

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

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Sperm morphological features play an important role in semen evaluation. Exposure to a variety of chemical compounds, especially environmental endocrine disrupters, elicit abnormalities in sperm of certain species. Baseline data on ultrastructure of normal sperm as well as abnormalities observed concomitantly, are required before causal links between such substances and abnormalities can be established. 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 normal sperm features and abnormalities. The following abnormalities of the acrosome and sperm head were documented from micrographs: Loose acrosome in various stages of disintegration, lip forming of the acrosome; bizarre head, crater defect, poor condensation of the nucleus and the Dag defect. The observed abnormalities were very similar to those reported for other members of the Bovidae. Different forms of a hollow sphere, formed by the nucleus and covered by an abnormal acrosome have not previously been described for other species.

Key words: impala sperm, acrosome and head anomalies, transmission electron microscopy, Kruger National Park

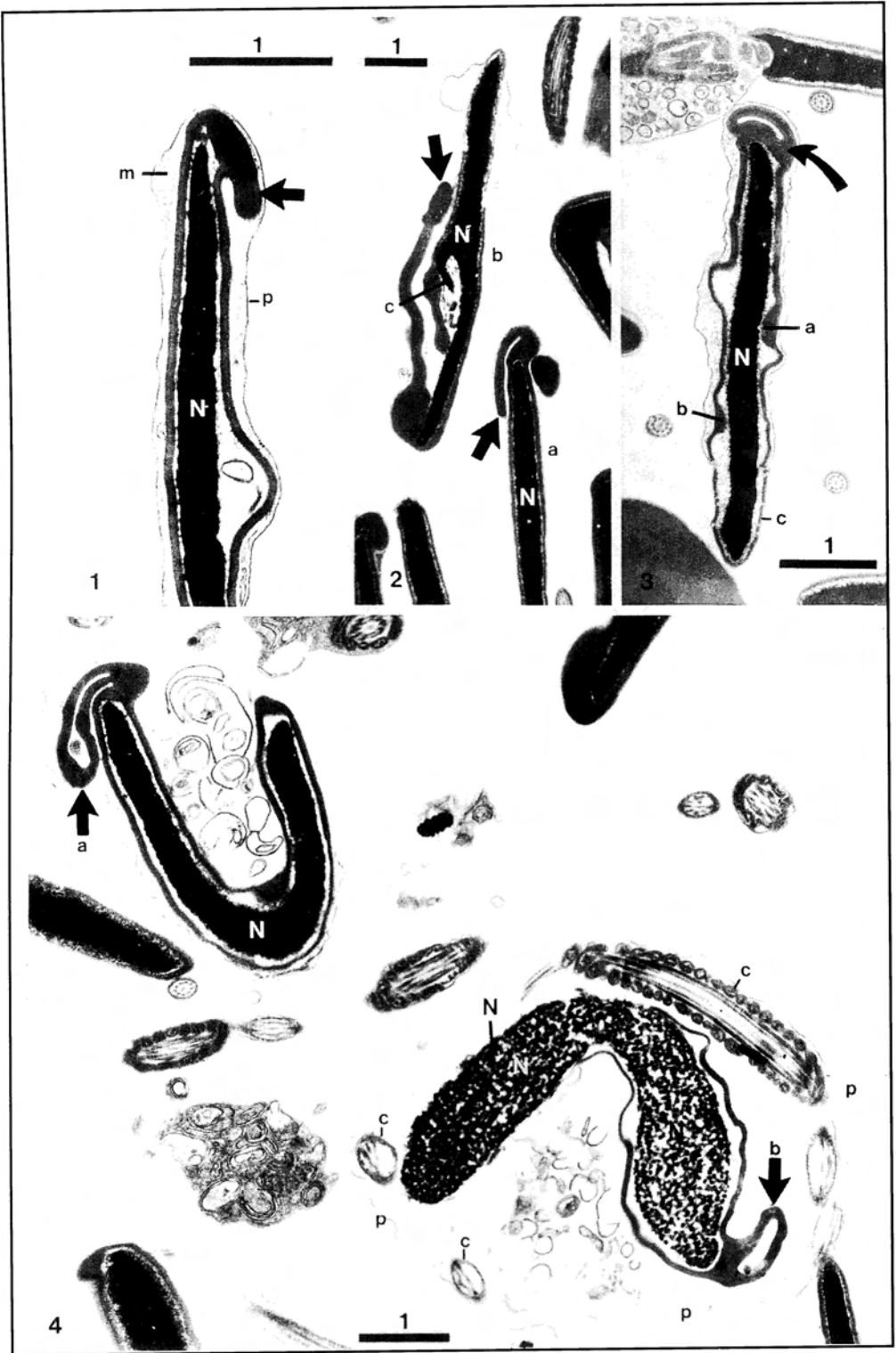
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Introduction

The percentage of normal sperm has an important role in fertilisation in humans (Kruger *et al.* 1986; De Yi Lui & Baker 1992). It seems logical that the same will hold true for other animals (Reinecke *et al.* 1995). In order to fertilise the ovum, sperm must remain motile while travelling over a relatively long distance to reach the ovum. It also requires a normal acrosome in order to penetrate the zona pellucida of the ovum. The sperm is equipped with specialised organelles which are formed during spermatogenesis in the testis. Any defect in these organelles can have an adverse influence on the function of the sperm (Fawcett & Phillips 1969; Fawcett 1975; Holstein & Roosen-Runge 1981; Holstein *et al.* 1988).

Knowledge of sperm ultrastructure is important to evaluate semen. Tests for sperm density, motility and morphology are well established for humans. Laboratory methods are fast, economical and generally provide an efficient quantitative evaluation. Sperm samples are easy to obtain and a small sample is required for analysis (Menkveld *et al.* 1991).

Since the percentage of sperm with normal morphological features is important in evaluating semen, it follows that knowledge of sperm abnormalities and ultrastructural damage will be of value to indicate possible causes of lowered sperm quality. Recent research showed that sperm of earthworms are very sensitive to the presence of foreign chemicals (Reinecke *et al.* 1995). Sperm abnormalities in mammals can occur during spermatogenesis. Genetic factors, temperature, toxic sub-



stances, hormonal changes and stress may be responsible in producing defects (Mann & Lutwak-Mann 1981; Holland & White 1982; Dadoune 1988; Holstein *et al.* 1988; Bonet *et al.* 1993; Facemire *et al.* 1995; Reinecke *et al.* 1995).

