Transmission electron microscopic observations of flagellum abnormalities in impala (*Aepyceros melampus*) sperm from the Kruger National Park

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Sperm must remain motile in order to reach and penetrate the ovum and defects in the ultrastructure of the tail can have an adverse influence on motility. Live spermatozoa were collected from the cauda epididymis of 64 impala rams in the Kruger National Park and studied by transmission electron microscopy to document sperm abnormalities. The following abnormalities of the flagellum were documented from micrographs: abnormal baseplate and neck attachments; neck vacuoles and displaced organelles; double or short flagella; bent flagella; principal-piece vacuoles; displaced axoneme and the Dag defect. The implications of these abnormalities for sperm motility are discussed.

Key words: impala sperm, flagellum anomalies, transmission electron microscopy, Kruger National Park

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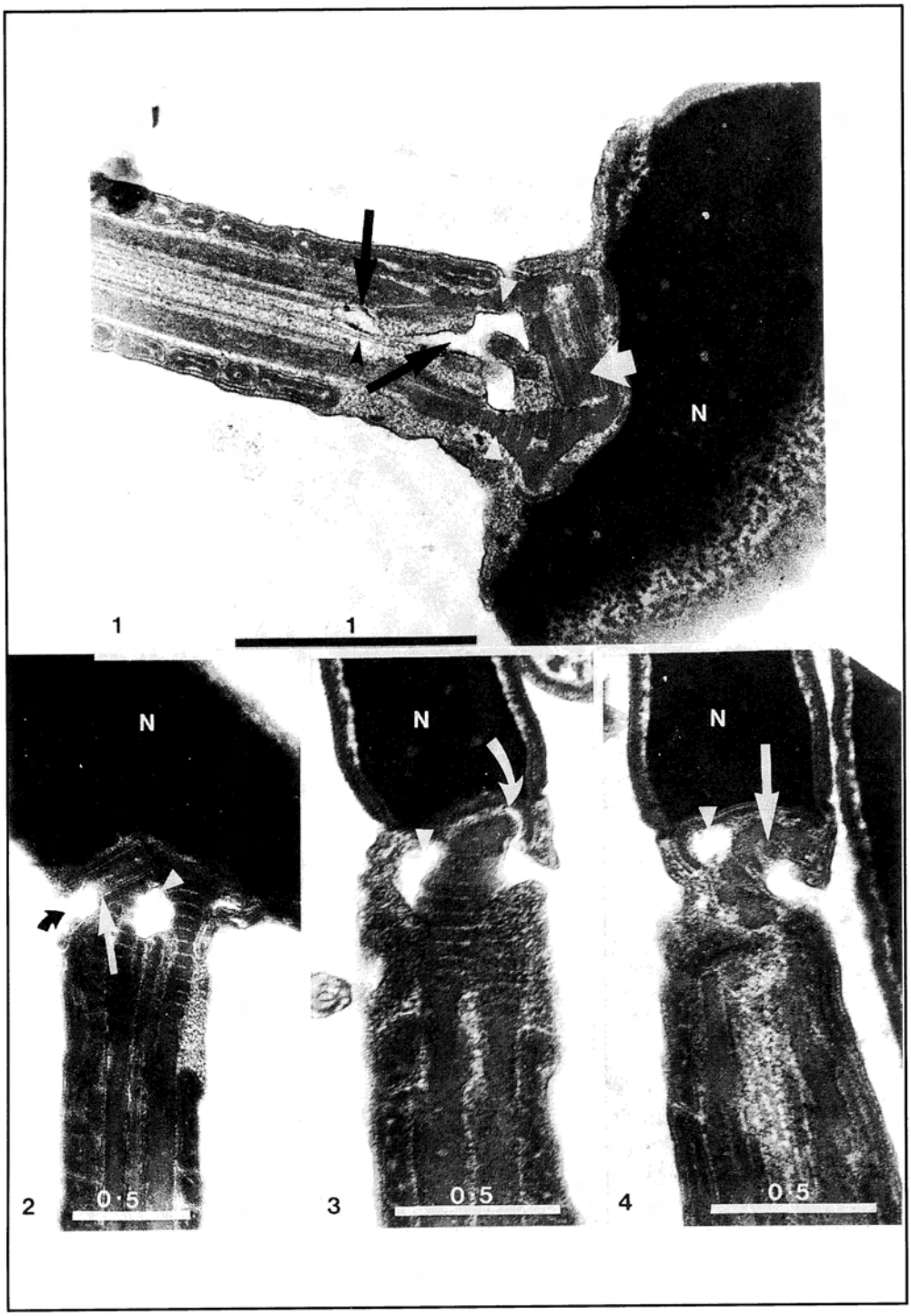
Introduction

The ultrastructure of normal impala sperm was recently studied to provide baseline data for future reference in ecotoxicological studies (Ackerman *et al.* 1996a, 1996b, 1996c). The percentage of normal sperm morphological features is important in fertilisation in humans (Kruger *et al.* 1986; De Yi Lui & Baker 1992). Fawcett (1975) and Holstein & Roosen-Runge (1981) indicated that defects can have an adverse influence on the functioning of the sperm. Ackerman *et al.* (1996c) studied the spectrum of abnormalities of the acrosome and head of impala sperm. They believe that knowledge of sperm abnormalities and ultrastructural damage can be useful in detecting possible causes of lowered sperm quality. Sperm abnormalities in mammals can occur during spermatogenesis. Genetic factors, temperature, toxic substances, hormonal changes and stress are considered to be possible causes (Mann & Lutwak-Mann 1981; Holland & White 1982; Dadoune 1988; Facemire *et al.* 1995; Reinecke *et al.* 1995). Before attempting to relate sperm abnormalities to possible causes, a thorough knowledge of generally occurring abnormalities is needed.

