Ultra-imaging in applied animal andrology: The power and the beauty

John T. Soley[a],* and Lizette du Plessis[b]

[a]Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, South Africa
[b]Electron Microscope Unit, Department of Anatomy and Physiology, Faculty of Veterinary Science, University of Pretoria, Onderstepoort 0110, South Africa

*Corresponding author. john.soley@up.ac.za

Highlights

- Sperm ultrastructure remains of clinical value, providing relevant complementary data.
- Knobbed acrosome defect in cheetah and rhinoceros described.
- Perinuclear theca illustrated in ratite spermatids.
- Technique assists in establishing phylogenetic and structural/functional relationships.
- Aesthetic qualities of male gamete ultrastructure highlighted.

Abstract

Ultrastructural studies of the male gamete provide relevant complementary data of value for the clinical assessment of semen quality and assist in determining phylogenetic and structural/functional relationships. This is illustrated using semen samples and testicular material from vulnerable wild animals (cheetah and rhinoceros), commercially exploited exotic birds (ratites and tinamou) and poultry (chicken and duck). Transmission electron microscopy (TEM) was employed to record sperm and spermatid ultrastructural detail on a comparative basis. The power of the technique was demonstrated using normal and abnormal (the knobbed acrosome defect) formation of the acrosome in the cheetah and rhinoceros. The structural similarities of the defect across species was apparent. The determination of phylogenetic associations was illustrated by comparing structural characteristics between ratites (ostrich, emu and rhea), the tinamou and poultry (chicken and duck), highlighting the morphological peculiarities evident in the midpiece and proximal principal piece of the sperm tail. A clear distinction was obvious between the ratites and tinamou on the one hand and the Galliform and Anseriform birds on the other. The potential power of using molecular techniques in conjunction with ultrastructural studies to explain structural/functional relationships was demonstrated by describing a transient elaboration of the perinuclear theca that occurs during a specific stage of spermiogenesis in ratites, and which can only be imaged using TEM. The inherent aesthetic appeal of the structurally complex normal and defective male gamete was also emphasised.

Keywords: Ultrastructure; Sperm; Phylogeny; Avian; Rhinoceros; Cheetah

1. Introduction

The discovery and description of the male gamete or spermatozoon in 1677 through the combined efforts of van Leeuwenhoek and Ham, and the subsequent illustration of human and canine (or rabbit and canine, depending on the reference source) sperm the following year in the Philosophical Transactions of the Royal Society of London (Meyer, 1938; Kempers, 1976), marked the beginning of man's fascination with this remarkable motile cell. These tiny "animalcules", as van Leeuwenhoek described them, with lashing tail and conspicuous head, lent themselves to artistic portrayal, from the remarkably accurate drawings of van Leeuwenhoek himself, to the fanciful depictions of followers of the homunculus theory who believed that the sperm head contained a pre-formed embryo of the species (Meyer, 1938; Andrade-Rocha, 2017). Interestingly, as Mortimer (2018) points out, some modern-day depictions of spermatozoa, influenced by artistic licence, have "led to many misconceptions about what spermatozoa look like and how they are constituted", a statement supported by Pacey (2018). Following the work of Spallanzani a century later that corroborated the findings of van Leeuwenhoek (Castellani, 1973), incremental improvements and sophistication of the compound microscope led to more accurate portrayals of sperm structure. Gustaf Retzius, for example, while meticulously describing the structure of over 400 animal species, provided exceptionally detailed illustrations of comparative sperm morphology. These studies, published in Biologische Untersuchungen, Neue Folge from 1904 laid the foundation for the determination of phylogenetic relationships based on sperm structure (Afzelius, 1995). However, it was only following the development of electron microscopes in the early 1940's and the rapid commercialisation of these instruments that the true structural intricacy of the sperm cell was revealed. The increased resolving power of this technique enabled researchers to accurately describe sperm ultrastructure, leading to a better understanding of structural/functional relationships, as well as providing additional data relevant to the assessment of sperm anomalies and the determination of phylogenetic relationships. Birkhead and Montgomerie (2009) provide a more comprehensive account of relevant milestones in the study of comparative sperm structure.

Sperm morphology forms one of the essential clinical parameters in the assessment of semen quality (see Mortimer, 2018) and numerous studies on various species have detailed "normal" sperm structure as a prerequisite to identifying abnormal forms. These studies have ranged from purely descriptive investigations of sperm structure in mammals and birds based on light and electron microscopy to sometimes complex morphometric analysis of head shape and size (Santiago-Moreno et al., 2016), whereas "strict criteria" are employed for the morphological assessment of human sperm under a light microscope with bright field illumination (Kruger et al., 1986; Menkveld et al., 1990). The use of the term "normal" sperm has been challenged due to the subjectivity involved in determining normality and the term "ideal" proposed as a more accurate descriptor of sperm morphology (Mortimer, 2018). It has been pointed out that morphological changes to the sperm also take place in the female reproductive tract in a wide range of taxa prior to fertilization (Zhang et al., 2015; Lüpold and Pitnick, 2018). Despite these valid criticisms pertaining to the description of normal sperm structure, a general consensus has been established regarding what is considered to be normal/typical morphology for a given species (Mortimer, 2002).