The aim of this study was to document abnormalities of the sperm head occurring in 64 impala from the Kruger National Park and to provide a qualitative evaluation of these abnormalities for 20 of the above mentioned animals which were not in contact with copper contaminated food. It was shown that the grazing in this park south of the Phalaborwa Gate is contaminated with copper and the mean copper concentration in the livers of impala was found to be abnormally high (Gummow *et al.* 1991). For a future quantification of the possible effect of this contamination on sperm ultrastructure, it was imperative to study sperm abnormalities in semen of animals from both contaminated and uncontaminated areas before attempting to relate cause and effect in a separate publication. Ackerman *et al.* (1996a, 1996b) have recently provided electron microscopic studies of the normal impala sperm.

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 from 14 animals originated from the uncontaminated area along the banks of the Nwaswitsbaka river. Six samples were also collected from animals in captivity at Skukuza after their termination. These animals were 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 operating at 80 kV was used to study the ultrastructure of abnormal sperm from the cauda epididymis of 64 impala.

Holstein *et al.* (1988) does not support a rigid classification of abnormalities because of the variety of combinations that may occur in a single sperm cell. The methods of Holstein *et al.* (1988) and Menkveld *et al.* (1991) for documenting sperm abnormalities in humans were followed in this study.

A TEM study was also made of 500 sperm sections of 20 mature impala rams (food uncontaminated by copper) to determine the percentage sections demonstrating abnormalities in the following groups:

- a. All abnormalities of the acrosome except lipping of the acrosome.
- b. Lipping of the acrosome.
- c. Dag defects associated with the sperm head.
- d. Abnormalities of the nucleus.
- e. Bizarre sperm heads.

A combination of abnormalities in a single sperm section from the different groups listed above were often observed.



Fig. 1. Sagittal section of a sperm head showing lipping (arrow) of the apical thickening of the acrosome. The material (m) directly beneath the plasmalemma (p) is probably remains of the weathered acrosome.

Fig. 2. Longitudinal sections of sperm heads (a) and (b) showing acrosome lipping of different lengths (arrows). The nucleus (N) of sperm (b) shows evidence of ridging (c) associated with acrosome thickening in this region.

Fig. 3. The apical acrosome showing an empty cyst and lipping (arrow). The main segment (a) and the equatorial segment (b) is also abnormally thickened. The acrosome and the post acrosomal dense lamina (PADL) (c) is dorso-ventral displaced from the nucleus.

Fig. 4. The apical acrosome of sperm (a) and (b) shows lipping as well as a cyst (arrows). In both, cytoplasmic material occurs in the bend of the sperm head. A Dag defect is present in sperm (b); a subcondensed nucleus (N) and segments of the flagellum (c) is enclosed by a plasmalemma (p).

