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

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Since knowledge of sperm morphological characteristics can play an important role in semen evaluation and fertilisation, baseline data on sperm ultrastructure are required. Live spermatozoa were collected from the cauda epididymis from 64 impala rams in the Kruger National Park and 5082 spermatozoa from 40 of these impala were studied by scanning electron microscopy. The mean length of impala sperm was 59.23 ± 2.7 µm. The morphology of normal sperm as well as the occurrence of abnormalities were documented. The morphology of impala sperm were compared with those of other mammals. New findings on appendages of the cytoplasmic droplet are described and interpreted.

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

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Introduction

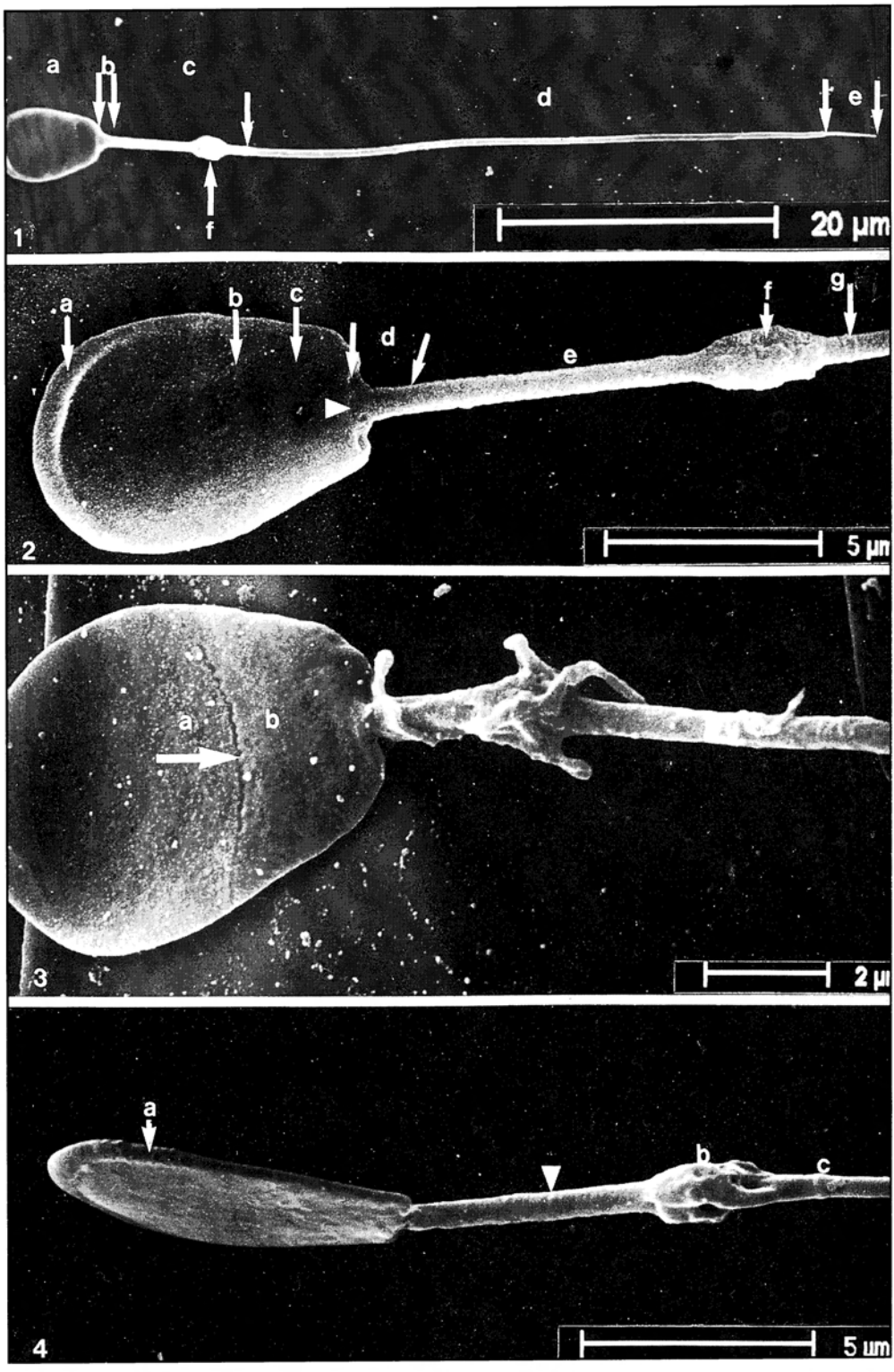
Apart from microscopical studies of impala sperm by Fairall (1971), Skinner (1971) and Dott & Skinner (1989) no electron microscopical studies of impala sperm ultrastructure have been done. Scanning electron microscopy provides a three dimensional image and high resolution of the surface structure allowing for more reliable and accurate dimensions and interpretation of various sperm structures (Fujita *et al.* 1970; Liakatas *et al.* 1982; Conradie *et al.* 1988; Bonet 1990; Soley 1992; Van der Horst *et al.* 1991). Knowledge of sperm morphology can play an important role in semen evaluation. The percentage sperm with normal morphology has been shown to play an important role in fertilisation in humans (Kruger *et al.* 1986; De Yi Lui & Baker 1992).

Ultrastructural abnormalities of sperm usually affect fertility adversely (Coubrough & Soley 1977; Dadoune & Fain-Maurel 1977; Coubrough & Soley 1981; Mahadevan & Trounson 1984; Thilander *et al.* 1985; Barthèlèmy *et al.* 1992; Bacetti *et al.* 1993). Reinecke *et al.* (1995) have observed sperm damage after exposure of earthworms to dieldrin. These observations open up the possibility of utilising sperm as sensitive biomarkers of environmental quality. This evaluation will, however, require reliable baseline data of sperm ultrastructure in order to interpret structural changes and abnormalities resulting from exposure to toxicants.

The aim of this study was to study the surface morphology of impala sperm with the aid of the scanning electron microscope in order to obtain reliable data for future reference.

Material and methods

Live sperm were collected from the cauda epididymis of 64 impala rams that were culled in official research projects by scientists from the Kruger National Park in South Africa. Fixation methods employed in the field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and preparation techniques for scanning electron microscopy were described fully by Ackerman *et al.* (1994) and Ackerman (1995).

The morphology of normal and abnormal impala sperm were studied in 40 of the above mentioned 64 animals using a Philips XL20 SEM. Measurements of the head, midpiece, principal-piece and end-piece were obtained from 95 normal sperm from 19 different healthy impala, using the image analysis software of the Philips XL20. An evaluation for the following abnormalities were made from 5082 sperm of 40 impala rams: Dag defects (all forms), kinked tail and tail-stump defects (Williams 1987; Oettlé & Soley 1988; Menkveld *et al.* 1991) and double flagella. These defects were readily detected and constituted the majority of defects occurring in our experimental animals.