Considering the above introductory remarks, this manuscript focusses on the value of ultrastructural studies within the context of applied animal andrology. The paper does not aim to provide a detailed description of sperm ultrastructure; this has been addressed in a number of comprehensive and concise reports and reviews (Fawcett, 1965, 1975; Zamboni, 1987; Jamieson, 2007; Pesch and Bergmann, 2006; Kaya et al., 2014; Mortimer, 2018). Neither does it present a chronological or historical account of the application of electron microscopy in the study of vertebrate spermatology. It attempts to define the continuing value and application of ultrastructural research in the expanding field of applied animal andrology. Although a variety of ultrastructural techniques are available, including immunocytochemical approaches, this account is based on routine transmission electron microscopy (TEM) (including fixation and preparation protocols (Asa et al., 1986; Phillips and Asa, 1989; Soley, 1997; Du Plessis and Soley, 2011, 2012)) as the most widely used routine form of ultrastructural study. Despite the criticism that morphological studies provide little complementary functional data (Lüpold and Pitnick, 2018), sperm ultrastructure forms the cornerstone around which sophisticated molecular techniques can be employed to determine functional relationships (this is illustrated towards the end of the paper). Additionally, electron microscopy continues to provide supportive evidence for a better understanding of sperm structure at the light microscopical level.

As a field of study, applied animal andrology is not restricted to work on production or companion animals. Climate change, loss of habitat and poaching have focussed attention on the need to preserve endangered species, specifically by utilising basic and applied research in reproductive biology (Comizzoli and Holt, 2019). Informed knowledge of species-specific sperm structure as part of semen assessment is essential when considering semen banking (the role of which in possibly saving the northern white rhino is pertinent (see Hildebrandt et al., 2018)) and the application of assisted reproductive technologies (ART). Similarly, the rise of niche farming enterprises utilising exotic birds and reptiles such as the ostrich, emu and crocodile illustrates the widening scope of applied animal andrology. To emphasise these points, this paper describes some salient ultrastructural features of sperm and spermatids from vulnerable wild species (cheetah and rhinoceros), exotic farmed birds (ostrich, emu and rhea), the tinamou and poultry (chicken and duck). Ethical approval for the work was obtained from the Animal Ethics Committee, Faculty of Veterinary Science, University of Pretoria.

2. Sperm ultrastructure: seeing is believing

The advent of TEM, when applied to the study of sperm structure and development, revealed the remarkable internal complexity of this specialised cell. The precise arrangement of its various components, for example, the aligned elements of the connecting piece and the intricate nature of the axoneme and associated structures, not only added to our understanding of sperm structure and function, but also engendered a certain aesthetic appeal. Moreover, by following the morphological transformations that occur during spermiogenesis, the processes involved in determining sperm structure, as well as the testicular origin of certain sperm defects, could be appreciated. A brief description of the structure, anomalies and formation of the acrosome observed in some of the animals and birds studied serves to illustrate this point.

Although open to debate (Khawar et al., 2019), the acrosome is described as a Golgi-derived structure with some distinct lysosome-like and secretory vesicle properties that, in a cap-like fashion, covers approximately the anterior two thirds of the sperm head in mammals. It is bound by a single membrane and divided into two basic parts, an anterior segment that varies in shape and size and a thinner equatorial region (Fig. 1a) which is not observed in some species, for example, the grasscutter (*Thryonomys swinderianus*) (personal observations) and bandicoot rat (*Bandicota indica*) (Dorman et al., 2014). In the animals studied, the anterior segment presented the general features associated with the acrosome of domesticated species. The apical tip of the acrosome in the cheetah was pointed while in the rhinoceros it was gently swollen (Fig. 1a). In the non-passerine birds the acrosome showed no regional specialisation and simply formed a short, cap-like structure covering the tapered tip of the nucleus (Fig. 7a). The outer acrosomal membrane lies beneath the plasmalemma and the inner acrosomal membrane abuts the nuclear envelope. Between the latter two membranes lies the sub-acrosomal space which in some species clearly demonstrates a thin layer of proteinaceous material, the perinuclear theca (discussed in more detail below). The homogeneous contents of the acrosome are moderately electron-dense (Fig. 1, Fig. 2) and represent various hydrolytic enzymes and a protein matrix (Toshimori, 2009; Khawar et al., 2019). The remaining part of the sperm head, the postacrosomal region, is characterised by the presence of a postacrosomal sheath (Toshimori, 2009) previously referred to as the postacrosomal dense lamina. (Fig. 1a). The function of these various components of the sperm head have been reviewed by Toshimori (2009) and Bedford (2014).

