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ULTRASTRUCTURAL STUDY ON SPERMATOZOA IN SOME FARM ANIMALS

(With 4 Plates)

By

MAHA S. ZIADA, TAFIDA M. WAHBA, M.F.A. ELSHELTAWY and G. M. DARWISH

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دراسة التركيب الدقيق للحيوانات المنوية فى بعض حيوانات المزرعة

مها سليمان زيادة ، تفيدا موريس وهبه ،
ماجد أحمد الشلتاوى ، جمال مصطفى درويش

لقد تم دراسة التركيب الدقيق للحيوانات المنوية فى الطلائق البقرى الفريزيان والطلائق الجاموسى والكباش البرقى والتيوس الزرايبى باستخدام الميكرسكوب الالكترونى يتكون الحيوان المنوى فى اى من الفصائل السابق ذكرها من الرأس والذيل. أما عن الاختلافات فتتلخص فى اللاتى: زيادة سمك الغشاء البلازمى الى كل من الطلائق البقرى والكباش عنها فى الطلائق الجاموسى والتيوس. يمكن تمييز القلنسوة فى جميع الحيوانات تحت الدراسة إلى ثلاثة أقسام وهى الجزء الامامى فى القمة والجزء الرئيسى والجزء المستوى. وقد لوحظ صغر حجم الرأس فى الطلائق الجاموسى عنها فى باقى الفصائل. أما المسافة ما بين القلنسوة والنواة فكانت أكثر وضوحا فى حيامن الكباش والتيوس. الجزء ما بعد القلنسوة فى الطلائق البقرى غير منظم ومتوسط الكثافة أما فى الطلائق الجاموسى فتميز بانه حبيبى. الكروماتين فى النواة فى الطلائق الجاموسى أقل امتدادا للامام عن مثيلة فى باقى الحيوانات. بالنسبة لعنق الحيمن فيتكون من جزء الاتصال والسنتريول. الجزء الاوسط من الذيل يتكون من الميتوكوندريا والتسع ألياف الخارجية والجزء المحورى الذى يتكون من التسع ثنائيات حول زوج من البيفيات الفردية. وفى الجزء الرئيسى من الذيل يظهر غلاف ليفى حول البيفيات المحورية بدلا من الميتوكوندريا. ويعتبر انتهاء الغلاف الليفى هو الحد الفاصل بين الجزء الرئيسى والجزء الاخير للحيوان المنوى .

SUMMARY

The comparative ultrastructure of ejaculated Friesian bull, Water buffalo, Barki ram and Zaraibi goat spermatozoa is studied by transmission electron microscopy. In all the species studied, two main regions could be distinguished in the paddle-shaped head of the sperm:

The head region (containing the nucleus, acrosome, perforatorium, equatorial segment and post-nuclear cap) and the tail region (containing the neck, midpiece, main piece and the end piece). In the present study, some species differences are encountered. The plasma membrane was observed to be thicker in both bull and ram spermatozoa than that in buffalo and goat spermatozoa. The acrosome could be distinguished into an apical segment, a main segment and equatorial segment. The smaller sized apical body in buffalo spermatozoa could be noticed than in other species. The subacrosomal spaces appeared more definable in the ram and the goat spermatozoa than in the bull sperm while the acrosomal dense area was more indistinct. Post-acrosomal dens lamina was found amorphous and moderately electron dense in bull spermatozoa while finely granular in buffalo spermatozoa. The nuclear chromatine was observed less taper anteriorly in buffalo spermatozoa than those in other species. The neck region contained the connecting piece and the centriole. The middle piece consisted of a compact and helically arranged mitochondrial sheath, 9 outer coarse fibers and the axoneme. This axoneme was found to be composed of 9 microtubule doublets surrounding a central pair of singlets. A fibrous sheath was surrounding the axoneme instead of the mitochondrial helix in the principle piece. The termination of the fibrous sheath demarcated the junction of the principle and end pieces.

