoka Universitiy, Iwata

each of the pair of medium radixes on each side twists 180 degrees and exchanges its position with each other. After this exchange of position of two pairs of the medium radixes, each radix loses the character of neck platelet and gradually changes into a peripheral fibril with high electron density. Therefore, in the anterior portion of the neck consists of a pair of large radixes at both the lateral sides, 3 small radixes at the dorsal and 2 small radixes at the ventral; these 7 radixes in relation to one another take a circular form in a transverse section. While in the posterior portion, it consists of each pair of medium radixes at both the lateral sides, 3 small radixes at the dorsal side (fig. 22) and 2 small radixes at the ventral (figs. 21 & 24), these 9 radixes forming a circle (text fig. 3). Each radix is composed of about 15 segmental structures as laminated columns with high electron density, the neck platelets.

TEXT FIGURE 3 *Schematic diagrams of neck*



N. B.: Left shows longitudinal section of flattened view and right shows cross sections at anterior, middle and posterior portions.

At the portion where the large radixes are reduced in their thickness, narrowest on the inside of these radixes, there is a centriole (fig. 19). As the centriole is very sensitive to various treatments, its characteristic feature can not be obtained in poor preparations. In successful sections, it shows a radial structure with high electron density, fine granules around a central point with low electron density. Sometimes, the centriole attaches in part, closely with the neck platelets or radixes surrounding it. Based upon many sections it may be assumed that the centriole extends toward the posterior direction, forming the central radix within a short distance and then branching off 11 fine fibrils; that is 9 peripheral fine fibrils (inner ring of axial fibril bundle) and 2 central fine fibrils.

In the neck region, each of the 11 fine fibrils already represents a fine tubular structure as seen in the middle piece (figs. 26~29). The portion of the central radix structures is

occupied with low electron dense matrix (figs. 17~29). The cell membrane continuing from the head has also a unit membrane system in this region, but covering around the neck with rather looseness (figs. 13 & 28).

DISCUSSION

In spite of numerous cytological investigations, little has been established in the structure of the sperm neck. Because of its complexity and fragility, the structure of the neck, especially the part connecting to the head has been attractive to many workers[38,86,102,106]. For many years, it has been reported that the neck contains a centriole and that the axial fibril bundle originates in this portion. Most electron microscopists studying the sperm morphology only remain in giving a magnification of the optical microscopic findings, while some of them describe more detailed structures of the neck, some of which are based on the assumption[26,53,63,81]. Although SCHULTZ-LARSEN considered the neck as an artifact which appeared at the anterior portion of the middle piece, ÅNBERG, who first examined the neck of human spermatozoa with sectioned materials, could distinguish the neck from the middle piece on the basis of its internal structures. His finding is now supported by many other authors[11,38,87,95]. Some authors, however, still consider the neck as a part of the middle piece, such as "implantation region" (BLOM & BIRCH-ANDERSEN[14]) or "centriolar region" of middle piece (NICANDER & BANE). According to the present author's opinion, the neck of the bull sperm should be completely distinguished from the middle piece, because of the following morphological characteristics; first, the neck has a centriole at its central portion, secondly, fibrils in the neck consist of many neck platelets, and thirdly, the arrangement of the mitochondrial sheath which is quite regular in the middle piece, represents an irregular form in the neck (figs. 13 & 23).

In the age of optical microscope, it has been suggested that the neck consisted of one or more fibrils or fibril bundles[86,102,106,108], and also the existence of the centriole was questioned[44]. In the early years of electron microscopy, and still today, the detailed structure of the neck is obscure. Some authors have described two fibrils in the neck[26,29,92], and others three[18,41,49] or more[37,53,62,81]. This discordance in number of fibrils is also observed in the case of whole cell preparations (fig. 14), where three have been the most frequent to be observed (fig. 16). The ultramicrotomized materials, each fibril bundle is clearly shown as a radix or gathering of radixes consisting of many neck platelets with a characteristic arrangement.

