Electron microscopy in the investigation of asthenozoospermia

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ABSTRACT

Asthenozoospermia, defined as low sperm motility, is a significant cause of subfertility in men. Its origins are diverse and in some instances cannot be ascertained. However, severely reduced motility can often be associated with abnormalities in the structure of the sperm tails, which can only be detected by transmission electron microscopy (TEM). In this respect, TEM is an important adjunct to the traditional methods of semen analysis. This review examines the development of the current state of knowledge of sperm tail abnormalities. These may be genetic in origin, or they may be acquired as a result of extrinsic factors. At present, consistent molecular markers are not available to characterise many of the genetic defects. However, TEM can distinguish specific defects of genetic origin and the non-specific structural anomalies that are typical of an acquired condition. It can also differentiate sperm structural anomalies from necrospermia, or sperm death, which is another significant cause of asthenozoospermia. In this modern era of assisted reproduction, it is possible in some instances to circumvent the problems of sperm immotility and to achieve fertilisation and pregnancy using intracytoplasmic sperm injection (ICSI). However, because of the possible genetic origin of asthenozoospermia, many scientists working in the field of infertility believe that it is of the utmost importance to investigate the causes of asthenozoospermia. This review considers the continuing relevance of TEM to the evaluation of sperm tail abnormalities in the context of current reproductive techniques.

KEY WORDS: Infertility, male.
Microscopy, electron.
Sperm motility.
Spermatozoa.

Introduction

Asthenozoospermia, or low sperm motility, is a frequent cause of male infertility.[1] Many cases of severe asthenozoospermia are thought to result from fine structural abnormalities of the sperm flagellum, or tail.[2] In some instances, these arise as a result of genetic factors.[2] These 'primary' aberrations tend to be homogeneous and affect all or most of the sperm in the ejaculate; clearly they are irreversible. In other cases, the causes of the sperm tail defects are unclear, although they sometimes relate to an underlying disorder. This may be due to infection, testicular injury or pathology, or the presence of antisperm antibodies.[3,4] These factors may induce multiple ultrastructural changes in the sperm, which are accompanied by a loss of motility.[1] In general, these 'secondary' or acquired effects are heterogeneous within the sperm sample. In contrast to the genetic defects, these changes may be reversible through treatment of the underlying pathology.[1,5] Electron microscopy is the only means to resolve the exact nature of the structural abnormalities in the sperm tail. Through the examination of many individual sperm within a sample, it is possible to differentiate between genetic and acquired abnormalities.

Structure of the normal sperm tail

The structure of the sperm tail is summarised below and illustrated in Figure 1; more detailed descriptions can be found elsewhere.[6] The tail of the human spermatozoon is approximately 50 µm in length. The central structure, the 'axoneme', arises from the distal centriole at the base of the sperm head. The axoneme comprises a system of nine outer microtubule doublets, circularly disposed around two central single microtubules; this is known as the '9+2' configuration. Each doublet consists of a complete tubule, designated 'A', that is fused to a partial C-shaped microtubule designated 'B'. Each 'A' subunit possesses two dynein arms that project towards the adjacent doublet. These arms are composed of the protein dynein, which possesses ATPase activity. A central sheath connects the two central singlet microtubules to one another. The outer doublets are connected to each other via nexin links and to the central sheath through radial spokes that arise from subunit 'A'.

The axoneme is common to other motile structures such as cilia, where movement depends on the relative sliding of adjacent microtubule doublets using the energy released from the hydrolysis of ATP.[7] The integrity of the axoneme is essential for the effective movement of the sperm tail. The axoneme extends through the full length of the tail, which has three segments: midpiece, principal piece and end piece. These segments are distinguished by the nature of the 'periaxonemal' components that surround the axoneme.

The most proximal of the three tail segments is the midpiece (Fig. 1A and 1C), which, in human sperm, is