Key words: *Ultrastructural, Spermatozoa, Farm Animal.*

INTRODUCTION

The freezing procedures were found to differentiate in its effect on different farm animal spermatozoa. Ultrastructure and sperm antigens were also found to be highly affected by the freezing procedures (Ziada, 1994). Mohamed *et al.* (1998) found that the buffalo spermatozoa are more sensitive and somewhat fragile than the bull spermatozoa under the effect of different freezing levels. On the other hand, goat spermatozoa appeared more powerful in their freezability, especially by adding some biological fluids, appeared in their post-thaw semen quality (Shehada *et al.* 2001). Abd-El Mohsen (2001) achieved satisfactory post-frozen and thawed ram semen quality by adding some anti-oxidants to the extender. Freezability differences between spermatozoa of different species was referred to many factors especially their ultrastructural configurations.

In the fresh state, buffalo, ram and goat spermatozoa although were shown (by light microscope) to be similar in appearance to those of

the bull spermatozoa, yet they differ in their fertilizing capacity especially in their frozen state. This was of particular interest to study the comparative ultrastructure of spermatozoa of different species of farm animals to provide a basis for comparative study to its fertilizing capacity.

Kruger *et al.* (1986) and De Yi Lui and Baker (1992) convinced that the percentage of normal sperm morphological features has an important role in fertilization. Van der Merwe *et al.* (1992) have also shown that sperm morphology plays a significant role in pregnancy outcome in humans.

Recent interest has been primarily focused on the sperm head, partly because of the many defects in this region and also due to the importance of the acrosome and post acrosomal sheath in fertilization (Bedford, 1982). In addition, Pillaja and Roth (1973), through the use of cell fractionation, have made significant contributions to an understanding of the ultrastructural relationships in the neck and tail regions.

Published reports on comparative study of domestic animal spermatozoa were reviewed in a number of studies (Fawcett and Philips, 1969; Fawcett, 1975; Bartoov *et al.*, 1980; Menckveld *et al.*, 1991 and Tingari, 1991). We hope that the present study enabled us to investigate the spermatozoal ultrastructure of different domestic animals in our native breeds in buffalo, sheep and goat in comparison with Friesian bull spermatozoa. This will form a descriptive base for further study on the ultrastructural effects of preservative techniques currently in use with farm animal semen.

MATERIAL and METHODS

Semen samples were collected from three animals from each of the four species under study (buffalo, bull, sheep and goat) using the suitable artificial vagina. Above averaged samples (motility > 70% and 600 x 10^6 / ml, concentration for buffalo and bull and 6 x 10^9/ml for ram and goat on the average) from each species were pooled together. Samples were then prepared for transmission electron microscopy as described by Kakar and Anand (1984).

One ml.of each semen sample was centrifuged at 3000 r/min. for 5 min. The supernatant was discarded and the pellet was resuspended and washed once with 0.1M KPO4 buffer pH 7.2 and recentrifuged. The pellet formed was then prefixed in 2% glutraldehyde solution in 0.1M KPO4 buffer (pH 7.2) for 1 hr. at 5°C. The sperm suspension was

centrifuged to remove the glutraldehyde and the sperm pellet was washed with KPO4 buffer and then fixed for 1hr in 1% buffered osmium tetroxide.

Samples were then dehydrated in alcohol 50, 70, and 90% each for 2 changes of 20 min. Clearance with 5 changes of propylene oxide were performed. Samples were incubated in pre-embedding medium (45 ml Dodecanyl succinate anhydride DDSA, 5-ml methyl nadic anhydride MNA with 100-ml propylene oxide) for 2 hours. Finally, the sperm pellets were fixed for 1 hr. in embedding medium (Tab embedding resin, 50 ml, DDSA, 45 ml, 2,4,6-dimethyl amino methyl phenol, 2.0 ml) and embedded in beam capsules and kept at 60°C for 18-20 hr.

The samples were ultra-thin sectioned with ultramicrotome, taken on 200 mesh copper grids and stained according to Reynolds (1963) with uranyl acetate for 10 min., rinsed with distilled water and dried on filter paper. The grids were then stained with lead citrate for 20 min. Finally, the grids were washed with distilled water and dried on filter paper. The sections were examined and photographed at transimission electron microscope (Electron microscope unit, Army Medical Academy). Electron micrographs were studied.

