

A transmission electron microscopic study of impala (*Aepyceros melampus*) sperm from the Kruger National Park

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Since knowledge of sperm morphology can play an important role in semen evaluation and fertilisation, baseline data are required. Live spermatozoa were collected from the cauda epididymis of 64 impala rams in the Kruger National Park and studied by transmission electron microscopy. The morphology of normal sperm was documented. The impala sperm shares characteristics with other members of the Bovidae. The occurrence of appendages on the cytoplasmic droplet of the flagellum of impala sperm is described for the first time. A total of 31 micrographs, showing typical features of impala sperm, in sections through various planes of the sperm, are presented.

Key words: sperm, ultrastructure, impala, *Aepyceros melampus*, transmission electron microscopy.

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Introduction

The mammalian sperm must conform to a number of special requirements in order to fertilise the ovum. For instance, it requires sufficient motility and a normal acrosome and nucleus. For each of these functions as well as the maintenance of its metabolism, the sperm is equipped with specialised organelles which are structurally formed during spermatogenesis in the testis. Any defect in these organelles can have an adverse influence on the functioning of the sperm (Fawcett 1975; Holstein & Roosen-Runge 1981; Holstein *et al.* 1988).

Recent research on the spermatozoa of earthworms (Reinecke *et al.* 1995) has shown that these cells are sensitive to the presence of foreign chemicals and that damage to the sperm nucleus could result from exposure to toxicants. Knowledge of sperm ultrastructure is therefore important to assess such effects and to evaluate semen quality.

The ultrastructure of mammalian sperm of a number of species has been investigated and reviewed by various researchers (Fawcett & Phillips 1969; Fawcett 1975; Bartoov *et al.* 1980; Mann & Lutwak-Mann 1981; Tingari 1991; Menkveld *et al.* 1991; Ackerman *et al.* 1994). Apart from microscopical studies by Fairall (1971), Skinner (1971) and Dott & Skinner (1989) the ultrastructure of impala sperm has not been researched.

The aim of this study was to document the ultrastructure of normal sperm of the impala, using transmission electron microscopy.

Materials and methods

Samples of live sperm were collected between June 1992 and May 1993 from the cauda epididymis of 64 impala in the Kruger National Park. Scrota of animals culled in various research projects by scientists of the National Parks Board were removed, labelled, and wrapped in paper and transported in a cool bag to the temporary laboratory. Spermatozoa were sam-

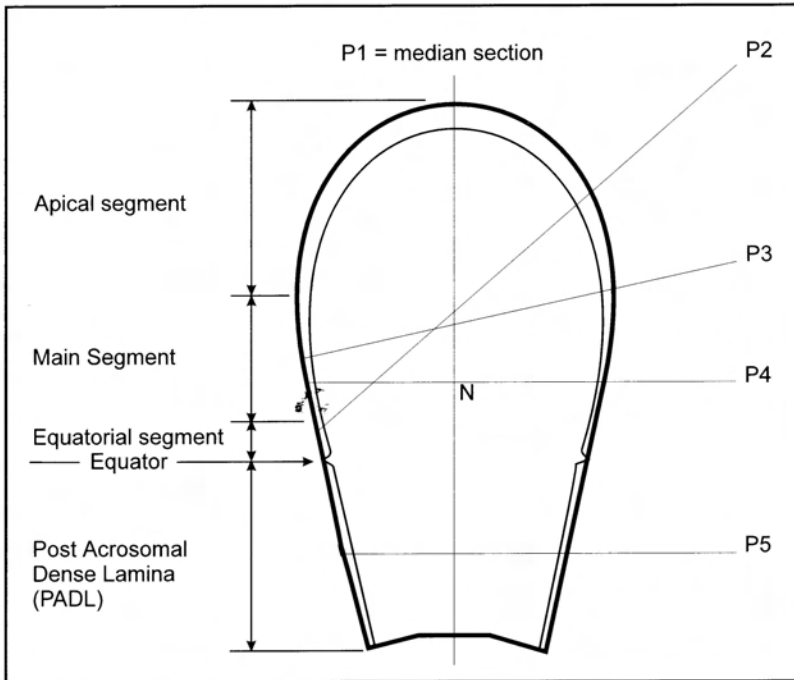


Fig. 1. Diagram of sperm head: planar section to show the relevant parts and planes of sectioning (P2 - P5).

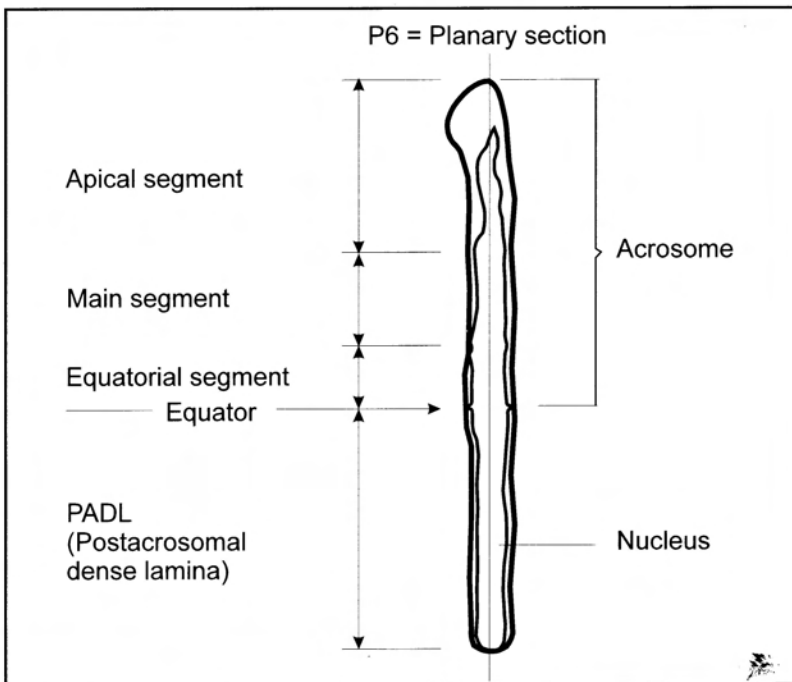


Fig. 2. Diagram of sperm head: median section to show the relevant parts.

pled from the cauda epidymis, fixed within five minutes after sampling, and prepared for transmission electron microscopy as described by Ackerman *et al.* (1994) and Ackerman (1995).

