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Tail stump spermatozoa: morphogenesis of the defect. An ultrastructural study of sperm and testicular biopsy

Spermatozoen mit Stummelschwanz: Morphogenese des Defektes. Elektronenmikroskopische Untersuchung von Hodenbiopsien und Spermatozoen

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Summary. We have studied the ultrastructure of testes biopsies and immotile spermatozoa from a supposed secondary infertile male. A wide range of tail defects has been described in testes and semen. Among these, tail stump spermatozoa occurs rarely. Although the origin of this defect is unknown, testes sections revealed that damage occurs during spermiogenesis at the latest stages during flagellum elongation in spermatids.

Zusammenfassung. Wir haben die Ultrastruktur der Hodenbiopsien und der unbeweglichen Spermatozoen von einem Mann studiert, der vermutlich eine sekundäre Unfruchtbarkeit präsentiert. Mehrere Arten von Anomalien des Schwanzes in dem Hoden und in der Samenflüssigkeit werden beschrieben. Dennoch sind Spermatozoen mit einem Stummelschwanz selten vorhanden. Obwohl die Herkunft dieser Anomalie unbekannt ist, zeigten die Biopsien der Hoden, daß sich diese Verletzung während der späteren Phase der Spermiogenese ereignet, bei der Entwicklung des Schwanzes in den Spermatiden.

Introduction

During recent years, electron microscopy (TEM) has contributed to our knowledge on the ultrastructural characteristics of spermatozoa in pathologic conditions, more particularly, in cases of male infertility. Different kinds of defects in human spermatozoa have been described in isolated case reports (Alexandre *et al.*, 1978; Bacetti *et al.*, 1979; Bisson *et al.*, 1979; Chemes *et al.*, 1987; McClure *et al.*, 1983; Nistal *et al.*, 1979; Pedersen & Hammen, 1982; Ross *et al.*, 1973; Williamson *et al.*, 1984) but their evaluation is necessary for the prognosis and management of infertility. Many of these defects are found in sperm with asthenozoospermia and/or teratozoospermia.

The present investigation of an infertile male describes ultrastructural features of

spermatozoa and testes biopsies. We attempt in this study to determine the morphogenesis of this defect causing total asthenozoospermia.

Patients and methods

Case report

The patient was a 33-year-old male who presented for investigation of a secondary infertile marriage. He was supposed to have previously fathered a 7-year-old boy from a first marriage, but he questioned his paternity during the divorce.

He had no history of significant illness: neither the patient nor anyone else in his family had a history of respiratory diseases or chronic sinusitis. His parents were not consanguineous and he had two sisters and brothers with children. Clinical examination showed a normal virilized male with normal testes and external genitalia. No varicocoele was detected.

Serum sample analysis

Two serum samples were analysed for follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone (T).

They were assayed by routine radioimmunoassay using international standards FSH: MRC 69/104, LH: MRC 68/40.

Semen analysis

Four semen samples were collected by masturbation into sterile containers after 3 days of sexual abstinence and were analysed within 1 h of collection. After liquefaction at 37 °C the sample volume, PH, sperm counts, per cent of motility and per cent alive forms were appraised with standardized methods (World Health Organization recommended procedure 1987). Polynuclear and spermatogenetic cells were differentiated by a peroxydase staining technique. Morphology of spermatozoa was studied on air-dried and fixed slides, abnormalities identified according to the classification of David *et al.* TEM was performed on the last semen sample.

Testes analysis

On both testes, a biopsy was performed and was examined by light and electron microscopy. For optic microscopy, samples were fixed in Bouin's solution, embedded in paraffin, and sections stained with haematoxylin-eosin.

Electron microscopy procedure

Semen. For ultrastructural study semen was mixed with glutaraldehyde 2% for 1 h in cacodylate buffer 0.3 mol l^{-1}. The fixed semen was centrifuged at 200 *g* for 15 min and the pellet washed in cacodylate buffer overnight at 4 °C. The post fixation was done with 1% OsO^4 for 1 h, followed by water washing and staining in 2% acqueous uranyl acetate for half an hour. Then semen was included in 2% agar, water-washed quickly several times, dehydrated through a graded ethanol series, cleared in propylene oxyde and embedded in epoxy resin (epon 812; Cipec, France). Ultrathin sections were made with a Reichert OMU2 ultramicrotome using a diamond knife and stained with a saturated methanol solution of uranyl acetate and with 2.5% citrate. Sections were examined using a Jeol 100 B electron microscope usually at 80 kV. A quantification of heads and flagellum abnormalities was made from at least two different blocks counting 100 heads and tails on one section only.