Impala sperm provided us with such an opportunity as it was shown by Gummow *et al.* 1991 that the mean copper concentration in the livers of impala from an area south of the Phalaborwa Gate in the Kruger National Park is abnormally high. In order to evaluate the possible effect of this contamination on sperm ultrastructure quantitatively, it was imperative to study sperm abnormalities in animals from both contaminated and uncontaminated areas beforehand in order to document the types of abnormalities present. The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aim of this study was to follow up on our previous qualitative study (Ackerman *et al.* 1996c) and to document abnormalities of the sperm flagellum occurring in 64 impala from the Kruger National Park and to provide a qualitative evaluation of the abnormalities for 20 of the above-mentioned animals which were not in contact with copper contaminated food. This was done as a precursor to the quantitative evaluation of the sperm from both contaminated and uncontaminated areas intended for a future contribution.

Materials and methods

Samples of live sperm were collected between June 1992 and May 1993 from the cauda epididymis of 64 impala as described by Ackerman *et al.* (1996b). Forty four of the impala were terminated by scientists of the Kruger National Park in a copper contaminated area in the vicinity of Phalaborwa, while samples of 14 animals originated from the uncontaminated area along the banks of the Nwaswitshaka River. Additionally, six samples were collected from animals which had been in captivity at Skukuza and then terminated. These animals had been fed with food not contaminated with copper. Preparation for transmission electron microscopy (TEM) was done as described by Ackerman *et al.* (1994). A Philips CM10 TEM operated at 80 kV was used to study the ultrastructure of abnormal sperm from the cauda epididymis. The methods of Holstein *et al.* (1988) and Menkveld *et al.* (1991) for documenting sperm abnormalities in humans were followed.

A TEM study was made of 200 sections of the neck and 500 sections of the flagellum of the sperm of 20 of the above-mentioned 64 mature impala rams from an uncontaminated area near Skukuza in the Kruger National Park to determine the percentage sections demonstrating the following groups of abnormalities:

a. Vacuoles of the neck.
b. Other abnormalities of the neck.
c. Vacuoles of the principal-piece.
d. Other abnormalities of the principal-piece.
e. Dag defects associated with the flagellum.

Some of the sections of the sperm cells reveal that malformed sperm show either a single abnormality or a combination of abnormalities from the above categories.

Results

Neck abnormalities including the presence of vacuoles are shown in Figs. 1-5. It is evident that vacuoles affected various organelles. Microtubules (Fig. 1) were displaced and the proximal centriole damaged (Fig. 2). The vacuoles may cause a weakening of neck connections (Fig. 3). Head base craters (Figs. 5 & 7) deform the attachment of the head to the neck. A sperm with a double flagellum is shown in Fig. 8. Sharply bent flagella occurred as well (Figs. 9-11). Vacuoles of the midpiece and principal-piece cause displacement and compression of

Fig. 1. Planar section of a sperm showing a vacuole (black arrow) enclosed by a membrane formed between the segmented columns (white arrow head) of the neck. The proximal centriole (white arrow) is not affected but the microtubuli is displaced (black arrow head).
Fig. 2. Planar section of a sperm with a segmented column vacuole (white arrow head) and a vacuole (black arrow) penetrating the side of the neck damaging the proximal centriole (white arrow).
Fig. 3. Sagittal section of a sperm through a neck vacuole opening externally and extending around the connecting piece and part of the capitulum (arrow). This vacuole and another one (arrow head) may cause weakening of the strength of the connection to the neck and its structure.
Fig. 4. Sagittal section of a sperm showing a vacuole in the central region of a proximal centriole (arrow) in transverse section. No elements of the connecting piece is visible under the baseplate (arrow head) due to the presence of another vacuole.

