

Phenotypes of Sperm Pathology: Genetic and Acquired Forms in Infertile Men

Review

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Asthenozoospermia and teratozoospermia are frequently responsible for infertility in men, yet they are poorly understood conditions that are often unrelated to any known andrological disorder. The cause of male infertility remains unclear in many individuals and numerous patients are believed to suffer from idiopathic infertility; however, recent developments have demonstrated that genetic abnormalities play a major role. The classic description by Scandinavian groups in the mid 1970s that absolute asthenozoospermia could be caused by genetic-related dynein (a structural protein with ATPase activity) deficiency in spermatozoa (Afzelius et al, 1975; Pedersen and Rebbe, 1975) opened up a new field that has been subsequently enriched by the recognition that other alterations such as congenital absence of the vas and classical forms of cystic fibrosis and, more recently, microdeletions in the long arm of the Y chromosome, can be responsible for male infertility. In the present review I will develop the concept that various forms of asthenozoospermia or teratozoospermia are also of genetic origin. Spermatozoa are terminally differentiated cells with organelles that are specialized in particular functions. They have a pathology of their own, which is not identified by routine semen analysis or functional tests because the deficiencies demonstrated by these methods do not reveal the underlying pathology but, rather, are secondary manifestations. The powerful resolution of electron microscopy overcomes the limitations of light microscopy and allows an excellent observation of sperm pathology because the internal

structure and spatial organization of sperm components can be discerned in detail.

During the last decade, the practice of intracytoplasmic sperm injection (ICSI) has demonstrated that the use of normal spermatozoa is not a prerequisite for fertilization. In extreme situations, an accurate diagnosis is essential because a genetic component is present in many patients with severe male factor infertility.

Flagellar Pathology in Asthenozoospermia

Severe asthenozoospermia is frequently caused by flagellar structural alterations, which are responsible for deficient motility in more than 70% of infertile men (Chemes, 1991). A study of spermatozoa in a large series of men with severe asthenozoospermia disclosed 2 kinds of tail abnormalities: nonspecific flagellar anomalies, which are random, secondary alterations that affect variable numbers of spermatozoa in different samples; and dysplasia of the fibrous sheath (DFS), which is a systematic, primary anomaly that affects most spermatozoa, with associated respiratory pathology and familial incidence (Chemes et al, 1987a, 1998). These findings demonstrate that severe asthenozoospermia is mainly the result of structural abnormalities of the tail, and have challenged the putative "functional" basis of sperm motility disorders.

The majority of patients with severe asthenozoospermia who consult their physicians have increased numbers of spermatozoa with nonspecific flagellar anomalies (NSFAs). These are characterized by modifications in the number, topography, and organization of axonemal microtubules that result in disruption of the normal 9 + 2 architecture of the flagellar axoneme (Figure 1A through C). These anomalies are not apparent on semen smears because microtubular alterations do not modify flagellar diameter and are discernible only at the ultrastructural level. In this group of patients, forward progression averaged $11.6\% \pm 10.2\%$, and rapid linear progression averaged $3.6\% \pm 4.7\%$. Longitudinal follow-up revealed that patients with NFSAs can experience improved sperm motility as a result of various etiologic or empiric treatments, and that they can expect reasonable fertility success, either through conventional methods or in vitro fertilization (IVF; Chemes et al, 1998). These observations indicate that the pathologic NSFA phenotype is reversible and most likely secondary

