Acta vet. scand. 1974, *15*, 620—630.

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ULTRASTRUCTURAL STUDIES OF BLUE FOX SPERMATOZOA

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ANDERSEN, KJELL: *Ultrastructural studies of blue fox spermatozoa.* Acta vet. scand. 1974, *15*, 620—630. — The fine structure of epididymal and ejaculated spermatozoa from blue fox is analyzed by electron microscopy. The result of this investigation seems to indicate that the blue fox spermatozoon is of the same type as that of the dog. The apical body appears, however, to have a rather characteristic shape and to be more well-developed than found in sperm cells from the usual domestic animals.

The other structures studied in the different parts of the blue fox spermatozoon including the head, neck and flagellum displayed the same principal characteristics as those in the sperm cells of other domestic species.

In a few cases double development of the middle piece could be observed.

blue fox spermatozoa; ultrastructure.

The introduction of artificial insemination in the breeding of blue fox (*Fougner et al.* 1973) has to a certain extent increased the necessity of spermatological studies in this species. In connection with investigations of the reproduction of the male blue fox, light microscopical studies have thus been performed of spermatozoa collected from a number of foxes (*Aamdal* 1972).

Electron microscopical studies of the fine structure of blue fox spermatozoa could however not be found in the literature.

The investigation described in the present paper was conducted to elucidate the main principles of the ultrastructure of the blue fox spermatozoa and further to compare the different structures with those found in earlier electron microscopical studies of mammalian spermatozoa (*Fawcett* 1958, *Nicander & Bane* 1966).

MATERIAL AND METHODS

Sperm cells were collected from cauda epididymidis immediately after castration of two fertile male foxes in the second half of the breeding season, which totally extends from February thoughout April and the first part of May.

The material was at first fixed in a 3 % glutaraldehyde solution in Millonig's phosphate buffer for 45 min. and subsequently in a solution of 1 % osmium tetroxide for 60 min. (*Millonig* 1961). After dehydration in alcohol the material was embedded in Epon (*Luft* 1961). The entire procedure took place at room temperature.

Some preparations of spermatozoa from fresh ejculates, obtained by digital manipulation (*Aamdal* 1972) of two other foxes, were also made, using the method described above.

Sectioning was accomplished by use of glass knives on an LKB ultrotome. Thin sections were picked up on polyvinyl formal coated copper grids and stained, first with lead citrate (*Dalton & Ziegel* 1960) and then with uranyl acetate (*Watson* 1958). Electron microscopy was performed with a Siemens Elmiscop I.A., at primary magnifications of 6000—35000 ×.

RESULTS

Head

The major component of this part of the spermatozoon is the flattened and elongated nucleus which in sagittal sections appears to have its greatest thickness in the posterior half, except for a slight "waist-like" narrowing at the level of the so-called equatorial segment which will be described below; whereas the anterior part of the nucleus is gradually tapering towards the apical region (Fig. 1). The nuclear substance constitutes a heavily condensed mass with no discernible details except for some small irregular lighter areas.

The nuclear membrane, often referred to as the nuclear envelope, consists of two triple layered elements of which the innermost is usually closely applied to the heavily stained nucleus thus making the double structure of nuclear envelope less discernible, except at the level of some indentations sometimes found in the nuclear substance (Fig. 2). Otherwise the nuclear envelope is closely covering the surface of the nucleus except at the base of the head where it forms pouch-like evaginations

protruding posteriorly around the neck of the spermatozoon (Figs. 8 and 9).

Centrally to the evaginations the envelope approaches the base of the nucleus joining the so-called basal plate (Fig. 9), a structure constituting the wall of the implantation grove in which the neck region is connected with the head. The basal plate, possibly being developed by accommodation of dense material on the outer component of the nuclear envelope, is separated from the nuclear base by a narrow space probably representing the space between the two components of the nuclear membrane (Fig. 9).