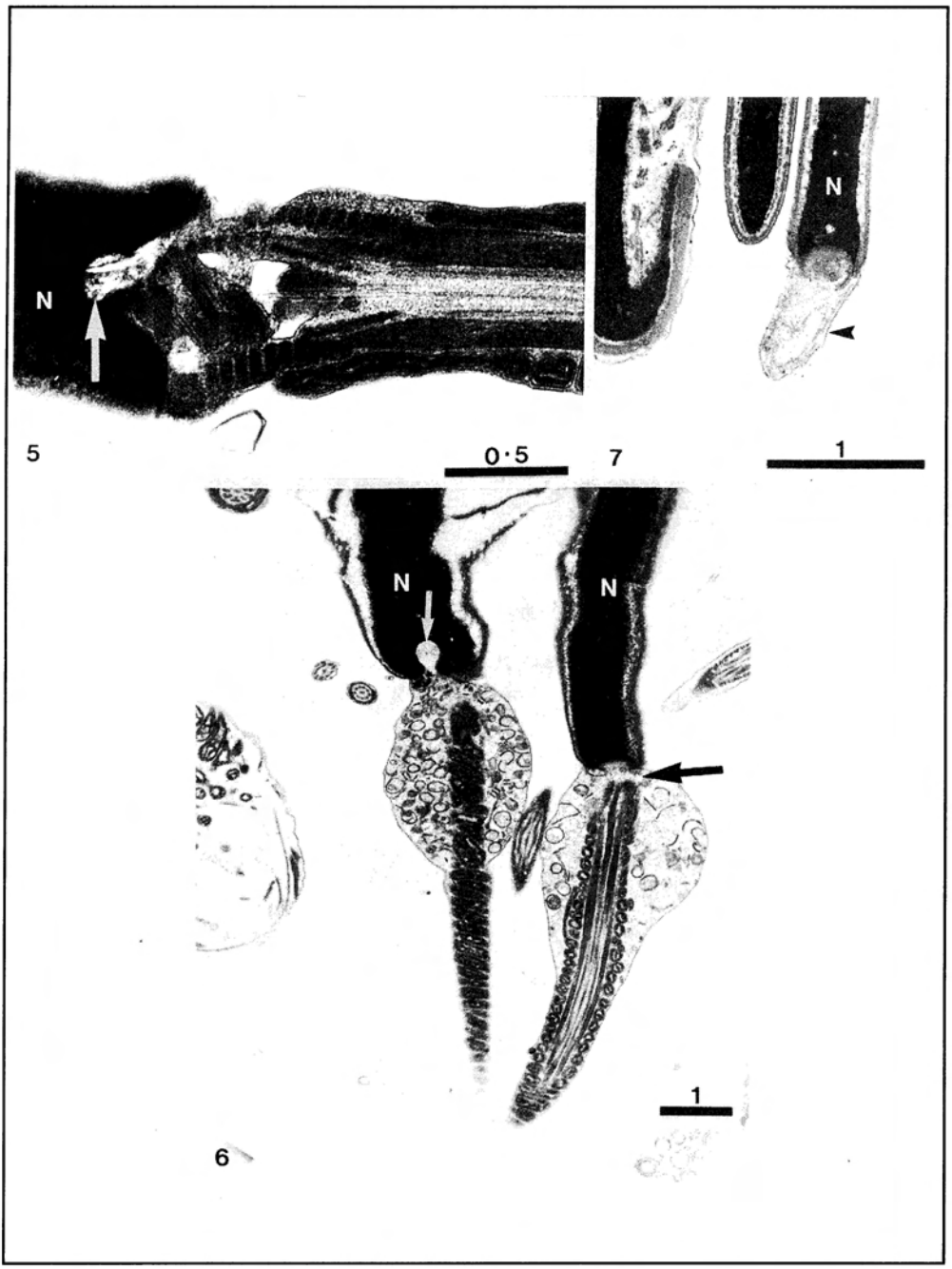


Fig. 5. Sagittal section of a sperm with segmented column vacuoles. A head base crater (arrow) deforms the connection of the head with the neck.

Fig. 6. Section of a sperm with a head base crater (white arrow) of which the flagellum is cut superficially. The other sperm's flagellum (black arrow) is not implanted. Proximal cytoplasmic droplets are present on both sperm.

Fig. 7. Section of a sperm with a base crater defect. Absence of neck organelles and a fully developed flagellum may result from the plain of section or represent a short flagellum (arrow head).