Observations:

Buffalo bull spermatozoa (Plate 1):

The cell membrane is found to be completely surrounding the head with loose attachment at acrosomal region (Fig. 1) and closely adherent to the post-acrosomal sheath (Fig. 2). The acrsome is divided, along, its length into inner acrosomal membrane, outer acrosomal membrane with homogenous, moderate electron density layer in between (Fig. 2). The acrosome could be distinguished into 3 segments; apical segment, main segment and equatorial segment (Figs. 1,2). The apical ridge is found as hook-like enlargement extending from the inner acrosomal membrane. Equatorial segment appeared of lower thickness and higher density than the main segment. There is also a lower dense space (subacrosomal space) between inner acrosomal membrane and outer nuclear membrane (Fig. 2) The post acrosomal dense lamina was found granulated and well defined in Fig. 3. The nucleus appeared of greater electron density, wedge-shaped, occupying the whole area of the head, except for the very anterior end taken by the apical crest of the acrosome (Figs. 1-3).

Beneath the nuclear fossa, the neck region appeared as a short transversely oriented proximal centriole in which nine sets of double

microtubules could be observed embedded in a ring-like structure composed of nine fused bundles of electron-dense material (Fig. 4). Mitochondria in the neck region were longitudinally dispersed along the long axis of the cell, forming a typical pars ascendens. The core of the mid-piece composed of the axoneme, consisting of the familiar 9+2 microtubule arrangement (Fig. 5). A fibrous sheath of the principal-piece extended between the distal portion of the annulus and the axoneme. The principal-piece tapered gradually towards the end-piece. The coarse fibers disappeared gradually, while the longitudinal columns were observed to make contact with the adjacent microtubular doublets by means of septum-like, inward extension (Fig. 6). The end-piece formed the short, thin termination of the tail and consisted of the axoneme covered by the plasmalemma. Towards the end of the tail, the organised structure of the axoneme become disrupted (Fig. 7).

Bull Spermatozoa (Plate 2):

The cell membrane (plasma membrane) appeared thicker than that in buffalo spermatozoa. It loosely envelops the head at the region of the acrosome cap termination with the exception of an attachment along its base as it is firmly attached to the posterior region of the head (Figs. 1, 2). The acrosome is composed of an apical segment, main segment and equatorial segment (Fig. 1). The acrosome of bull spermatozoa is found to cover two-thirds of the nucleus. It is a membrane limited structure enclosing an electron-dense material. The equatorial segment is shown to extend over the nucleus to the post-acrosomal region. Post-acrosomal sheath is appearently covering the posterior part of the sperm nucleus from the caudal limit of the acrosome to the basal belt. It is obvious that a subacrosomal space (Fig. 2) can be defined between inner acrosomal membrane and outer nuclear membrane. Post acrosomal dense lamina (Fig. 3) is also found extending from the posterior margin of the acrosome to the base of the head. The dense chromatin material of the nucleus is thicker near the junction of the midpiece and gradually more tapers anteriorly (Fig. 3).

The neck (connecting piece) is considered as the junction between the head and middle piece. It is composed of cross-banded connecting pieces, which was attached anteriorly to the basal plate of the nucleus and posteriorly to the nine outer dense fibers of the flagellum (Fig. 3). The mitochondrial helix with the microtubules is illustrated in cross sectioned neck region (Fig. 4). The midpiece consisted of the axoneme, which is formed from nine microtubule doublets surrounding a central pair of singlets and a compact, helically arranged mitochondrial

sheath which is closely abutted by plasmalemma (Fig. 5). The principal piece resembles the longest part of the tail in the bull spermatozoa. It is surrounded by fibrous cover replacing the mitochondrial layer (Fig. 6). At the termination of the principal piece, the structure of the sperm tail was similar to a flagellum, composed of the two central and the nine peripheral double fibers, but at its very end, only the cell membrane surrounded the remaining filaments (Fig 7). The junction between the principal piece and the end piece is clearly demarcated by the termination of the fibrous sheath.