The acrosome constitutes a double walled structure covering approx. ¾ of the nucleus. As found in the spermatozoa of other species it consists of a rather electron dense homogenous matrix limited by a triple-layered membrane. The innermost part of this, usually called the inner acrosomal membrane, is normally found close to the nuclear membrane except at the tip of the nucleus where it projects anteriorly forming a kind of invagination into the acrosome (Figs. 1 and 3). When studied at higher magnifications, this structure, usually referred to as the perforatorium or apical body, seems to contain anteriorly an electron dense element, of a rodlike appearance in sagittal sections, and more posteriorly a lighter, almost triangular area with a heavier stained substance centrally not seen constantly, thus possibly being an artifact (Fig. 3).

Transverse sections of the foremost part of the acrosome seem to indicate that the apical body has a wedge-shaped structure forming a kind of an edge, rather than being strictly conical (Fig. 4).

Posteriorly the inner membrane is reflected and continues as the so-called outer acrosomal membrane, which extends anteriorly, forming the outer limit of acrosome.

In the posterior region of the acrosome the outer membrane is found in close apposition to the inner, the two membranes thus forming the so-called equatorial segment (Fig. 1). More anteriorly the membranes deverge to a certain extent, forming a somewhat greater space between them, which contains most of the acrosomal matrix. At the apical margin of the spermatozoa the acrosome displays a moderate enlargement.

Posteriorly to the acrosome the nucleus is surrounded by a dense lamina commonly referred to as the postnuclear cap, a

structure which according to *Zamboni* (1971) should be denominated as the postacrosomal cap, a designation which is more relevant to the localization of this structure.

Except in the region near the base of the sperm head, where the postacrosomal cap and the nuclear envelope seem to be rather closely joined to each other, there is a space of varying width between these two structures that sometimes contains concentric vesicles of membranous material, equivalent in density and appearance to the nuclear envelope (Fig. 5), a phenomenon also observed in bull spermatozoa (*Wooding & O'Donnell* 1971). The postacrosomal cap seems to end at the base of the head, and a ring-like or concentric space appears to be formed between the cap and the basal plate through which the evagination of the nuclear envelope mentioned above is protruding (Fig. 9).

The entire surface of the sperm head is covered by a triple-layered cell membrane or plasmalemma. The plasmalemma is more or less separated from the outer acrosomal membrane, but appears to adhere closely to the outer surface of the postacrosomal cap (Fig. 1).

Neck

As in sperm cells from other species (*Fawcett* 1958) this part of the spermatozoon is found to constitute the connection between the sperm head and the flagellum, the term connecting piece therefore being applied to it.

The connecting piece encloses centrally the proximal centriole, a cylindrical structure located transversely in a plane approximately parallel to the head flattening, with its longitudinal axis at an angle of about 75° to that of the spermatozoan flagellum (Fig. 6). The centriole consists of a rather solid electron dense wall with longitudinal indentations or groves arranged parallelly (Figs. 6 and 8). Between these groves, embedded in the wall of the centriole, nine tubular triplets can be observed which are somewhat obliquely inserted giving the structure a whorl-like appearance (Fig. 7).

The connecting piece is further characterized by the presence of longitudinal cross-striated columns surrounding the centriole and constituting the structural framework of sperm neck (Figs. 7, 8 and 9). These columns seem to converge upon and merge partly with the so-called capitulum, a kind of articular structure

Figure 1. Sagittal section of a sperm head showing the heavily condensed nucleus (n) with its waist-like narrowing in the equatorial region (e). The acrosome, consisting of one inner (i) and one outer (o) membrane, is rather symmetrically developed. Anteriorly the inner acrosomal membrane separates from the nuclear envelope (ne) thus forming the so-called apical body (ap). Posteriorly the two membranes of the acrosome lie close together constituting the equatorial segment (e). The plasmalemma (p) is more or less detached from the acrosome but is found in close apposition to the postacrosomal cap (pa) which seems to be separated from the nuclear envelope except near the base of the sperm head. El. micr. 30,000 ×.