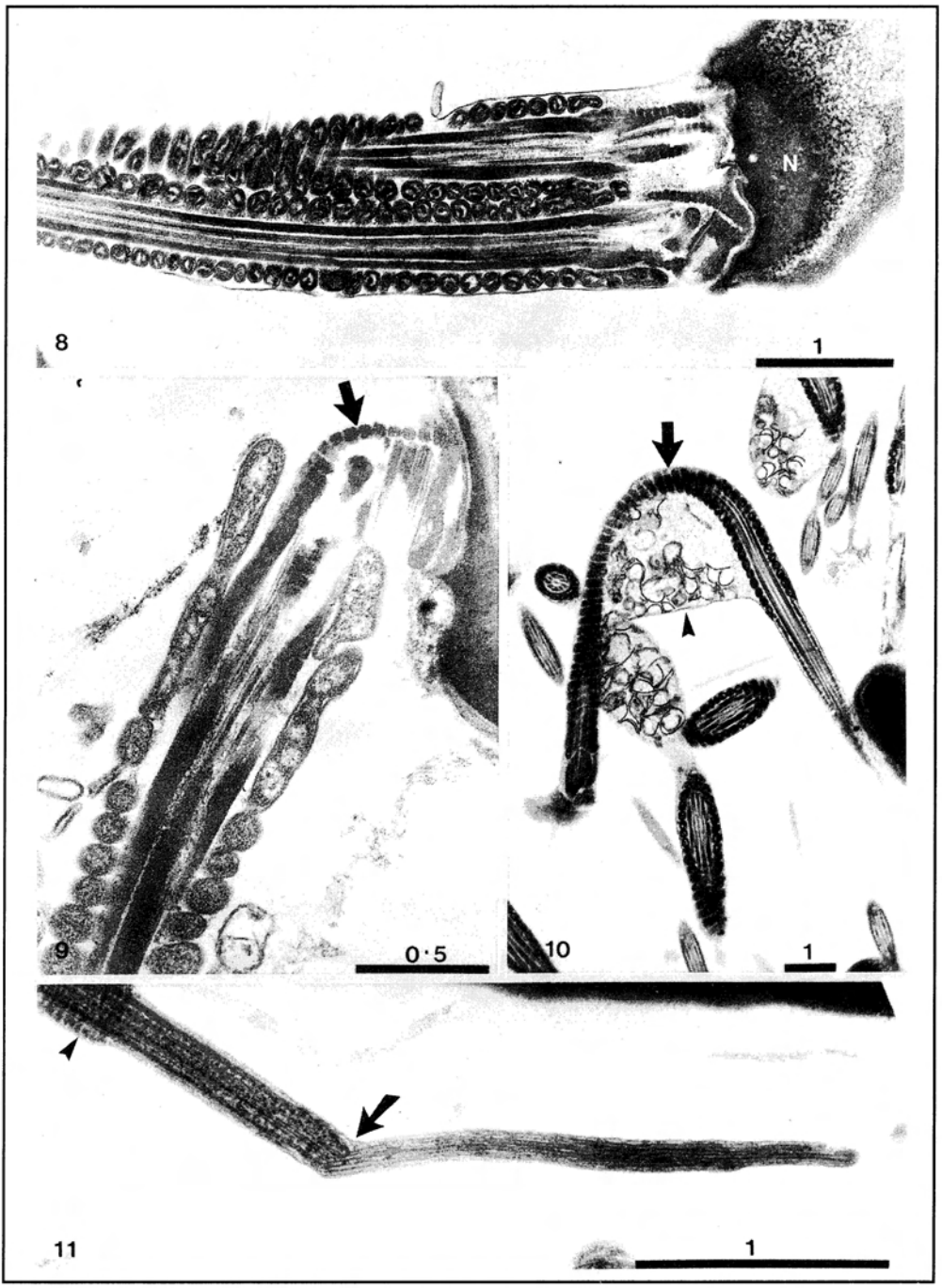
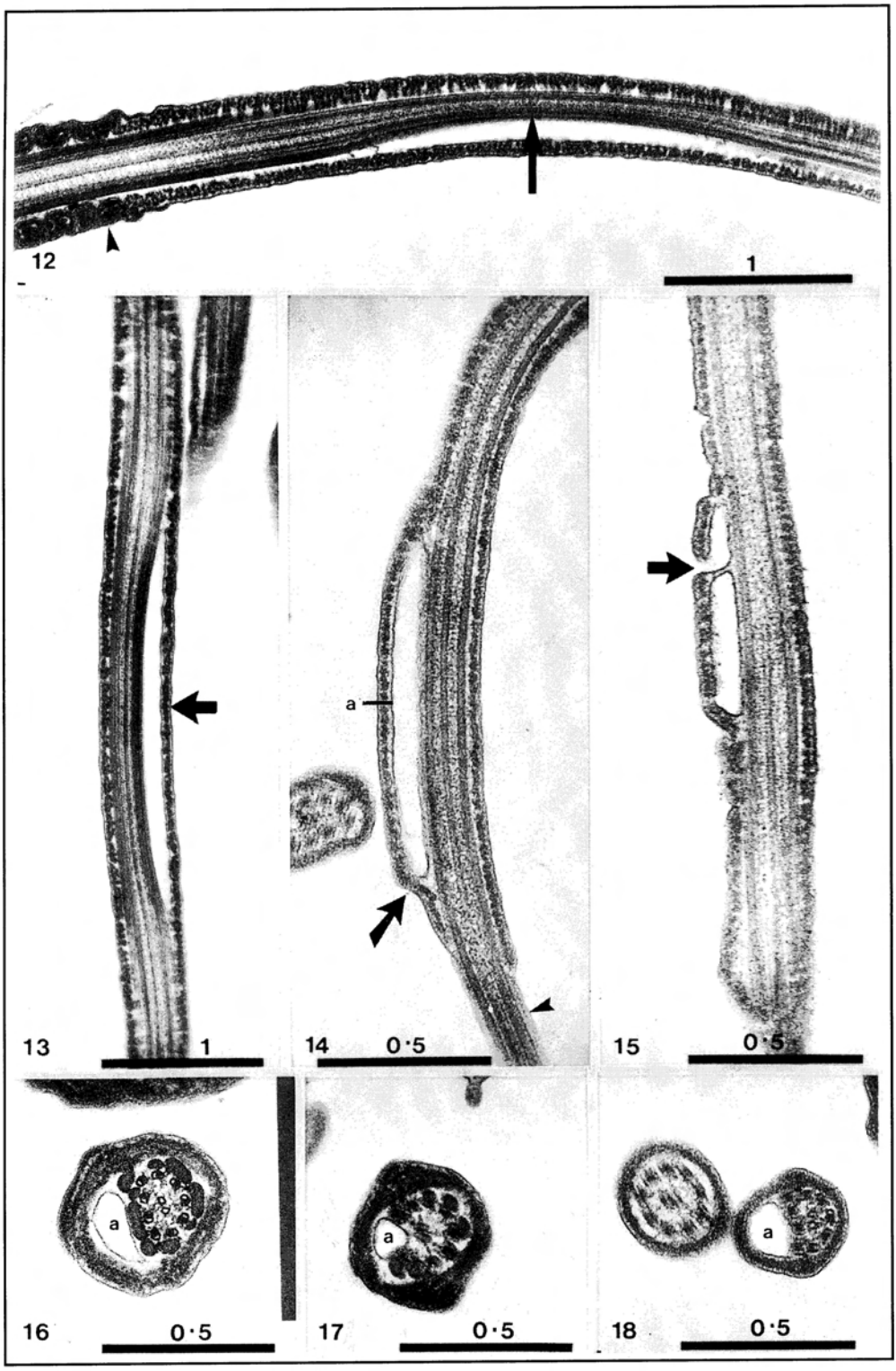


Fig. 8. Section of a sperm displaying two flagella but with normal organelles.
Fig. 9. Longitudinal section of a flagellum with an angularly bent neck (arrow).
Fig. 10. Longitudinal section of a bend in the midpiece region (arrow) containing a cytoplasmic droplet (arrowhead).
Fig. 11. Longitudinal section of a principal-piece (arrow head) with an angularly bent end-piece (arrow).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organelles (Figs. 12-18). This may affect the fibrous sheath of the principal-piece adversely. The coarse fibres and axoneme were sometimes also compressed (Fig. 16). The Dag defect is characterised by varying degrees of coiling of the flagellum within an extended intact plasmalemma which occasionally included a cytoplasmic matrix. The flagellum may also be coiled on the periphery of the dorsal or ventral side of the sperm head. Sometimes the flagellum was haphazardly coiled on one side of the head (Figs. 19-28).