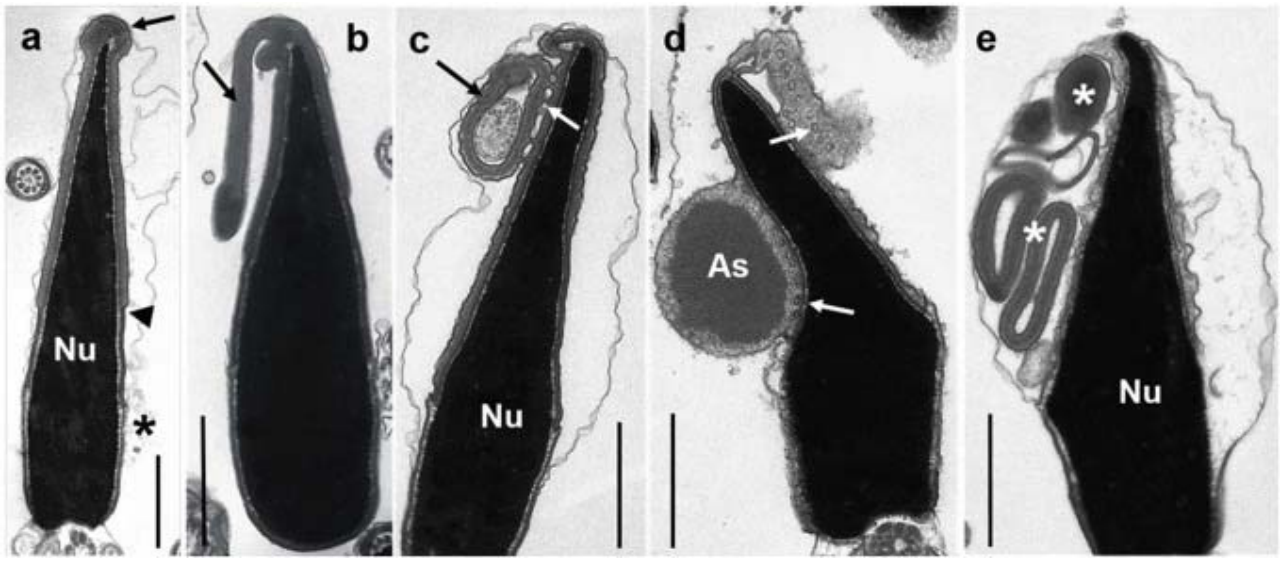

Fig. 1. Sections of normal and defective rhinoceros sperm showing the knobbed acrosome defect. (a) Cell displaying typical morphological features of the acrosome. Note the gently swollen apical ridge (arrow) and the junction between the anterior and equatorial regions (arrowhead) and equatorial region and post-acrosomal sheath (asterisk). (b) The knob is formed by a lip-like extension of the acrosomal apex (arrow). (c) The knob consists of excess acrosomal elements enclosing a cyst (arrow). The acrosome itself appears relatively normal. (d, e) The acrosome is missing and replaced by a sphere of acrosomal material (As) or by a combination of solid and rolled up acrosomal elements (asterisks). In each of the abnormal cells note the eccentric positioning of the defect, signs of vesiculation in c and d (white arrows), and the basic similarities across species when compared to Fig. 2 below. Nucleus (Nu). Bars =1 µm.

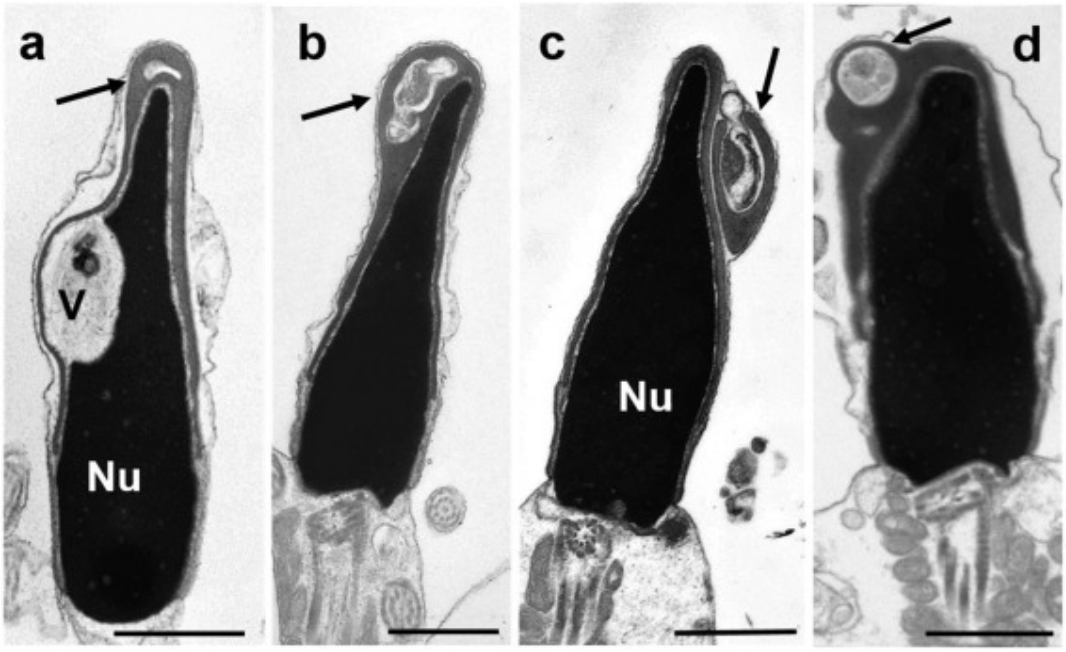

Fig. 2. The knobbed acrosome defect in cheetah sperm. (a) This cell displays a discrete, apically-positioned knob containing a small, compressed cyst (arrow). A large vacuole (V) indents the nucleus (Nu) and deforms the overlying acrosome. (b – d) Cells showing various forms of the defect. In each cell the knob (arrows) is eccentrically positioned, shows an accumulation of acrosomal material and displays a cyst filled with cellular material of unknown origin. Bars =1 µm.

Although "As spermatologists we all appreciate the beauty of [normal] spermatozoa" (Mortimer, 2002), abnormal forms have a certain allure. A particular sperm defect involving the acrosome has been referred to as the "knobbed acrosome defect". It has been categorised as a genetic sperm defect associated with impaired fertility/sterility and thus viewed as a major sperm defect (Blom, 1973; Chenoweth, 2005). This abnormality has been observed in various domestic species such as the bull, boar and ram (see Chenoweth, 2005 for relevant references) as well as in the impala (Ackerman et al., 1997). The defect appears to be prevalent in other wild species and has been identified in the rhinoceros and cheetah (personal observations). In both animals the defect displays the typical morphological features described in the literature (Blom, 1973; Cran and Dott, 1976; Toyama, 1993; Chenoweth, 2005). The apex of the sperm head shows an eccentric knob-like thickening of the acrosome, often accompanied by cystic inclusions filled with cellular material of unknown origin (Figs. 1b–e, 2 a-d). In some cells there appeared to be abnormal fusion of acrosomal membranes reminiscent of the vesiculation described by Jones (1973) (Fig. 1c,d). Occasionally the knob manifested as a slightly flattened apical extension of the acrosome that was reflected posteriorly in a lip-like fashion (Fig. 1b). In the rhinoceros the actual acrosome was occasionally absent but its contents abnormally concentrated to form a knob (Fig. 1d,e). In some defective cells in the cheetah the knob was situated at the apex of the acrosome and not eccentrically positioned (Fig. 2a). Whereas the testicular origin of the knobbed acrosome defect has been described by Toyama (1993) and confirmed in the cheetah (personal observations), other defects involving the acrosome may obviously also arise during spermiogenesis. This is illustrated in the cheetah where the forming acrosome at the Golgi phase of development is shared by two undivided spermatids (Fig. 4).