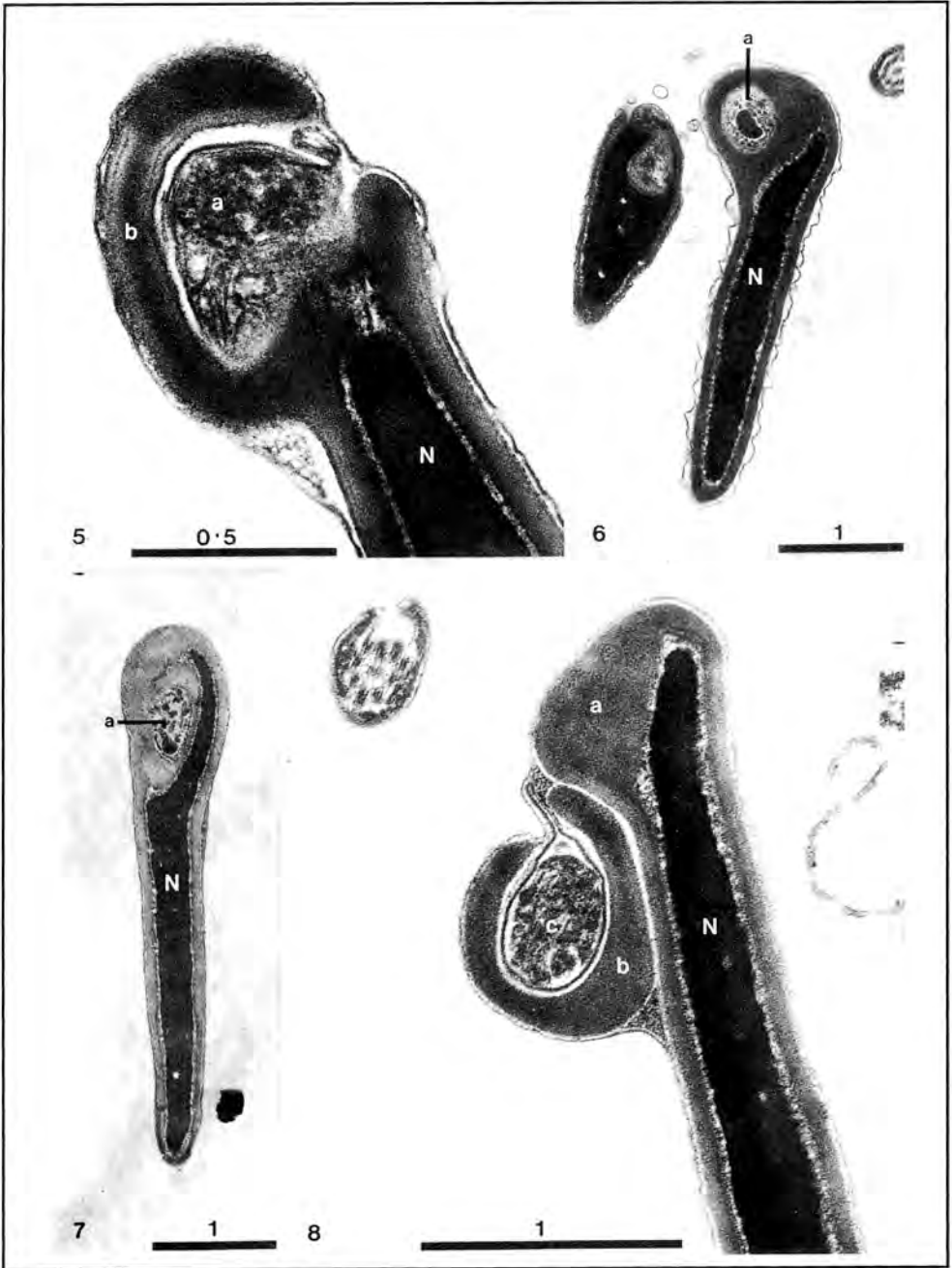


Fig. 5. A cyst (a) of the apical acrosome (b), filled with granular material seen in a median section of the sperm head.
 Fig. 6. An apical cyst (a) filled with granular material. The electron dense inclusion is probably remnants of the acrosome. The tip of the nucleus (N) is slightly flattened due to pressure caused by the cyst.
 Fig. 7. Sagittal section with a cyst (a) of the apical acrosome deforming the tip of the nucleus (N).
 Fig. 8. Sagittal section of the apical segment. An abnormal thickening (b) with a cyst (c) filled with granular material associated with acrosome (a) is shown.

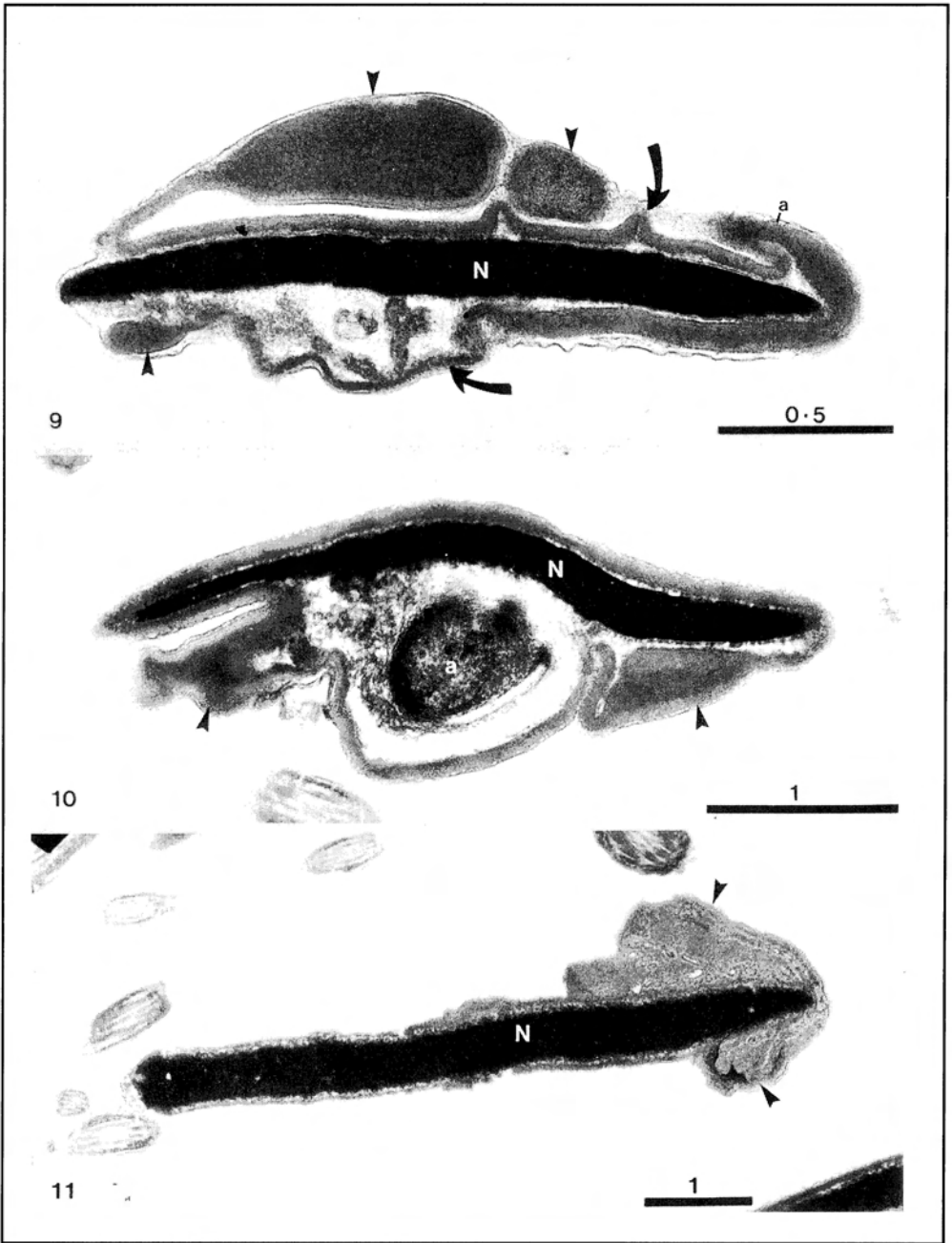
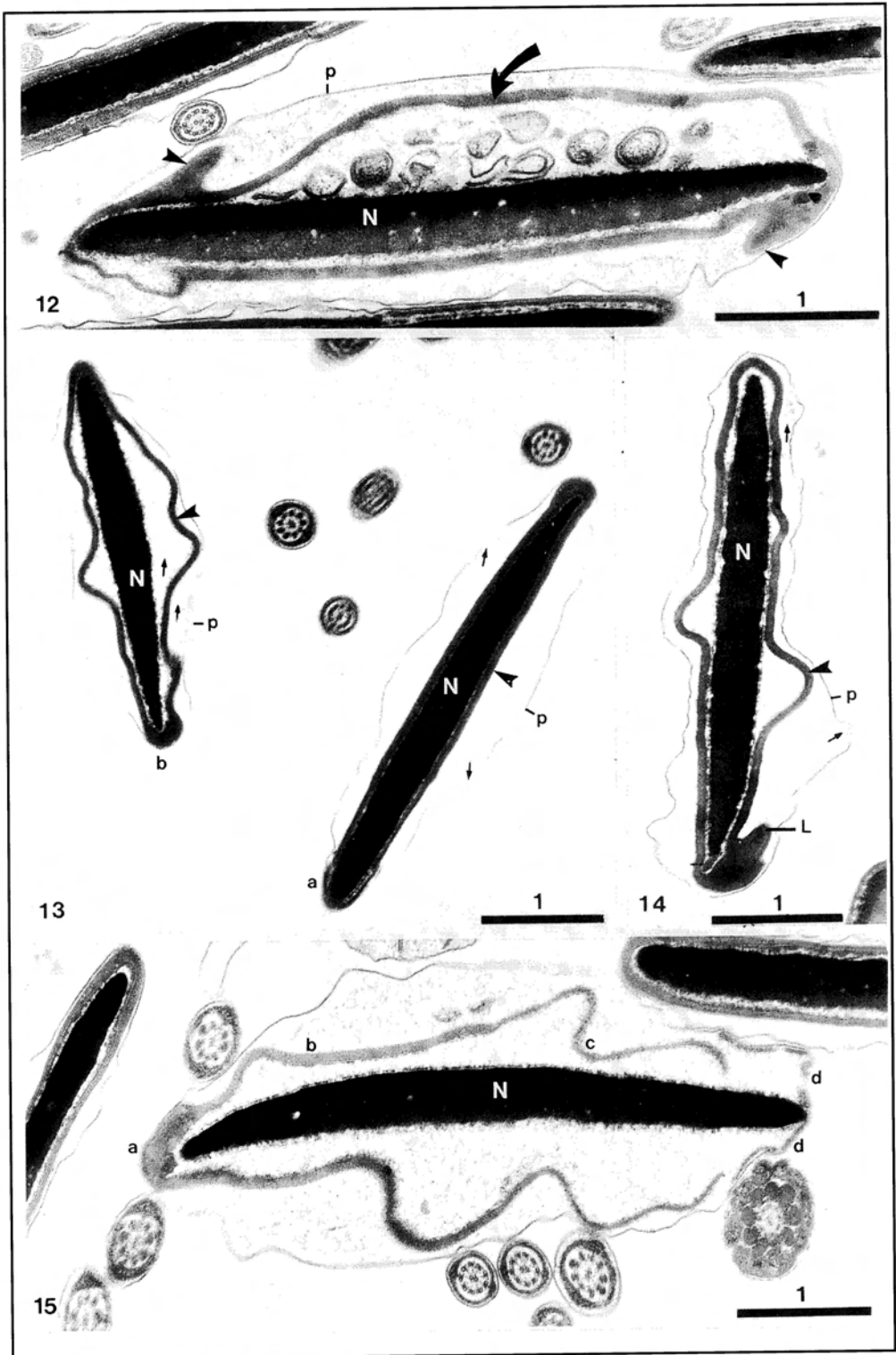