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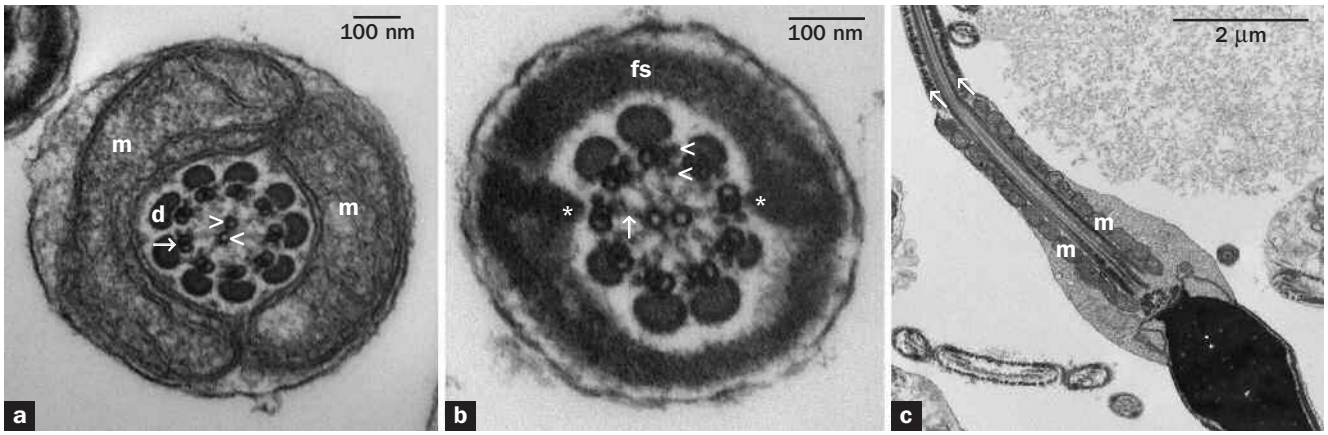

Fig. 1. Normal sperm tail. **a)** cross-section through the midpiece: mitochondria (m), outer dense fibres (d), microtubule doublets (arrow) and central singlet microtubules (><). **b)** Cross-section through the level of the principal piece: fibrous sheath (fs), longitudinal columns (*), dynein arms (<) and radial spokes (→). **c)** Longitudinal section through the midpiece and proximal segment of the principal piece: mitochondria (m) and rib structure of the fibrous sheath (arrows).

4–5 μm in length. This is the location of helically arranged mitochondria, which provide a source of energy for sperm motility through the generation of ATP. Between the mitochondria and the axoneme, nine outer dense fibres are also present. The mitochondrial helix continues to the base of the midpiece, which terminates in a ring-shaped structure called the annulus.

The principal piece extends distally from the end of the midpiece for approximately 45 μm. The distinctive characteristic of this tail segment is an outer fibrous sheath that surrounds the axoneme and the dense fibres and is present throughout its length (Fig. 1B and 1C). The fibrous sheath comprises two longitudinal columns situated in the plane of the central microtubules and a series of transverse ribs which are connected to the columns (Fig. 1C). The nine outer dense fibres that arise in the midpiece are of varying lengths and terminate at different levels in the principal piece.[6,8] The two dense fibres that are adjacent to the longitudinal columns of the fibrous sheath terminate first, leaving the other seven to continue further.[9] Stereological analysis of the human sperm tail has shown that some dense fibres remain through 60% of the length of the principal piece.[8] The fibrous sheath, however, extends distally to the end of the principal piece. All periaxonemal elements terminate at this point; the axoneme is surrounded only by a plasma membrane in the short end piece of the tail.

Although the exact function of some of the sperm tail components is unclear, the studies that are presented below indicate that a defect in any component can have a profound effect on the motility of the sperm. The defects, described as 'axonemal' or 'periaxonemal', are grouped according to the predominant type of anomaly. In the case of periaxonemal anomalies, these often associate with axonemal defects.[10,11] Axonemal defects, however, are frequently observed in a background of normal periaxonemal structure.

Axonemal defects

Specific axonemal defects

In the mid-1970s, two reports appeared in the literature that described a sperm defect in which all axonemal dynein arms were absent (Fig. 2A); this resulted in complete lack of sperm motility.[12,13] This was later recognised as one of the manifestations of an inherited condition that was named the 'immotile cilia syndrome'.[14] This affects both cilia and sperm flagella, as a result of their common axonemal structure. In many instances, infertility due to absent or impaired sperm motility is found in association with chronic respiratory conditions, which arise because of the defective cilia.[14] About half of these cases present with situs inversus, bronchiectasis and sinusitis, which together make up the triad of Kartagener's syndrome.[15]

Soon after the reports on dynein arm deficiencies appeared, other specific axonemal defects were found that resulted in immotility of both cilia and sperm. They included the absence of radial spokes, which was sometimes accompanied by the displacement of the central tubules and some of the doublets.[16,17] One rare and extreme cause of immotile sperm and cilia was found to be a complete absence of the axoneme.[18] In this case, the periaxonemal structures of the sperm, which comprise the fibrous sheath, dense fibres and the mitochondrial helix, were all normally assembled.