We followed the procedure of Holstein *et al.* (1988), Oettlé & Soley (1988) and Menkveld *et al.* (1991) to describe sperm abnormalities directly with the use of micrographs. Abnormalities of sperm were divided into the following categories:

acrosome: abnormal thickening and distribution; nipple defect; ridging or wing forming; disintegration and loss of acrosome;

head: malformed (micro, megalo, elongated, double heads and bizarre forms), loose heads and agglutination of sperm heads;

flagellum: tail-stump defect; Dag defects; angularly bent flagellum (at the neck, inside cytoplasmic droplet or at annulus); simple loops and terminally coiled-up flagellum; double flagellum and abnormal attachment of the neck to the base of the head.

Results

Normal sperm

A normal impala sperm has two prominent regions consisting of a head and flagellum (Fig. 1) The head is superficially divided into an acrosomal region and a post acrosomal region by the equator (Figs. 2 & 3). The flagellum is divided into four parts consisting of the neck, midpiece, principal-piece and end-piece (Fig. 1) The mean length of impala sperm was 59.23 ± 2.7 µm.

The head

The head of the impala sperm is flattened to form a paddle-shaped structure with a flattened base (Fig. 4). The mean measurements with standard deviations for 95 sperm were: head length: 7.59 ± 0.61 µm; head width (halfway between apex and base): 4.81 ± 0.49 µm; head thickness (dorso-ventrally with SEM): 0.89 ± 0.15 µm; head thickness (TEM): 0.65 ± 0.05 µm; head base width: 2.13 ± 0.33 µm; acrosome lip thickness (from apex to base): 0.58 ± 0.12 µm.

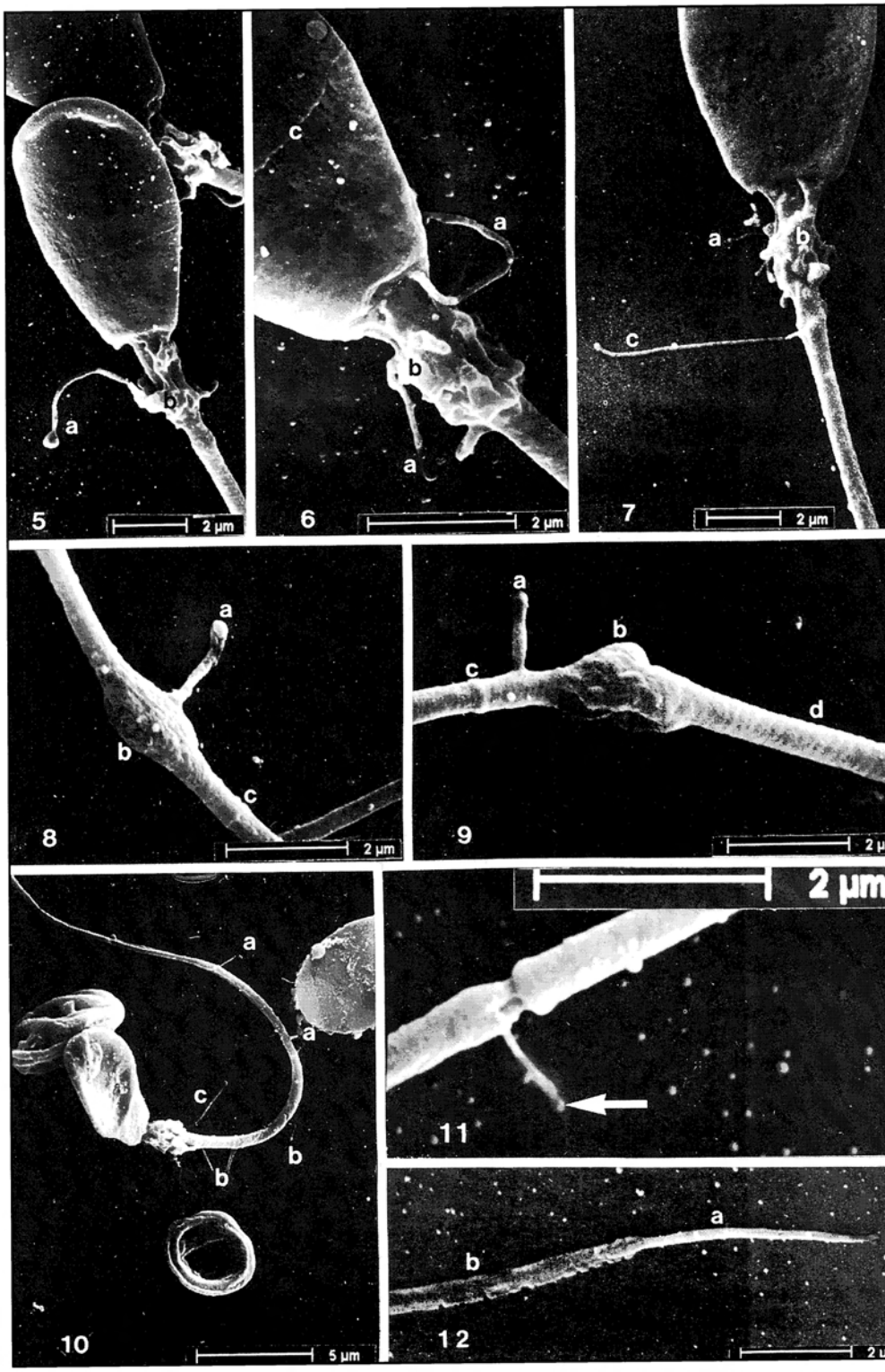
The acrosome formed approximately two thirds of the apical region of the head, with a prominent one-sided thickening on the periphery of the head in one plane. This thickening was the largest on the frontal periphery of the head and decreased along the sides until it disappeared close to the equator. The latter region sometimes exhibited small irregular millings or protrusions under high magnification and distinctly divided the acrosome and the post acrosomal dense lamina (PADL) from each other (Fig. 3).

Fig. 1. Normal impala sperm from the cauda epididymis divided into a head (a) and a flagellum consisting of a neck (b), midpiece (c), principal-piece (d) and end-piece (e). A cytoplasmic droplet (f) usually occurs on the midpiece close to the annulus, the separation between the midpiece and principal-piece.

Fig. 2. The paddle-shaped head showing an one-sided acrosomal thickening (a) and the equator (b) separating the acrosome from the PADL (c). Note the flattened base of the head (arrow), the neck region (d) between the two arrows, the *pars spiralis* (c), the cytoplasmic droplet (f) and the annulus (g).

Fig. 3. Sperm showing the irregular knurling of the equator (arrow) which separates the acrosome (a) from the post acrosomal lamina (b).

Fig. 4. Edge-on view showing the thickness (a) of the flattened head. Note the *pars spiralis* (arrow head) partially visible beneath the plasmalemma of the midpiece, and the cytoplasmic droplet (b) close to the annulus (c).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The flagellum

The flagellum tapered from the neck to the end-piece (Fig. 1).