Testes. Each biopsy, during surgery, was immediately immersed in the fixative of McDowel & Trump, containing 4% of paraformaldehyde and 1% glutaraldehyde, for at least 2 h. The samples were then post-fixed in OsO4 cacodylate 2%, dehydrated, embedded in Epon, cut and stained like sperm.

Results

Endocrine profile

Each sample was found within the normal range: FSH: 2.9 and 4 mUi (< 8.8 mUi; LH: 3.7 and 2.6 mUi (3–12 mUi; T: 25.4 and 25 nmol l^{-1} (10.4–30 nmol l^{-1}).

Semen analysis

Biochemical analysis. No disturbance was found in fructose, acid phosphatase, and L-carnitine content. The complete asthenozoospermia was not explained by an alteration of biochemical content of seminal plasma.

Semen analysis. This showed in the four samples studied: a volume between 4 and 5.8 ml; PH: 7.4–7.8; sperm counts 9.6–32 10^6/ml, 30.7–144.2 10^6 per total ejaculate; % alive forms: 70–87; % motility: 0.

The most striking feature of this semen is the total absence of motility in all the samples.

Teratozoospermia: all air-dried slides showed the same profile: mainly tail disturbances (Fig. 1). Absence of flagellum: 21–33 %; short tail spermatozoa: 14–29 %; coiled tails: 11–26 % and an abnormal proportion of spermatids, spermatocytes and cytoplasmic residues.

Electron microscopy of sperm

Sperm head. Among all the different sections, only 15 % of the sample were normal with an acrosome covering correctly two thirds of nucleus. Most sperm heads were abnormally shaped (Fig. 1): 6 % were microcephalic, 5 % duplicated, 74 % were irregular (abnormal wawy acrosome, 22 %; degenerative acrosome reaction, 12 %; dispersed acrosomial matrix, 12 %; incomplete acrosome, 11 %; total absence, 14 %).

The nucleus had a high degree of maturity with a well-condensed karyoplasm (91 %).

The post-acrosomial sheath seemed more preserved with a normal location on most sperm heads.

Sperm tail. On the different sections, the examination was rather difficult. The flagellum was absent in 14 % of cells, appeared coiled in 18 % and was reduced in length in 20 % (short tail spermatozoa abnormally thick). Most cells (48 %) consisted of an accumulation of cytoplasm containing a variety of axonemal and periaxonemal structures like those seen in testicular biopsy. There were often remnants of coarse fibres, fibrous sheath and microtubules. Mitochondria were dispersed without any helical arrangement.

There is no transition from midpiece and principal piece: the annulus is often absent or displaced. Studies of transversal sections showed an axoneme which was always disturbed: 65 % had no central pair, in other sections one or two doublets were missing or in excess with axonemal disorganization.

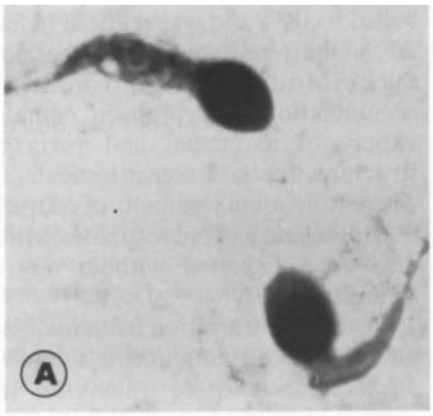
Periaxonemal structures showed a very thick fibrous sheath, without symmetry, absence or malposition of longitudinal columns, with abnormalities of coarse fibres (Fig. 1).