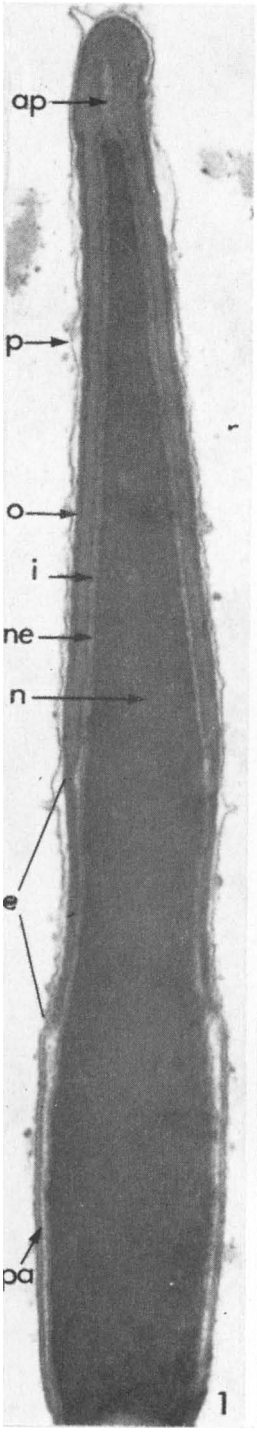
Figure 2. Sagittal section of a part of the sperm head, showing the double structure of the nuclear envelope (ne) at the level of an indentation in the nucleus (n). The acrosome is seen on one side of the section with the inner (i) and outer (o) membrane. El. micr. 95,000 ×.

Figure 3. Sagittal section of the apical region of a sperm head. In this section the apical body, limited anteriorly and laterally by inner acrosomal membrane (i) and posteriorly by the nuclear envelope (ne) appears anteriorly to consist of a rod-like dense structure (r) and posteriorly of a triangular lighter area (t) with a central density. El. micr. 90,000 ×.

Figure 4. Transverse section of the apical region of a sperm head showing the edge-formed nature of the anterior part of the apical body (ap). El micr. 45,000 ×.

Figure 5. Sagittal section of the postacrosomal region of a sperm head showing concentric vesicles of membranous material between the nuclear envelope (ne) and the postacrosomal cap (pa) covered by the plasmalemma (p). El. micr. 105,000 ×.