Ram spermatozoa (Plate 3):

The cell membrane is found to be detached from the acrosome anterior to the nuclear cap. It was nearly always intact and adherent over the rest of the sperm surface (Fig. 1, 2) It is found as electron dense as in bull spermatozoa (Fig. 2). The acrosome is divided into three parts: a posterior, thin, region; an anterior, thicker region and a terminal ridge-like part, containing a denser body, found only in the region near the apex of the sperm head (Figs. 1, 2). Sagital sections through the head reveal a structure very similar to that of the bull, the description of the acrosome region and nucleus applying equally to both species. The subacrosomal substance in ram spermatozoa appears slightly more definable (Fig. 1).

The concavity at the caudal surface of the nucleus, the implantation fossa, that provides an attachment to the conforming capitulum of the connecting piece could be well defined in (Fig. 2). Examination of longitudinal sections of the mid-piece showed that the structure of mitochondria found in transverse sections should usually be indicative of state of other mitochondria in the same mid-piece (Figs. 3, 4). Cross section in the principal piece containing its axoneme and associated coarse fibers is presented in (Fig. 5).

Goat spermatozoa (Plate 4):

Goat sperm ultrastructure was found, in high extent, resembling that of the other studied mammalian spermatozoa. The cell membrane appeared surrounding the whole sperm, wavy in contour, loose in attachment to the head region and close in contact to the rest of the sperm. It is found as electron dense as in bull spermatozoa (Fig. 1). The acrosomal bulge was observed along the apical edge of the head. This bulge gradually decreased in size along the edges of the head until it ended in the vicinity of the equatorial region. The equatorial region demarcated the acrosome from the post-acrosomal dense lamina (Fig. 1).

The nucleus appeared flattened containing highly compact chromatin (Figs. 1,2).

The tail composed of the neck, middle, principal and end piece. The neck forms a basal plate that fits into a depression in the posterior surface of the nucleus (Fig. 2). In different sections, the basal plate of the neck appeared to be continuous with the nine coarse fibers that project posteriorly throughout most of the tail (Figs. 3-6). The central core of the middle piece with the entire length of the tail make up the axoneme. It is composed of 9 pairs of microtubules that are arranged radially around 2 central filaments. In the middle piece (Fig. 4), nine coarse fibers appeared to be associated with the nine doublets inclosing two central doublets. The mitochondrial sheath is found to be arranged in a helical pattern around the longitudinal fibers of the tail and terminated at the annulus. The principal piece extended from the annulus to near the end of the tail. It composed of the axoneme and its associated coarse fibers (Fig. 5). The end piece, which appeared posterior to the termination of the fibrous sheath, contains only the axoneme covered by the plasmalemma (Fig. 6).

DISCUSSION

Mammalian spermatozoa exhibit considerable species differences in their size and shape, yet they all possess the same set of cellular organelles assembled on a common architectural theme (Olson and Winfrey, 1991). The plasma membrane of spermatozoa was briefly studied by many authors as it is the first barrier can be exposed to any factor affecting sperm integrity. The present results substantiate the earlier claim of Hancock (1965) that in all mammalian species the firmness of attachment of the plasma membrane enveloping the whole spermatozoon differs at different regions of the cell. In agreement with Saacke and Almquist (1964a) and Heath and Gupta (1976) the cell membrane of buffalo and bull spermatozoa was found, in the present study, loosely envelops the head with the exception of an attachment along the base of the head. In another report by Saacke and Almquist, (1964b), they denoted that there was a constant attachment of the plasma-lemma in the region of the annulus. The cell membrane of ram and goat spermatozoa was also found wavy in contour, loose in attachment to the head region and close in contact to the rest of the sperm. The same findings were observed by Quinn *et al.* (1969) who found that the plasma membrane of ram spermatozoa appeared stripped

away from the underlying acrosome but it was nearly always intact and adherent over the rest of the sperm surface. The sperm plasma membrane of studied spermatozoa in bull and ram was found thicker and electron denser than those in buffalo and goat spermatozoa. It was found by Rahlmann (1961) in more details that bull spermatozoa consisted of double walls. This may explain the good freezability character of bull and ram spermatozoa. However, species differences have become in need to more research using more cyto- or immuno-chemical ultrastructural investigations, dealing with more details in sperm plasma membrane for its important role in keeping sperm integrity.