In some forms of specific axonemal defect, sperm motility can be detected, albeit often severely impaired. This led to a proposal that the term 'immotile cilia syndrome' should be renamed 'ciliary dyskinesis',[19] more recently termed 'primary ciliary dyskinesia' (PCD). There are a number of descriptions in the literature of partial absence of dynein arms; for example, where either the inner or the outer arms are consistently missing.[20,21] These reports indicate that a total lack of inner arms results in complete immotility of the sperm. However, the selective absence of only the outer dynein arms appears to be compatible with some degree of movement.[10,20,21]

The absence of the central microtubules and the central sheath from the axoneme of both sperm tails and respiratory cilia has also been described and is associated with reduced sperm motility.[19] In some of the axonemal cross-sections observed, this was accompanied by the transposition of an outer doublet into the central region that is normally occupied by the singlet microtubules.[19] In the author's laboratory, there have been several cases where the central tubules were seen in a proportion of sperm tail cross-sections, but the central sheath was consistently absent

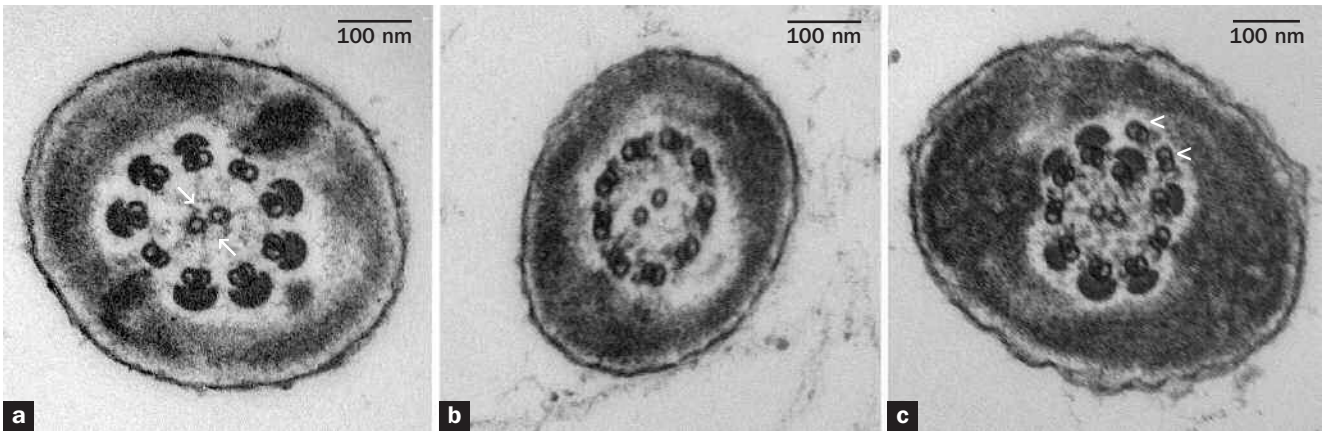

Fig. 2. Axonemal anomalies. **a)** Primary ciliary dyskinesia (PCD): cross section of a sperm tail with complete absence of dynein arms. Arrows show the central sheath. **b)** Probable PCD: cross-section of a sperm tail that lacks a central sheath but shows the normal 9+2 configuration of microtubules. **c)** Non-specific anomaly: cross section through a sperm tail that shows two additional extra-axonemal doublets (<).

(Fig. 2B) (T. A. Ryder, personal communication). These cases resemble that described by Afzelius and Elliason[20] who also noted short radial spokes. In the author's examples, the sperm defects were associated with severely reduced (forward motility <5%) or absent motility. These cases may represent a further manifestation of PCD, although no clinical information was available on associated respiratory symptoms.

Some publications describe cases where central tubules were missing from the sperm tails, but with no apparent respiratory disease present.[20–23] However, the images or descriptions in these publications suggest that they may represent a condition described as 'dysplasia of the fibrous sheath' (DFS),[24] which will be discussed later in this review. The recognition of DFS as a cause of asthenozoospermia post-dates the above case reports.[1]

Where sperm defects are specific, affect the whole population of sperm and do not respond to medical intervention, these are suspected to have a genetic origin.[25] This is particularly so where a familial pattern of inheritance is evident; certain sperm anomalies are found to be especially prevalent in the offspring of consanguineous relationships.[25] The examples of PCD, described above, have comprised specific axonemal anomalies that have been present both in sperm flagella and in the ciliated cells of the respiratory tract. However, some male patients whose respiratory cilia are structurally defective may still possess normal sperm.[26,27]

The diversity of the manifestations of ciliary dyskinesia illustrates the genetic heterogeneity of these disorders. Research has been carried out to investigate the molecular basis of PCD with two genes, *DNAI1* and *DNAH5*, known to be involved in almost half of the cases of outer dynein arm defect.[28] The role in PCD of four other genes under investigation is either very minor or remains to be determined,[28] thus more research is needed to establish reliable molecular markers for this condition. In most cases of PCD, inheritance is considered to be autosomal recessive.[29] However, there is some evidence that the disease can show an autosomal dominant inheritance pattern.[30,31] This is of particular concern in the current era of assisted reproductive technology, as these genetic defects may be transmitted to the offspring. This underlines the importance of transmission electron microscopy (TEM) as a tool for the diagnosis of PCD.