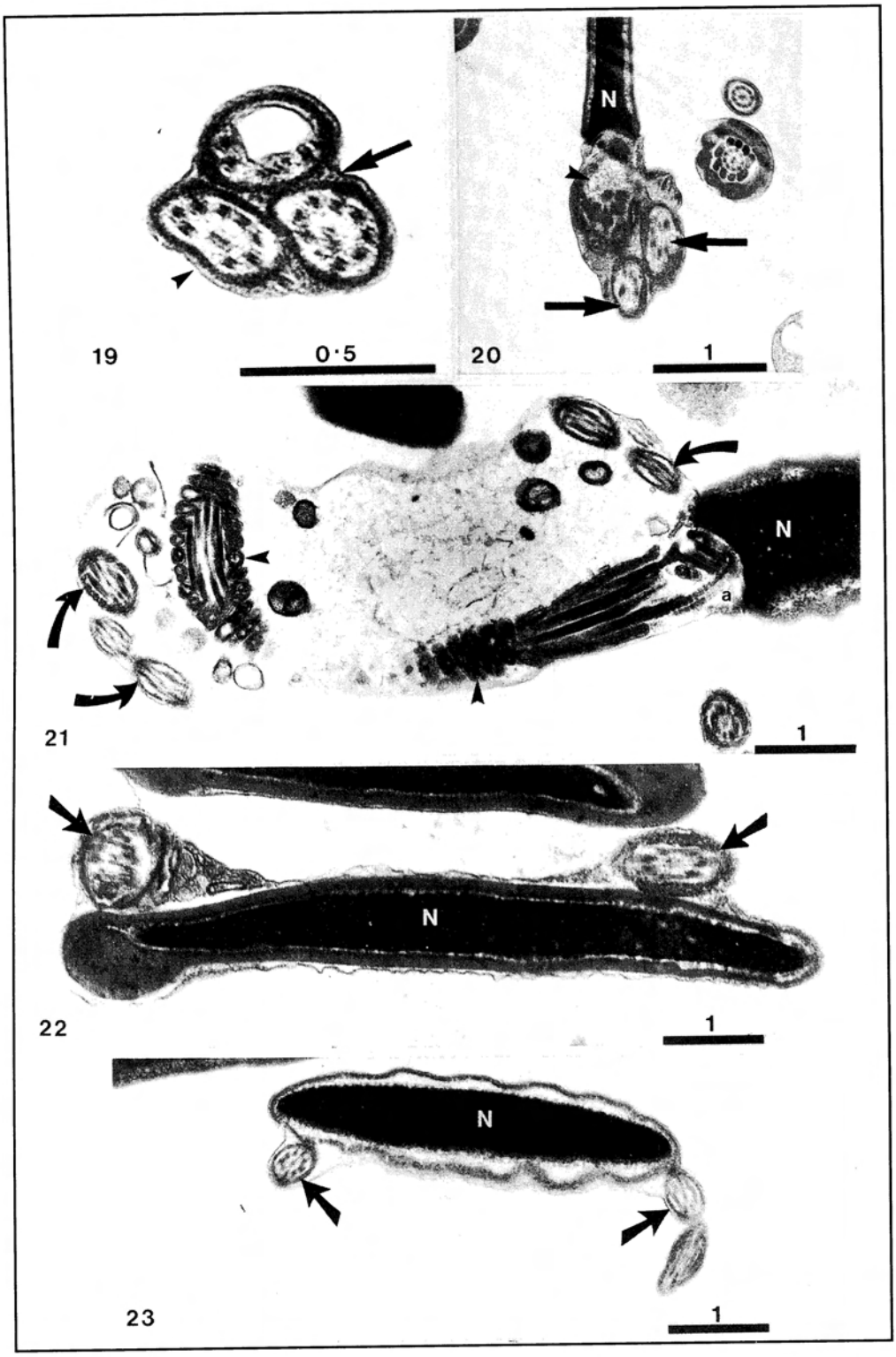
From the evaluation of 200 neck and 500 flagellum sections of sperm of 20 impala rams the following percentages were calculated for the different groups of abnormalities:

a. Vacuoles of the neck : 31.5 %
b. Other abnormalities of the neck : 29.5 %
c. Vacuoles of the principal-piece : 8.8 %
d. Other abnormalities of the principal-piece : 5.6 %
e. Dag defects associated with the flagellum : 7.6 %

Discussion

Sperm must remain motile in order to travel the required distance to reach and fertilise the ovum (Zamboni 1987, 1992). There are numerous reports of structural defects, particularly of the sperm tail, which are associated with low sperm motility. The loose head defect is considered by Zamboni (1987, 1992) as the most important abnormality of the neck of human sperm. This author described flagella with normal motility that occurred in the ejaculate together with sperm heads without flagella and these latter sperm could not fertilise the ovum. Oettlé and Soley (1988) and Menkveld *et al.* (1991) referred to vacuoles in the segmented column. These vacuoles may weaken the ultrastructure of the sperm neck and subsequently may also play a role in defective motility and detachment of the flagella (Figs. 1-5). Defects of the mitochondria, paired central microtubuli, radial spokes, peripheral microtubuli and their dynein arms and the fibrous sheath, all adversely influence the motility of the flagella (Fawcett 1975; Zamboni 1987; Ryder *et al.* 1990; Zamboni 1991, 1992). Total sperm immotility is usually the result of the above-