A Philips CM10 transmission electron microscope (TEM) operated at 80 kV was used to study the sperm of each animal. Only sections of sperm which did not exhibit known structural abnormalities were used to describe the normal impala sperm. We followed the methods of Holstein *et al.* (1988) and Menkveld *et al.* (1991) to document sperm abnormalities directly from the transmission electron micrographs.

Results

Ultrastructure of the normal sperm

Sperm head

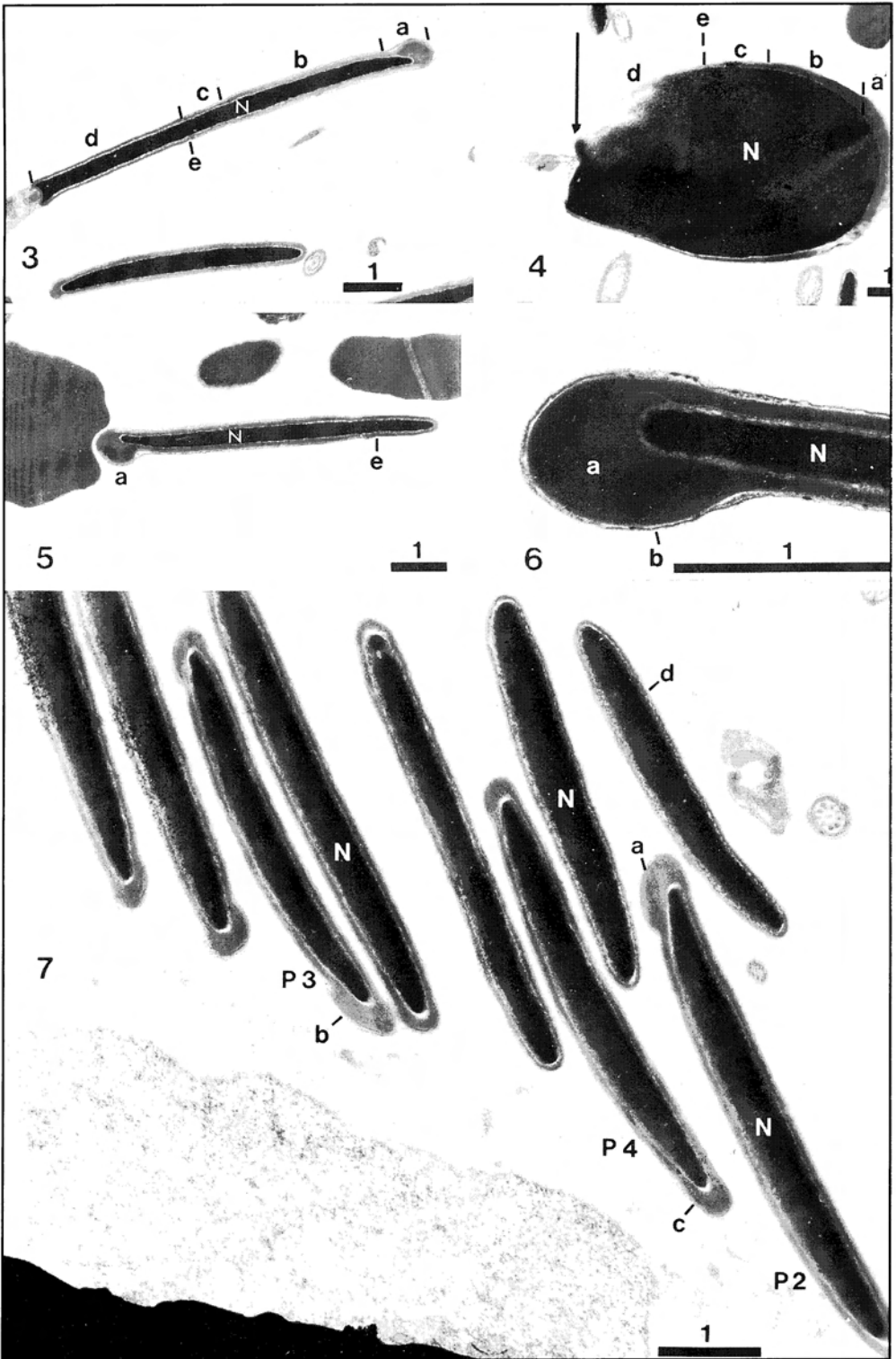
The head was covered by a plasmalemma covering the acrosome's apical segment, main segment, equatorial segment and the post acrosomal dense lamina (PADL) (Fig. 3). Median sections of the sperm head (Fig. 3) showed that the paddle-shaped head was dorso-ventrally flattened. This was also confirmed by planar sections (Fig. 4). The nucleus resided beneath the acrosome and the PADL. Approximately two thirds of the apical part of the nucleus was covered by a well developed acrosome consisting of a fine, homogenous substance of average electron density. The apical segment of the head had a prominent thickening of the acrosome on one side (Figs. 3-6).

Transverse sections of the posterior parts of the apical and main segments and planar sections of the sperm head showed that the one-sided thickening of the acrosome gradually disappeared towards the equator (Figs. 1, 2 & 7). The main acrosomal segment was laterally thicker than either the dorsal or ventral side (Fig. 7). Indications of a sub-acrosomal space (SAS) or perforatorium in the apical segment were not found. However, a SAS was frequently observed

laterally in transverse sections between the nucleus and acrosome of the main segment (Fig. 8). The equatorial segment of the acrosome was visibly thinner than the main segment in median sections of the head and terminated at the equator, demarcating the division between the acrosome and the PADL (Figs. 3 & 9). The outer and inner membranes of the acrosome merged at the equator from where the plasmalemma surrounded the surface of the PADL posteriorly (Fig. 9). The PADL enclosed the remaining part of the nucleus from the equator to the base of the head. A space containing floccular material (Figs. 9 & 10) was present between the PADL and the nucleus.