The acrosomal morphology, in the studied mammalian spermatozoa (buffalo, bull, ram and goat) was found to be divided into three major layers, an inner membrane, an outer membrane and a homogeneous moderately electron dense middle layer. The description of the acrosome of freshly ejaculated mammalian spermatozoa recorded in this study is in close agreement with the previous report of Heath and Gupta (1976). Many studies indicated that the acrosome of buffalo spermatozoa is structurally similar to bovine spermatozoa (Blom and Birch-Andersen, 1965; Bernstein and Teichman, 1972 and Barth and Oko, 1989).

Jones and Martine (1973) found that most spermatozoa in samples from freshly collected ram semen possessed intact acrosomal membranes and the electron density of the content of the acrosome was homogeneous. In the present study, the electron microscopic observations of mammalian spermatozoa revealed that the acrosomal cap could be distinguished into an apical segment, a main segment and equatorial segment. In agreement with Hancock (1966) the apical ridge was observed as hook-like enlargement present on one side of the acrosome near its anterior border. Meanwhile, Saacke and Almquist (1964a) denoted that the hook like apical ridge of the bovine spermatozoon was progressively less pronounced in parasagittal sections away from the median line. In cross sections just posterior to the apex of the head, slight enlargements on both ends of the section indicated the subtle appearance of the apical ridge near its lateral extremities.

In the present study, the apical ridge of buffalo spermatozoa was comparatively smaller than that in other studied mammalian spermatozoa. Heath and Gupta (1976) postulated that the smaller sized apical body in buffalo spermatozoa might be responsible for the smaller sperm head size in buffalo spermatozoa. In ram spermatozoa, the present findings were confirmed by Quinn *et al.* (1969). The acrosome divided

into three parts; a posterior, thin smooth region, an anterior, thicker region often showing a somewhat corrugated surface and a terminal ridge-like part, containing a dense body, found only in the region near the apex of the sperm head. The apical ridge has been reported as a constant feature of ram spermatozoa (Randall and Friedlander, 1950).

The subacrosomal space in buffalo spermatozoa was observed in the present study, a narrow but somewhat distinct area of low-electron density, intervene between the inner acrosomal membrane and the outer nuclear membrane almost along the length of the acrosome. In accordance with our findings, Ziada (1994) reported the lower density of the subacrosomal area. The subacrosomal substance in the ram spermatozoa of the present study appeared to be slightly more definable than in the bull and the acrosomal dense area more indistinct. The post-acrosomal dense lamina (termed by Fawcett, 1970) or post-nuclear cap (termed by Hancock, 1966) was observed directly abutted the equatorial segment of the acrosome where the anterior end of the post acrosomal sheath extended over the posterior part of the equatorial acrosomal segment. In bull spermatozoa, this material has been found, in accordance with Barth and Oko (1989), as amorphous, moderately electron dense and continuous anteriorly with the material of the sub-acrosomal space. In the present study, the post-acrosomal dense lamina of buffalo spermatozoa was found to be finely granular and also of moderate electron density, which is close in agreement with Ziada (1994).

In the present observations, the nucleus was found in all studied mammalian spermatozoa to occupy the whole area of the head except for the very anterior end taken by the apical crest of the acrosome. The degree of anterior taper of nucleus in buffalo spermatozoa was less than in bull spermatozoa. Nuclear chromatin was found to be virtually homogeneous. Meanwhile, Saacke and Almquist (1964 a) found that vaculation of nuclear material was fairly regular feature of bull spermatozoa while it could not be demonstrated in buffalo spermatozoa. In accord with our findings, Ziada (1994) failed to demonstrate the presence of any nuclear vacules as those early reported by Heath and Gupta (1976). The nuclear membranes were prominent in bull spermatozoa but appeared closely applied to the nuclear chromatin of buffalo spermatozoa. The same findings were confirmed by Saacke and Almquist (1964 a) for bull spermatozoa and Ziada (1994) for buffalo spermatozoa. The nuclei of ram and goat spermatozoa were found to high extent similar to bull spermatozoa.