Fig. 9. Sagittal section including part of the apical segment. Abnormally thickened acrosomal material (arrow heads) can be seen in different places. Lipping (a) is present and the acrosome is partially displaced (arrow) from the nucleus (N).

Fig. 10. Transverse section of the sperm head through the main segment of the acrosome. The acrosome is abnormally thickened (arrow head) and an acrosomal cyst (a) has indented the nucleus (N) slightly.

Fig. 11. Sagittal section of a sperm head with an abnormal thickening (arrow head) on both sides of the apical part of the nucleus.



Results

Acrosome

Acrosome lipping (Figs. 1-4). This is an abnormality of the apical region of the acrosome. The degree and form of the abnormality can vary. The sagittal section of a sperm head (Fig. 1) shows material directly beneath the plasmalemma which is probably remains of the acrosome. Lengthening in varying degrees of the acrosome lip occurred frequently (Figs. 2-4) as well as ridging and abnormal thickening of the acrosome. Fig. 3 shows the apical acrosome with apparently an empty cyst. Sometimes lipping was associated with subcondensation of the nucleus (Fig. 4).

Acrosome cysts (Figs. 5-8). Each of the cysts was filled with granular material. The formation of a cyst usually results in a deformity at one end of the nucleus (Fig. 38).

Abnormal thickening (Figs. 9-11). This was often associated with lengthening and deformation of the acrosome.

Disintegration of the acrosome (Figs. 12-19). The acrosome appeared wavy and separated from both the plasmalemma and the nucleus in the apical segment of the sperm head. Within these abnormal dilated spaces on

both its outer and inner aspects weathered acrosomal material was frequently observed. Various stages of acrosomal disintegration were present and in some cases a broken plasmalemma or an advanced stage of PADL disintegration was observed.

Nucleus

The occurrence of vacuoles was sometimes observed (Fig. 20) while crater defects occurred in the nucleus or at its base - pouch formation (Figs. 21-23).

Malformed heads

Wing forming or ridging (Figs. 24-26) occurred in which the nucleus was often diploid and the result of abnormal development due to incomplete sperm separation during spermatogenesis. Instances where the sperm heads became spherical (Figs. 27-29) and folded (Figs. 30-32) were also observed.

Karyoplasm

Chromatin condensation is a chemical process due to macromolecular interaction between DNA and base proteins to form complexes which are stabilised by bisulphate bonding (Bartoov *et al.* 1980). Chromatin subcondensation occurred in varying degrees (Figs. 33-35).



- Fig. 12. Transverse section of the apical segment of the sperm head. Cytoplasmic and degenerated acrosomal material occurs between the nucleus (N) and an abnormally displaced acrosome (arrow). The dilated space between the plasmalemma (p) and the acrosome also contains acrosomal material. Acrosome lipping (arrow head) occurs dorso-ventral on the sperm head.
- Fig. 13. Sagittal section through a sperm head (a) showing an abnormally loose plasmalemma (p) to which remains (arrow) of the acrosome (arrow head) are connected. In the slightly oblique sagittal section (b) of the sperm head the plasmalemma (p) separated from the acrosome (arrow head) and the latter in turn detached from the nucleus. Degenerated acrosomal material (arrow) surrounds the acrosome. This is an example of advanced degeneration of the acrosome.
- Fig. 14. Sagittal section of a sperm head with a displaced plasmalemma (p) and acrosome (arrow head). Degenerated acrosomal material (arrow) is clearly shown. The apical acrosome forms a lip (L).
- Fig. 15. The whole acrosome, consisting of an apical segment (a), the main segment (b) and the equatorial segment (c) as well as the post acrosomal dense lamina (PADL) (d) is displaced from the nucleus (N).