Since ÅNBERG had first reported the neck platelet in the human sperm, other authors noticed this structure not only in the human[87,95] but also in farm animals[14]. ÅNBERG considered it as a discoid plate having a hole in its center and he called

it the "basal plate", which in the present study is the name given to the structure at the base of the head (text figs. 1 & 2). The neck platelets of the bull sperm do not show such a discoid plate but rather are trapezoid or hemispheroid in shape, arranged in a circular or elliptic form. There are some reports describing neck platelets of spermatocytes[30,56,64], but the three-dimensional structure of this region has not yet been elucidated. BLOM & BIRCH-ANDERSEN[14] reported that in bull sperm the neck platelets form radixes, however, they could not show accurately the number of the radix or their arrangement.

On the basis of the findings obtained from a number of microphotographs, it is noticed that in the anterior portion of the neck there are two large radixes at both lateral sides, each of which branches off 2 medium radixes, respectively, in the middle portion, and that there are 3 small radixes at the dorsal side and 2 small radixes at the ventral (figs. 22 & 21).

In a flattened section of the bull sperm's neck, the large radix generally appears as an inverted triangle. In 224 of 720 specimens, this triangle appeared at the left side (fig. 17), and in 223 did at the right side (fig. 18), while the remaining 273 included those in which it appeared at both side or did not appear. From this point of view, it can be said that the arrangement of the radixes in flattened view is entirely symmetrical as same as it is proven in transverse view.

It is very interesting to note the mode of junction between the medium radixes of the neck and the peripheral fibrils of the middle piece. Each medium radix does not simply connect with the corresponding peripheral fibrils, but "mosaic junction" occurs at this portion (figs. 13 & 17). Since this "mosaic junction" appears in almost all photographs, it may not be due to artifact caused by the sectional angle. On the other hand, 3 small radixes locating at the dorsal side (fig. 22) and 2 ventral ones (fig. 21) in the neck change into the respectively corresponding peripheral fibrils of the middle piece, where "mosaic junction" does not occur. The reason why "mosaic junction" appears only in the case of medium radixes, perhaps may be due to the fact that at the middle portion of the neck each of the pair of medium radixes which originates from a pair of large radixes twists and exchanges its position with the other one (fig. 20), and that, in the case of small radixes, such exchange of position does not occur. As for the mode of inside and outside ones as shown in text figure 3, may be considered theoretically. In order to clarify this problem, however, much more work will be required. It can be said that this exchange of position of the medium radixes is not owing to artifacts made in the course of fixation or sectioning, but it is undoubtedly one of actual structures, because many microphotographs of flattened view through the head, neck and middle piece clearly demonstrate this figure, and the spermatozoon seems to be protected such influence of fixation by the cell membrane and

the mitochondrial sheath.

The number of the neck platelets in a large radix was counted as more than 9 by SCHULTZ-LARSEN in human sperm and 13~14 by BLOM & BIRCH-ANDERSEN[14] in the bull sperm. In the present work, a few microphotographs showed more than 15 platelets (figs. 7 & 13). The determination of the number of the neck platelets must depend on serial sections because this may be influenced by the sectioning angle. The present author considers the number of the neck platelets of a large radix to be more than 15, however, it is not known whether this number is uniform in all radixes.

The researchers who denied the existence of the neck portion considered that the axial fibril bundle connects directly to the head[96]. In 1909, RETZIUS noticed 2 or 3 of the small granules in this region in various mammalian sperm. Thereafter, as the advance in the study of spermatogenesis and sperm morphology progressed, this granule came to be called a centriole and was recognized in many species[11,108]. Some workers could not identify this structure[87] or denied its existence[96], and also the number of such structures varied considerably among observers[6,10,26,29,49,98]. The discordance in number may be mainly due to differences in materials or methods.

On the other hand, some electron microscopists had detected a centriole in the center of the sperm neck in human[3,87,95] and bovine sperm[14], however, they could show this structure only in sagittal or oblique sections, but not in flattened sections. In spite of this, they illustrated the centriole in their schemata, as if the structure appeared in a flattened section including the head.