Neck and midpiece

In planar sections, the neck at the base of the head broadened to form a strong link with the head (Fig. 11). The neck was considered to terminate at the junction of the distal end of the *pars ascendens* and the proximal end of the *pars spiralis* at which point it was of the same thickness as the latter (Fig. 12). Planar and median sections of the head showed a cavity at the base of the nucleus covered by a baseplate. The proximal part of the neck connecting the tail with the head was implanted in this cavity like a ball in a socket and kept in position by a lip of the baseplate (Fig. 11). The capitulum was formed by the proximal fusion of the connecting components of the nine longitudinally segmented columns (Fig. 11, 17). The segmented columns, each with approximately 14 segments were peripherally situated in the neck. The segmented columns fused in the distal region of the neck with the outer dense fibres which surrounded the axoneme in the flagellum. Usually one of the two main segmented columns was observed together with the proximal centriole in planar sections. These main columns possessed a more prominent structure and a more distinct bell-shaped connecting piece than the other columns (Fig. 11). Two of the other thinner segmented columns, which were





- Fig. 3. Sagittal section showing the dorso-ventral flattening of the head. The acrosome, consisting of the apical segment with a one-sided thickening (a), main segment (b) and equatorial segment (c), is separated from the post equatorial dense lamina (PADL) (d) by the equator (e). The equatorial segment of the acrosome is thinner than the main segment. N = nucleus.
- Fig. 4. Planar section showing the paddle-shaped head. The one-sided thickening of the acrosome is limited to the apical segment (a). The acrosome, consisting of the apical segment (a), the main segment (b) and the equatorial segment (c) covers approximately 60% of the head. The PADL (d) is separated from the acrosome by the equator (e). N = nucleus.
- Fig. 5. A slightly oblique sagittal section of the head showing the nucleus (N), equator (e) and the one-sided thickening of the acrosome (a) of the apical segment.
- Fig. 6. A sagittal section of the apical acrosome thickening (a), covered by a plasmalemma (b).
- Fig. 7. Transverse sections approximately through the plains P2, P3 and P4 (Fig. 1). The peripheral thickening of the acrosome (a), (b) and (c) disappears gradually from the top to the bottom. Note the transverse section of the PADL (d).

proximally connected, could usually be seen in median sections (Fig. 17). They were in close association with the peripheral microtubules of the axoneme and followed a track through the midpiece partially into the principal-piece (Figs. 17, 20 & 22).

A cylindrical proximal centriole was observed at the base of the head at an angle between 35° and 70° with the longitudinal axis of the flagellum (Fig. 11). The proximal centriole (Fig. 11) consisted of nine longitudinal circumferential bars of electron dense material in which nine sets of triplet microtubules were embedded (Figs. 18 & 19). A distal centriole was not observed.

Mitochondria beneath the enclosing plasmalemma were observed lying parallel to the longitudinal axis of the tail on the periphery of the neck to form a typical *pars ascendens* (Figs. 12, 17 & 20). A few transversely orientated mitochondria were occasionally observed in the *pars ascendens* (Figs. 20 & 21). Below this region, transversely orientated mitochondria formed a characteristic sheath of peripherally orientated mitochondria which were placed end-to-end in a dense spiral (Fig. 22). This sheath of mitochondria, the *pars spiralis* of the midpiece, was enclosed between the plasmalemma of the midpiece and the outer dense fibres of the flagellum (Fig. 23). The *pars spiralis* had approximately 48 spirals between the neck and annulus (Fig. 22).

The majority of sperm had a distally situated cytoplasmic droplet on the midpiece (Fig. 13). The knob or baton shaped appendages of the cytoplasmic droplets, midpiece and principal-piece (Figs. 13-16) were described by Ackerman (1995) and Ackerman *et al.* (1996) by scanning electron microscopy and confirmed by transmission electron microscopy. The content of the appendages of the cytoplasmic droplet was of cytoplasmic origin and the droplet and its appendages were an integral part of the