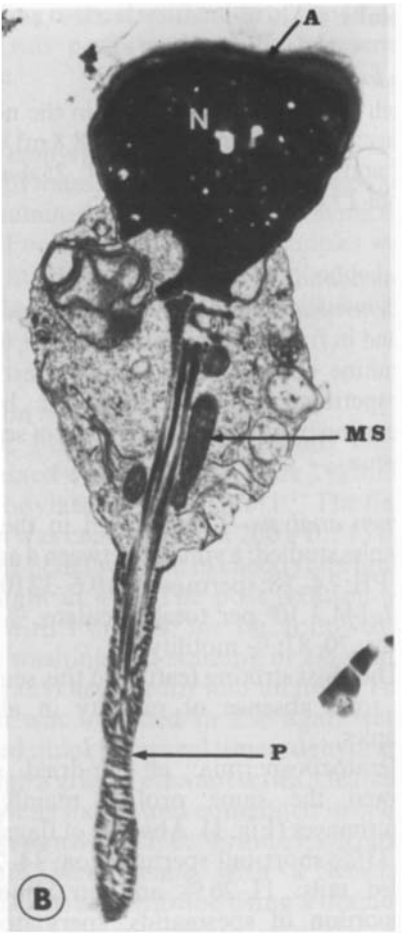
Light microscopy of testes. In both testes spermatogenesis was markedly impaired. Few spermatozoa were found in the seminiferous tubules lumen with a high percentage of teratozoospermia with mainly degenerative spermatocytes and spermatids. The peritubular wall appeared thickened by a fibrous process with a beginning of hyalinization. In the interstitium Leydig cells appeared slightly hypertrophic, and the whole tissue seemed oedematous.

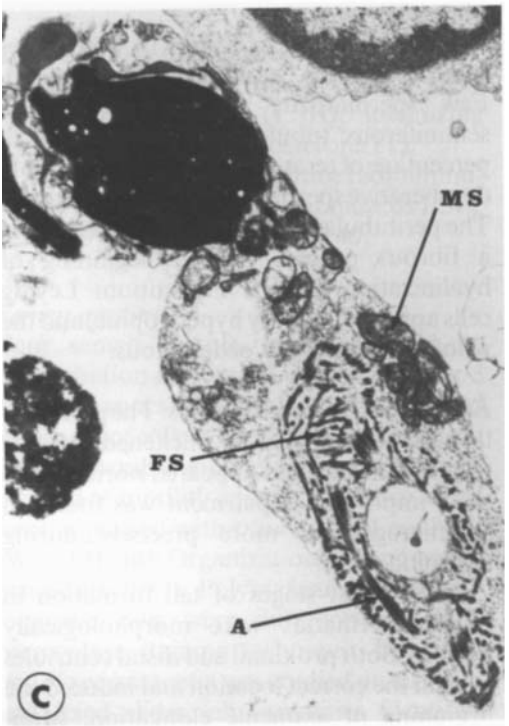
Electron microscopy of testes. The peritubular wall seemed slightly thickened in some tubules. Leydig cells appeared normal. The most important impairment was found in spermatogenesis, more precisely during spermiogenesis.

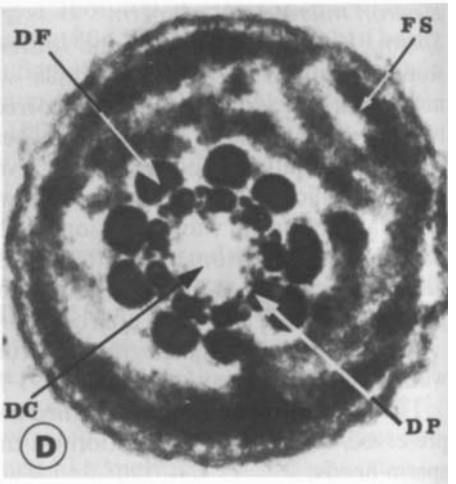
The earliest stages of tail formation in round spermatids were morphologically normal. Both proximal and distal centrioles were in the correct location and induced the beginning of axoneme elongation. Man-

FIGURE 1









chette formation was well visualized as well as the annulus being in the correct location in the first stages (Fig. 2). The elongation of sperm heads and chromatin condensation proceeded correctly, concomitantly to acrosome development. During further stages of development (stages 7 to 8 in spermatids) many disturbances occurred: the middle piece was never complete, the mitochondrial sheath was absent or foreshadowed without any alignment.

During flagellum organization, annulus migration was impaired and no organization of mitochondrial sheath along the flagellum occurred. There was a perturbation of all the axoneme microtubules, dense fibres and fibrous sheath formation. At the final stages of spermiogenesis, the spermatid tails were replaced by stumps with different shapes containing axonemal and periaxonemal structures, vesicles, mitochondria and sometimes structures of the neck region. In tubular lumen, spermiation showed stump spermatozoa, tail-less spermatozoa and many cytoplasmic residues (Fig. 3).

Discussion

The tail stump defect studied here had been described elsewhere in human cilia (Afzelius & Eliasson, 1979; Escalier *et al.*, 1982) or spermatozoa (Alexandre *et al.*, 1978; Bacetti *et al.*, 1979; Bisson *et al.*, 1979; Escalier & David, 1984; McClure *et al.*, 1983; Nistal *et al.*, 1979; Ross *et al.*, 1973; Zamboni, 1987) and also in mammals: bulls (Vierula *et al.*, 1987), mice (Dooher & Bennet, 1977).