Figure 6. Horizontal section of the base of the sperm head and the neck region. The striated columns (sc) of the connecting piece terminate in the implantation grove (ig) and are enclosing the proximal centriole (pc) which can be seen sectioned through its longitudinal axis. Centrally and further backwards possible remnants of the distal centriole (dc) can be discerned. El. micr. 50,000 ×.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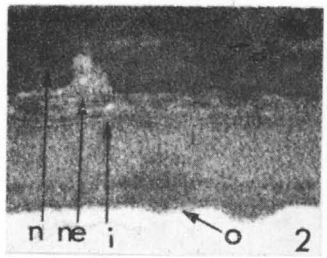
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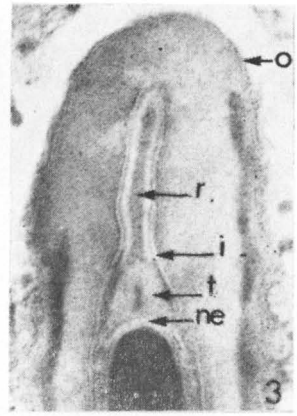
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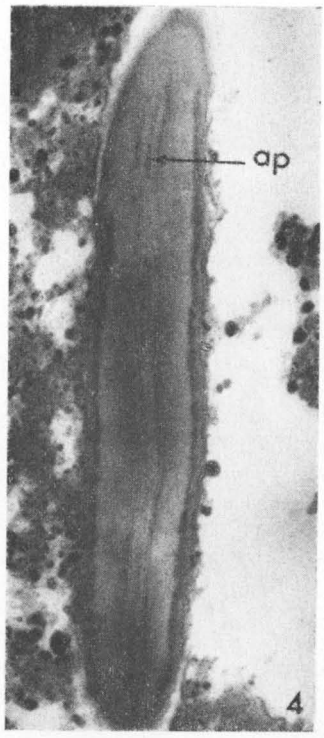
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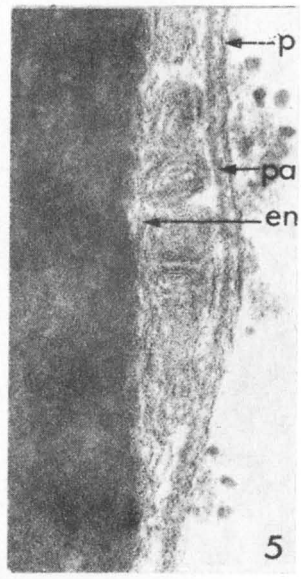
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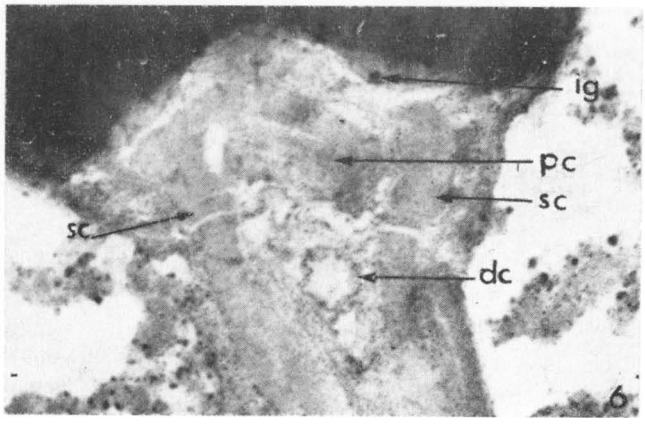
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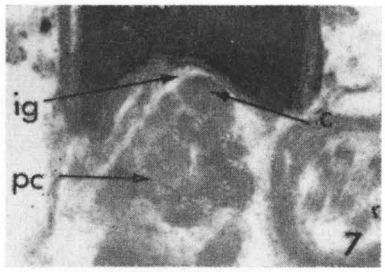
Figure 7. Sagittal section of the base of the sperm head and the proximal part of the neck. The nine triplets are clearly seen, embedded in the wall of the proximal centriole (pc) which is sectioned transversely. Anteriorly, connected with the wall of the centriole the capitulum (c) is forming a part of the articular facet which connects the neck to the base of the head in the implantation grove (ig). El. micr. 50,000 ×.

Figure 8. Longitudinal section of the nuclear base and the neck. One of the striated columns (sc) is seen connected with the capitulum (c) and the proximal centriole (pc) which is sectioned obliquely. El. micr. 40,000 ×.

Figure 9. Parasagittal section of the nuclear base and the neck. The nuclear envelope protrudes through the gap between the postacrosomal cap (pa) and the basal plate (b) forming an evagination or diverticulum (d) around the neck. Terminating anteriorly in contact with the capitulum, a striated column (sc) is seen, probably formed by fusion of two separate elements which continues posteriorly as two of the coarse fibres of the flagellum. El. micr. 40,000 ×.

Figure 10. Transverse section of the middle piece with the 9+9 +2 constellation of filaments. The coarse fibre number 2 (or 9 when seen opposite direction) appears to be intermediate in size compared with the numbers 1, 5 and 6 and the others. The filaments system is enclosed by the mitochondrial helix (m) and the plasmalemma (p). El. micr. 50,000 ×.