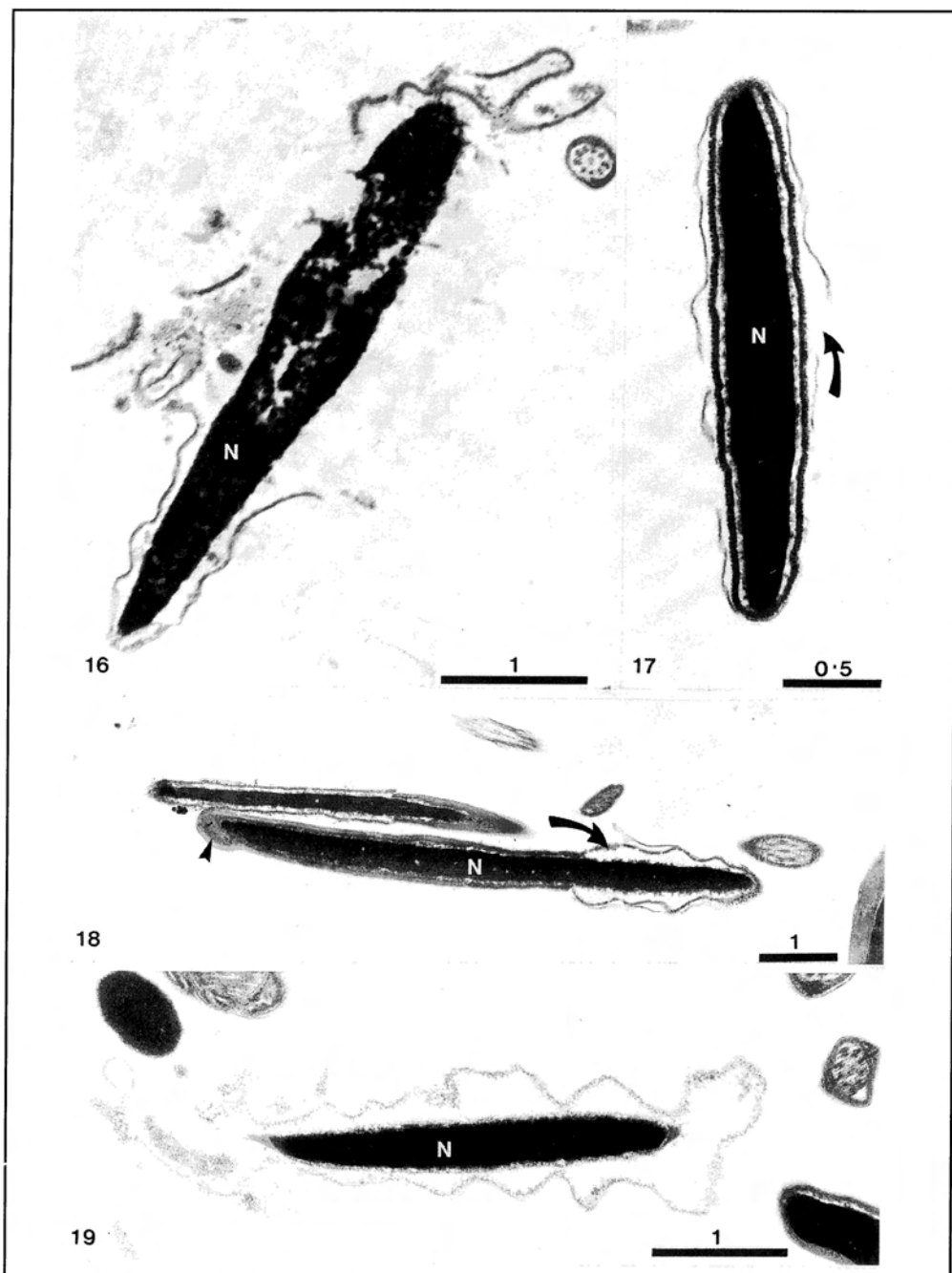


Fig. 16. Sperm head showing a broken plasmalemma and a disintegrated acrosome.
 Fig. 17. Transverse section of the post acrosomal area showing damage of the plasmalemma surrounding the (PADL) (arrow).
 Fig. 18. The acrosome (arrow head) seems normal but the plasmalemma is broken and the PADL (arrow) is damaged.
 Fig. 19. Transverse section showing an advanced stage of PADL disintegration.

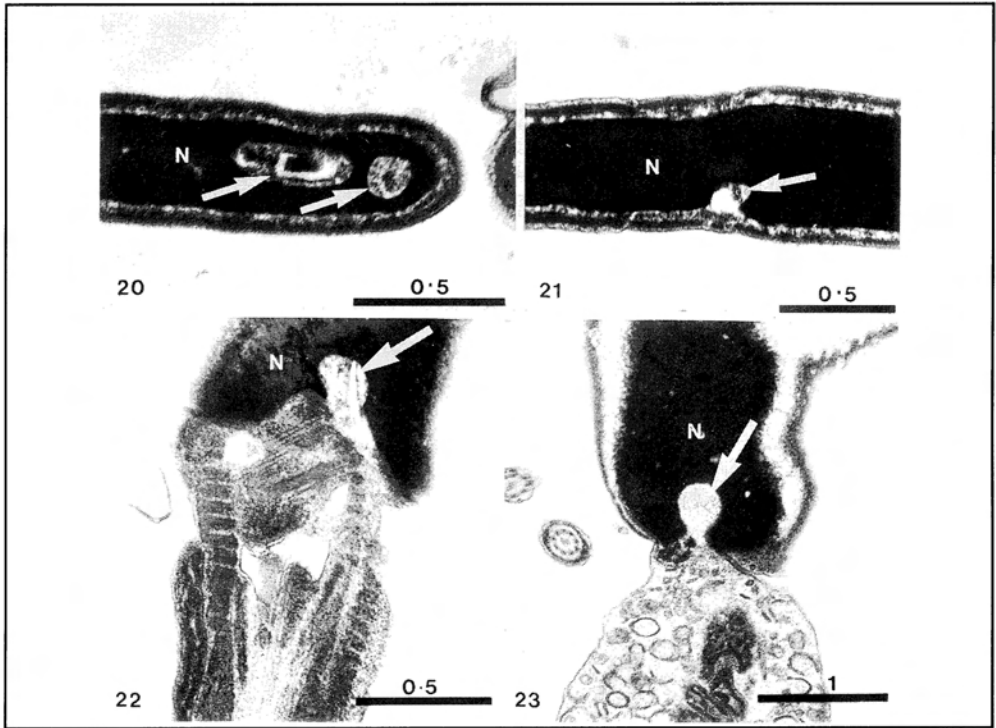


Fig. 20. Transverse section through the nucleus with two vacuoles (arrow). One vacuole is filled and the other is partially filled with electron dense material.