It has ever been believed that the centriole is cylindrical[38] or spherical[3] and it consists of 9 granules with high electron density[14]. SCHULTZ-LARSEN illustrated 11 granules of the centriole in the human sperm. The present author also believes that the centriole is a spherical structure consisting of fine granules with high electron density as same as the neck platelet, but he could not obtain any clear findings sufficient to determine the number of the granules. Furthermore, he has an opinion that the centriole is not floated freely in the central portion of the neck as mentioned by many investigators[3,14,87,95], but it has a close connection with the surrounding radixes and releases 11 fine fibrils toward the middle piece. A variable tendency in the space between the centriole and the fibrils may depend not only on the position of the sperm at fixation process but also on some artifacts caused by contraction or separation of these fine structures.

In the present work, only one true centriole was observed. BRADFIELD[22] has the opinion that each complex of the basal plates or each fibril radix should be considered as a centriole which is modified in morphological characteristic, by analogy with the fact that several basal granules in a cillium of an epithelial

cell[34,40], are located corresponding with each fibril consisting of a cillium and they represent a special striated appearance similar to the fibril radix of the sperm neck. Likewise, NICANDER & BANE offered a similar opinion studying stallion sperm; they assume two centrioles corresponding with a pair of central fine fibrils, besides a centriole which is located in the central portion. If these assumption may be allowed to conform to the structure of the bull sperm, the number of centrioles should be calculated until 3 or 8, because besides the true centriole each of 2 large radixes and of 5 small radixes may be able to possess its own centriole, respectively. In order to clarify this question, much more study should be needed.

The mitochondrial sheath which loosely surrounds the neck is likely to be broken down or collapsed and so its number appearing in cross section is variable from 0 to 4. On the other hand, the mitochondrial sheath is tightly wound with the basal granule of the head (figs. 7 & 13). This finding resembles with the schema of BLOM & BIRCH-ANDERSEN[14] in the bull sperm, but as they could not illustrate the basal granule at the basal part of the head the relationship between the mitochondrial sheath and the basal granule is quite obscure in their schema. According to NICANDER & BANE, however, the collar of the mitochondrial sheath in this part is estimated to be an elongated extension of the nuclear sheath of the head and they call it an "evaginated nuclear membrane". In connection with the vigorous movement of the living sperm, the role of the structural details in the neck portion should be taken into further consideration.

CONCLUSION

The results obtained about the neck of the bull sperm will be summarized as follows:

1) The principal internal structures of the neck consist of a centriole large, medium and small radixes surrounding it. The mitochondrial sheath which originates in the basal granule of the head form 2 spiral structures, the mitochondrial helixes, irregularly surrounding these radixes as a collar.

2) At the anterior end of the neck 2 large and 5 small radixes form 3 protrusions corresponding with the 3 fossula-like depressions of the head.

3) The arrangement of the radixes is quite regular. In a transverse section, they take an elliptic form around the centriole; 2 large radixes at both the lateral sides, 3 small radixes at the dorsal side and 2 small radixes at the ventral, respectively.

4) In the middle portion of the neck each of the large radixes branches off 2 medium radixes respectively, and at that portion each of the pair of medium radixes on each side twists and exchanges its position with the other one.

5) Each of the large, medium and small radixes is composed of about 15 segmental structures with high electron density, the neck platelets.

6) In the posterior portion, the centriole extends toward the posterior direction, forming the central radix within a short distance.

7) These all radixes change into a fibrillar bundle at the transitional region from the posterior portion of the neck to the middle piece; 4 medium radixes change to 4 thicker peripheral fibrils, 5 small radixes to 5 peripheral fibrils, and the central radix to a pair of central and 9 peripheral fine fibrils, the latest ones forming the inner ring of the axial fibril bundle.

8) It may be said that the neck portion of the bull spermatozoon is symmetrical on flattened view, but difference between the dorsal and ventral sides can easily be distinguished.