The mean measurements with standard deviations for 95 sperm were: total length: 51.72 ± 2.36 µm; length of midpiece (neck included): 10.39 ± 0.7 µm; midpiece thickness: 0.62 ± 0.11 µm; length of principal-piece: 38.27 ± 2.2 µm; thickness of principal-piece: 0.46 ± 0.09 µm; length of end-piece: 3.32 ± 0.7 µm; length of cytoplasmic droplet: 2.51 ± 0.31 µm; thickness of cytoplasmic droplet (center): 1.64 ± 0.44 µm.

Midpiece and neck

Scanning electron micrographs clearly revealed the connection between the head and neck. The neck region appeared smooth and the neck did not differ in thickness from the midpiece (Fig. 2). Subsurface structures were in general not clearly visible beneath the plasmalemma except the *pars spiralis* which was sometimes distinctly discernible (Figs. 4 & 9).

Approximately 78% of the sperm possessed a distal cytoplasmic droplet on the midpiece (Figs. 2 & 4). In a few cases the cytoplasmic droplet occurred in the neck region. In the latter case these droplets occasionally exhibited appendages (Figs. 5-7). In some cases these appendages were also present on cytoplasmic droplets located near the annulus (Fig. 8) where they were normally shorter and more robust. Transmission electron micrographs showed that these appendages had the same plasmalemma as the cytoplasmic droplet and that their content was of cytoplasmic origin (Ackerman *et al.* 1996, in press). Similar appendages were sometimes found on the midpiece and principal-piece (Figs. 9-11). In these cases transmission electron micrographs also revealed that the content of the appendages was enclosed by the plasmalemma of the relevant structure and continuous with the content of the midpiece or principal-piece involved. Slender appendages that could rather be described as filaments were sometimes observed on the midpiece and principal-piece.

The annulus demarcating the border between the midpiece and principal-piece was clearly visible at low magnifications since the principal-piece was slightly thinner at this point than the midpiece. At higher magnifications the annulus exhibited a slightly thickened ring at the distal end of the midpiece (Figs. 4 & 9).

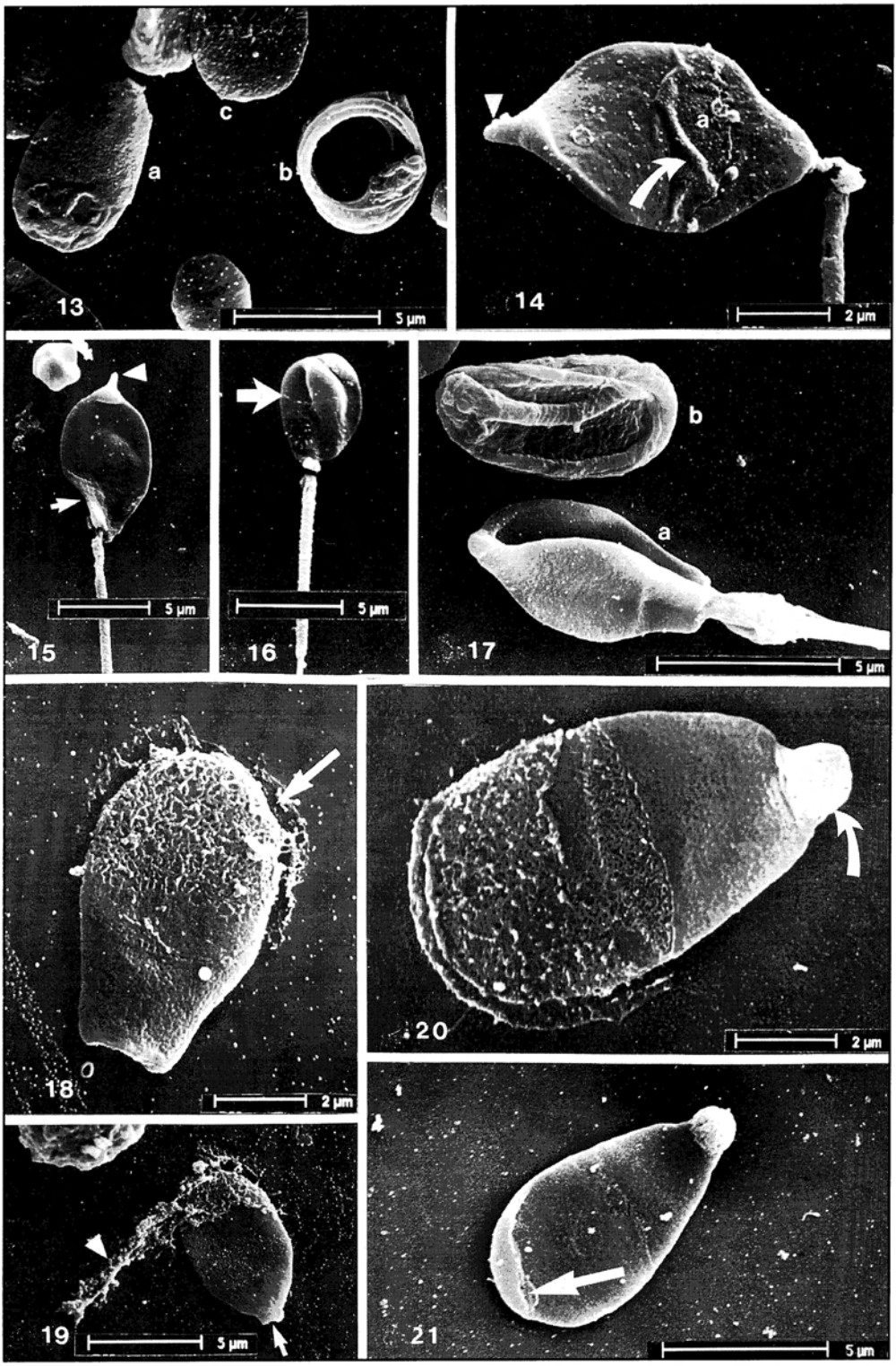
The principal-piece

The principal-piece of the flagellum appeared relatively smooth; it was much longer than the midpiece and became thinner towards the end-piece (Fig. 1). Appendages similar to those described for the midpiece were sometimes observed (Figs. 10 & 11).

The end-piece

The end-piece of the flagellum was clearly distinguishable, even at relatively low magnifications (1000 x) appearing much thinner

Fig. 5. Slender appendage (a) of a cytoplasmic droplet (b) in the neck region.
Fig. 6. Short appendages (a) of a cytoplasmic droplet (b) in the neck region. Note the characteristic knurling of the equator (c).
Fig. 7. Shorter appendages (a) of a cytoplasmic droplet in the neck area (b). The long appendage (c) probably developed from the distal part of the cytoplasmic droplet but could be an appendage of the midpiece.
Fig. 8. A more robust appendage (a) of the cytoplasmic droplet (b) close to the annulus (c).
Fig. 9. Appendage (a) of the midpiece between the cytoplasmic droplet (b) and the annulus (c). Note also the *pars spiralis* (d) of the midpiece.
Fig. 10. Appendage of the principal-piece (a), midpiece (b) and cytoplasmic droplet (c).
Fig. 11. Appendage (arrow) of a principal-piece with abnormal fibrous sheath close to the annulus.
Fig. 12. End-piece (a) half the thickness of the principal-piece (b).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(± 50%) than the principal-piece. It resembled a whip-lash. (Figs. 1 & 12).