Fig. 12. Longitudinal section of the flagellum showing a vacuole in the principal-piece. The fibrous sheath remain in position while the vacuole compresses the coarse fibres and the axoneme (arrow); midpiece: arrow head.
Fig. 13. Longitudinal section of a principal-piece with a vacuole further away from the annulus. The fibrous sheath (arrow) remains in position and the vacuole displaces the coarse fibres and microtubuli.
Fig. 14. Longitudinal section of the principal-piece close to the end-piece (arrow head). The fibrous sheath (arrow) is now pressed outward by a membrane-lined vacuole (a) while the coarse fibres remain in position.
Fig. 15. Longitudinal section of a vacuole which damaged the fibrous sheath of the principal-piece (arrow).
Fig. 16. Transverse section of a midpiece. The membrane-lined vacuole (a) compresses the coarse fibres and axoneme. The central pair of microtubuli have lost their 3:8 orientation apparently due to the pressure exerted by the vacuole.
Fig. 17. Transverse section of a vacuole in the principal-piece (a) which wedged in between the coarse fibres and the peripheral microtubuli compressing the organelles to both sides.
Fig. 18. Transverse section of a principal-piece close to the end-piece with a vacuole (a) disturbing the symmetry of the axoneme.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mentioned defects, either singly or in various combinations. Infertility in humans with a high sperm count but showing one or more of these defects, usually results because of the inability of the sperm to reach the ovum (Zamboni 1987).

The majority of known defects of the axoneme were observed in impala sperm. However, impala sperm with these abnormalities were present in very low numbers, leading to the conclusion that their influence on fertility and reproduction will be negligible in this case. However, the presence of vacuoles was of more significance. Similar vacuoles to those observed in transverse sections of some principal-pieces of impala sperm were also described for the bull and pig (Uzu *et al.* 1976; Kojima 1981). Segretain & Roussel (1988) described the formation of vesicles of the periaxoneme during spermiogenesis in mice. These vesicles appeared during step 10 in the development of spermatids and increased in numbers while some increased in size and length along the axoneme during the following stages. Under normal circumstances the vesicles disappeared at step 16. If the latter step is omitted, it could possibly lead to the formation of permanent vacuoles next to the axoneme. The presence of vacuoles in flagella of impala indicate that a similar process may be involved.

Vacuoles in impala and buffalo sperm probably exert pressure on the axoneme, disrupting the orderly arrangement of microtubules and pressing them together (Ackerman *et al.* 1994). The sliding movement of the peripheral microtubules could possibly be inhibited affecting the motility of the sperm. In some cases the vacuoles press the fibrous sheath of the principal-piece outward possibly inhibiting the supporting role of this organelle in respect of the axoneme (Figs. 14 & 15). Zamboni (1991) states that an abnormal fibrous sheath affects sperm motility adversely. Chemes *et al.* (1987) found that sperm with abnormal fibrous sheaths which are usually accompanied by defects of the axoneme, were 95-100 % immotile. It is thus likely that sperm with a high percentage of vacuoles in the flagella will have a lowered ability to fertilise the ovum.

Vacuoles of the sperm neck were responsible for the vast majority of other abnormalities of the neck that were recorded. The vacuoles usually caused damage to organelles of the neck or were responsible for the absence of some of them.

Fig. 19. Transverse section of a Dag defect where the principal-piece folded back on itself inside a common plasmalemma (arrow). The region of the principal-piece containing the vacuole exhibits loss of some axonemal microtubuli. Only 8 of 9 peripheral microtubuli are visible in another region of the axoneme (arrow head).

Fig. 20. Longitudinal section of a sperm head with a flagellum extensively coiled up in the neck region. Some of the organelles of the midpiece (arrow head) and principal-piece (arrow) are absent.

Fig. 21. Longitudinal section of a sperm head with a flagellum loosely coiled up with cytoplasm and enclosed by a plasmalemma. Organelles of the neck (a) and sections of the midpiece (arrow head) and principal-piece (arrow) are present.

Fig. 22. Sagittal section of a sperm head with regions of the principal-piece (arrow) coiled around the periphery.

Fig. 23. Transverse section of the post equatorial area of a sperm head with the principal-piece (arrow) coiled around the periphery of the head.