Figure 11. Longitudinal section of the posterior part of the middle piece and the anterior part of the main piece. The helically arranged mitochondria (m) of the former and the ribs in the fibrous sheath (fs) of the latter are cross sectioned. At the junction of the two parts of the flagellum the anular ring (ar) displays a triangular form in cross section. The plasmalemma (p) is intact on both sides of the section. El. micr. 45,000 ×.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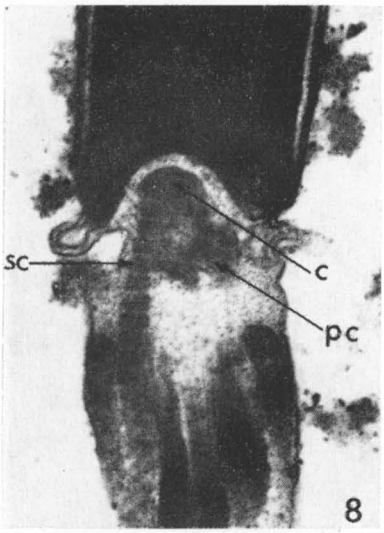
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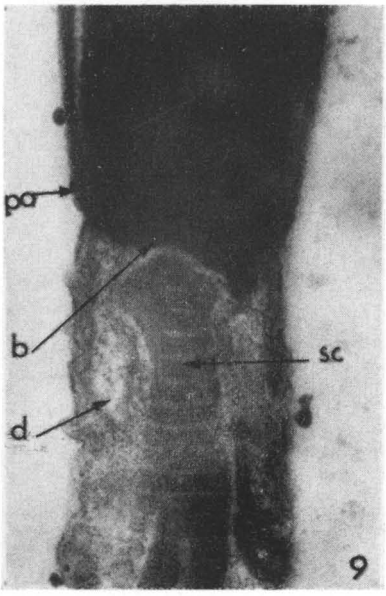
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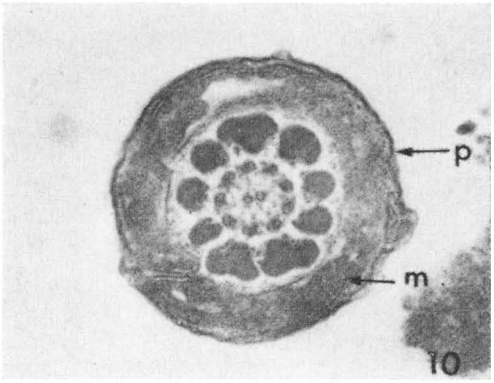
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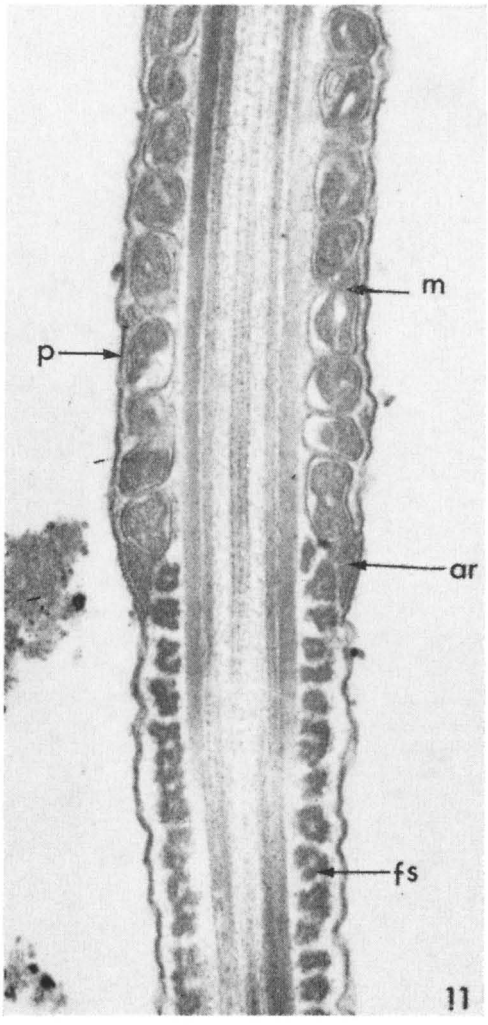
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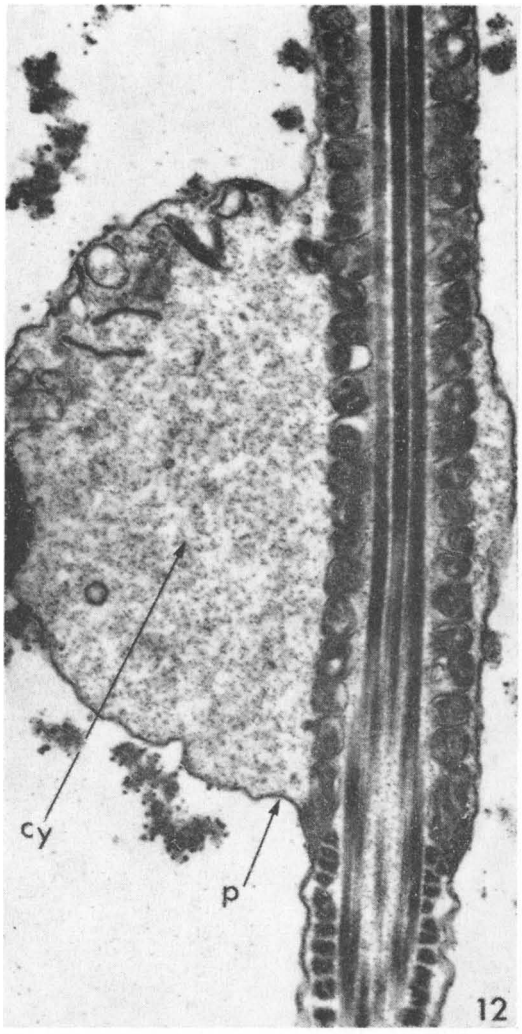