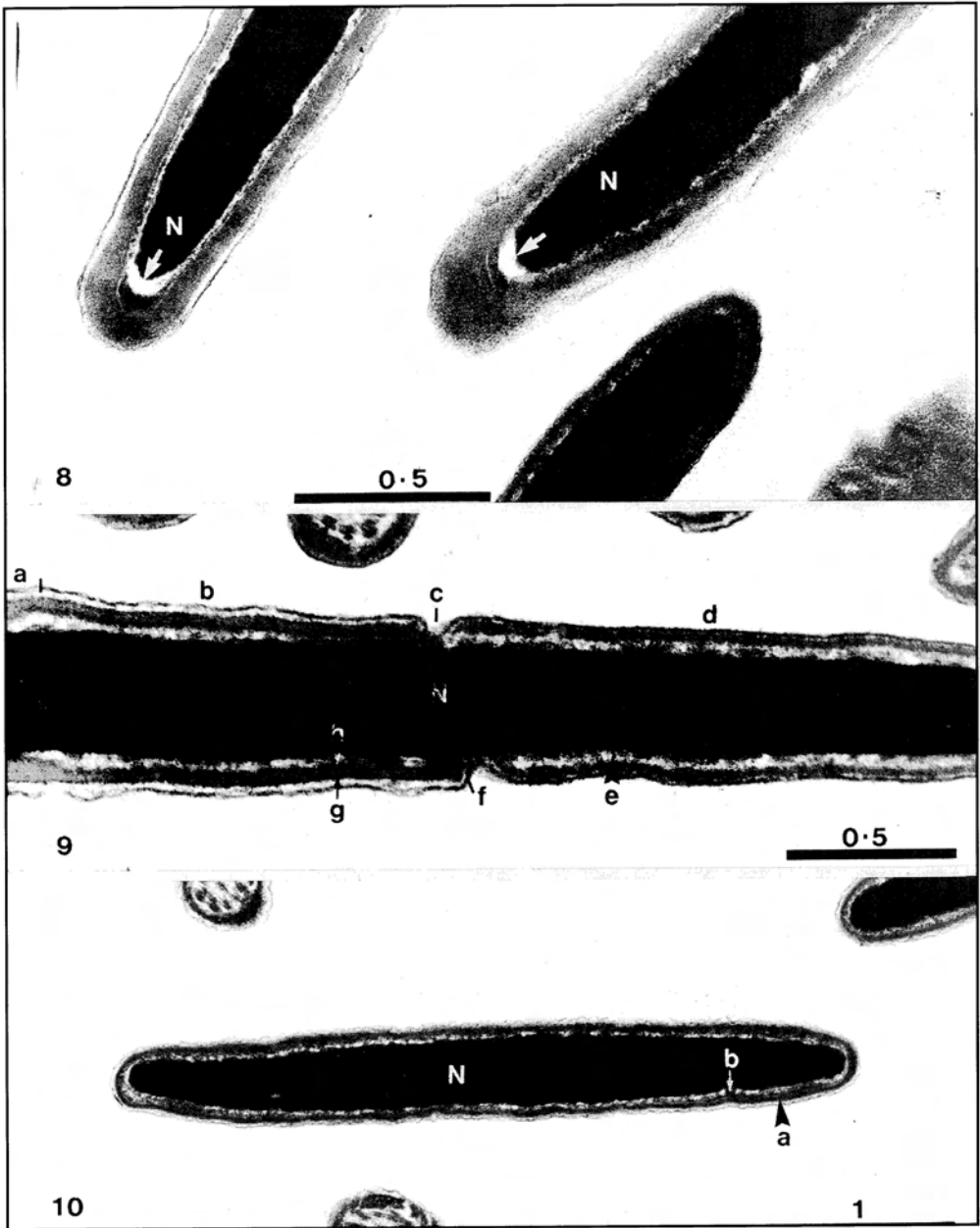
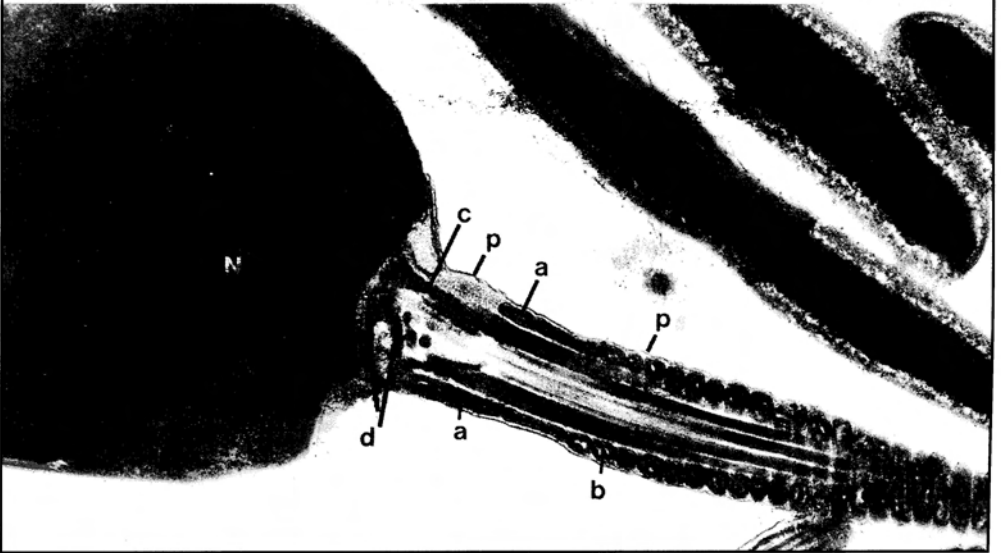
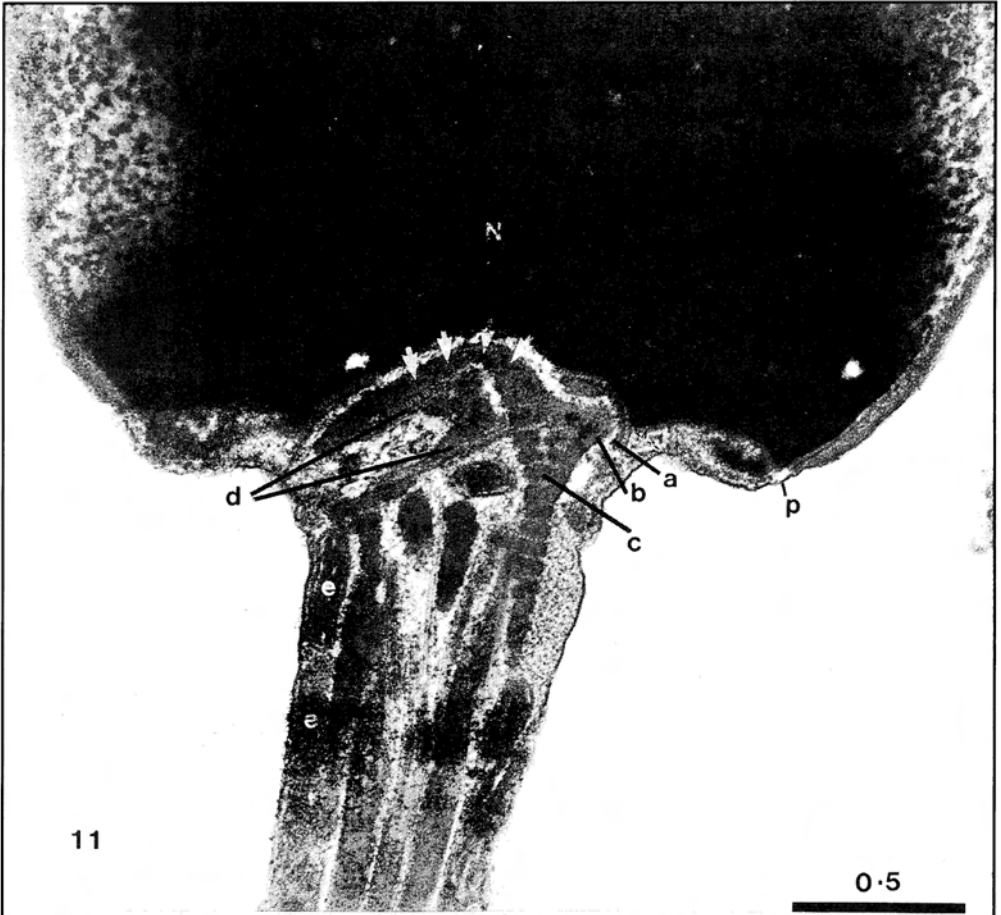


Fig. 8. A sub-acrosomal space (arrow) between the nucleus (N) and the acrosome in two transverse sections of the main segment of the acrosome.
 Fig. 9. Median section of the nucleus (N) showing part of the main segment (a), the whole equatorial segment (b), the equator (c) and part of the PADL (d). The inner (h) and outer (g) acrosomal membrane meets at the equator to separate the acrosome from the PADL. Note the indent formed by the plasmalemma (f) at the equator and the floccular material (e) between the PADL and nucleus.
 Fig. 10. Transverse section through the PADL region of the head showing the floccular material (b) between the PADL (a) and the nucleus (N).



Figs. 11 & 12 (caption on next page).

sperm cell. (Fig. 14). At the midpiece and principal-piece the appendages were also covered by the plasmalemma of the sperm cell and the appendages shared the content of the sperm cell directly beneath the plasmalemma (Fig. 15, 16).

The cone-shaped annulus consisted of an electron-dense ring which was closely associated with the last spiral of the *pars spiralis* and formed a pointed lip distally (Fig. 24A). The fibre sheath of the principal-piece was wedged in beneath the lips of the annulus and was separated from the mitochondria by the base of the annulus. In some instances the plasmalemma followed the contours of the last spiral of the *pars spiralis* and the lip of the annulus to form a retro-annular recess (Fig. 24b).