Abnormal sperm

The abnormalities of impala sperm were documented in the micrographs and legends of this study. A low incidence of flagellum anomalies was generally observed (Figs. 13-14).

Discussion

In respect of shape and size mammalian sperm exhibit great variation but they all have the same set of cellular organelles and are based on a common design (Olsen *et al.* 1991). Additional observations (Ackerman *et al.* 1994) show that the general morphology of mammalian sperm is very similar. Unpublished observations by the senior author on the sperm of *Alcelaphus buselaphus* (red hartebeest), *Tragelaphus strepsiceros* (kudu) and *Damaliscus dorcas phillipsi* (blesbok) confirm this similarity which is expected since they are all members of the Bovidae (Skinner & Smithers 1990).

The head of the impala sperm was slightly longer (7.59 µm) than that of the buffalo (5.9 µm) (Ackerman *et al.* 1994) but shorter than that of the bull (9.0 µm) (Saacke & Almquist 1964). The small but typical differences between sperm of closely related species confirm the earlier observations by Wagner and Leuckhart (Mann & Lutwak-Mann 1981). The dorso-ventrally flattened head of the impala sperm is shared by many other mammals (Saacke & Almquist 1964; Fawcett 1975; Tingari 1991; Ackerman *et al.* 1994)

The apical horseshoe-shaped thickening of the acrosome on the periphery and only on the one plane of the head, is very similar to those observed in other species. Saacke & Almquist (1964) and Barth & Oko (1989) found the same phenomenon in bull (*Bos tauris*) sperm and described it as a hook-shaped apical body bent back over itself. Lipping, an abnormality observed in sperm of impala and buffalo can easily be confused with this condition.

In most cases the equator of the sperm head is clearly visible. The appearance varies from a row of small pore-like cups to a ring of irregular knurlings or protrusions which demarcated the acrosome from the PADL. These variations can either be attributed to artefacts of processing or to structural changes in the end connections of the acrosome.

A perfect side-view of sperm heads is seldomly obtained because they usually adsorbed dorso-ventrally on the mica substrate during the preparation. The thickness of the head as determined by SEM was therefore questionable. Sagittal sections of

Fig. 13. Loose head with the acrosome abnormally thickened and showing crater defects (a); ring-shaped form of a Dag defect; the head is visible beneath the coiled tail (b); part of a sperm head with an acrosome showing signs of disintegration (c).

Fig. 14. Nipple defect (arrow head) with abnormally thickened, acrosome (arrow) in the abnormal equatorial segment (a). Note the abnormal pear-shaped head and abnormal attachment of the flagellum.

Fig. 15. Sperm showing a nipple defect (arrow head) and a post acrosomally deformed head (arrow).

Fig. 16. Ridging of the nucleus; the acrosome follows the abnormal contour of the nucleus and covers only one third of the head surface area in stead of the normal 60%. Note the abnormal position of the equator (arrow).

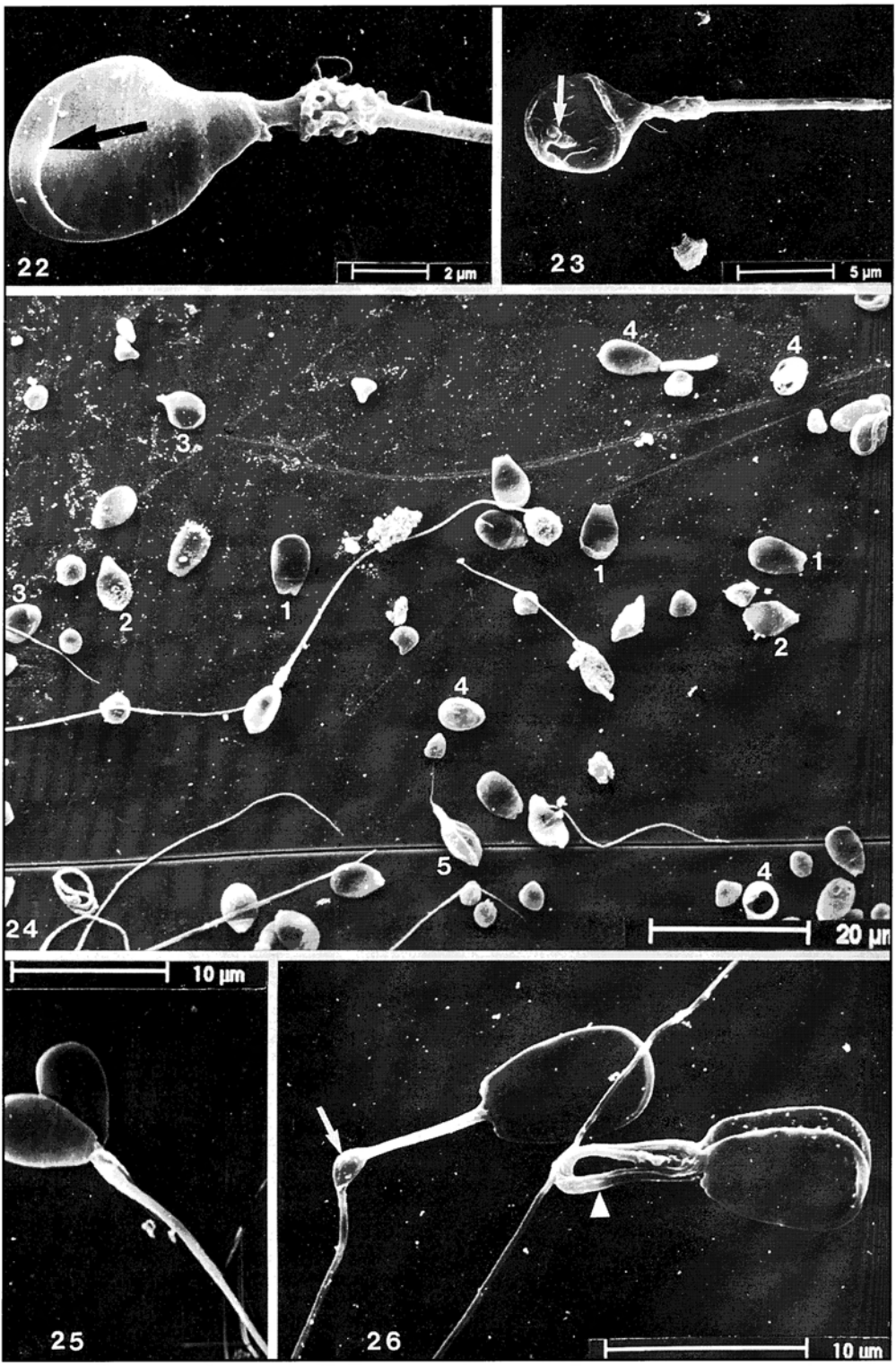
Fig. 17. A typical example of ridging observed frequently (a). A sperm showing the Dag defect (b).