Fig. 21. Nucleus (N) with a crater defect (arrow). Floccular material originating from the area between the nucleus and the acrosome (arrow head) remains in the crater.

Fig. 22. Crater defect located at the base of the nucleus (pouch defect, arrow).

Fig. 23. A narrow opening connecting the crater defect (pouch) at the base of the nucleus with the neck (arrow).

Multiple head-malformation

Multiple nuclei (Figs. 36-40) also occurred. Fig. 36 shows a sagittal section of the sperm head with two nuclei. The presence of cytoplasm between the two nuclei is shown in Fig. 37. In Fig. 38 the presence of a cyst between the two nuclei and a common acrosome can be seen. Fig. 39 shows a transverse section of a sperm head with the Dag defect. Fig. 40 shows a sperm head with three nuclei sharing a common acrosome.

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

- a. Acrosomal abnormalities (except lipping): 53.0 %
- b. Lipping of the acrosome: 17.8 %
- c. Dag defect associated with the sperm head: 2.8 %
- d. Abnormalities of the nucleus: 1.2 %
- e. Bizarre heads: 6.0 %

Discussion

Preliminary studies of sperm abnormalities of other members of the Bovidae such as buffalo, red hartebeest, kudu and blesbok showed that similar abnormalities occurred. Although many variations of the same abnormality may occur in impala, there is a simi-



Fig. 24. Transverse section (oblique) of the sperm head. The nucleus with central axis (N) has formed three wings (white arrows); PADL (arrow head).

Fig. 25. Transverse section of a nucleus (N) and absent acrosome with three apparent disordered wings (white arrow) embedded in cytoplasmic material enclosed by a plasmalemma (arrow).

Fig. 26. Transverse section of a nucleus (N) with four wings (white arrow). The presence of a plasmalemma (arrow head) and cytoplasmic material (black arrow) associated with the nucleus indicates that at least a part of the sperm head was covered with cytoplasmic material. The acrosome is absent.

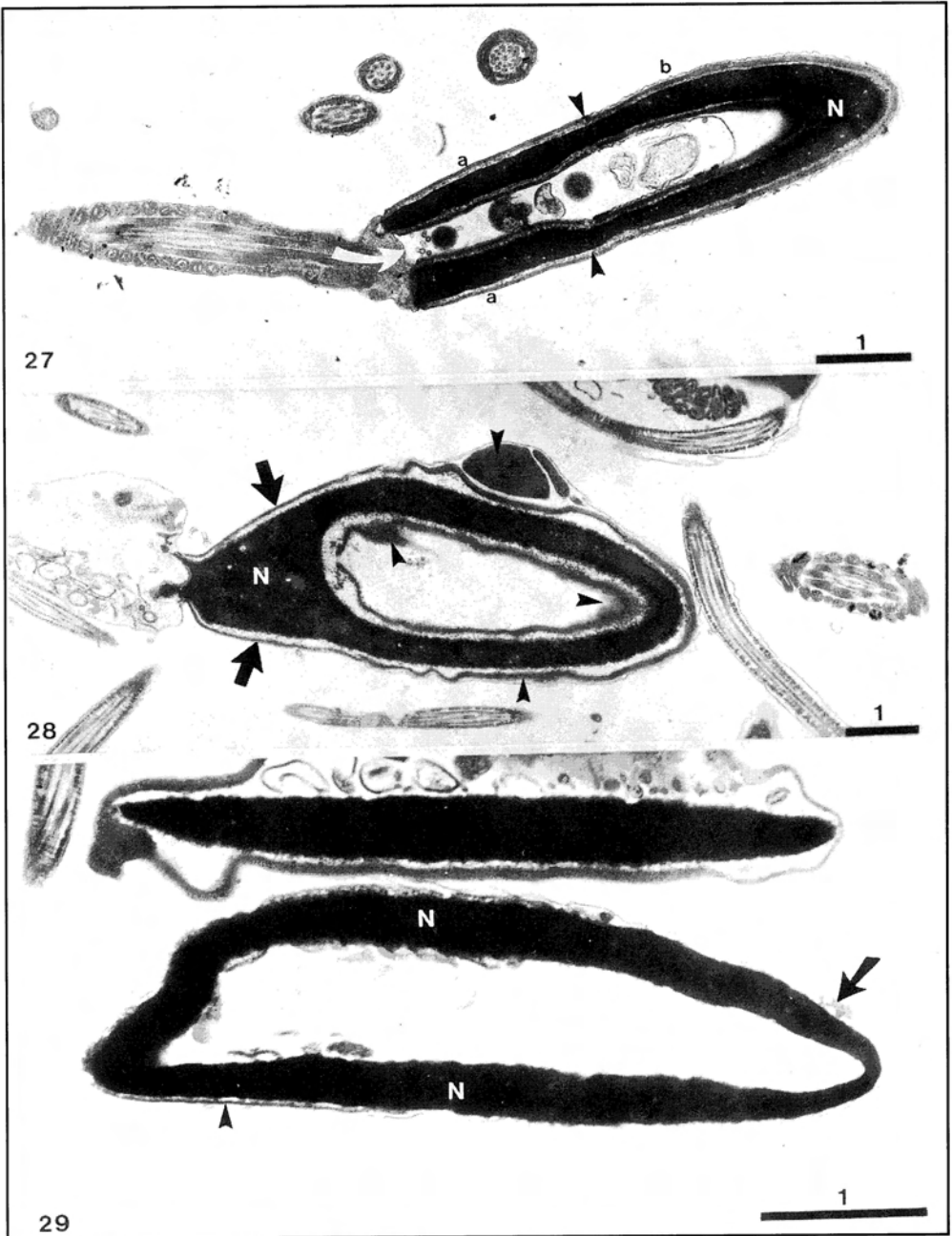


Fig. 27. Median section of a sperm head which formed an elongated hollow sphere opening (arrow) onto the neck. The equator is in the region of the arrow head which separates the PADL (a) and the acrosome (b) on both sides of the nucleus.