The term "neck" was applied by classical cytologists to a poorly-defined region between the sperm head and the beginning of the middle piece, but few details of its structure could be resolved with the light microscope (Fawcett and Philips, 1969). Fawcett (1965) described the traditional definition of the neck as the region between the nucleus and the first gyre of the mitochondrial helix of the midpiece, including mitochondria of different orientation, as a normal component of this region (neck). The presence of several coaxially oriented mitochondria, with occasional cicumferentially arranged ones interspersed between them seemed to be a regular feature of the neck region of mammalian spermatozoa studied in the present paper. The presence of two or more mitochondria in the neck region of mammalian spermatozoa is well known (Fawcett, 1965, 1975 and Fléchon et al, 1976).

Tingari (1991) proposed to redefine the midpiece by including these longitudinal mitochondria as part of the mitochondrial sheath. Thus, he assumed that the midpiece would be defined as the region between the neck and the annulus (without longitudinal mitochondria) In accordance with Ackerman and Reinecke (1994), the core of the mid-piece of studied mammalian spermatozoa was composed of the axoneme, consisting of the familiar 9+2 microtubule arrangement. The axoneme traversed the full length of the flagellum. Generally, in the neck region seemed to reveal a basal plate and a connecting piece. Beneath the nuclear fossa, lay a short proximal centriole in which nine sets of triplet microtubules could be observed. They were embedded in a ring-like structure composed of nine fused bundles of electron dense material. Both longitudinal and transverse sections demonstrated in the present study that the pars ascendens formed a sheath around the structures of the neck region. In accord with our observations, Ackerman and Reinecke (1994) described the mitochondria in the neck region as longitudinally dispersed along the long axis of the cell, forming a typical pars ascendens. They were coaxially arranged and occasionally interspersed with circumferentially arranged mitochondria.

The mid-piece of studied spermatozoa is observed as consisted of the axoneme which is formed from nine microtubule doublets surrounding a central pair of singlets. Meanwhile, a compact and helically arranged mitochondrial sheath is closely abutted by plasmalemma. The present findings were previously reported in buffalo by Heath and Gupta (1976), Barth and Oko (1989) and Ziada (1994), in bull by Saacke and Almquist (1964 b) and in ram by Jones and Martin (1973).

Cross sections of different regions of studied mammalian sperm tail appeared comparable to those published by Bishop and Walton (1960); Hanckock (1966); Quinn *et al.* (1969;)Fawcett (1970); Jones and Martin (1973); Heath and Gupta (1976); Ackerman and Reinecke (1994) and Ziada (1994).

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Plate 1: Ultrastructure of buffalo bull spermatozoa.

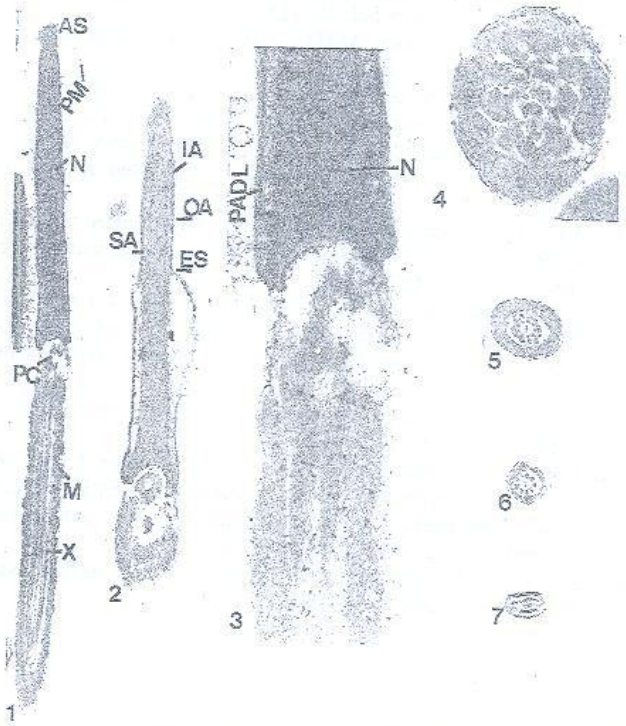

Fig. 1: Sagittal section in the head and neck showing the plasma membrane (PM),the apical segment (AS) of the acrosome, the nucleus (N) which accommodating the transversely oriented proximal centriole (PC) and the mitochondrial helix (M) surrounding the axoneme (X). (X10,000).