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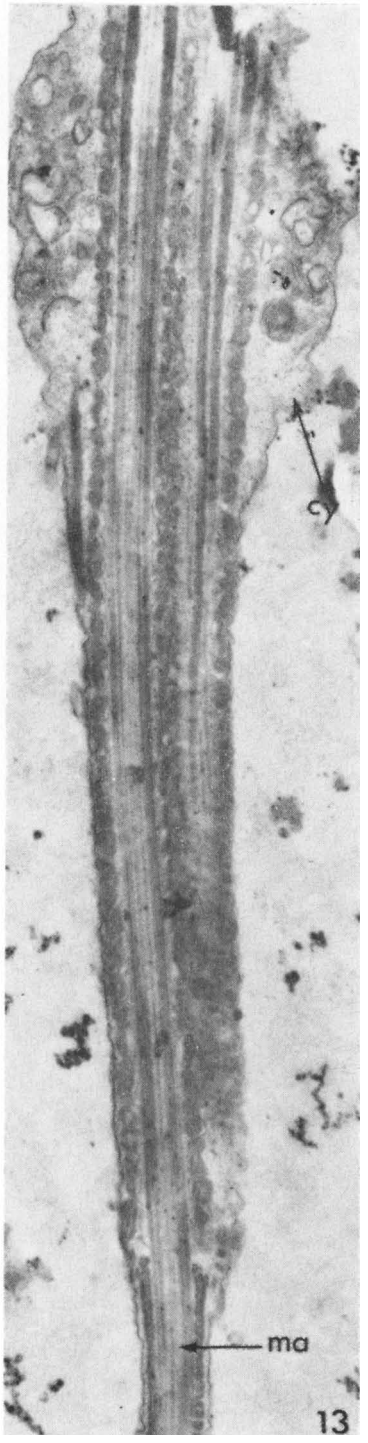

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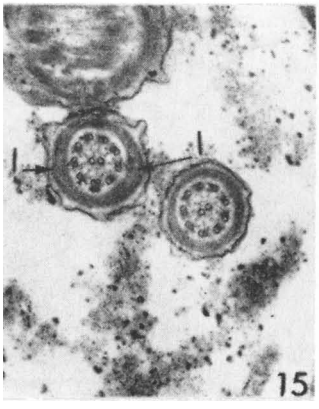

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Figure 12. Longitudinal section of a sperm tail showing a cytoplasmatic droplet (cy) enclosing the posterior part of the middle piece near the junction to the main piece. The droplet contains desintegrated cytoplasmatic structures and is limited by an intact plasmalemma (p). El. micr. 30,000 ×.