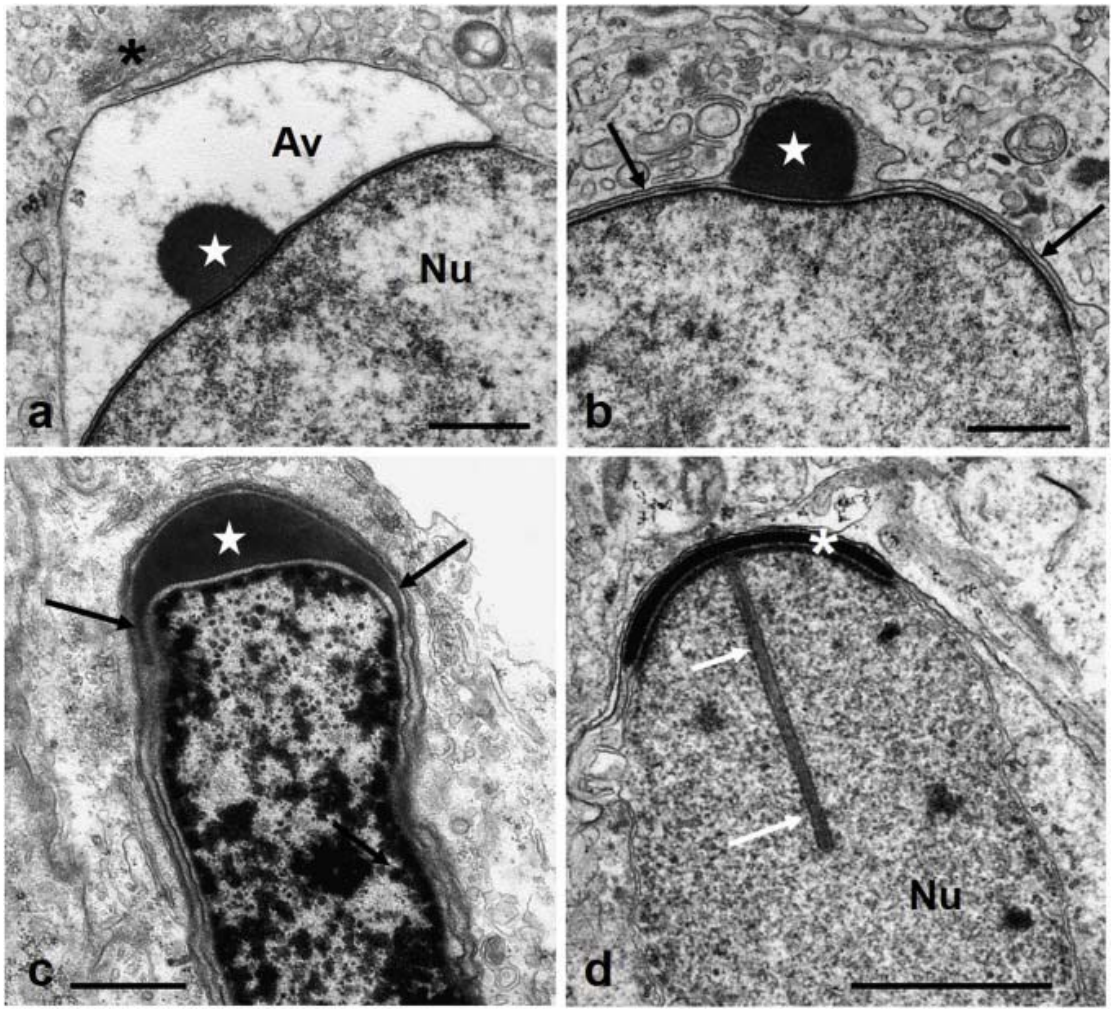

Fig. 3. Early development of the acrosome in the cheetah (a–c) and ostrich (d). (a) Cheetah spermatid at the Golgi phase of acrosome development. Note the closely associated elements of the Golgi complex (asterisk), the acrosome granule (star) and acrosome vesicle (Av). Nucleus (Nu). (b) Cap phase showing the collapsed and spreading acrosomal vesicle (arrows) and the intact acrosomal granule (star). Note the subacrosomal perinuclear theca compressed between the inner membrane of the forming acrosome and the nuclear envelope. (c) Early acrosome phase. The contents of the acrosomal granule (star) begin to spread (arrows) between the expanding membranes of the collapsed acrosomal vesicle. (d) Elongating ostrich spermatid showing the crescent-shaped acrosome (asterisk) at a stage of development similar to that of the acrosome phase in mammals. Note the elongating endonuclear canal (arrows) extending into the nucleus (Nu). Bars =0.5 µm.