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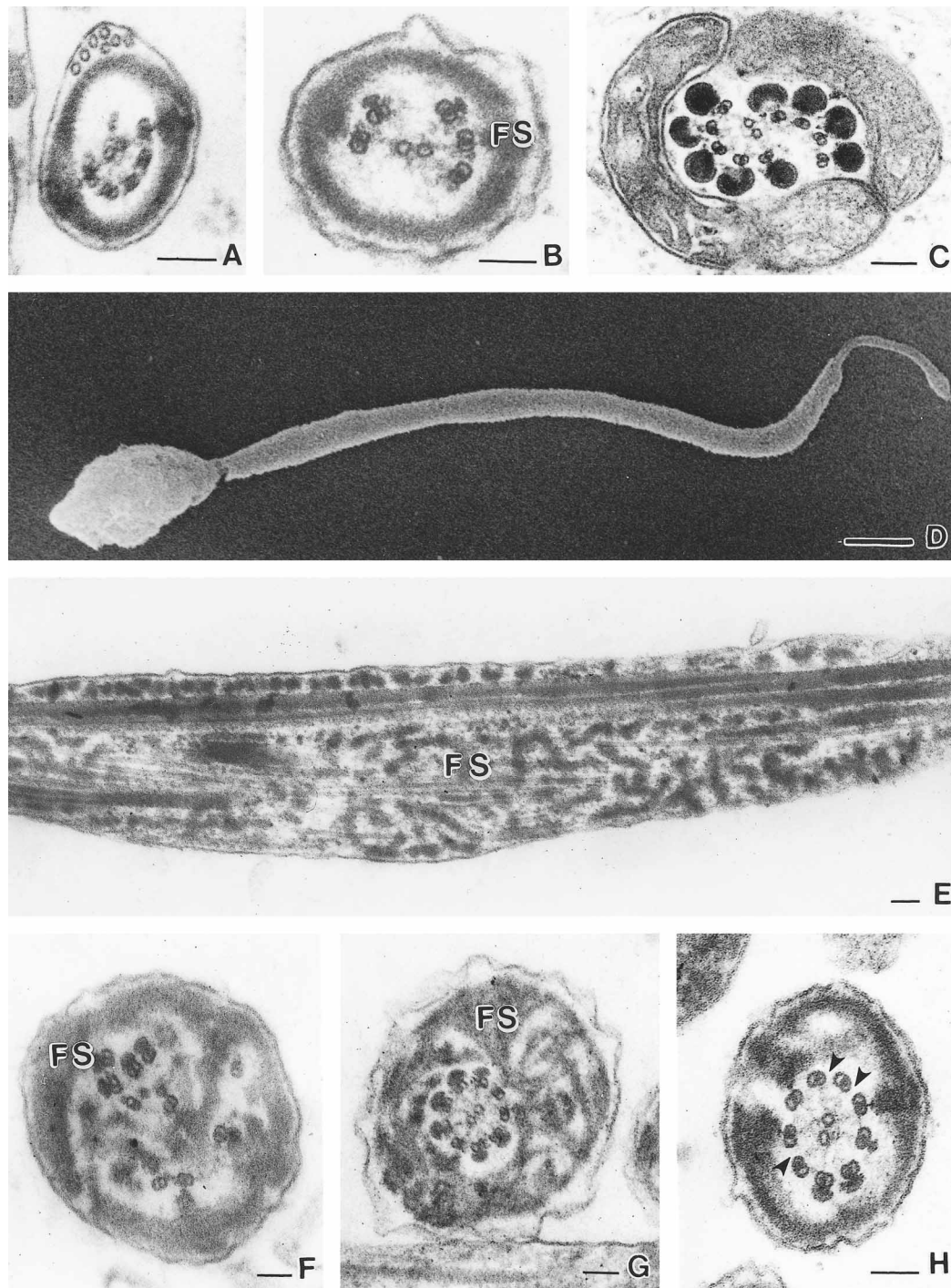


Figure 1. (A–C) Nonspecific flagellar anomalies. Lack (A, B) or translocation (A) of microtubules and “fractured” axonemes (C) can be observed. Fibrous sheaths (FS) have a normal configuration. (D–G) Dysplasia of the fibrous sheath (FS). The tail is short, thick, and irregular. The FS is disorganized and redundant. Distorted (F) or normal (G) axonemes can be seen in association with amorphous FS. (H) Sperm flagellum in the classic form of ICS. Note complete absence of dynein arms in the microtubular doublets (arrowheads). The patient had sperm immotility, chronic respiratory disease, and dextrocardia (Kartagener syndrome). Bars represent 0.1 μm (A–C and F–H) and 1 μm (D).

to different conditions that affect fertility, such as varicocele, infections of the seminal pathway, immunologic factors, and so on, even though in most patients it is impossible to identify any of these conditions.

In summary, NSFA is the most frequent flagellar pathology underlying severe asthenozoospermia, and its structural phenotype of random microtubular alterations is characteristically heterogeneous. NSFA has no

familial incidence, and is sometimes secondary to different andrological disorders, which are potentially reversible or responsive to different treatments. In more severe cases or when there is no response to treatment, the use of spermatozoa with NSFA in microfertilization techniques does not pose serious risks in view of the lack of a genetic component in this pathology.