Fig. 18. Loose head. No loose flagella were found in this sample. Note the disintegrating acrosome (arrow).

Fig. 19. Loose head with a narrow head base (arrow). The disintegrated acrosome is almost disposed of (arrowhead).

Fig. 20. Tail-stump defect (arrow). The head shows a disintegrated acrosome. A knob (stump) developed in place of the flagellum.

Fig. 21. Tail-stump defect. The head shows an abnormal acrosome thickening (arrow) covering the whole apical area of the head.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the head observed by TEM (Ackerman 1995; Ackerman *et al.* 1996, in press) showed a mean thickness of 0.65 ± 0.05 µm while a measurement of 0.89 ± 0.15 µm was obtained with SEM. Confirmation of the thickness of sperm head measurements obtained by SEM with TEM is therefore advisable in this instance.

Immature sperm from the testis and corpus epididymis usually exhibits a cytoplasmic droplet around the neck. The presence of the cytoplasmic droplet around the midpiece of sperm from the cauda epididymis is considered to be a normal phenomenon (Bonet 1990). However, its presence around the midpiece of sperm in the ejaculate is considered to be an abnormal condition in humans. This abnormality is used as a morphological characteristic for the evaluation of sperm (Holstein *et al.* 1988; Dadoune 1988; Menkveld *et al.* 1990). The general contention is that the droplet will occur further down in more mature sperm closer to the annulus and eventually disappear.

The appendages observed on the cytoplasmic droplet and on other regions of the midpiece and principal-piece have, as far as we could ascertain, not been described before except for our own observations on buffalo sperm (Ackerman *et al.* 1994). Bonet *et al.* (1993) described a filament-like cytoplasmic extension between the head and connecting piece of sperm. This structure was not an integral part of the cytoplasmic droplet but could be compared to filament-like appendages which we sometimes observed on the midpiece and principal-piece of impala and buffalo sperm.

Accepting that the cytoplasmic droplet migrates along the neck and midpiece, it probably loses the more delicate appendages first before losing the more robust ones later on. Consequently the cytoplasmic droplet will normally have no appendages when occurring close to the annulus or will contain only the more robust structures. It is expected that the sperm will lose the appendages of the cytoplasmic droplet, the midpiece and principal-piece on its way to the ejaculate. Thus appendages will normally be absent in the ejaculate. The appendages of the cytoplasmic droplet are probably formed when the connection of the remaining droplet of the sperm to the cytoplasm of the Sertoli cell is stretched and broken during the process of spermiogenesis (Fawcett & Phillips 1969; Mann & Lutwak-Mann 1981).

Evaluation of human semen samples normally shows a heterogeneous sperm morphology while evaluation of the semen of other animals reveals generally homogeneous sperm morphology (Menkveld *et al.* 1991). Just as mammals exhibit similarities in terms of normal morphological features, they also exhibit similarities in sperm abnormalities. This holds true for abnormalities observed by scanning as well as transmission electron microscopy (Nicander & Bane 1966; Ross *et al.* 1973; Coubrough & Soley 1981; Holstein *et al.* 1988; Oettlé & Soley 1988; Menkveld *et al.* 1991; Bonet *et al.* 1993).

Minute abnormalities of the acrosome, nucleus and other internal structures require transmission electron microscopical evaluation while external flagellum anomalies are easily detected by scanning electron

Fig. 22. Pear-shaped head with an abnormal acrosome thickening (arrow) and uneven distribution.
Fig. 23. Pear-shaped head with a severe thickening of the acrosome (arrow).
Fig. 24. All the sperm heads in this sample were abnormal. Note the loose head (1), tail-stump head with disintegrating acrosome (2), pear-shaped tail-stump head (3), Dag defects of various degrees (4) and bizarre ridging (5).
Fig. 25. Double head with common flagellum.
Fig. 26. Double head with a lengthy, coiled common flagellum. Dag defect indicated by arrow head. Note the angularly bent flagellum (arrow) of the second sperm.