Classically, in mammals, the acrosome develops through a number of stages as originally proposed by Clermont and Leblond (1955) and illustrated at the ultrastructural level by Fawcett (1994). During the Golgi phase proacrosomal granules emanating from the Golgi complex merge to form a large granule housed within an acrosomal vesicle. The membrane of the vesicle contacts and adheres to the subacrosomal perinuclear theca coating the nuclear envelope from where it spreads outwards from its point of contact (Fig. 3a). The collapse and expansion of the acrosomal vesicle around the anterior aspect of the nucleus characterises the cap phase. The acrosomal granule, situated at the pole of the nucleus, remains intact (Fig. 3b). It is during this phase in the boar that the changes characterising the knobbed acrosome defect materialise and progressively develop (Toyama, 1993). During

the acrosome phase the contents of the granule dissipate and spread throughout the cap formed by the acrosomal vesicle (Fig. 3c). At this stage during spermiogenesis in the ostrich the forming acrosome is accompanied by a gradually deepening endonuclear canal housing an acrosomal rod (perforatorium) (Soley, 1996) (Fig. 3d). During the maturation phase, extension of the acrosome around the anterior aspect of the nucleus and consolidation of its contents is completed.

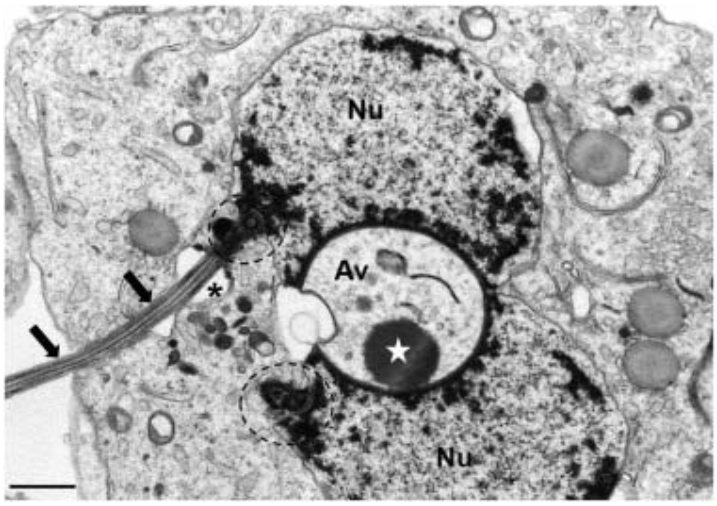

Fig. 4. The bizarre beauty of twin (undivided) cheetah spermatids sharing what appears to be an enlarged acrosomal vesicle (Av) containing a single acrosomal granule (star). The vesicle typically indents each nucleus (Nu) at the point of contact and the nuclear envelope correspondingly appears thickened due to the accumulation of chromatin material. Two centriolar complexes are present (encircled), one of which demonstrates the emerging flagellum (arrows). Flagellar canal (asterisk). Bar = 1µm.

Whereas the acrosome defect outlined above severely affects fertility, TEM has also been instrumental, along with complementary clinical data, in defining minor sperm defects. The "chipped midpiece" defect or apical mitochondrial aplasia (Bertschinger et al., 1988; Rocha et al., 2006) reported in bulls has also been observed in the cheetah. Compared to the normal mammalian midpiece (Fig. 5a), the defect typically displays one or more missing gyres of mitochondria in the vicinity of the connecting piece and anterior midpiece without disruption of the plasmalemma (Fig. 5b). Intriguingly, in the cheetah, segments of the fibrous sheath of the principal piece just beneath the annulus, were also missing (Fig. 5c). As both studies in the bull concluded that this defect did not affect fertility, it is classified as a minor sperm defect.

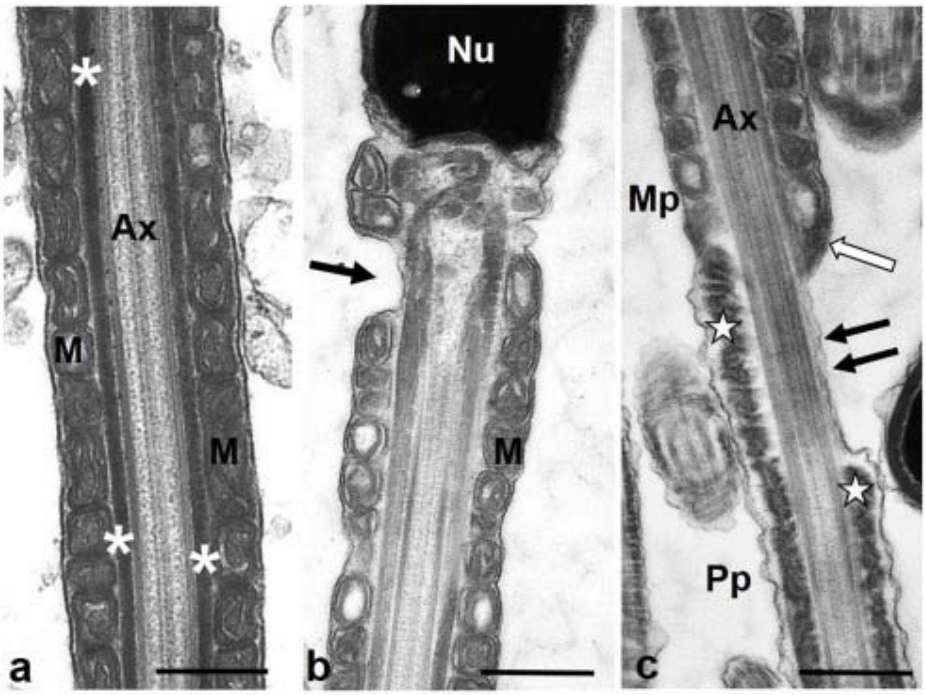

Fig. 5. (a) Cheetah sperm midpiece displaying typical morphological features. Mitochondria (M), outer dense fibres (asterisks), axoneme (Ax). (b) Connecting piece and midpiece of a cheetah sperm showing segmental aplasia of the mitochondrial sheath (arrow). (c) Junction of the midpiece (Mp) and principal piece (Pp) of a cheetah sperm. A section of the fibrous sheath (stars) immediately below the annulus (white arrow) is missing (double black arrows). Note the continuity of the plasmalemma in b and c which now lies against the outer dense fibres. Nucleus (Nu). Bars =0.5 µm.