DFS is an altogether different condition. We have diagnosed 50 such patients with extremely poor motility (Chemes et al, 1987a, 1998). Forward progression averaged $1.1\% \pm 3.1\%$ and rapid linear progression averaged $0.1\% \pm 0.9\%$, values that were significantly lower than those corresponding to patients with NSFA ($P < .001$). In most cases, spermatozoa were completely immotile and depicted short, thick, and irregular tails. This particular appearance has originated the denomination of “stump tails” or “short tails” to refer to this pathology. However, these terms are misnomers because either they fail to provide an insight into the underlying nature of these tail abnormalities or they encompass a heterogeneous array of sperm defects in which a short and thick tail is the common feature. We propose the more comprehensive denomination of DFS that identifies the main defect involved, and which refers to DFS as a developmental anomaly. Ultrastructural studies are important for distinguishing sperm with DFS from other spermatozoa with short and thick tails, which are sometimes present in patients with necrozoospermia or partial obstruction of the seminal tract, and which results in aging and death of spermatozoa and subsequent flagellar fragmentation and thickening. The diagnosis of DFS is made on the basis of fibrous sheath modifications that are present in all affected spermatozoa (Figure 1D through G). These modifications consist of a marked hypertrophy and hyperplasia of random fibrous sheath constituents that form thick rings around the microtubules of a normal or distorted axoneme. Missing axonemal central pairs, dynein arms in the microtubular doublets, or both, are also apparent in some but not all cases. The middle piece is not formed, and mitochondria are poorly assembled or absent.

These flagellar modifications and extreme asthenozoospermia were present in all patients studied and were stable in time, regardless of numerous treatments. We identified five pairs of brothers among our patients. Familial incidence may be even higher because this information is not available for all men. Moreover, other authors (Bisson et al, 1979; Baccetti et al, 1993) have also reported familial incidence, ethnic predominance, or both in various series of patients.

Studies on testicular biopsies or in immature spermatids in semen have demonstrated that these defects arise in spermiogenesis as a failure of the fibrous sheath to properly organize (Ross et al, 1973; Chemes et al, 1987a, 1998; Barthelemy et al, 1990). These features and the

familial incidence (Bisson et al, 1979; Chemes et al, 1987a, 1998; Baccetti et al, 1993) configure a phenotype that suggests a genetic origin of the syndrome. Some patients with DFS (10 out of 50) have suffered from chronic respiratory disease since early childhood. In 2 patients, ultrastructural studies of bronchial cilia demonstrated a lack of dynein arms, a characteristic finding in immotile cilia syndrome (ICS), from which this subset of 10 patients with DFS is a variant (Chemes et al, 1990).

DFS is a systematic sperm abnormality with extreme asthenozoospermia or total sperm immotility. It has a homogeneous and distinctive phenotype characterized by distortions in the fibrous sheath and other axonemal and periaxonemal structures. The condition is not secondary to any andrological disorder and does not respond to medical therapies. Frequent familial incidence has been recorded in all published series, but no genetic anomalies have been demonstrated. DFS has an ominous fertility prognosis. Classical IVF methods fail to achieve fertilization and pregnancy. ICSI may be the treatment of choice, but genetic counseling is required.

An account of sperm defects that are responsible for severe asthenozoospermia would be incomplete without a reference to the classical forms of ICS. Patients with ICS have a history of chronic respiratory disease from childhood and infertility due to sperm immotility. Afzelius et al (1975) reported a lack of dynein in the axonemes of respiratory cilia and sperm flagella as the primary defect responsible for immotility. Subsequent publications have identified various axonemal anomalies that are responsible for ICS (reviewed by Afzelius and Eliasson, 1979). We have seen only 5 of these patients, who presented with severe asthenozoospermia or sperm immotility. Three of them had severe respiratory disease; another had albinism (an alteration that has not been reported to date in cases of ICS). Kartagener syndrome (ICS + dextrocardia) was present in 1 patient. Ultrastructural examination of spermatozoa disclosed lack of dynein arms in sperm axonemes in 4 patients (Figure 1H), and lack of the central pair of microtubules in the fifth. The incidence of these classical forms of ICS in our population appears to be lower than that reported for other geographic areas. Some patients with DFS also have chronic respiratory disease due to lack of dynein in respiratory cilia. This makes them a mosaic variant of ICS in which fibrous sheath and periaxonemal anomalies combine with classical dynein deficiency in sperm axonemes and respiratory cilia (Chemes et al, 1990).