Non-specific axonemal defects

In addition to the genetic defects described above, there are many reports of non-specific axonemal anomalies in sperm from patients with asthenozoospermia. Typically, there is a heterogeneous range of aberrations affecting different sperm within an ejaculate that also contains structurally normal forms.[32] The typical array of anomalies includes disorganised microtubule arrangements, numerical aberrations such as additional or reduced doublets (Fig. 2C), microtubule translocations and missing central tubules.[1,33,34] Alterations in the number or arrangement of outer dense fibres may also be present.[32] As suggested previously, these mixed patterns may represent acquired defects, although in many cases no obvious cause can be identified.[2] However, mixed axonemal defects have sometimes been observed in patients who have suffered testicular injury or pathology.[34] Alternatively, these may be associated with varicocoele, genital tract infection or the presence of antisperm antibodies.[1] It has been suggested that the sperm defects may be potentially reversible through treatment of the underlying pathology.[1,5]

The diagnosis of these secondary defects is often made difficult by the innate heterogeneity of human sperm samples. Even in a 'fertile' semen sample, a proportion of abnormal sperm will be found and the defects may be the same as those present in the sperm of an infertile patient.[35,36] This has led several groups to recommend a quantitative approach to the examination of sperm samples from infertile men. In this case, individual samples are compared to a set of reference data derived from fertile donors.[37–39] These quantitative methods may, in some cases, enable the identification of subtle defects in men with asthenozoospermia.[37,38] However, their application is time-consuming and may not always be considered practical in a routine diagnostic setting.

Periaxonemal defects

The sperm anomalies that are considered here are those that involve the fibrous sheath, the mitochondrial sheath and the

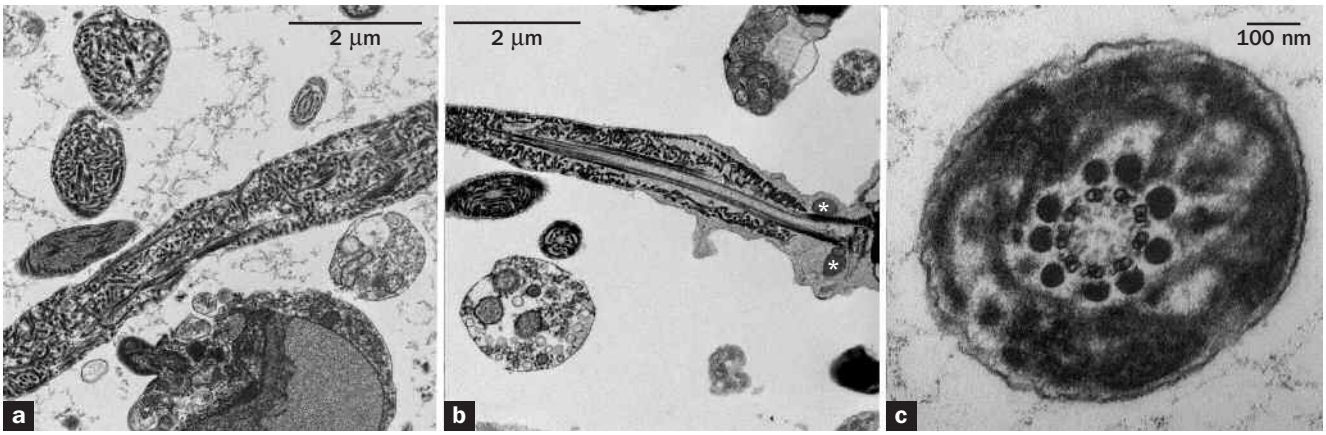

Fig. 3. Dysplasia of the fibrous sheath. **a)** Segments of sperm tail showing thickened and highly irregular fibrous sheath, with complete disruption of normal rib structure. **b)** Longitudinal section of tail and midpiece: fibrous sheath is thickened and irregular; midpiece is short with scanty mitochondria (*). **c)** Cross-section of tail showing thickened fibrous sheath and absence of central microtubules.

dense fibres that surround the axoneme. Although it is not the main defective component, in many cases the axoneme itself is also involved in these conditions. A number of the descriptions in this section concern isolated reports based solely on one or two patients. However, their inclusion is considered pertinent, as they show many of the hallmarks of a genetic origin.