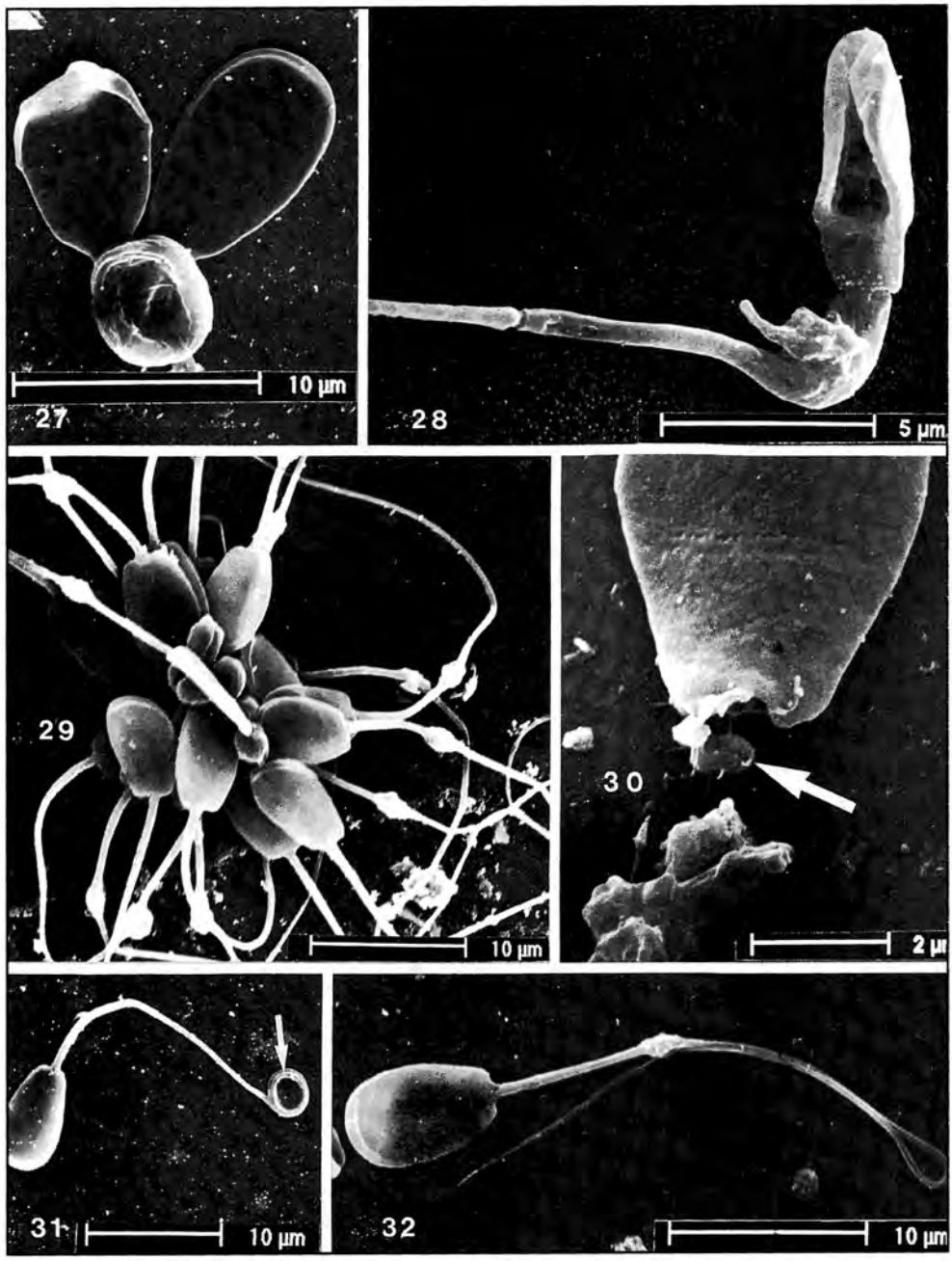

Fig. 27. Double head with a simple, coiled up common flagellum (Dag defect).
Fig. 28. A bizarre head.
Fig. 29. Agglutination of sperm heads.
Fig. 30. Unidentified structure (arrow) shown in the neck region of a sperm with a broken off flagellum.
Fig. 31. Terminally coiled flagellum (arrow).
Fig. 32. Flagellum forming a loop (simple Dag defect) and folding back over itself. Sometimes cytoplasmic material occurs in the loop. The plasmalemma surrounds the region where the flagellum joins on itself. This may be the precursor of the Dag defect.

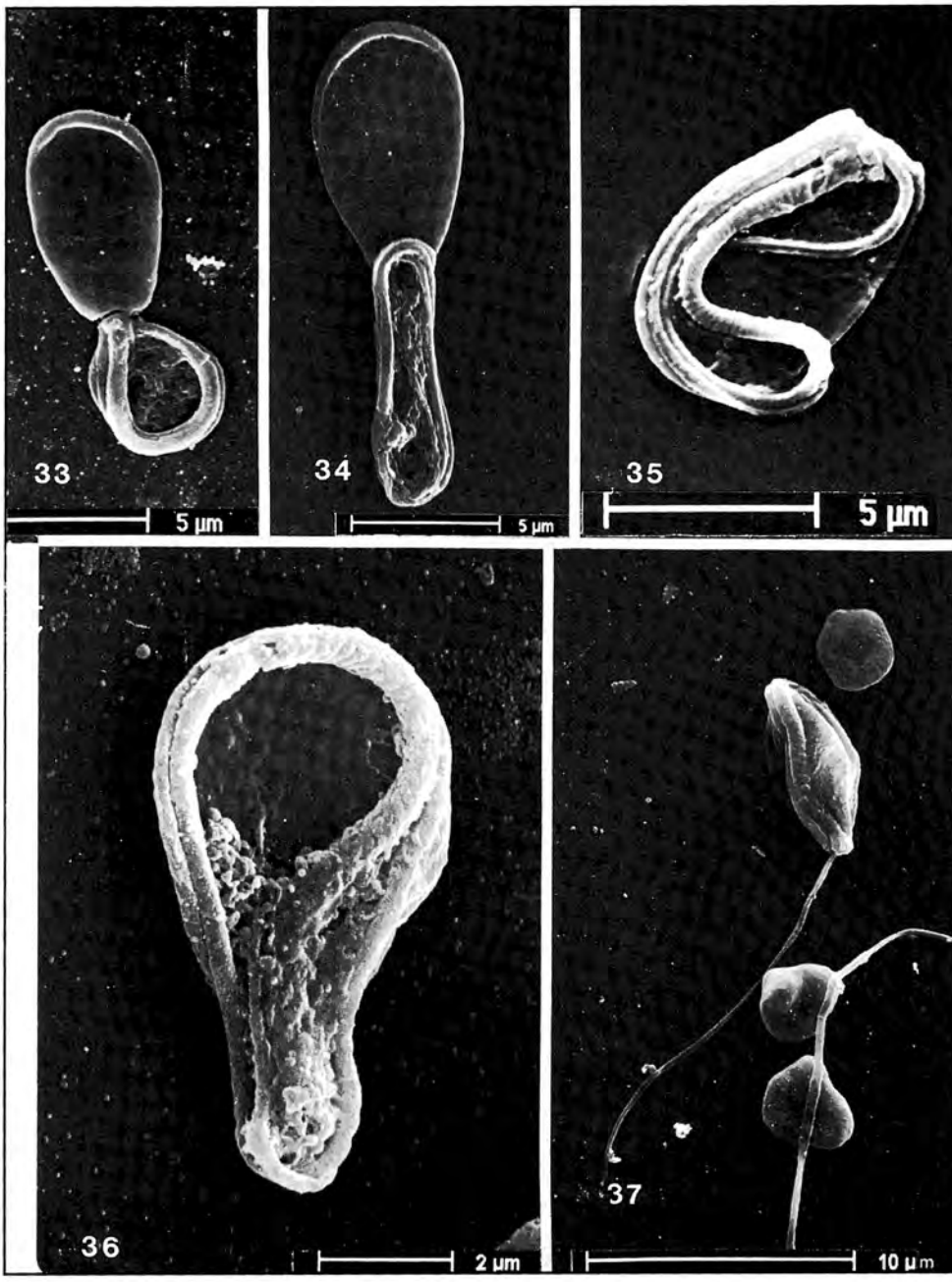

Fig. 33. A Dag defect may be associated with different degrees of coiling of the flagellum inside the plasmalemma. In this case the flagellum is coiled up loosely.
Fig. 34. A Dag defect with the flagellum coiled up lengthwise.
Fig. 35. A Dag defect with the flagellum coiled up loosely on the head.
Fig. 36. A Dag defect with the flagellum coiled around the periphery of the head. This variation and the examples shown in figures 35 and 36 were the most commonly observed.
Fig. 37. A Dag defect with the midpiece coiled around the periphery of the head but the principal-piece and end-piece dangles free.