3. Sperm ultrastructure: a phylogenetic tool

Sperm morphology has been used as a phylogenetic tool, possibly unwittingly, since the early descriptions of a number of vertebrate species by van Leeuwenhoek, and then on a more structured basis following the prodigious comparative studies of Retzius and the work of Ballowitz (see references in Jamieson, 2007 and Birkhead and Montgomerie, 2009). In a comprehensive review of avian sperm ultrastructure, Jamieson (2007) concludes that “the taxonomic and phylogenetic value of sperm characters is indisputable”. Clearly, the more morphological characters that can be identified, the more accurately phylogenetic relationships can be determined and possibly even cladistic analysis attempted. TEM would obviously be indispensable in this regard. This is illustrated (Fig. 6) in a brief comparison of the main morphological characteristics identified in the midpiece (excluding the connecting piece) and proximal principal piece of selected non-passerine birds, the ostrich, emu, rhea, tinamou, chicken and duck. The description is based on personal observations supplemented by data from the relevant literature (Bakst and Howarth, 1975; Maretta, 1975; Asa et al., 1986; Phillips and Asa, 1989; Soley, 1993; Du Plessis and Soley, 2014)

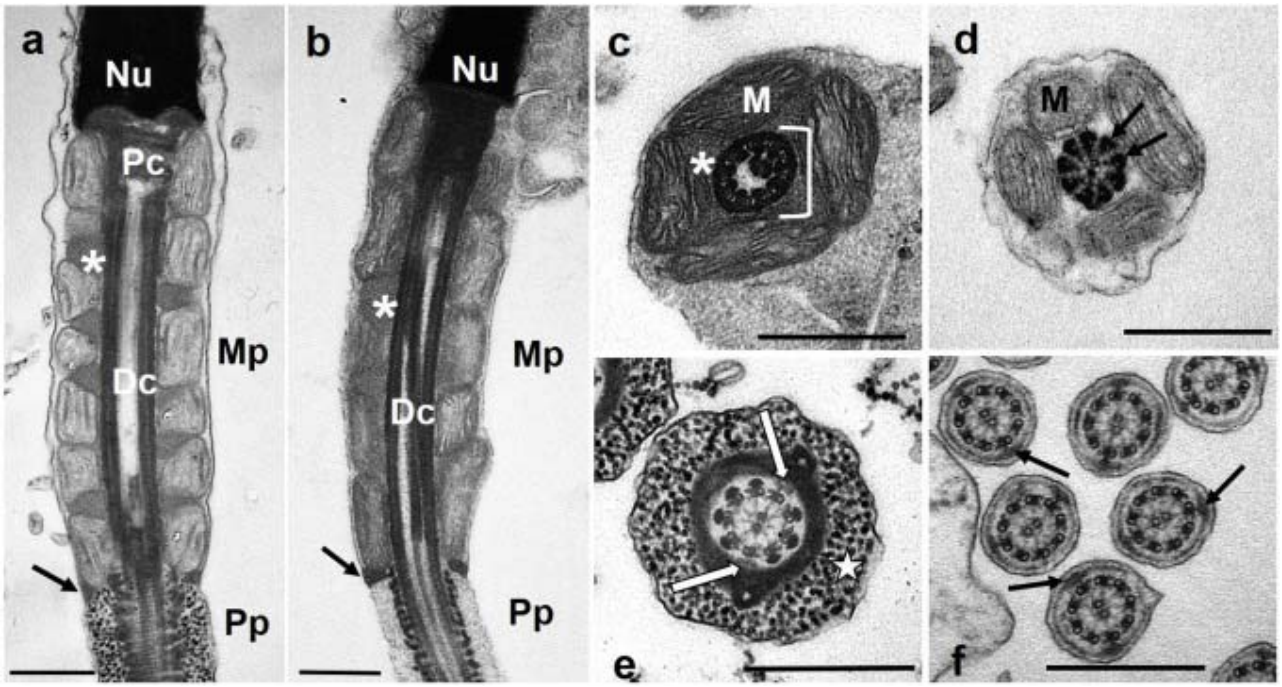

Fig. 6. Longitudinal sections through the midpiece (Mp) and proximal principal piece (Pp) of a tinamou (a) and ostrich (b) sperm. In both cells note the long distal centriole (Dc), intermitochondrial cement (asterisks) and the granular nature of the tinamou principal piece. Nucleus (Nu), annulus (arrows). (c–d) Transverse sections through the midpiece of an ostrich (c) and chicken (d) sperm. Mitochondria (M) surround the distal centriole (bracket) in the ostrich, and the dense fibres (arrows) and axoneme in the chicken. Note the absence of intermitochondrial cement in the chicken. (e–f) Transverse sections through the proximal region of the tinamou principal piece (e) and rhea distal principal piece (f). Small outer dense fibres surround the axoneme in the tinamou of which two fibres (3 and 8) are incorporated (arrows) into the hollow longitudinal columns of the fibrous sheath. Granular material (star) is present in the surrounding cytoplasm. In the rhea, longitudinal columns tenuously connected to the underlying microtubular doublets (arrows) remain visible more distally in the flagellum. Bars =0.5 µm.