The axial filament complex (axoneme) formed in the neck, consisted of a central pair of single microtubules and nine peripheral microtubule doublets. The characteristic axoneme stretched over the whole length of the flagellum from the neck to the end-piece (Figs. 23, 25, 26 & 28).

Microtubules exhibited the characteristic 9 + 2 arrangement of flagella. The nine pairs of peripheral microtubules were numbered as shown in Fig. 23. The centres of the two single central microtubules were connected and the axis was lengthened on both sides through the microtubules and adjacent outer dense fibres. Number 1 of the peripheral microtubules is associated with the adjacent outer dense fibre located on the axis perpendicular through the central pair. The dynein




Fig. 11. Planar section through the base of the sperm head enclosed by a plasmalemma (P). The base plate (a) covers the cavity in the base of the nucleus and forms an articulation plane for the capitulum (arrows). The bell shape of the connecting piece (b) of one of the main segmented columns (c) is kept in position by the lip of the base plate. The cylindrical proximal centriole (d) in longitudinal section forms an angle of approximately 50° with the longitudinal axis of the flagellum. Two co-axially orientated mitochondria (e) of the *pars ascendens* are shown.

Fig. 12. Planar section of the sperm head, the neck and a part of the midpiece showing the plasmalemma (P) covering the *pars ascendens* (a) of the neck as well as the *pars spiralis* of the midpiece (b). One of the secondary segmented columns (c) is present, extending distally to become an outer dense fibre. In this case the proximal centriole (d) forms an angle of approximately 60° with the longitudinal axis of the flagellum.


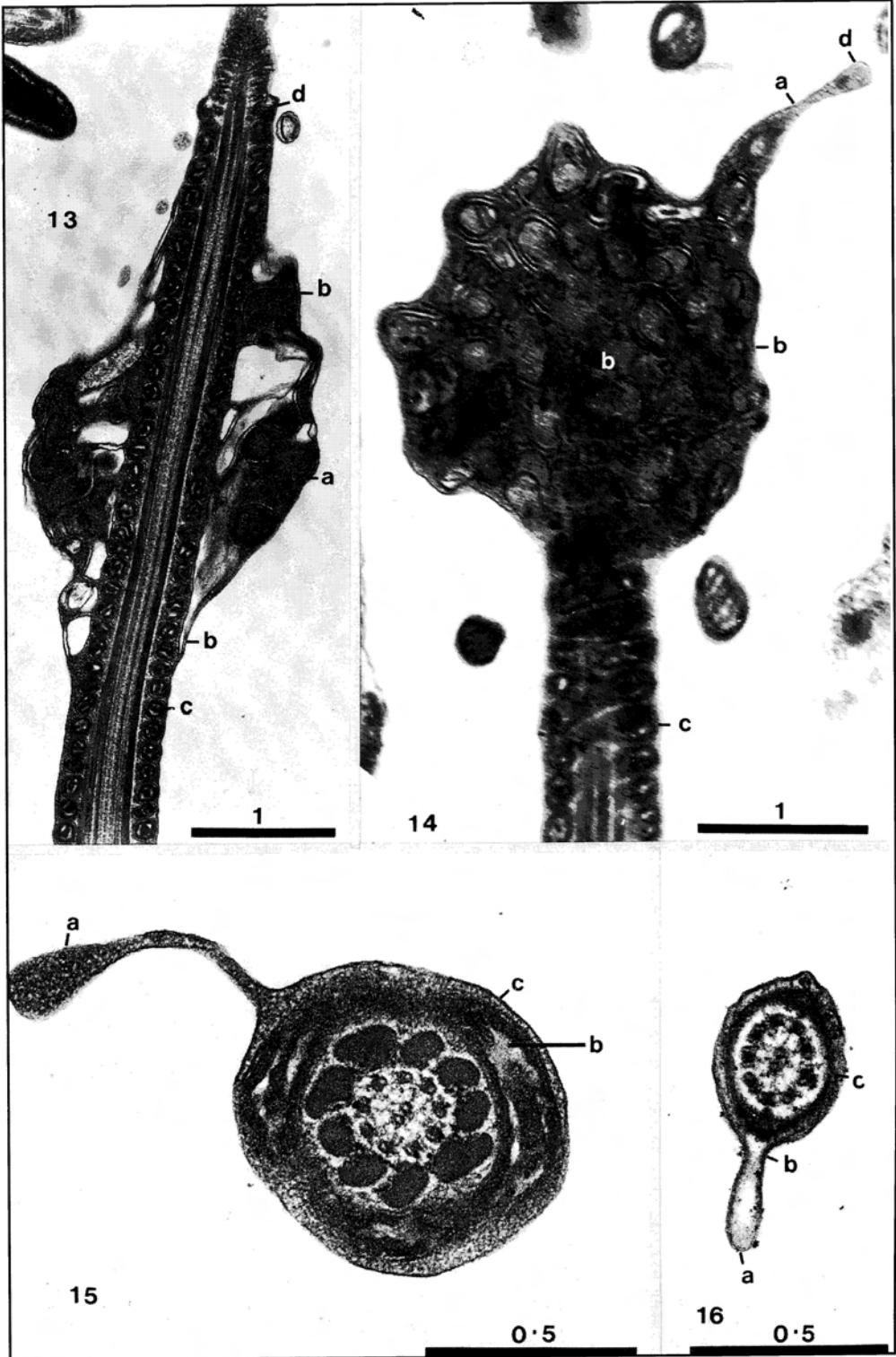


Fig. 13. Longitudinal section of the midpiece (c) showing a distal cytoplasmic droplet (a), covered by the plasmalemma (b). The location at the annulus (d) represents the destination of the cytoplasmic droplet on its migratory route along the shaft of the neck and midpiece.

Fig. 14. Longitudinal section of the midpiece of a sperm cell. A knob-shaped appendage (a) of a cytoplasmic droplet (b) around the midpiece (c) is covered by the plasmalemma (d). The knob-like structure is filled with cytoplasmic material.

Fig. 15. Transverse section of the midpiece. A knob-shaped appendage (a) of the midpiece (b) is covered by the plasmalemma (c) of the sperm cell. The content of this structure is continuous with the material between the plasmalemma (c) and the mitochondria of the *pars spiralis* (b).