Fig. 28. Median section of a sperm head which probably also formed a hollow sphere. An opening onto the neck is absent in this plane. An acrosome (arrow head) and a PADL (arrow) is unevenly distributed across the inner and outer sides of the nucleus (N).

Fig. 29. Transverse section of a sphere-shaped sperm head. A small portion of the acrosome (arrow) and PADL (arrow head) is also present.

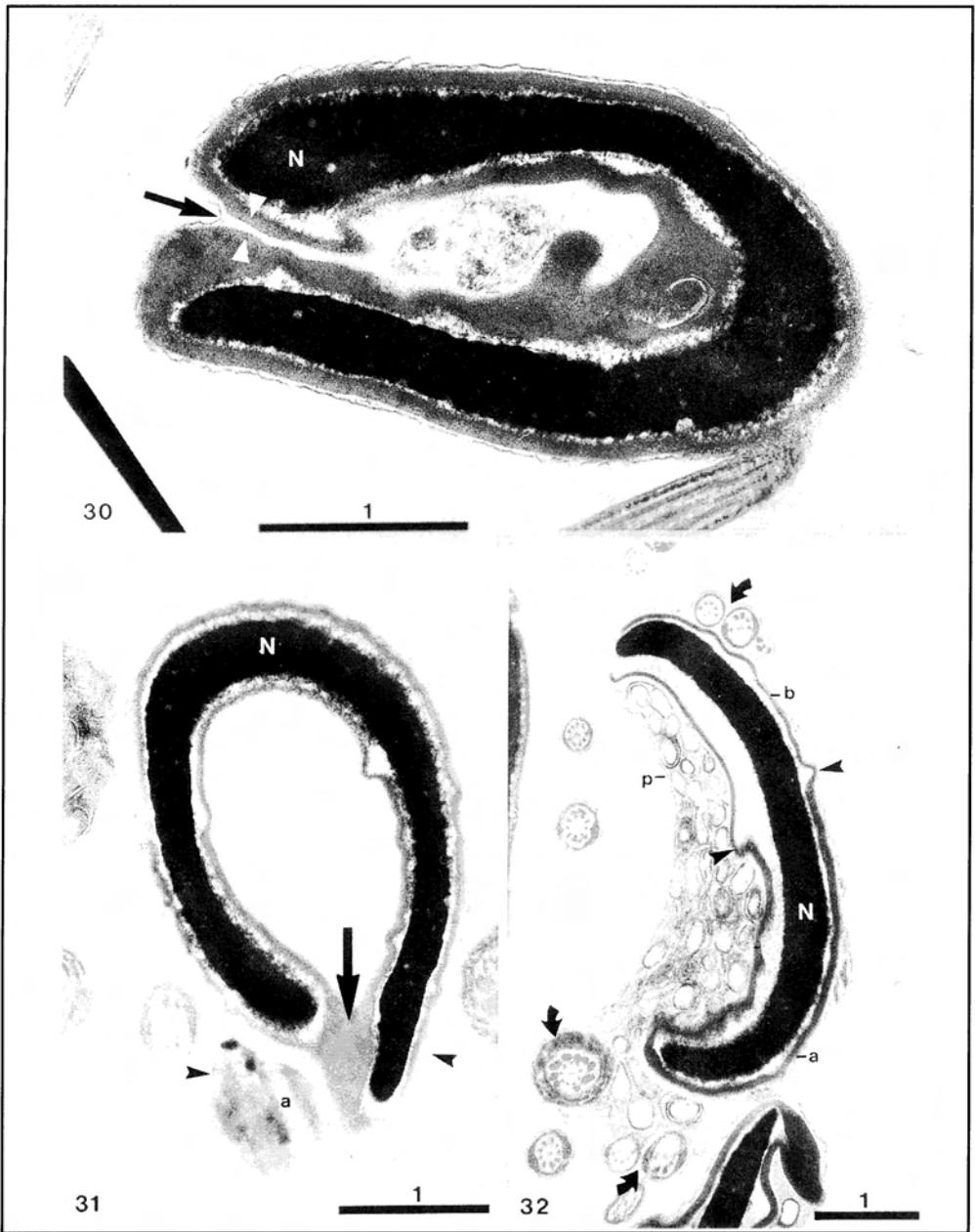
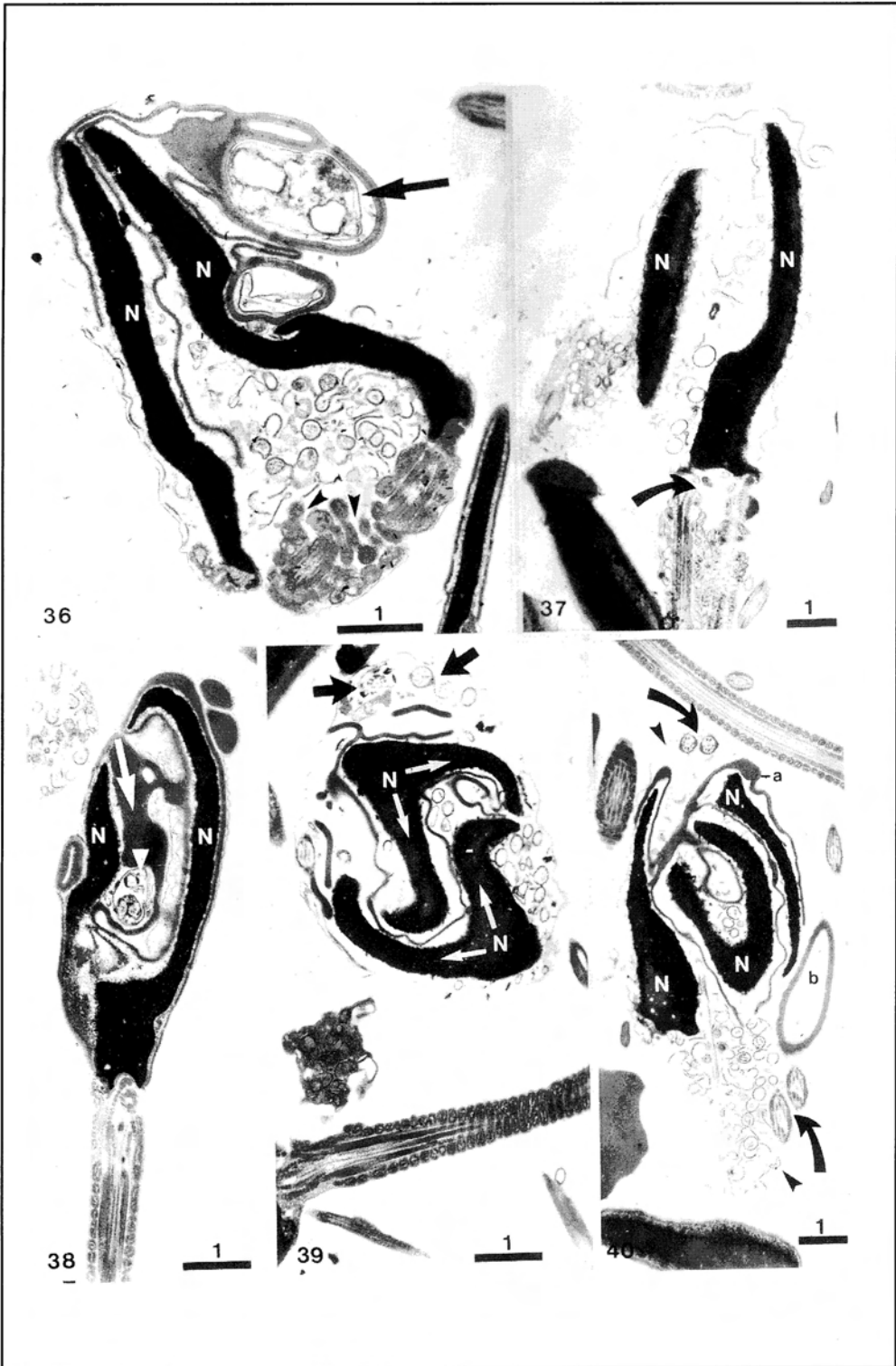