Fig. 2: Sagittal section in the head illustrating the equatorial segment (ES), the subacrosomal space (SA), the inner acrosomal membrane (IA) and the outer acrosomal membrane (OA). (X 16,000).

Fig. 3: Sagital section in the post-acrosomal region of the head showing the granular post-acrosomal dense lamina (PADL) and the nucleus (N). (X 40,000).

Fig. 4: Transverse section in the neck. (X 40,000).

Fig. 5: Transverse section in the middle piece. (X 40,000).

Fig. 6: Cross section in the principal piece. (X 30,000).

Fig. 7: Cross section in the end piece. (X 16,000).

Plate 2: Ultrastructure of bull spermatozoa.

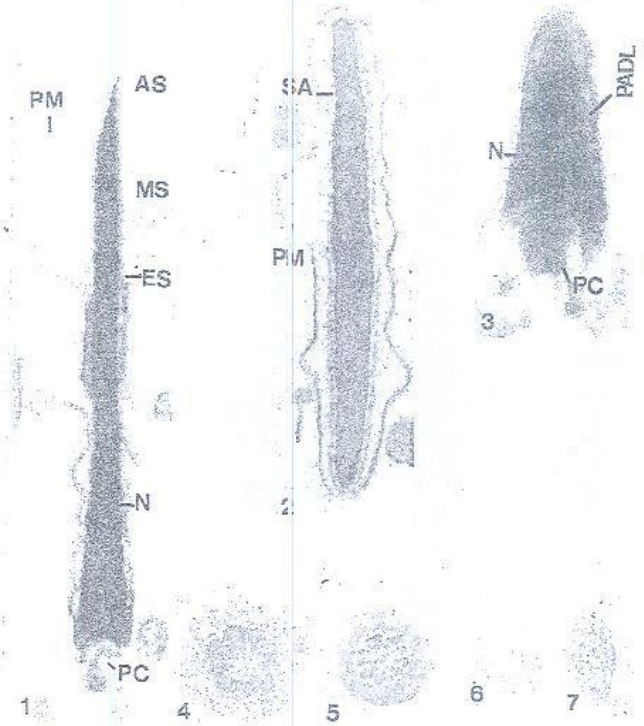

Fig. 1: Sagittal section in the head region illustrating plasma membrane (PM). The acrosomal segments are appeared as apical segment (AS), main segment (MS) and equatorial segment (ES), the nucleus (N) and the proximal centriole (PC). (X 20,000).

Fig. 2: Oblique near median section showing the loose plasma membrane (PM) and the subacrosomal space (SA). (X 30,000).

Fig. 3: Sagittal section in the head showing the nucleus (N), the post acrosomal dense lamina (PADL) and the proximal centriole (PC). Note the evaginated nuclear membrane at the base of the head. (X 30,000).

Fig. 4: Cross section in the neck region. (X 30,000).

Fig. 5: Cross section in the mid-piece illustrating the usual 9+9+2 pattern. (X 30,000).

Fig. 6: Cross section in the principal piece. (X 30,000).

Fig. 7: Cross section in the end piece. (X 30,000).

Plate 3: Ultrastructure of ram spermatozoa.