Dysplasia of the fibrous sheath

Dysplasia of the fibrous sheath is one of the most widely reported of the periaxonemal sperm defects. The features of this defect were described as early as 1973,[40] although the terminology was not introduced until much later.[41] The most consistent manifestation of DFS is a thickened and disorganised fibrous sheath (Fig. 3A), in which the longitudinal columns are often absent and the rib structure is severely distorted.[41,42] At the light microscope level, the sperm usually appear short and thickened; an appearance that has led to the terminology of 'stump tail' and 'short tail'.[42,43]

Dysplasia of the fibrous sheath is a heterogeneous condition in which defects of the fibrous sheath are associated with a number of other features (Table 1). Absence or disruption of the mitochondrial sheath is a common feature (Fig. 3B) and has sometimes been attributed to a lack of caudal migration of the annulus.[42,44] Abnormal lengths of the dense fibres have also been described in some cases.[1,10]

In addition to the disturbed periaxonemal features, anomalies of the axoneme are frequently observed in DFS. These may be of a variable nature,[45,46] or one type may predominate. The range of anomalies has included the absence of dynein arms.[47] The absence of central microtubules as a predominant feature (Fig. 3C) is also common in this condition.[1,20,42,43,48] This feature may be present in as many as half of all cases.[24]

Although there is no ciliary equivalent of either the sperm fibrous sheath or the mitochondrial sheath, a proportion of the patients with DFS exhibit long-term respiratory symptoms (Table 1).[1,41,44,47,48] Indeed, chronic respiratory conditions have been estimated to affect as many as 20% of patients suffering from DFS.[2] In some studies, where nasal biopsies have been examined, the cilia showed axoneme anomalies.[47,48] Interestingly, a few cases have been reported in which the axonemal defects in the cilia were different from those found in the sperm.[47] In cases where ciliary defects were found in association with the characteristic sperm anomalies of DFS, this condition was considered to be a variant form of ciliary dyskinesia.[47]

There appear to be two forms of DFS, which differ in the proportion of sperm affected.[1] In the more commonly reported 'complete' form, almost all of the sperm show the characteristic features of DFS. However, only 70–80% of the sperm in the 'incomplete' form have abnormal fibrous sheaths.[1] Immunofluorescence studies have revealed that a lesser proportion of sperm show anomalies of the mitochondrial sheath compared with the 'complete' form.[44]

Many of the characteristics of DFS have led to its acceptance as a genetic defect.[2] It appears to be irreversible with time and unresponsive to medical treatment, in contrast to many 'acquired' defects of sperm flagella.[1] It frequently shows a familial pattern of occurrence (Table 1) and an increased incidence in cases of consanguinity.[25,49,50] Its association with respiratory defects and the tendency to affect a high proportion of the sperm population also point towards a genetic origin. The existence of the 'incomplete' form of DFS is itself an anomaly and it is unclear whether or not this, too, represents a genetic form of the condition. However, it can be associated with long-term respiratory symptoms,[44] albeit less frequently than the 'complete' form.[1]

Some molecular studies have been carried out to investigate the genetic basis of DFS.[51–53] Two structural proteins, A-kinase anchoring proteins 3 and 4 (AKAP3 and AKAP4), are major components of the fibrous sheath. Partial deletions in the genes coding for these proteins have been identified in a patient with DFS.[51,52] However, in most cases, no differences in these genes were detected between individuals with DFS and normal controls.[51,53] Some studies have also examined chromosomal anomalies in sperm. In 1997, two patients with DFS were found to have similar pericentric inversions in chromosome 9.[46] Several reports have indicated an increase in the frequency of disomy in the sex chromosomes and of diploidy in the sperm of patients with DFS, when compared with controls.[51,52,54] So far, however, no consistent or specific genetic marker has been identified for DFS.

Dysplasia of the fibrous sheath is generally associated with poor fertility potential.[1] There have, however, been some successful intracytoplasmic sperm injection (ICSI)

fertilisations that have resulted in pregnancy and live births.[49,55,56] In view of the likely genetic origin of DFS, an accurate diagnosis is considered to be important, so that counselling can be offered to the patients.[51] The light microscopy appearance of short thickened sperm tails can be indicative of other conditions such as necrospermia.[2] However, if vitality testing does not indicate a high proportion of dead sperm (necrospermia), then electron microscopy is the only reliable means to confirm a diagnosis of DFS.