In both ratites and the tinamou, the distal centriole runs the entire length of the midpiece (Fig. 6a,b) whereas in the chicken and duck it is much shorter and restricted to the anterior part of the midpiece immediately beneath the connecting piece. The implication is that the axoneme in the chicken and duck originates within the midpiece (Fig. 6d) whereas in the three other birds it begins at the midpiece/principal piece junction. In the ostrich and tinamou a rod of moderately electron dense material lies in the centriolar lumen (Fig. 6b,c) and contains a pair of longitudinally oriented microtubules that are continuous with the central pair of axonemal microtubules at the annulus. A similar situation is observed in the rhea although the dense material around the microtubules is absent. The lumen of the short distal centriole in the chicken and duck is empty. In Galliform and Anseriform birds nine independent outer dense fibres surround the axoneme (Fig. 6d) in the midpiece below the distal centriole and extend into the anterior part of the principal piece. In the ratites and tinamou typical outer dense fibres are only observed for a short distance in the anterior aspect of the principal piece (Fig. 6e). However, it would appear, as also noted by Jamieson (2007), that the dense fibres in these birds are present in the midpiece but are incorporated into the wall of the distal centriole (Fig. 6c). The number of mitochondria differ with 40 or more being present in the emu, 20–25 in the ostrich, 20 in the tinamou, 24–30 in the duck and 30 in the chicken. They also differ in appearance, those of the ostrich being rectangular

with parallel cristae, round in the tinamou with irregularly disposed cristae, or of irregular shape with flattened sides and parallel cristae in the duck. In the tinamou, ostrich (Fig. 6a–c) and rhea flocculent material, referred to as inter-mitochondrial cement, is seen between the mitochondria. Although not specifically confirmed, this material appears to be absent in the emu and is not present in the chicken (Fig. 6d) or duck. An annulus separating the midpiece from the principal piece was a consistent feature in each of the birds (Fig. 6a,b). The fibrous sheath of the principal piece also differs markedly between the birds studied. In the duck and chicken it formed an amorphous sheath whereas in the ratites and tinamou two longitudinal columns (hollow for much of their length in the tinamou) (Fig. 6e) connected by rib-like fibrous elements, as in mammals, formed a more organised structure. The columns maintained a close association with coarse fibres/microtubular doublets 3 and 8 throughout the entire principal piece (Fig.6 e,f).

4. Matching ultrastructure and function: fitting the pieces

Lüpold and Pitnick (2018) note, with some exceptions (Lindemann and Lesich, 2016), that "apart from biophysical studies linking sperm morphology to swimming velocity, surprisingly little is known about the adaptive significance of sperm form and the selective processes underlying its tremendous diversification throughout the animal kingdom." This is a legitimate statement considering the numerous, sometimes subtle, structural modifications in sperm ultrastructure exhibited between species. In some instances this occurs between closely related families. A case in point is the difference observed in the appearance of the perinuclear theca (PT) during spermiogenesis between Anseriform and Galliform birds on the one hand and members of the Struthioniformes on the other. During spermiogenesis in mammals, the PT appears as a continuous layer of detergent resistant material that covers the entire surface of the nucleus with the exception of the implantation fossa (the site of sperm head-tail attachment). This material, composed of a variety of cytosolic and nuclear proteins (Dadoune, 2003; Longo et al., 1987; Meistrich, 1993; Oko and Sutovsky, 2009; Toshimori, 2003), can be subdivided into a sub-acrosomal layer (SAL) and a post-acrosomal sheath (PAS) or calyx (Clermont et al., 1993; Longo et al., 1987; Oko, 1995; Oko and Sutovsky, 2009; Toshimori, 2003; Tovich et al., 2004). The proteinaceous nature of the PT, together with its specific location, would suggest that it serves to bind the acrosome and post-acrosomal plasmalemma to the nucleus, thereby assisting in maintaining the shape of the sperm head (Longo and Cook, 1991). The PT is also involved in the delivery of essential activating factors to the oocyte at fertilization, and failure to do so prevents the defective spermatozoa from inducing normal embryo development as a result of an abnormal structure or molecular composition of their PT (reviewed by Oko et al., 2017). Additionally, new, unpublished data indicates that PT proteins undergo structural remodelling/processing during mammalian sperm capacitation (personal communication, Dr Peter Sutovsky, University of Missouri), a process that endows spermatozoa within the female reproductive tract with hyperactivated motility and the ability to fertilize the ovum.

Based on the results of recent studies (Du Plessis and Soley, 2013, 2016), it is clear that material similar in location and arrangement to that of the mammalian PT is present during spermatid differentiation in ratites and can also be divided into a SAL and PAS. Information in the literature and personal observations indicate that this layer is also present in other avian orders such as the Galliformes (eg. chicken) and Anseriformes (eg. duck) although not

identified as such. However, of significance is the obvious difference in structure exhibited by the PT in late stage elongated spermatids of non-passerine birds. Whereas in the chicken and duck the PT appears as an amorphous, flocculent layer throughout spermiogenesis (Fig. 7c), in all ratites studied thus far (ostrich, emu and rhea), the "zone of cytoplasm between the manchette microtubules and the nuclear membrane [the PT] was occupied by a continuous array of small, regularly-positioned, finger-like projections which appeared to emanate from the cytoplasmic surface of the nuclear membrane." (Du Plessis and Soley, 2013) (Fig. 7b). This unique (in respect of birds) structural modification of the PT appeared during specific phases of spermiogenesis, was particularly prominent in a limited zone immediately beneath the base of the acrosome in late-stage elongated spermatids at the longitudinal manchette stage of development (Fig. 7a), and disappeared prior to spermiation (Du Plessis and Soley, 2013, 2016).