Fig. 16. Transverse section showing a baton-shaped appendage (a) of the principal-piece (c) covered by the plasmalemma (b). The content of this structure contains elements of or is continuous with the material between the plasmalemma (b) and the fibrous sheath.



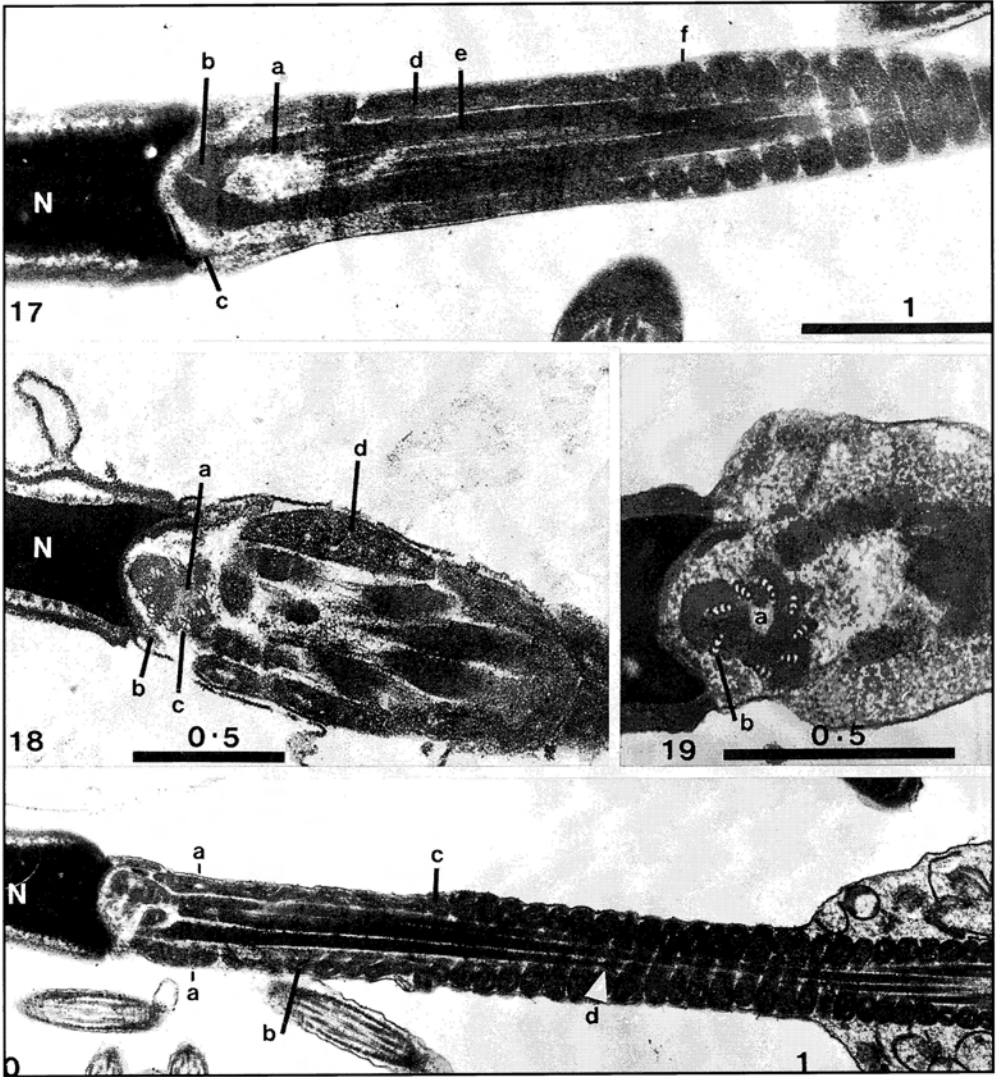


Fig. 17. Median section of the neck and part of the midpiece showing the connecting pieces of two of the longer, segmented columns (a). The columns unite proximally to form a capitulum (b) and continue as outer dense fibres (e). Co-axially orientated mitochondria (d) of the *pars ascendens* are present in the neck but not in the *pars spiralis* (f) of the midpiece. Note the lip of the base plate (c).

Fig. 18. Median section of the head and neck of a sperm cell showing a transverse section of the cylindrical proximal centriole (a). The nine sets of triplet microtubules (c), a lip of the base plate (b) and a co-axially orientated mitochondrion (d) is shown.

Fig. 19. Transverse section of the proximal centriole (a) with only eight of the usual nine sets of triplet microtubules.

Fig. 20. Co-axially orientated mitochondria (a) associated with the circumferential orientated mitochondria (b) in the neck of the flagellum. Position (c) shows the starting point of the *pars spiralis*. An indication of the spiral (d) formed by the midpiece mitochondria can be observed.

arms (d) of the peripheral microtubules (e) indicate the direction to follow from number one when numbering the other microtubules and their associated outer dense fibres (Fawcett 1975).

The principal-piece

The principal-piece became gradually thinner from the annulus to the end-piece. The fibrous sheath of the principal-piece was covered by the plasmalemma and consisted of transversal dorsal and ventral columns which were connected with each other with ringlike bands of electron dense material. (Figs. 26 & 30). Outer dense fibres 3 and 8 disappeared abruptly in the principal-piece close to the annulus. The dorsal and the ventral columns were formed in place of outer dense fibre number 3 and 8 respectively. These columns were respectively connected by a short spina to microtubules 3 and 8 of the axoneme (Figs. 26 & 27). The outer dense fibres and columns disappeared gradually, one after the other. Close to the end-piece the fibrous sheath formed a thin ring around the axoneme (Fig. 27).

The end-piece

Since the end-piece was much thinner than the midpiece, the distal border of the principal-piece could easily be determined. The plasmalemma also covered the end-piece (Fig. 31). The whole of the sperm cell was covered by a single plasmalemma (Figs. 3, 9, 11, 13-16, 19 & 31). The end-piece formed the short end of the flagellum and consisted mainly of the plasmalemma and axoneme (Fig. 28). Close to the tip of the end-piece the pair of peripheral microtubules were separated so that 20 single microtubules, including the central pair, could be distinguished (Fig. 29). The microtubules in the end-piece were often disorganised and partially or completely absent.