Absent fibrous sheath

There have been two reports each describing a single patient who displayed a rare condition in which the fibrous sheath was absent from the sperm flagellum.[4,57] In both cases the defect was associated with an abnormally long midpiece in almost every sperm. In one patient, additional microtubules were commonly observed either as isolated doublets or in supplementary axonemes.[4] Evidence from testicular biopsy suggests that this condition arises during spermiogenesis.[57] In the case reported by Baccetti and colleagues, the fibrous sheath was absent from all the sperm, which suggested a genetic defect.[4]

Rare anomalies of fibrous sheath assembly

A recent report described two unrelated patients with varying degrees of impaired sperm motility. All the sperm from these patients exhibited an unusual form of fibrous sheath anomaly.[58] The two longitudinal columns of the fibrous sheath were irregular and differed in thickness, with one column showing discontinuity along its length. The associated ribs were also incomplete and in some areas of the tail they appeared to have a longitudinal or oblique orientation; axonemal anomalies were frequently present where the rib structure was deficient.[58] The fact that this anomaly affected 100% of the sperm examined suggests a genetic basis for this defect. This is further supported by the fact that both patients had a consanguineous ancestry.

A rare discontinuous tail anomaly

One isolated report concerned an asthenozoospermic patient, in whom 95% of sperm showed differing anomalies in the midpiece and the principal piece.[59] The midpiece was elongated to about three times the normal length and often contained supernumerary microtubule doublets. The principal piece completely lacked both axonemes and dense fibres, consisting solely of fibrous sheath. The fact that these anomalies affected most of the sperm, in repeated samples over a period of time, has led the authors to believe that this, too, is a genetic defect.

Mitochondrial sheath defects

The function of the mitochondria in the midpiece is to provide ATP, the essential source of energy for sperm motility, through oxidative phosphorylation. As ATP has a major role in cell metabolism, the presence of functional mitochondria also has a bearing on sperm viability.[60,61] Aberrations of the mitochondrial sheath have already been described above, where they were frequently associated with fibrous sheath anomalies. There is evidence, however, that anomalies of the mitochondrial sheath also occur in the absence of other sperm defects in asthenozoospermic patients. The number of mitochondrial gyres that constitutes the normal midpiece of human sperm has been variously estimated between 11 and 14 gyres.[62,63] Deviations from this range can occur in the form of abnormally short (Fig. 4A) or long (Fig. 4B) midpieces, both of which can be associated with asthenozoospermia.

Mundy and colleagues studied 10 patients in whom no obvious cause of asthenozoospermia had been established.[62] The study, undertaken by scanning electron microscopy (SEM) and TEM, demonstrated a significant reduction in the length of the midpiece and in the number of mitochondrial gyres compared with a fertile control group. Some variation in mitochondrial size was also observed in the asthenozoospermic patients. Other authors have also found that a proportion of patients with low sperm motility

Table 1. Features observed in dysplasia of the fibrous sheath.

Reference	Midpiece defects	Axoneme anomaly	Motility (%)	Familial pattern	Respiratory symptoms	Number of patients
1	Yes	Yes	Ave.1.1	6/42	10/42	42
10	Some	Yes: various	NA	NA	NA	27
20	Yes	Central tubules and sheath	NA	2 brothers	No	3
40	Yes	In 40–60% of tails†	0–6	NA	NA	2
41	NA	Yes	0–5	2 brothers	2/5	5
42	Yes	Central tubules + other	0	NA	No	1
43	Yes	Central tubules + other	0	3/8	NA	8
44	Yes	Yes	0–<5	NA	1/6	6
45	Yes	Yes: some tails	0	NA	No	1
46	Yes	Yes: various	0	*	NA	2
47	NA	Axoneme disrupted or absent	0	NA	2/2 ciliary defects	2
48	Yes	Central tubules	0	NA	No‡	1

NA: information not available.

* Associated with pericentric chromosomal inversions.

† Predominantly central tubules.

‡ 15% of cilia in nasal brushing lacked central microtubules; compared to 0% in a healthy controls.