The possibility that DFS and ICS are due to gene anomalies is now strongly suggested by widespread familial incidence (Afzelius and Eliasson 1979; Bisson et al, 1979; Chemes et al, 1998). Even though the fibrous sheath seems to be the main flagellar component involved in cases of DFS, lack of dynein in microtubular doublets,

absence of axonemal central pairs, or abnormal outer dense fibers indicate that this is a disease that affects the cytoskeleton of the sperm tail, a suggestion that was advanced by Escalier and David (1984). This particular phenotype probably indicates a polygenic origin rather than a point mutation as the responsible factor. To date, single fibrous sheath proteins and chromosomal loci have been identified as good candidates to search for genetic alterations (Carrera et al, 1994; Pilder et al, 1997). Multicenter collaborations are currently being carried out to try to identify gene anomalies in patients with DFS. Afzelius and Eliasson (1979) have already proposed a polygenic origin for the various phenotypes of ICS.

Results of microfertilization techniques have been encouraging when applied to patients with severe primary sperm pathologies such as DFS or ICS. Fertilization and pronuclear formation rates are comparable with those reported for other groups of patients with severe male factor infertility. There have been publications of live births (Stalf et al, 1995; Brugo Olmedo et al, 1997) and more recent personal observations with numerous successful microfertilizations and live births (Chemes et al, 1998; Bisioli et al, 1999; Gallo et al, 1999; Brugo Olmedo et al, 2000). Because DFS and ICS have genetic components, the problem arises as to the convenience of using these spermatozoa to obtain fertilization in view of the risk of transmission to the next generation. To date there are no reports of gene anomalies in men with DFS. Until this issue is clarified it is important to make patients aware of the potential risks involved in using abnormal spermatozoa to attain fertilization that would not have taken place if the natural mechanisms of sperm selection had operated as they do in nonassisted conception. Until more genetic knowledge becomes available it is necessary to conduct continued assessments on newborns, which in our hands and the scarce literature so far available, has not disclosed any kind of genetic-related abnormalities. However, it would be wise to wait until these children attain adulthood to confirm these preliminary observations.

Ultrastructural study of spermatozoa has a diagnostic and prognostic value in asthenozoospermia by identifying various kinds of flagellar alterations with different fertility potential. In fact, while 33% of patients with NFSA were able to achieve fertilization and pregnancy after medical treatment or classical techniques of assisted fertilization, there are no such reports in men with DFS or ICS. These latter patients may be good candidates for ICSI, but the transmission of the defect remains a possibility. These considerations not only apply to DFS and ICS, but to all severe cases of sperm pathology with a known or potential genetic component such as various forms of teratozoospermia.

The conflict between the classical notion that asthen-

ozoospermia is mainly a "functional" condition and the more recent recognition that various forms of tail abnormalities are responsible for most sperm motility disorders has now started to unravel. The fibrous sheath that was classically considered a structure with mainly structural-mechanical properties is now known to be composed of proteins such as AKAP4 that anchor protein kinase A to the fibrous sheath and is instrumental in the phosphorylation of tail proteins, an essential step in flagellar motility (Carrera et al, 1994). This and the known ATPase function of dynein, another axonemal structural protein, indicate a dynamic interaction between structural components of the sperm cytoskeleton and functional biochemical events that are essential for sperm movement.

Sperm Defects in Teratozoospermia

Teratozoospermia has been reported as an important cause of deficient fertility in men. Two main kinds of teratozoospermia can be identified. In the first and most frequent variety, morphological examination discloses numerous anomalies in different sperm components. This heterogeneous pattern shows no common defect and results from random alterations in spermatozoa. The type of NFSA described in the previous section belongs to this category. The second variety shows a homogeneous microscopic pattern with a systematic sperm defect present in most spermatozoa. To this variety belong *acephalic spermatozoa* (Perotti et al, 1981; Chemes et al, 1987b, 1999), *round head acrosomeless spermatozoa* (Holstein et al, 1973; Nistal et al, 1978; Florke Gerloff et al, 1984), the *miniacrosome sperm defect* (Baccetti et al, 1991), DFS or *stump tail defect* (Chemes et al, 1987a, 1998), and the dynein-deficient axonemes of ICS (Afzelius et al, 1975).

Acephalic Spermatozoa and Defects in the Head-Neck Attachment—Pinheads are a rare variety among the multiple forms of spermatozoa with abnormal heads. They have received little attention and their characteristics are poorly known. In 1981, Perotti et al reported an infertile patient in which most spermatozoa showed no heads at all and called them *decapitated spermatozoa*. We have reported 3 men with this trait and noted that decapitated (or acephalic) spermatozoa are of testicular origin and derive from spermatids in which the tail anlage develops independently from the nucleus (Chemes et al, 1987b). The finding of loose heads lacking the implantation fossa (the normal site of flagellar attachment) and of immature spermatids with no connection between nucleus and flagellar anlage supports this interpretation. With the more recent study of 7 additional patients, we have broadened the concept of acephalic spermatozoa as a condition derived from pathologic development of the neck region during spermiogenesis, and included in this syndrome

other forms of abnormal sperm displaying abnormal head-middle piece attachments (Chemes et al, 1999).