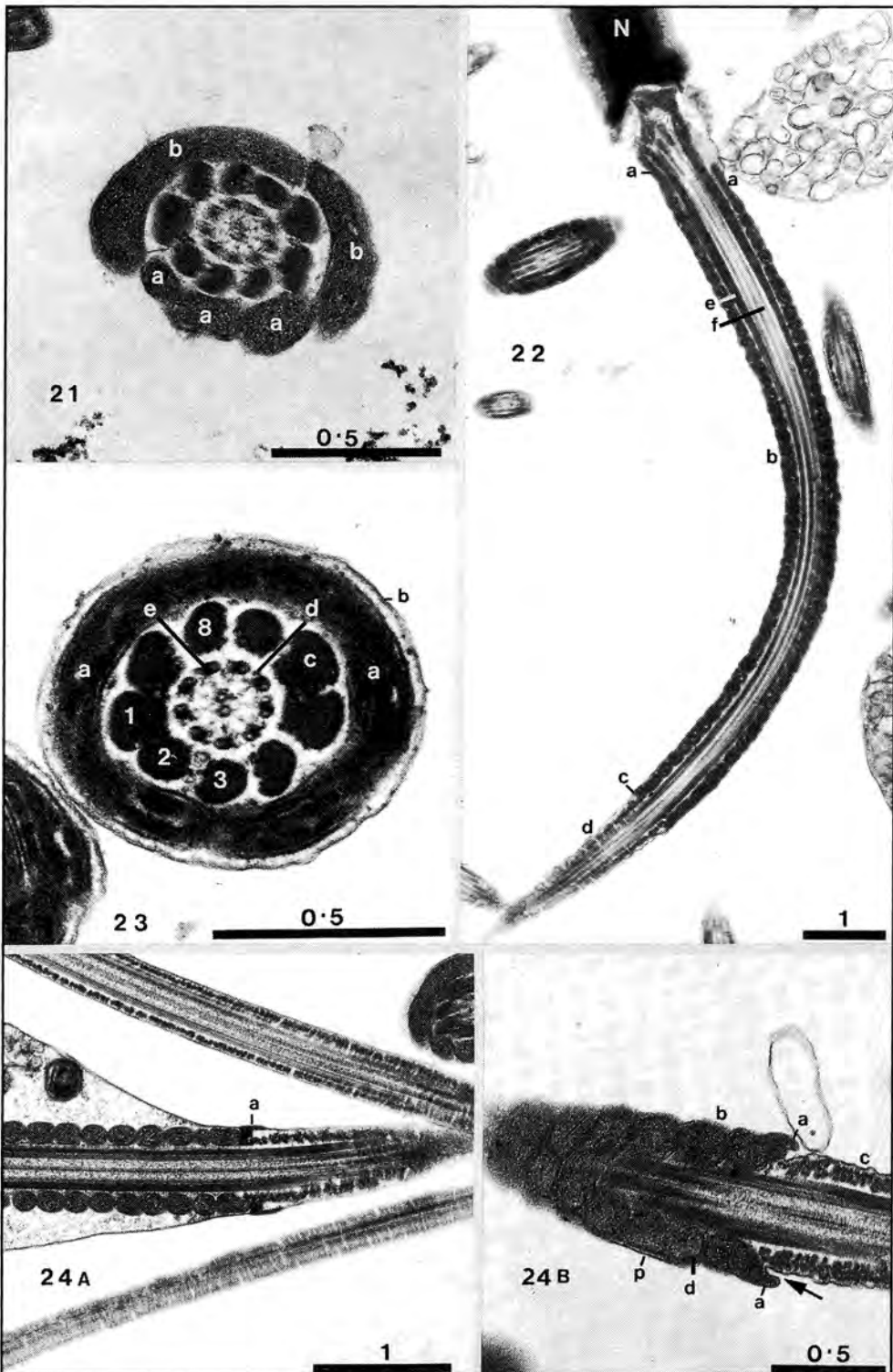
Discussion

The dorso-ventrally flattened head of the impala sperm is a characteristic shared with other members of the Bovidae and most other mammals (Saacke & Almquist 1964a; Fawcett 1965; Fawcett 1970; Roldan *et al.* 1992; Ackerman *et al.* 1994). Although Blom (1963), Saacke & Almquist (1964a), Blom & Birch-Andersen (1961a) and Barth & Oko (1989) observed that the acrosome of bull sperm folds back over itself at the apical end, we did not see this in the normal impala sperm.

The acrosome of impala and buffalo sperm thickens gradually on the periphery on one side of the apical part of the head only. Median sections through the apical segment showed no folding or hook forming of the acrosome in normal impala or buffalo sperm (Ackerman *et al.* 1994). Preliminary observations of the sperm of red hartebeest, kudu and blesbok gave the same results. Blom & Birch-Andersen (1961b) described the presence of an apical vacuole in the apical segment between the acrosome and the tip of the nucleus. Saacke & Almquist (1964a) could not confirm this for bull sperm and our study also did not reveal the presence of such a vacuole in the apical segment of impala sperm.

The proximal centriole was prominent in sagittal and planar sections but the distal centriole was absent as described for mature mammalian sperm (Fawcett & Phillips 1969; Holstein & Roosen-Runge 1981).

The presence of various coaxially orientated mitochondria with one or two peripheral mitochondria between them, seems to be a general characteristic of the mitochondrial sheath of the sperm neck of impala. The occurrence of two or more mitochondria in the sperm neck is well known for other mammals (Fawcett 1965, 1975; Fléchon *et al.* 1976; Holstein & Roosen-Runge 1981).



Fawcett (1965) described the neck of a sperm as that part of the flagellum between the nucleus and the first spiral of the mitochondrial spiral (*pars spiralis*) of the mid-piece. All the mitochondria between these points are considered to be normal components of the neck. A similar arrangement of mitochondria was observed by Tingari (1991) in the neck of camel sperm but he suggested that the neck should be defined as the region of the flagellum between the head and the first mitochondrion. The midpiece will then include the coaxial (*pars ascendens*) and all the circumferential orientated (*pars spiralis*) mitochondria. The authors described the neck of the impala sperm according to the view of Fawcett (1965) because his terminology for the ultrastructure of mammalian sperm was used in this study. However, the definition of Tingari (1991) is not without merit.

The functional role (if any) of the appendages or filaments of the cytoplasmic droplet, mid-piece and principal-piece which were regularly observed in this study on impala sperm and also on buffalo sperm (Ackerman *et al.* 1994) is unknown. No mention of these appendages could be found in the literature. Woolley (1995) reported the development of extremely fine threads from the tips of sperm flagella for four avian and two mammalian species. It is not certain whether this observation could be related to the structures we found in buffalo and impala sperm. They could represent membrane extensions resulting from the spermiogenesis process. A micrograph of a sagittal section of a Holstein bull sperm published by Saacke & Almquist (1964b) showed a structure which resembled a rudimentary filament of the midpiece. No explanation of, or reference to this structure was made by the author. It is probable that similar structures will be found on the sperm of other mammals.