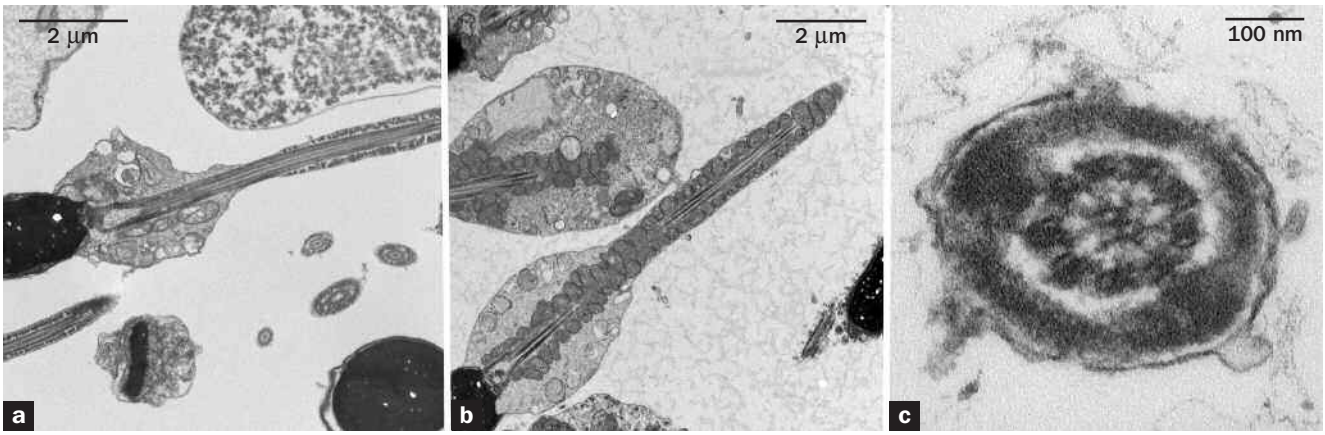

Fig. 4. Midpiece anomalies (a and b). **a)** Longitudinal section showing short midpiece with reduced number of mitochondria. **b)** Long midpiece, showing in excess of 25 mitochondrial gyres. **c)** Necrospermia. Cross-section of sperm tail showing degeneration of the plasma membrane and necrosis of microtubules.

exhibited disorders of the mitochondrial sheath in the absence of axonemal anomalies.[11,37,64] In some cases, the occurrence of short midpieces may be associated with the failed development or migration of the annulus during spermiogenesis.[37]

A few less-commonly described defects of the midpiece include one report that concerned a patient whose sperm had abnormally long and disorganised midpieces.[63] This resulted in very low and non-progressive sperm motility. As a high proportion of sperm were affected, this was believed by the authors to represent a genetic defect. Although there appears to be only one published report of this appearance, a few cases of long midpieces have also been observed in the author's laboratory (Fig. 4B) (T. A. Ryder, unpublished data). There is also an isolated report involving structurally aberrant mitochondria in a patient suffering from an inherited mitochondrial encephalomyopathy.[65]

Necrospermia

Sperm death appears to be a significant cause of asthenozoospermia in healthy individuals, as well as sufferers of polycystic kidney disease or spinal cord injuries.[66] The recently updated World Health Organization protocol for the examination of semen recommends that an assessment of viability should be carried out if progressive sperm motility falls below about 40%.[67] However, experience shows that the most commonly-used dye exclusion tests often give results that are inconsistent with the findings of electron microscopy (T. A. Ryder, personal communication). This is probably because the former tests are not sufficiently sensitive to detect the early stages of sperm degeneration.[33]

The features of necrospermia that are recognisable by ultrastructural examination include necrosis of the microtubules and fragmentation of the membranes (Fig. 4C) and mitochondrial cristae.[33,68] The prevalence of sperm death in individuals with asthenozoospermia is evident in the combined results of three studies involving a total of 480 patients; necrospermia was identified in 23% of cases.[33,64,68] If necrospermia is left untreated, there appears to be little prospect of success in either natural conception or assisted reproductive techniques. In many instances, there is evidence that necrospermia arises as a result of the hostile environment of the epididymis.[66,69] Moreover, it has been shown that improvements in sperm viability may be achieved by increasing the frequency of ejaculation, so that the period within the epididymis is reduced.[66,69] This has been proposed as a useful approach for the management of fertility in patients with epididymal necrospermia.[66,69] If necrospermia is potentially treatable then the accurate diagnosis of this condition is most important. Thus, it may be considered that electron microscopy is invaluable for the detection of necrospermia where viability tests are not predictive. It provides a sensitive means of detecting the early stages of sperm degeneration and is capable of distinguishing it from other structural causes of asthenozoospermia.