Figure 13. Longitudinal section of a sperm tail with a double middle piece enclosed by a cytoplasmatic droplet (cy) in a proximal position. The middle piece to the left continues with its filaments into the main piece (ma), while the other seems to terminate abaxially in the region of the anular ring. El. micr. 18,000 ×.

Figure 14. Transverse section of the anterior part of main piece. The ribs of the fibrous sheath are connected by two longitudinal columns (l) which can be seen at opposite points on the diameter defined by the central pair of filaments. The doublets of the inner ring of filaments which are found on this diameter have lost their corresponding outer fibres while the other fibres can still be observed close to the doublets. The tail is enclosed in an intact plasmalemma (p). El. micr. 20,000 ×.

Figure 15. Transverse sections of the posterior part of the main piece. Linear densities are connecting the concentric doublets with the two central single tubules. In the left section, remnants of the coarse fibres numbers 1 and 2 can still be seen fusing with the corresponding doublets, and the longitudinal columns (l) of the fibrous sheath are also discernible. In the right section, which is made through the tail further backwards, no coarse fibres are found and the two opposite thickenings representing the longitudinal columns of the fibrous sheath are less conspicuous. In both sections the fibrous sheath is covered by an intact plasmalemma. El. micr. 30,000 ×.

occupying the central part of the sperm head. The capitulum is further connected with the anterior surface of the centriolar wall (Fig. 7).

Between the striated columns and the cell membrane the diverticulum of the nuclear envelope mentioned above is found to contain varying amounts of clumps of nuclear material far less dense than the rest of the nucleus (Fig. 9).

Tail

As found in sperm cells from other species (*Fawcett*) the tail or flagellum consists of three components: the middle piece, the main piece and the end piece.

In the middle piece the filament system displays the usual $9+9+2$ pattern (Fig. 10). Centrally two single tubules are found, surrounded by a concentric ring of nine doublets. Each doublet consists of one tubular and one appearingly non-tubular unit. The two central filaments seem to be connected by a kind of linear densities in the surrounding matrix, similar densities can also be seen as lines radiating from the central pair to the concentrically arranged doublets. Outside the ring of the nine doublets, the nine coarse fibers, which connect the tail to the base of the sperm head via the striated columns in the neck, form the outer concentric ring of the filament system (Fig. 10). As further shown in Figs. 10, 11 and 12, the filaments are surrounded by the helically arranged mitochondria also found directly beneath the cell membrane in the middle piece of other mammalian spermatozoa (*Challice* 1952).

At the end of the middle piece the mitochondrial helix terminates in an anular structure, approximately triangular in cross sections (Fig. 11), usually called the Jensen's ring (*Bishop & Walton* 1960). In some spermatozoa a so-called cytoplasmatic droplet is found in a distal position enclosing the posterior part of the middle piece and containing elements of desintegrating endoplasmatic reticulum and some vesicles probably representing involution stages of the Golgi apparatus and mitochondrial material (Fig. 12).

In some cases double development of the middle piece is found, usually within a cytoplasmatic droplet situated in a proximal position (Fig. 13).

In the main piece the $9+9+2$ constellation of units is main-

tained in the foremost part, the outer coarse fibres however exhibiting a successive decrease in size as they become gradually closer to the inner ring of filaments (Fig. 14). Further backwards each coarse fibre terminates close to corresponding doublet at different levels of the main piece (Fig. 15).

In further accordance with observations made in other species (*Bradfield* 1955), a fibrous protoplasma sheath is found between the filament system and the cell membrane (Figs. 11, 12, 13, 14 and 15). The framework of this sheath seems to be made up by series of semicircular ribs which are held together by two longitudinal columns running at opposite sides in the plane of the central filaments and being especially conspicuous in the anterior part of the main piece (Fig. 14).