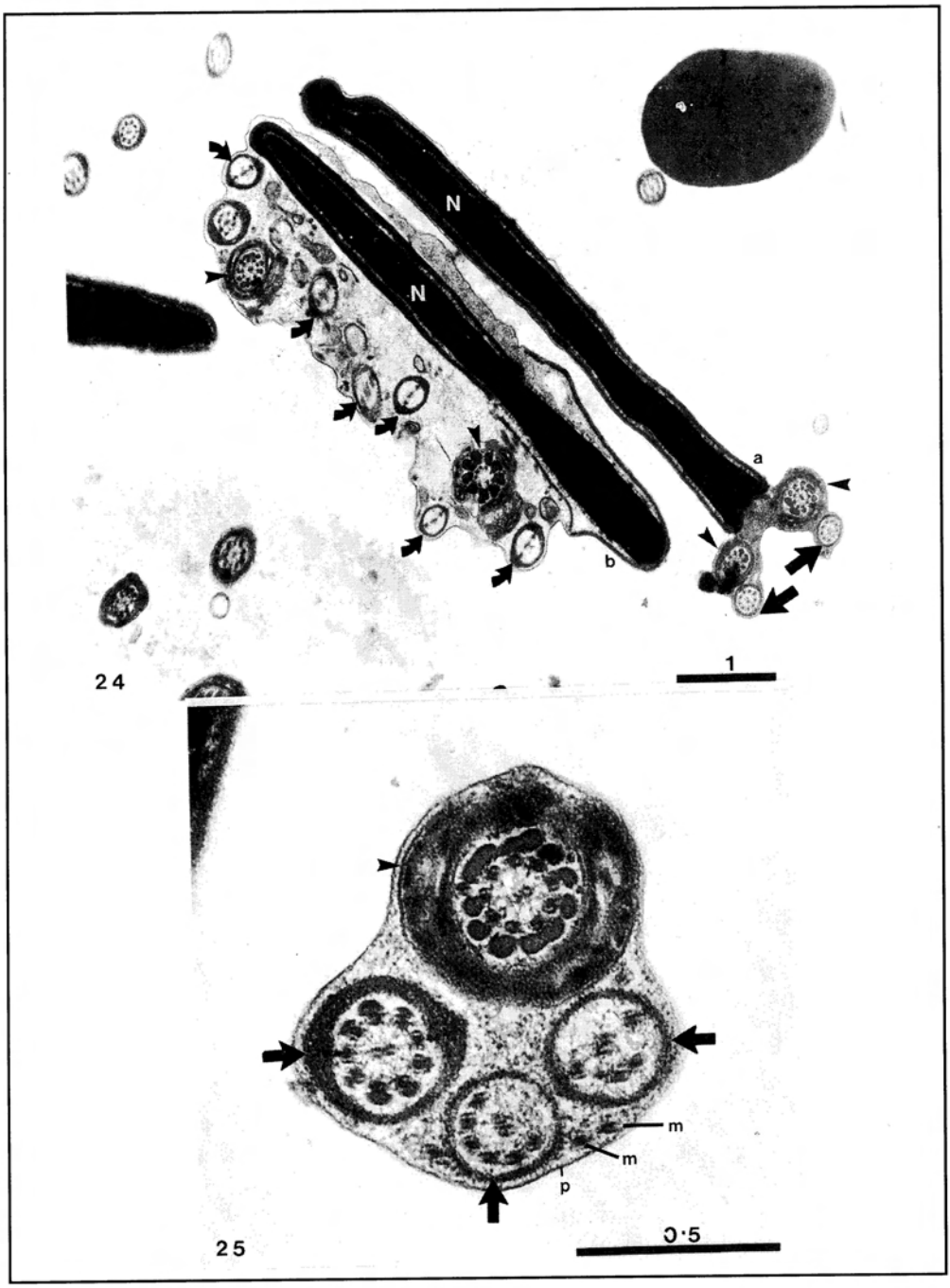

Fig. 24. Sections through sperm with the Dag defect. The midpiece (arrow head) and the principal-piece (arrows) are tightly coiled up against the head base (a) or loosely coiled in a cytoplasmic matrix (b).

Fig. 25. Transverse section of a flagellum showing the Dag defect. Sections of the midpiece (arrow head) and principal-piece (arrow) are present in the coiled flagellum. Some peripheral microtubuli of the axoneme in the principal-piece are absent. Peripheral microtubuli (m) which formed outside the flagellum are present in the cytoplasm below the plasmalemma (p).