Fig. 30. Transverse section of the apical segment of a lengthwise folded sperm head. The acrosome continues from the outer aspects along the inner aspects of the sperm head without fusion (arrow head). Note that the plasmalemma (arrow) is absent from the interior region.

Fig. 31. Transverse section of the main segment of a lengthwise folded sperm head. The acrosome fuses at the sides of the head (arrow). The plasmalemma (arrow head) surrounds the sperm head as well as the additional acrosomal material (a) but is absent from the interior cavity.

Fig. 32. Sagittal section of a slightly bent sperm head with an acrosome (a), equator (arrow head) and a PADL (b). Inclusion of segments of the flagellum (arrow) amid cytoplasm covered by a plasmalemma (p) indicates presence of a Dag defect.



larity with abnormalities observed in humans and animals such as the bull, pig, dog and other mammals (Coubrough & Barker, 1964, Fujita *et al.* 1970; Holstein 1975; Soley *et al.* 1985; Coetzee *et al.* 1985; Williams 1987; Zamboni 1987; Menkveld *et al.* 1991; Tingari 1991; Bonet *et al.* 1993 and Ackerman *et al.* 1994).

The ultrastructural pathology of human sperm as a cause of infertility is documented in isolated case studies as well as in overviews (Zamboni 1987, 1991, 1992). The latter author classified the pathology of the acrosome in three main categories viz. acrosomal hypoplasia, structural defects and absence of organelles. Examples of all three can be seen in Figs. 1 - 16 for the acrosome of impala. Most of these sperm will, depending on the seriousness of the defect, probably be unable to penetrate the ovum (Zamboni 1987, 1991, 1992).

Most of the nuclear defects also include acrosomal abnormalities (Holstein *et al.* 1988; Schill 1991; Zamboni 1991, 1992). Large vacuoles in the nucleus, severe forms of crater defects and subcondensation of chromatin are responsible for subfertile or infertile sperm (Zamboni 1991, 1992).

Vacuoles and crater defects seldom occurred in impala sperm (Figs. 20, 21) but subcondensation of chromatin varied from minimal

to severe (Figs. 33-35). All sperm, in which the nucleus formed ridges (Figs. 22-26) or were spherical or folded (Figs. 27-32) or showed multiple nuclei (Figs. 36-40) or showed a Dag defect, could be considered as bizarre forms. In addition these sperm almost always had an abnormal acrosome. Therefore, they would in general not be able to fertilise the ovum (Bartoov *et al.* 1980; Oettlé & Soley 1988). Different forms of a hollow sphere, formed by the nucleus and covered by an abnormal acrosome (Figs. 27-29) had not previously been described for mammals.

A percentage of 70.8 sperm sections with acrosomal abnormalities, including lipping of the acrosome, seems high for mature animals in an uncontaminated environment. This may be due to various reasons such as environmental influences on impala sperm and sensitivity of detection by TEM.

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- Fig. 36. Sagittal section of a sperm head with two abnormal nuclei (N) enclosing a cytoplasmic region. A cyst (arrow) is present in the common lipped apical acrosome. A flagellum with disarranged mitochondria (arrow head) forms part of the right hand nucleus.
- Fig. 37. Sagittal section of a sperm head with two nuclei, one with a flagellum displaying absent and disorganised organelles in the neck (arrow). Cytoplasm occurs between the nuclei.
- Fig. 38. Sagittal section of a sperm head with two nuclei. The abnormal common acrosome (arrow) has a cyst (arrow head) between the two nuclei.
- Fig. 39. Transverse section of a malformed binucleated sperm head in which each nucleus (N) has two wings (white arrows) and showing a Dag defect (arrow).
- Fig. 40. Longitudinal section of a malformed sperm head with three nuclei (N) sharing a common acrosome (a). A malformed acrosome (b) is probably part of the structure. Sections through the flagellum (arrow) beneath a plasmalemma (arrow head) show that a Dag defect is present.

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