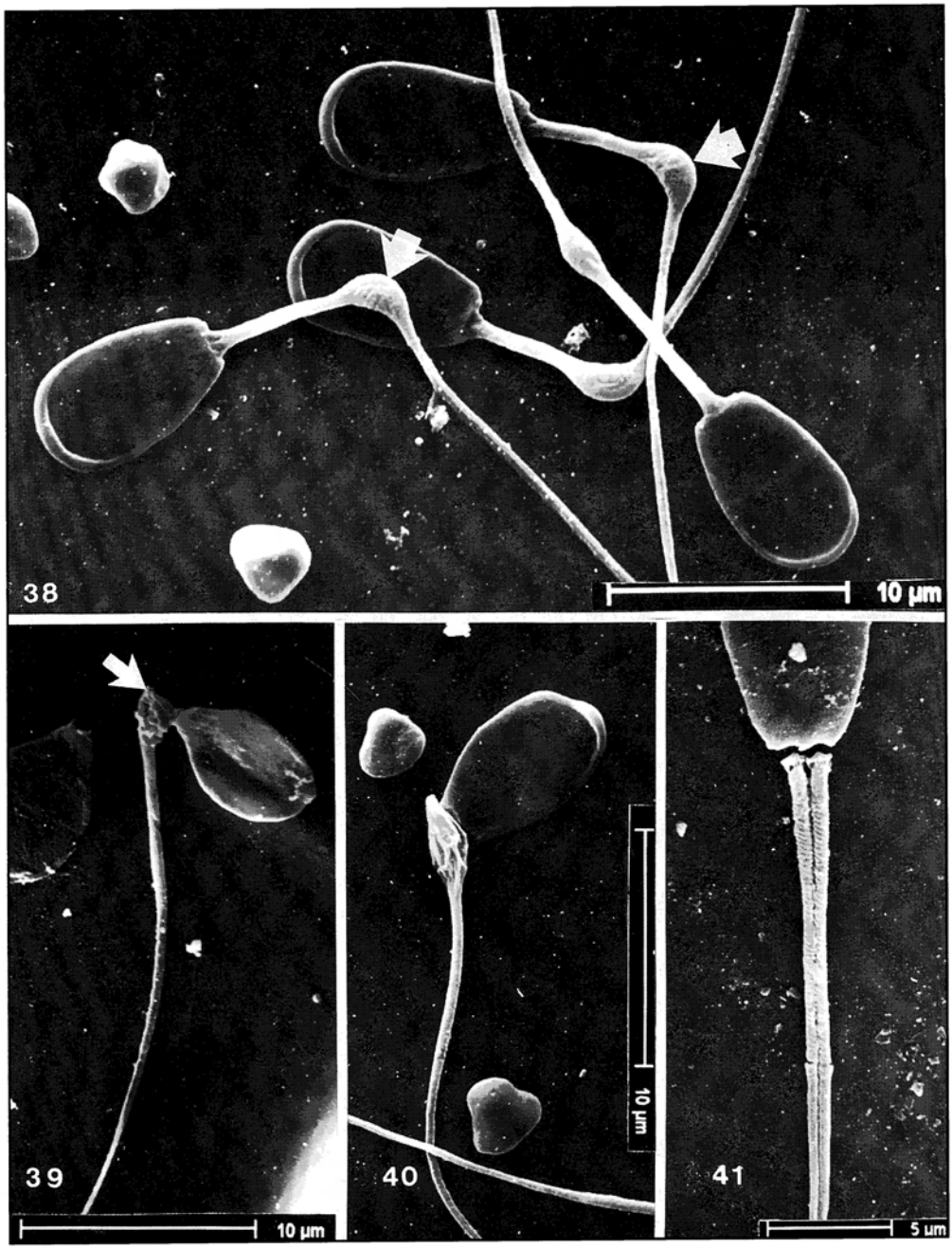

Fig. 38. Angularly bent flagella in the midpiece region beneath the cytoplasmic droplet (arrow).
Fig. 39. Angularly bent neck (arrow).
Fig. 40. Abnormal implantation of the flagellum.
Fig. 41. Double implantation of the flagellum. The flagella possibly share a common plasmalemma.

microscopy. Scanning electron microscopical evaluation of buffalo sperm (Ackerman *et al.* 1994) showed 87.4% of the sperm samples of healthy mature buffalo in the Kruger National Park to be without observable, known flagellum abnormalities. In the case of impala sperm flagella, 93.4% appear to be free from the abnormalities reported here.

Prominent abnormalities of mammalian sperm are relatively easy to detect by scanning electron microscopy. In particular, the presence of loose sperm heads (decapitated sperm head defect) are often used by fertility clinics to evaluate sperm morphology. This defect develops during the spermatid phase of spermiogenesis (Bacetti *et al.* 1984), and should be observable, if present, in sperm from the cauda epididymis. The occurrence of this anomaly is subject to different views: this can be a sperm with a disconnected flagellum (Fig. 18) or it can be a sperm of which the flagellum developed separately from the head or not at all (Holstein *et al.* 1988). The flagellum could also have been lost in the corpus or caput epididymis due to a defective connection. Centrifugation of semen during preparation could also have resulted in broken-off flagella. The decapitated head defect was described by Blom & Birch-Andersen (1970) for the bull and by Perotti *et al.* (1981) for humans. This defect is characterised by the occurrence of loose heads in the ejaculate with an equal number of separate flagella which are in most cases still motile. However, such a severe case was not observed in sperm from the cauda epididymis of the impala; only small numbers were observed in a few animals (Figs. 13, 18 & 19). The detection of the tail-stump defect, on the other hand, does not present any difficulties since a clearly distinguishable stump develops in place of the flagellum (Figs. 20, 21 & 24).

References

ACKERMAN, D.J. 1995. Die ultrastruktuur van sperme van die rooibok *Aepyceros melampus* (Lichtenstein, 1812) in die Nasionale Krugerwildtuin met spesiale verwysing na die invloed van koperbesoedeling. Ph.D thesis, University of Stellenbosch, Stellenbosch.

ACKERMAN, D.J., A.J. REINECKE & H.J. ELS. 1994. The ultrastructure of spermatozoa of African buffalo (*Syncerus caffer*) in the Kruger National Park. *Animal Reproduction Science* 36: 87-101.

ACKERMAN, D.J., A.J. REINECKE & H.J. ELS. 1996. A transmission electron microscopic study of impala (*Aepyceros melampus*) sperm from the Kruger National Park. *Koedoe* 39(2): 105-120.

BACCETTI, B., M.G. SELMI & P. SOLDANI. 1984. Morphogenesis of "decapitated" spermatozoa in a man. *Journal of Reproduction & Fertility* 70: 395-397.

BACCETTI, B., A.G. BURRINI, S. GAPITANI, G. COLLODEL, E. MORETTI, P. IOMBONI & T. RENIERI. 1993. Notulae seminologicae. 1. New combinations of Kartagener's syndrome. *Andrologia* 25: 325-329.

BARTH, A.D. & R.J. OKO. 1989. *Abnormal morphology of bovine spermatozoa*. Ames: Iowa State University.

BARTHÈLEMY, C., G. FRICOT, S. HAMAMAH, C. LEBOS, J. LANSAC & M.J. THARANNE. 1992. Ultrastructural comparison of human spermatozoa along a Percoll density gradient. *International Journal of Fertility* 37(6): 362-367.

BLOM, E. & A. BIRCH-ANDERSEN. 1970. Ultrastructure of the "decapitated sperm defect" in Guernsey bulls. *Journal of Reproduction and Fertility* 23: 67-72.