In the end piece no symmetrical arrangement of filaments could be found.

DISCUSSION

The present study of sperm cells from blue fox does not reveal any ultrastructural details which differ fundamentally from those found in other mammalian spermatozoa (*Fawcett* 1958, *Bishop & Walton* 1960, *Nicander & Bane* 1962).

The shape of the nucleus seems to resemble that of the dog spermatozoa (*Nicander & Bane* 1966), apart from being somewhat more slender.

The incompletely condensed nuclear material found in the basal evagination of the nuclear envelope, referred to as basal knobs in bull and moose spermatozoa (*Wooding & O'Donnell* 1971, *Andersen* 1973) was not so conspicuous as in those species, and no distinct pores could be found in the evaginated membranes.

The shape of the apical body, which is generally suggested to contain rests or specially modified elements of endoplasmatic reticulum (*Hadek* 1969), seemed to deviate somewhat from that found in the spermatozoa from the various domestic animals, this structure being more distinct and more well-developed.

The acrosome displayed the same regular symmetrical shape as that of dog spermatozoa, no pronounced marginal enlargement, as in the sperm cells of for instance domestic ruminants and moose, being found. Nor could there be found any difference in the level of termination of the equatorial segment on the two sides of the spermatozoon when the cell was sectioned sagittally.

In the connecting piece the structure and position of the proximal centriole as well as the striated columns exhibited the same principal characteristics as in other mammalian spermatozoa. The proximal centriole which in the blue fox spermatozoa is clearly discernible both in longitudinal and transverse sections (Figs. 6 and 7) is generally suggested to be a kind of basal body or kinetic center of the sperm flagellum (*Zamboni* 1971). In some rodent spermatozoa the central pair of microtubules have been found to extend through the central matrix of the neck to contact the posterior wall of the centriole (*Fawcett & Phillips* 1969). These central microtubules could accordingly be assumed to constitute a form of conductive element for impulses generated in the centriole to produce contractions in the filament system of the tail. No evidence based on similar structural manifestations could however be found in the present investigation to support this hypothesis.

As in other mammalian species the distal centriole seems to have disintegrated during maturation of the spermatozoon, presumably contributing to the formation of the columnar structures of the connecting piece and the filament system of the tail (*Fawcett & Phillips*). A rather inconsistant structure of concentric appearance, occasionally observed centrally in the posterior part of the neck, could however possibly be interpreted as a remnant of this centriole (Fig. 6).

The principal structure of the tail was in full accordance with the findings in other mammalian spermatozoa (*Bishop & Walton* 1960).

As no sections of the sperm head and neck of cells with double middle piece could be found, probably due to the extremely low frequence of this abnormality, a comprehensive study of this phenomenon could not be made.

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SAMMENDRAG

Ultrastrukturelle undersøkelser av blårevspermier

Blårevspermienes ultrastruktur blir beskrevet på grunnlag av elektronmikroskopiske undersøkelser av de forskjellige celle-avsnitt.

De observasjoner som er gjort synes å tyde på at sædcellene fra blårev er av samme type som dem en finner hos hund. Spesielt tydelig er likheten når det gjelder utformningen av kjerne og akrosom. En struktur som i sin oppbygning synes å avvike noe fra dem en finner i spermier fra de vanlige husdyr er det såkalte apical-legeme eller perforatorium, som hos blårevspermier viser en svært karakteristisk utformning.

Når det gjelder de øvrige strukturer i hode, forbindelsesstykke og flagell er det ikke påvist prinsipielle avvik fra de forhold som er funnet i spermier fra tidligere undersøkte husdyrarter.

Abnorm utvikling av spermienes flagell i form av dobbelt midtstykke er påvist, men i likhet med andre abnormiteter er frekvensen av denne spermiedefekten svært lav i det materialet som her er undersøkt.

(Received July 3, 1974).

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