Testicular origin of this defect, named short tail spermatozoa, or absence of the central complex, has been suggested (Escalier & David, 1984; Ross *et al.*, 1973). This case confirmed the dysfunction of spermiogenesis at the latest stages when spermatids and spermatozoa were affected in the same way. It was sometimes rather difficult to quantify normal from abnormal structures in ultrathin testicular sections but in our observation longitudinal and transversal sections of mid-piece or main piece of spermatids always demonstrated many disturbances of mitochondrial sheath, flagellum elongation and mainly absence of central pair in the axoneme.

According to different opinions, many explanations could be proposed. (1) In the testicular tissue studied, no spindle shape body was seen: according to Holstein & Schirren (1979) its appearance occurs at the stage 3 of the differentiation process and it disappears when the fibrous sheath is gradually established. (2) The manchette has been claimed to be responsible for the elongation and final formation of head and acrosome shape (Pedersen & Hammen, 1982); the manchette in different sections in our case had a normal appearance but only 14 % of heads had a normal shape. Later in spermiogenesis the manchette's function is unknown: it could play a role in mitochondrial alignment. (3) The distal centriole function was also incriminated (Alexandre *et al.*, 1978; Vierula *et al.*, 1987) when elongation is perturbed. Centrioles produce centriolar adjunct, cross-striated columns of the connecting piece, and beginning of formation of outer dense fibres: in our case, all these functions of centriole seemed normal except axoneme formation. (4) These disturbances were known to be a consequence of defective migration of annulus (Alexandre *et al.*, 1978; Escalier & David, 1984; Vierula *et al.*, 1987). The annulus induces probably mid-piece consti-

Figure 1. Electron and light microscopy of sperm. (a) Aspect of Schorr staining sperm cells in light microscopy showing short tail spermatozoa (5000). (b) Longitudinal section of spermatozoon (TEM): acrosome (A) on an irregular head (15000), nucleus with a normal degree of condensation (N), disorganized mitochondrial sheath (MS), short principal piece (P). (c) Longitudinal section of a spermatozoon: principal piece with a complete derangement of axoneme (A), mitochondria (MS), and fibrous sheath (FS) (13500). (d) Transversal section of axoneme with a thickened fibrous sheath (FS), nine dense fibres (DF) and nine peripheral doublets (DP) of the central complex (DC) (90000).