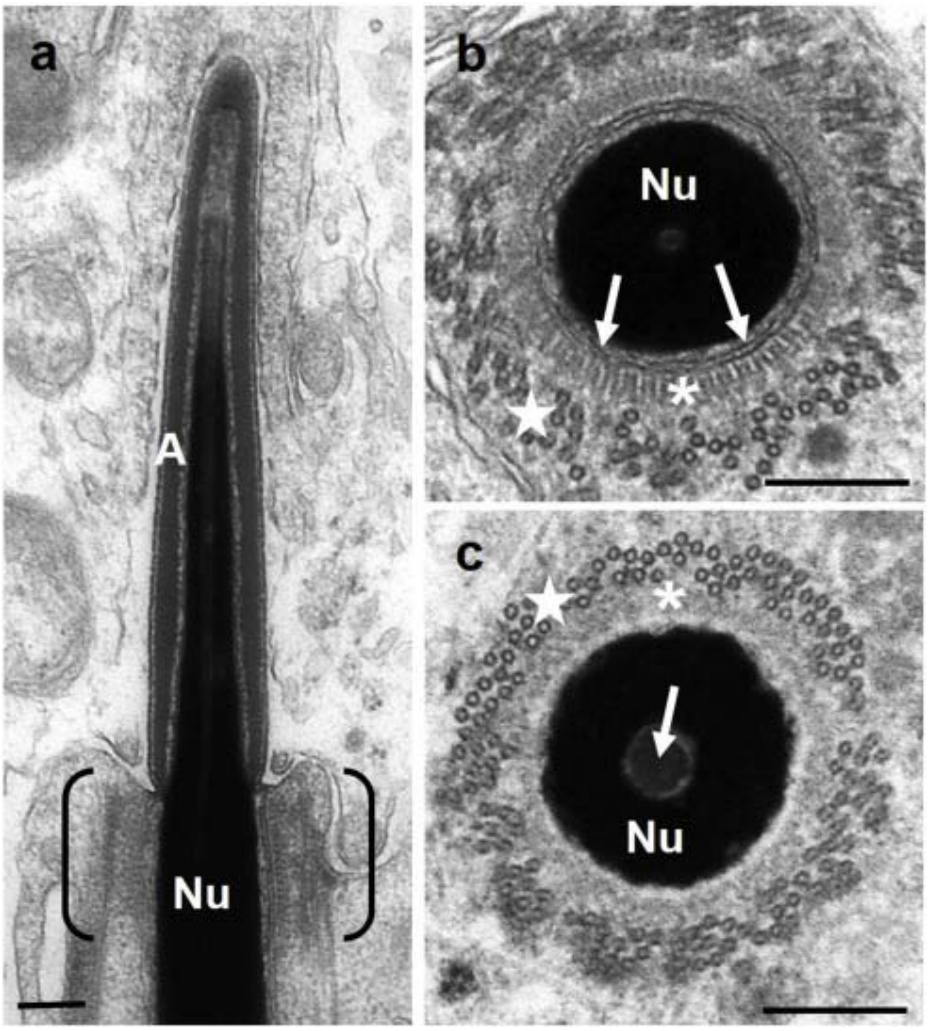

Fig. 7. TEM of developing spermatids in the ostrich (a, b) and duck (c) demonstrating the perinuclear theca (PT). (a) The finger-like elaborations of the PT (brackets) concentrate around the nucleus (Nu) of an elongated ostrich spermatid just beneath the termination of the acrosome (A). (b) Transverse section of the area bracketed in (a) to show the relationship between the projections (asterisk), and the nuclear envelope (arrows) and manchette microtubules (star). The projections are only clearly visible in the correct plane of section. (c) The PT of a similar stage duck spermatid shows no elaborations, only a layer of amorphous material (asterisk). The perforatorium (arrow) indicates that the nucleus is sectioned at approximately the same level as in the

ostrich, while the degree of chromatin condensation, tight envelopment of the nuclear envelope and the presence of the longitudinal manchette (star), indicates the stage during which the projections appear in the ostrich. Bars =0.25 μm.

Although TEM graphically illustrated the uniform and symmetrical arrangement of the projections and their close association with the nuclear envelope and manchette microtubules, identification of the proteins forming the PT in birds, and the reason for the precise arrangement of this structure at a specific stage of spermiogenesis in ratites, remain unknown and present an ideal opportunity for the marriage of ultrastructural imaging and molecular techniques for their resolution.

5. Conclusions

The power - Ultrastructural studies of animal spermatozoa continue to play an important role in the clinical assessment of semen quality of production animals (in the widest sense) thus supporting breeding programmes and ensuring food security. Additionally, such studies provide vital information relevant for the preservation of endangered species. As an academic exercise, electron microscopy supplies detailed morphological data that can be employed in a complementary role for the determination of both phylogenetic and structural/functional relationships. The beauty – The morphological intricacy of the developing male gamete lends itself to graphic portrayal, radiating an aesthetic quality that, arguably, is unique. Ultrastructural studies, supported by advanced preparatory techniques, will continue to be relevant in animal andrology, particularly in combination with powerful new imaging technologies such as super-resolution microscopy and image-based flow cytometry.

Declaration of Competing Interest

The authors declare no conflict of interest. The authors contributed equally to the review.

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