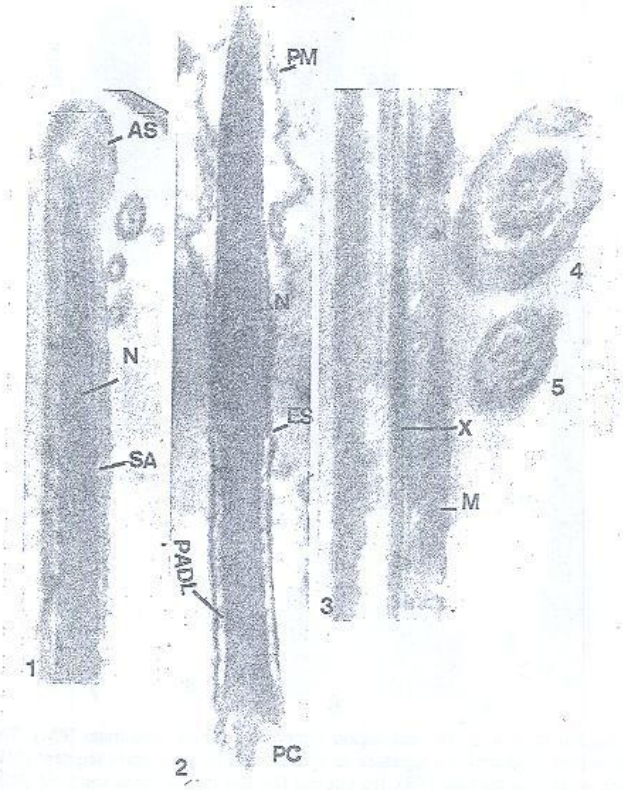

Fig. 1: Sagittal section of the sperm head showing the apical segment of the acrosome (AS), the subacrosomal space (SA) and the nucleus (N). (X 40,000).

Fig. 2: Sagittal section of the sperm head showing the plasma membrane (PM), equatorial segment (ES), post acrosomal dense lamina (PADL) and the nucleus (N) with the proximal centriole (PC). (X 30,000).

Fig. 3: Longitudinal section through the mid-piece showing the mitochondrial helix (M) surrounding the axoneme (X). (X 40,000).

Fig. 4: Cross section in the mid-piece piece. (X 40,000).

Fig. 5: Cross section in the principal piece. (X 40,000).

Plate 4: Ultrastruture of goat spermatozoa.

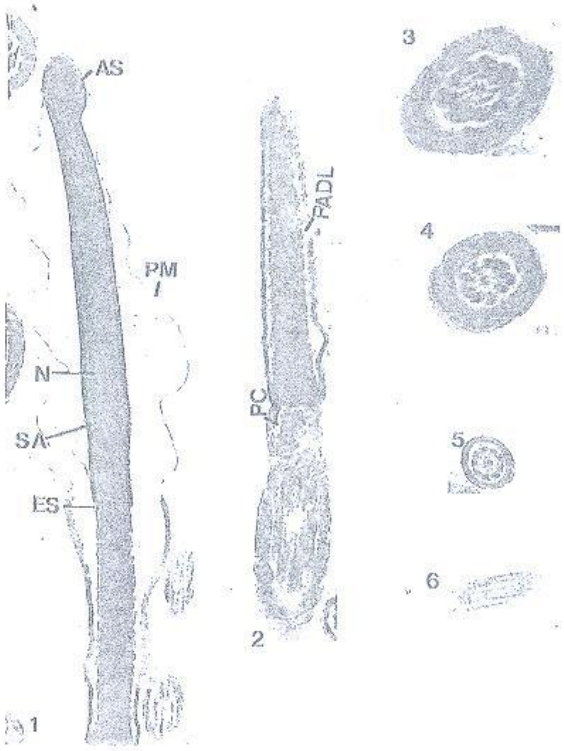

Fig. 1: Sagittal section in the sperm head showing the plasma membrane (PM), the nucleus (N), the acrosome with its 3 regions; the apical segment (AS), equatorial segment (ES) and the main segment in between them. Note the appearance of the sub-acrosomal space (SA). (X 20,000).

Fig. 2: Sagittal section in the sperm head and neck showing the plasma membrane which is closely apposed to the post-acrosomal dense lamina (PADL) and the proximal centriole (PC). (X 20,000).

Fig. 3: Cross section in the neck region illustrating the microtubules surrounded by the mitochondria . (X 30,000).

Fig. 4: Cross section in the mid-piece . (X 30,000).

Fig. 5: Cross section in the principal piece. (X 30,000).

Fig. 6: Cross section in the end piece (X 20,000).