BONET, S. 1990. Immature and aberrant spermatozoa in the ejaculate of *Sus domesticus*. *Animal Reproduction Science* 22: 67-80.

BONET, S., M. BRITZ & A. FRADERA. 1993. Ultrastructural abnormalities of boar spermatozoa. *Theriogenology* 40: 383-396.

CONRADIE, E., P.J. SELBY, K. COETZEE & R. MENKVELD. 1988. The comparison of techniques for the preparation of spermatozoa for scanning electron microscopy. *Medical Technology South Africa* 2(2): 152-154.

COUBROUGH, R.I. & J.T. SOLEY. 1977. An acrosome defect in a subfertile bull. *Proceedings Electron Microscopy Society of Southern Africa* 7: 117-118.

COUBROUGH, R.I. & J.T. SOLEY. 1981. The "Dag defect" in mammalian spermatozoa. *Proceedings Electron Microscopy Society of Southern Africa* 12:75-76.

DADOUNE, J.J. 1988. Ultrastructural abnormalities of human spermatozoa. *Human Reproduction* 3(3): 311-318.

DADOUNE, J.J. & M.A. FAIN-MAUREL. 1977. A routine technique for processing human ejaculate spermatozoa for scanning electron microscopy with special reference to their abnormal forms. *Biology of the Cell* 29: 215-218.

DE YI LUI & H.W.G. BAKER. 1992. Tests of human sperm function and fertilization *in vitro*. *Fertility and Sterility* 58(3): 465-483.

DOTT, H.M. & J.D. SKINNER. 1989. Collection, examination and storage of spermatozoa from some South African mammals. *South African Journal of Zoology* 24(2): 151-160.

FAIRALL, N. 1971. Die geslagsfisiologie van die rooibok (*Aepyceros melampus* Licht.) D.Sc (Agric) thesis, University of Pretoria, Pretoria.

FAWCETT, D.W. 1975. The mammalian spermatozoon. *Developmental Biology* 44: 394-436.

FAWCETT, D.W. & D.H. PHILLIPS. 1969. Observations on the release of spermatozoa and on changes in the head during passage through the epididymis. *Journal of Reproduction and Fertility* (Suppl.) 6: 405-418.

FUJITA, T., M. MIYOSHI & J. TOKUNAGA. 1970. Scanning and transmission electron microscopy of human ejaculate spermatozoa with special reference to their abnormal forms. *Zeitschrift für Zellforschung* 105:483-497.

HOLSTEIN, A.F., E.C. ROOSEN-RUNGE & C. SCHIRREN. 1988. *Illustrated pathology of human spermatogenesis.* Berlin: Grosse.

KRUGER, T.F., R. MENKVELD, F.S.H. STANDER, C.J. LOMBARD, J.P. VAN DER MERWE, J.A. VAN ZYL & K. SMITH. 1986. Sperm morphology features as a prognostic factor *in vitro* fertilization. *Fertility and Sterility* 46: 1118-1123.

LIAKATAS, M.D., A.E. WILLIAMS & T.B. HARGREAVE. 1982. Scoring sperm morphology using the scanning electron microscope. *Fertility and Sterility* 38(2): 227-231.

MAHADEVAN, M.H. & A.O. TROUNSON. 1984. Relationship of fine structure of sperm head to fertility of frozen human semen. *Fertility and Sterility* 41(2): 287-293.

MANN, T. & C. LUTWAK-MANN. 1981. *Male reproductive function and semen*. Berlin: Springer.

MENKVELD, R., F.S.H. STANDER, T.J.V.W. KOTZE, T.F. KRUGER & J.A. VAN ZYL. 1990. The evaluation of morphological characteristics of human spermatozoa according to stricter criteria. *Human Reproduction* 5: 586-592.

MENKVELD, R., R.J. SWANSON, E.E. OETTLÉ, A.A. ACOSTA, T.F. KRUGER & S. OEHNINGER. 1991. *Atlas of human sperm morphology*. Baltimore: Williams & Wilkens.

NICANDER, L. & A. BANE. 1966. Fine structure of the sperm head in some mammals with particular reference to the acrosome and the subacrosomal substance. *Zeitschrift für Zellforschung* 72: 496-515.

OETTLÉ, E.E. & J.T. SOLEY. 1988. Sperm abnormalities in the dog: a light and electron microscopic study. *Veterinary Medical Review* 59: 28-70.

OLSEN, G.E. & V.P. WINFREY. 1991. A comparison of mammalian sperm membranes. Pp.51-62. *In*: B.S. DUNBAR & M.G. O'RAND (eds.). *A comparative overview of mammalian fertilization, 3*. New York: Plenum Press.

PEROTTI, M.L., A. GIAROLA & M. GIORIA. 1981. Ultrastructural study of the decapitated sperm defect in an infertile man. *Journal of Reproduction and Fertility* 63: 543-549.

REINECKE, S.A., A.J. REINECKE & M.L. FRONEMAN. 1995. The effects of dieldrin on the sperm ultrastructure of the earthworm *Eudrilus eugeniae* (Oligochaeta). *Environmental Toxicological Chemistry* 14(6): 961-965.

ROSS, A., S. CHRISTIE & P. EDMOND. 1973. Ultrastructural tail defects in the spermatozoa from two men attending a subfertility clinic. *Journal of Reproduction and Fertility* 32: 243-251.

SAACKE, R.G. & J.O. ALMQUIST. 1964. Ultrastructure of bovine spermatozoa. I. The head of normal ejaculated sperm. *American Journal of Anatomy* 115: 143-162.

SKINNER, J.D. 1971. The sexual cycle of the impala ram *Aepyceros melampus* Lichtenstein. *Zoologica Africana* 6(1): 75-84.

SKINNER, J.D. & R.H.N. SMITHERS. 1990. *The mammals of the Southern African subregion.* Pretoria: University of Pretoria.

SOLEY, J.T. 1992. A histological study of spermatogenesis in the ostrich (*Struthio camelus*). Ph.D thesis, University of Pretoria, Pretoria.

THILANDER, G., I. SETTERGREN & L. PLÖEN. 1985. Abnormalities of testicular origin in the neck region of bull spermatozoa. *Animal Reproduction Science* 8: 151-157.

TINGARI, M.D. 1991. Studies on camel semen, III. Ultrastructure of the spermtozoon. *Animal Reproduction Science* 26: 333-344.

VAN DER HORST, G., P.T. CURRY, R.M. KITCHIN, W. BURGESS, E.T. THORNE, D. KWIATKOWSKI, M. PARKER & R.W. ATHERTON. 1991. Quantitative light and scanning electron microscopy of ferret sperm. *Molecular Reproduction and Development* 30: 232-240.

WILLIAMS, G. 1987. "Tail-stump" defect affecting the spermatozoa of two Charolais bulls. *Veterinary Record* 121: 248-250.