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; Conradi 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; Barthélémé 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
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; Oetlé & 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), Oetlé & 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).
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 mid-piece (neck included): 10.39 ± 0.7 μm; mid-piece 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
(± 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 morpholgy 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 taurus) 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
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. 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 microposcopy 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