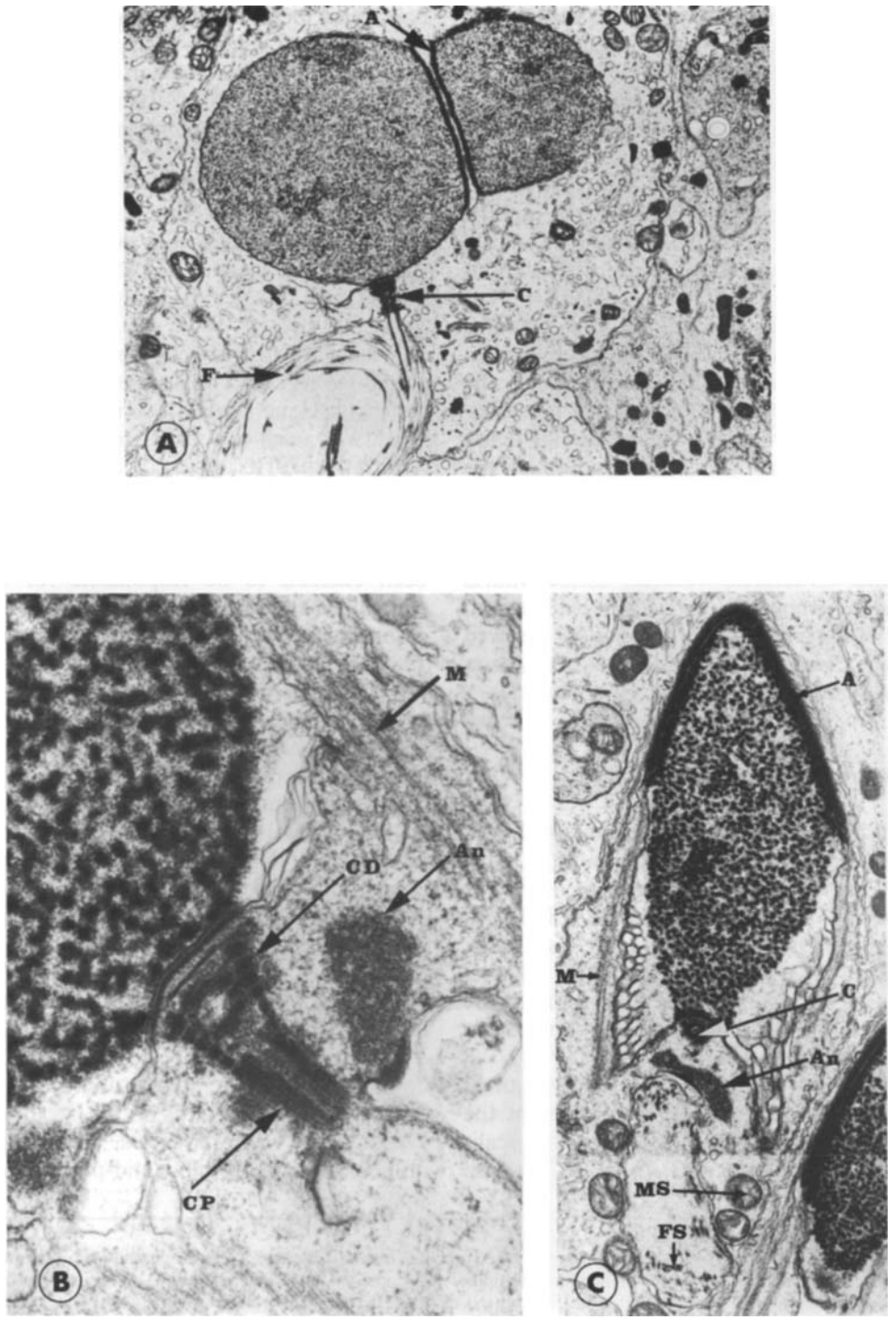


FIGURE 2

tution, and location of proximal limits of fibrous sheath and its thickness. We always saw annulus at the earlier stages in correct location but not after.

These different explanations could be involved in the defective elongation of flagellum either together or alone, each one operating at a particular moment of spermiogenesis.

This defect was then shown at testicular level but the true origin is debated. Sperm and cilia disturbances are often supposed to be genetically determined (Afzelius & Eliasson, 1979). Familial cases have been described in brothers (Afzelius & Eliasson, 1979; Alexandre *et al.,* 1978; Bisson *et al.,* 1979) or in consanguineous weddings (Bisson *et al.,* 1979). Vierula (1987) describing a similar defect in bulls confirmed the recessive autosomal inheritance: mutation affecting a single gene in his case. A mutation named 'quaking' was also described in mice (Dooder, 1977). Afzelius (1979) has incriminated many flagellar mutants in man: immotile cilia syndrome is a heterogenous condition i. e. different genes might be responsible for the disease.

In our case, no familial history was described and without any confirmation, he was considered to be the father of a 7-year-old boy, but the divorce has not been finalized and genetic investigations on serum blood tests were refused by the mother so the secondary infertility of the patient has not been ascertained. (This case could be worth publication with this information.) Thus, if he was the real father, we could suppose that tail ultrastructural defect could be acquired. Such an explanation was discussed by Williamson (1984) in a man with a history of testicular injury and antisperm antibodies with ultrastructural abnormalities. In other cases he also explained impairment of motility by genital infection. Neither genital infection (normal biochemical seminal analysis and sperm culture) nor antisperm antibodies were detected in our case.

The absence of central complex and perturbations of the elongation can also originate from abnormalities of tubulin polymerization like those induced by colcemid in drosophila (Escalier *et al.,* 1982) and induced different disturbances of intracellular movements: for example ribosome-like granules which are not eliminated during spermiogenesis according to Holstein (1979, 1981). And many ribosome-like granules were found in our case at later stages. The real cause of this defect has not been determined.

This observation underlines the importance of detailed electron microscopy analysis in the evaluation of male infertility, particularly in patients with motility diseases. The diagnosis and prognosis of infertility can be made more easy in cases of axonemal defects. Complete asthenozoospermia associated with ultrastructural perturbations of flagellum structures could affirm definitive male infertility.