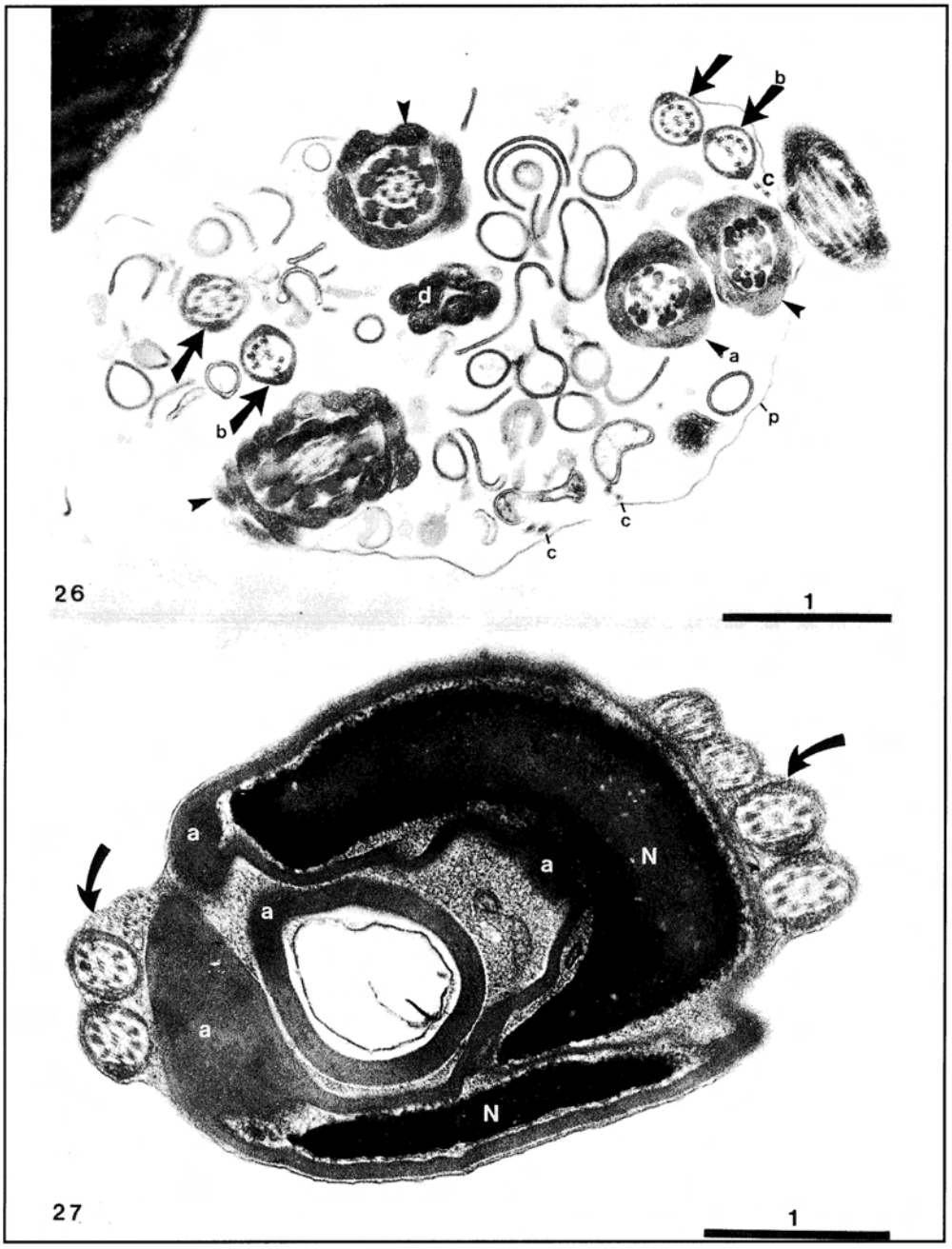

Fig. 26. Section of a Dag defect through a cytoplasmic droplet showing transverse sections of the midpiece (arrow head) and principal-piece (arrow). The organelles of midpiece (a) are incomplete and disarranged. Some of the peripheral microtubuli of the principal-piece (b) are absent. Loose microtubuli pairs (c) occur directly beneath the plasmalemma. Displaced mitochondria (d) occur amid the normal cytoplasmic organelles.

Fig. 27. Transverse section of a bizarre binucleated sperm head (N), an abnormal acrosome (a) and a flagellum, showing features of a Dag defect, coiled around the bizarre head (arrow).

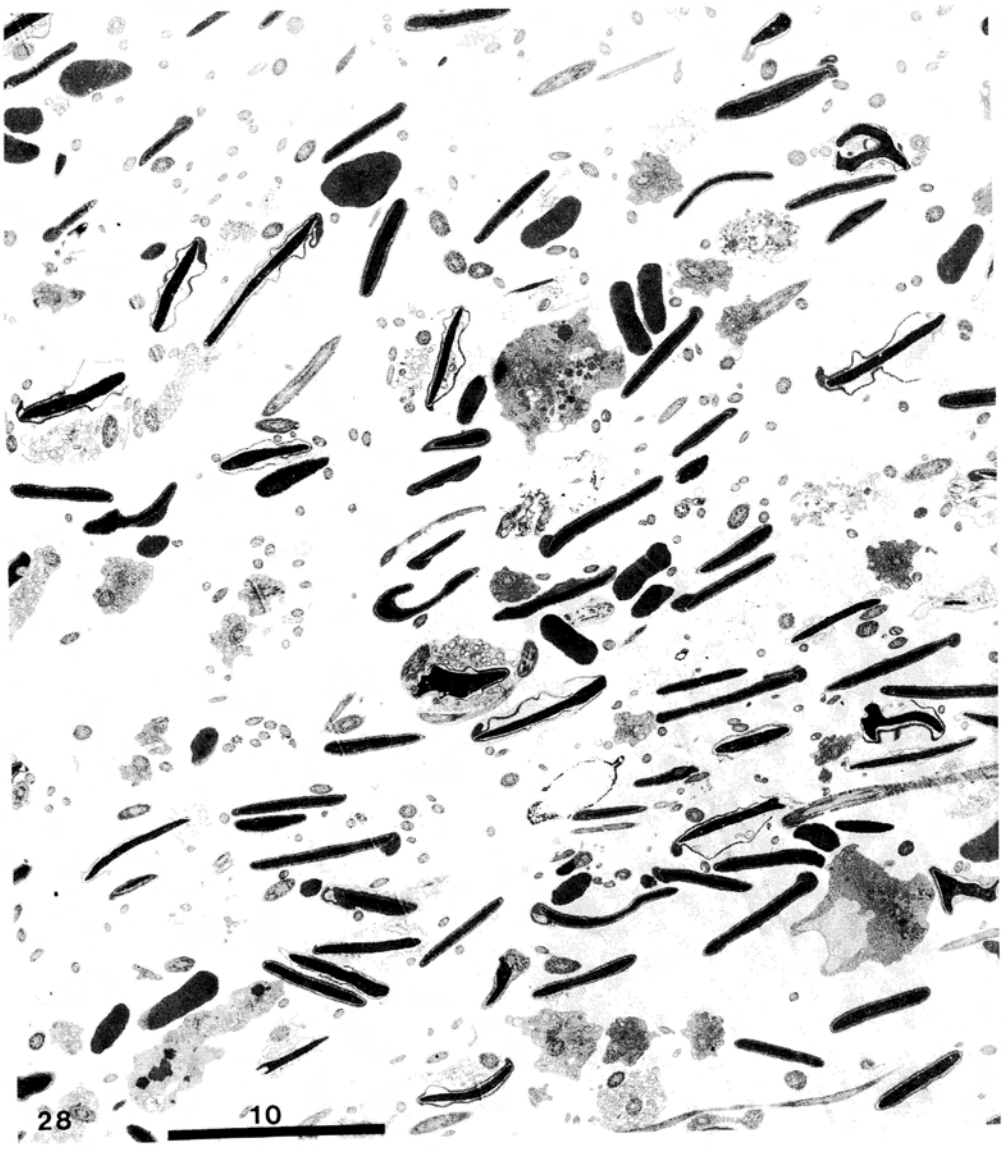

Fig. 28. Micrograph of a section of a sperm sample with a high percentage of abnormal sperm heads. Approximately 25% of the head sections appear normal.

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