Concluding comments

While *in vitro* fertilisation (IVF) was the main assisted reproductive technique (ART), the motility of the sperm remained an important parameter. However, since its development in 1992, the ICSI technique has been used increasingly to overcome the problems associated with sperm abnormalities.[70] In this technique, the tail structure and thus the motility of the sperm are less relevant to the success of the procedure. The ICSI technique has now been successfully utilised to produce fertilisation using sperm with severe structural tail defects. These include dysplasia of the fibrous sheath[49,55,56] and ciliary dyskinesia.[71,72]

With the emergence of such methods to overcome the natural barriers to fertilisation, the investigation of male infertility may nowadays be considered less important.[73] This review has highlighted a wide range of sperm tail defects that are associated with asthenozoospermia (summarised in Table 2). There is evidence that many of these are genetic in origin and thus could be passed on to future generations through ICSI. Concerns about the transmission of sperm defects are shared by many scientists working in the field of male infertility. They stress the importance of elucidating the causes of male subfertility, where this is possible, so that patients can make informed choices about assisted reproduction[24,46,55,68,72] The transfer of genes linked to infertility is just one of the issues involved. However, as discussed earlier, many of the genetic defects

that affect sperm tails are also associated with chronic respiratory disease; these may be severely debilitating or even life-threatening.[74] This highlights the continuing importance of accurate diagnosis of the causes of male subfertility.

For the investigation of asthenozoospermia, electron microscopy is an important adjunct to the established methods of routine semen analysis. With its 'catch all' ability, it is an effective method of screening for a range of structural alterations, many of which have distinct clinical implications. It is, however, an expensive and highly specialised technique that is only available in major hospitals and research institutions. Moreover, the analysis of semen is a specialised area within electron microscopy, which is not widely available in the UK. Consequently, in many instances it is necessary to refer cases of asthenozoospermia to a specialist unit; careful selection of appropriate patients is therefore required.

The main value of electron microscopy is to distinguish inheritable defects from potentially reversible problems. Chemes states[2] that severe asthenozoospermia is often caused by structural alterations, which are responsible for deficient motility in more than 70% of infertile men. Francavilla and colleagues[32] studied 120 infertile men whose sperm analysis showed greater than 50% vitality and up to 20% forward motility. Their results revealed that approximately 23% of 73 patients with a forward motility between 0% and 10% exhibited complete forms of PCD or DFS. Furthermore, their data suggested that all genetic defects should be detected by selecting samples with forward motility ≤ 7%. However, by extending the forward motility cut-off to 20%, these authors identified three cases of incomplete PCD and DFS. This implies that these incomplete forms do not represent genetic defects, although, from the available literature, it appears that their significance has not been ascertained.

The selection of samples with >50% vitality was based on the observation that all samples below this value would show degenerative changes in the sperm.[32] However, the use of this criterion did not totally exclude patients with necrospermia; this was present in 17% of individuals in a group with 11–20% forward sperm motility.[32] This observation supports the premise that the dye exclusion techniques lack sensitivity compared with electron microscopy. Ideally, electron microscopy should be capable of detecting both incomplete PCD or DFS and cases of previously undiagnosed necrospermia, in addition to the clearly defined genetic defects. For this purpose, it may be considered more appropriate to select samples with a forward motility ≤ 20%, in conjunction with vitality >50%. However, in view of the cost implications, electron microscopy should only be considered if extrinsic factors, which may adversely influence sperm motility, have been eliminated. Such factors may include illness or medication, the nature of the specimen container, abstinence of sexual activity, and the storage conditions and time between collection and analysis of the semen sample. Quality assurance in the referral laboratory is also an important consideration.

Future genetic research may ultimately provide reliable molecular markers for conditions such as ciliary or flagellar dyskinesia and DFS. However, studies to date show genotypic variation in patients with primary sperm defects. This variation is consistent with the heterogeneous phenotypes described earlier.[29,46,52] Currently, electron microscopy is the most effective method for the diagnosis of the structural defects that lead to asthenozoospermia.

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Table 2. Summary of the main specific axonemal and periaxonemal defects.

Axonemal		Periaxonemal	
Main anomaly	Associated anomalies†	Main anomaly	Associated anomalies†
Absent dynein arms: total	–	Dysplasia of fibrous sheath‡	Midpiece and axoneme
Absent outer dynein arms*	–	Absent fibrous sheath	Long midpieces
Absent inner dynein arms	–	Short midpieces	Variation in mitochondrial size
Absent radial spokes	Doublet/central tubule displacement	Long midpieces	–
Absent axoneme	–	–	–
Absent central tubules/sheath	Transposition of doublet to central region	–	–
Absent central sheath	Short radial spokes	–	–

* Almost half of cases associated with defects in *DNAI1* and *DNAH5*; other genes may also play a role.[28]

† These anomalies have sometimes been associated with the main anomaly, but may not always be present.

‡ Possible increases in sex chromosome disomy and sperm diploidy associated with DFS.[51,52,54] Specific anomalies of AKAP3/AKAP4[51,52] in one patient and of chromosome 9 in two patients.[46]

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