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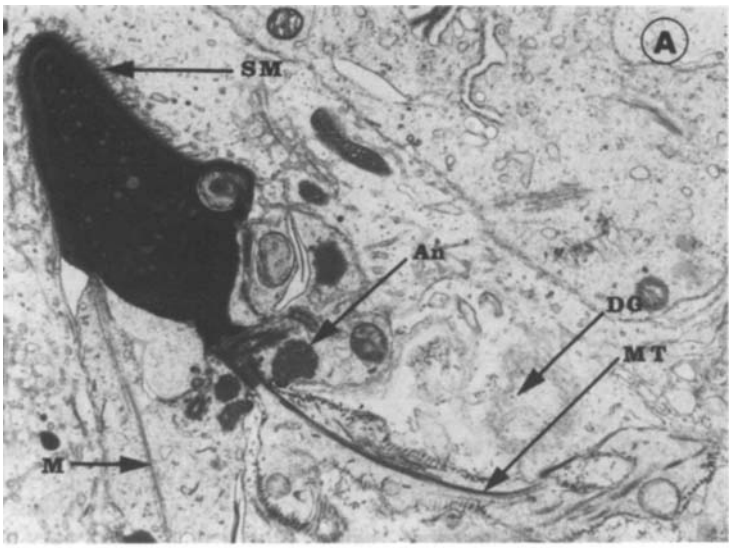
Figure 2. TEM testicular biopsy. (a) Binucleated spermatid step 2 showing a centred acrosome (A) with a not yet aligned coiled flagellum (F). Implantation fossa are seen on the nucleus and centrioles (C) (7500). (b) Spermatid at step 5: detail of the neck; centrioles distal (CD) and proximal (CP), annulus (An) and microtubules of the manchette (M) (37500). (c) Spermatid step 5: nucleus (N) with chromatin condensed in coarse granules, acrosome (A) elongated by microtubules of the manchette (M) showing centriole (C), annulus (An) still near nucleus, and a disorganized flagellum fibrous sheath (FS) and mitochondria (MS) (12000).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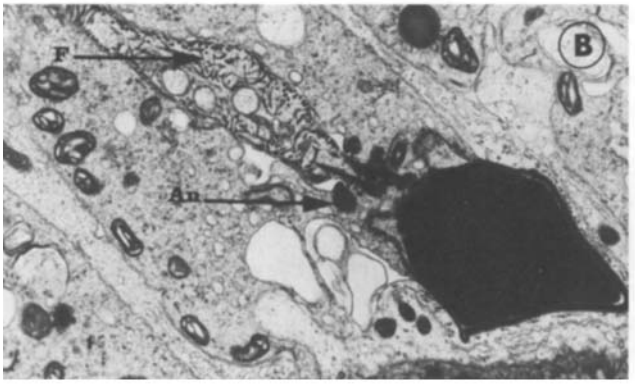
B
F
An

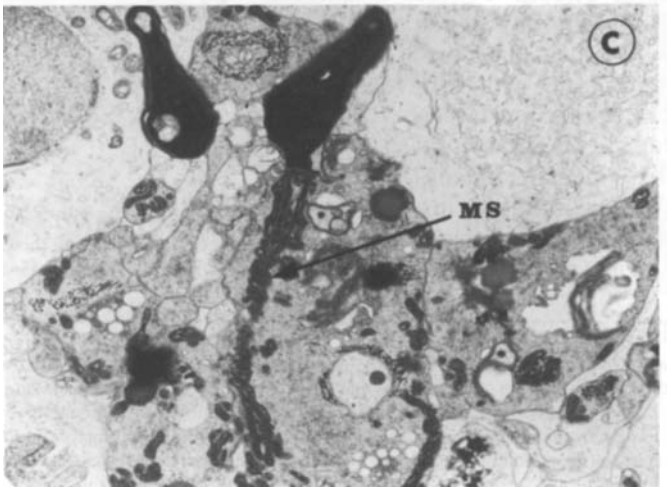
C
MS

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Figure 3. TEM testicular biopsy. (a) Spermatid step 6: the manchette is still well visualized (M), sub-plasmalemmal microfilaments surrounding head of spermatid (SM), distal and proximal centrioles, annulus (An) still close nucleus and mitochondrial sheath (MS) not developed then microtubules (Mt) and fibrous sheath seemed disorganized. Complexes of dense granules are seen in the cytoplasm (DG) (10500). (b) Spermatid step 8: annulus is still closed the nucleus (An) and all the flagellum showing clustered axonemal structures without mitochondrial sheath (F) (9000). (c) Spermatids close to spermiation showing many disorders of axoneme and mitochondrial sheath (MS) (6000).