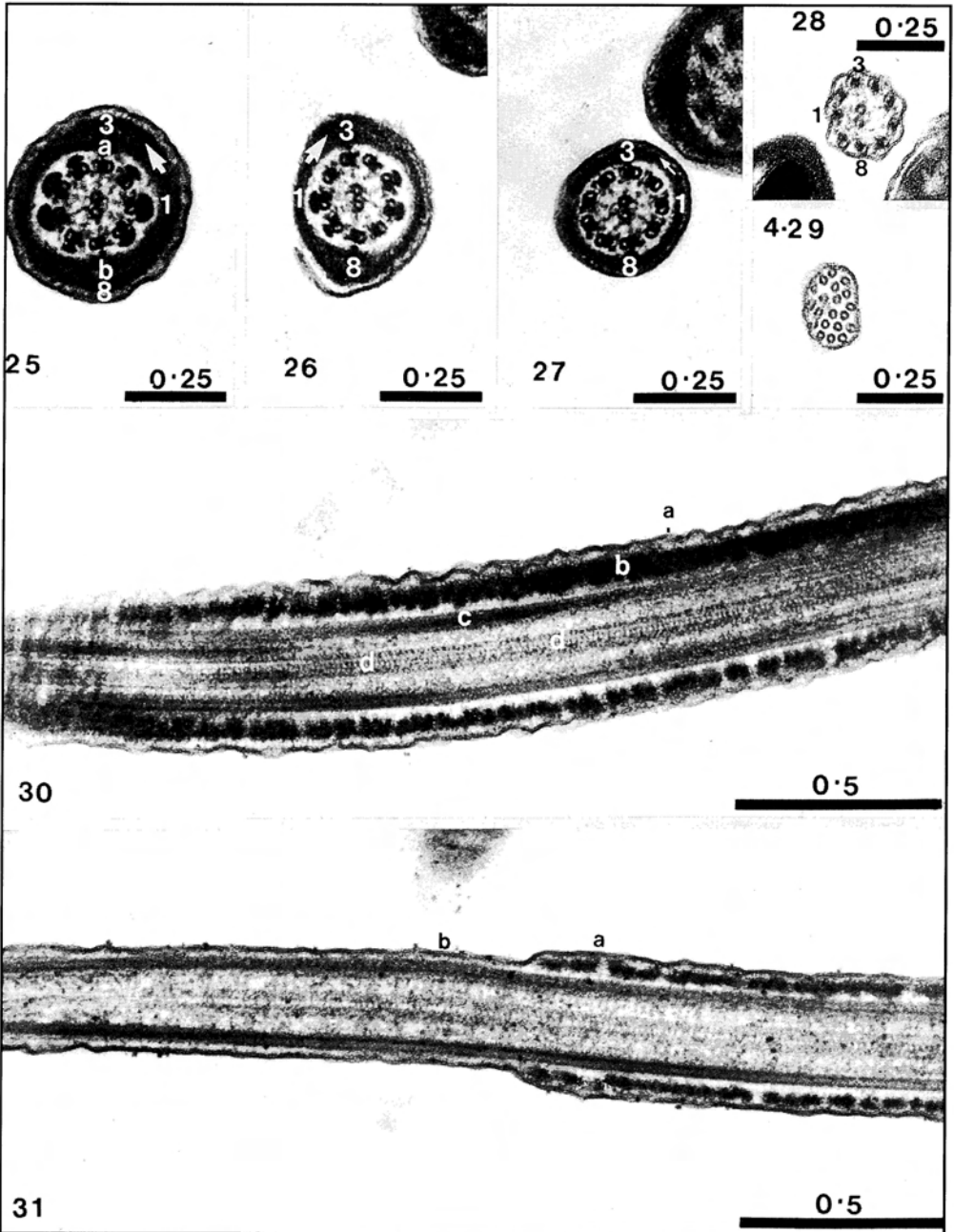
Fig. 21. Transverse section of the neck with three co-axially orientated mitochondria (a) and circumferential orientated mitochondria (b). All nine outer dense fibres and the axoneme is present indicating a transverse section through the distal part of the neck.

Fig. 22. Longitudinal section of the neck and mid-piece showing a number of co-axially orientated mitochondria (a) of a poorly developed neck. The *pars spiralis* of the mid-piece mitochondria (b) forms a sheath of approximately 46 windings around the outer dense fibres (c) and the axoneme and ends against the annulus (c). Then follows the principal-piece (d), the longest part of the flagellum.

Fig. 23. Transverse section of the midpiece showing the position of the *pars spiralis* (mitochondria sheath) (a) between the plasmalemma (b) and the outer dense fibres (c). The axis through the centres of the two central microtubules cuts through the peripheral microtubules and outer dense fibres 3 and 8 (see text for numbering of the microtubules).

Fig. 24A. Longitudinal section of a normal conical shaped annulus (a). No retro-annular recess is present.

Fig. 24B. Longitudinal section of the flagellum showing how the annulus (a) forms the border between the midpiece (b) and the principal-piece (c). In some instances the plasmalemma (p) follows the contours of the *pars spiralis* (d) and the annulus to form a retro-annular recess (arrow).



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- Fig. 25. Transverse section of the principal-piece close to the annulus showing the dorsal (a) and ventral (b) columns respectively, in the positions of outer dense fibre number 3 and 8. The peripheral microtubules are still associated with outer dense fibres.
- Fig. 26. Transverse section close to the center of the principal-piece. Note that the principal-piece except for outer dense fibres 3 and 8 further away from the annulus, has now also lost outer dense fibres 2, 4, and 7.
- Fig. 27. Transverse section closer to the end of the principal-piece. The fibrous sheath forms a thinner ring around the axoneme but is still in contact by a short spine with the peripheral microtubules 3 and 8. All the outer dense fibres have now disappeared.
- Fig. 28. Transverse section of the proximal part of the end-piece. The microtubules still maintain a doublet formation.
- Fig. 29. Transverse section of the distal end of the end-piece with the peripheral and central microtubules separated.
- Fig. 30. Longitudinal section of the principal-piece showing the plasmalemma (a), fibrous sheath (b), outer dense fibres (c) and microtubules (d). Note the ringlike bands (e) connecting the dorsal and ventral columns.
- Fig. 31. Longitudinal section at the transition of the principal-piece (a) to the end-piece (b).

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