We studied 10 patients who consulted us for primary sterility. Their ages ranged from 25 to 40 years. On clinical examination there were no significant andrological disorders. Sperm concentration was normal and motility ranged from severe asthenozoospermia to normal. Most spermatozoa displayed alterations in the head-neck region that adopted 3 main configurations. The first variety has normal flagella and a minute cephalic end with no nuclear material. The second variety exhibits a normal middle piece surrounded by a cytoplasmic droplet, which can be confused with a sperm head (Figure 2A and B). The third variety consists of spermatozoa with heads abnormally implanted in the middle piece. The heads attach either to the tip or to the sides of the midpiece without a linear alignment with the sperm axis. This misalignment ranges from complete lack of connection to a lateral positioning of the nucleus at a 90°–180° angle.

In testicular biopsies from affected individuals, early step 1 spermatids depict round euchromatic nuclei and a normal Golgi apparatus in the process of acrosomal formation. The centrioles are located away from the nucleus, and the axoneme grows from the distal centriole. In succeeding steps of spermiogenesis nuclear elongation-condensation and acrosomal development proceed normally, but the flagellar anlage develops independently from the nucleus and fails to establish contact with it and becomes separated at the end of spermiogenesis (Figure 2C). We found signs of increased Sertoli cell phagocytosis in a testicular biopsy from 1 of our patients and believe that in cases of complete separation, the heads remain connected to the residual bodies at spermiation and undergo phagocytosis by Sertoli cells. This explains why loose heads are so rare in semen. In our last series we found 2 brothers with this anomaly, which indicates that the responsible mechanism is most probably genetic, as has also been noted by Baccetti et al (1989). This concept gains support from the observation that seminal characteristics do not change in successive spermiograms performed over long periods, or under pharmacologically induced spermatogenic regression-restoration due to parental testosterone administration (Chemes et al, 1999).

Separation of heads and tails can occur within the testis at spermiation or in the seminal pathway due to an increased instability of the head-middle piece junction. This last possibility is supported by recent observations that highlight the fragility of the head-neck junction as seen by the increase in the percentage of loose heads and acephalic spermatozoa after centrifugation of semen samples containing spermatozoa with abnormal head-midpiece attachments (Chemes et al, 1999). It is important to distinguish acephalic from microcephalic spermatozoa. The investigation of deoxyribonucleic acid (DNA) content by

the Feulgen reaction and a careful electron microscopic study certify the diagnosis, but the typical appearance is identified in light microscopic smears.

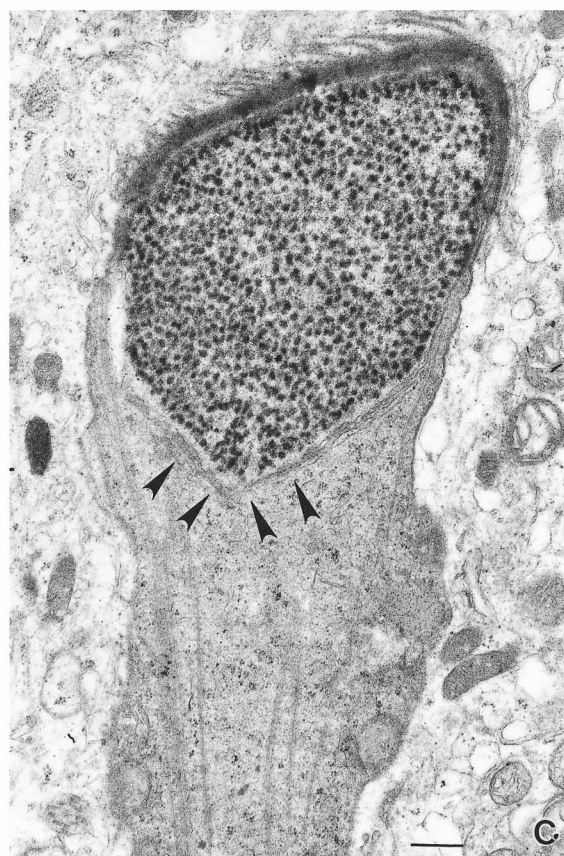
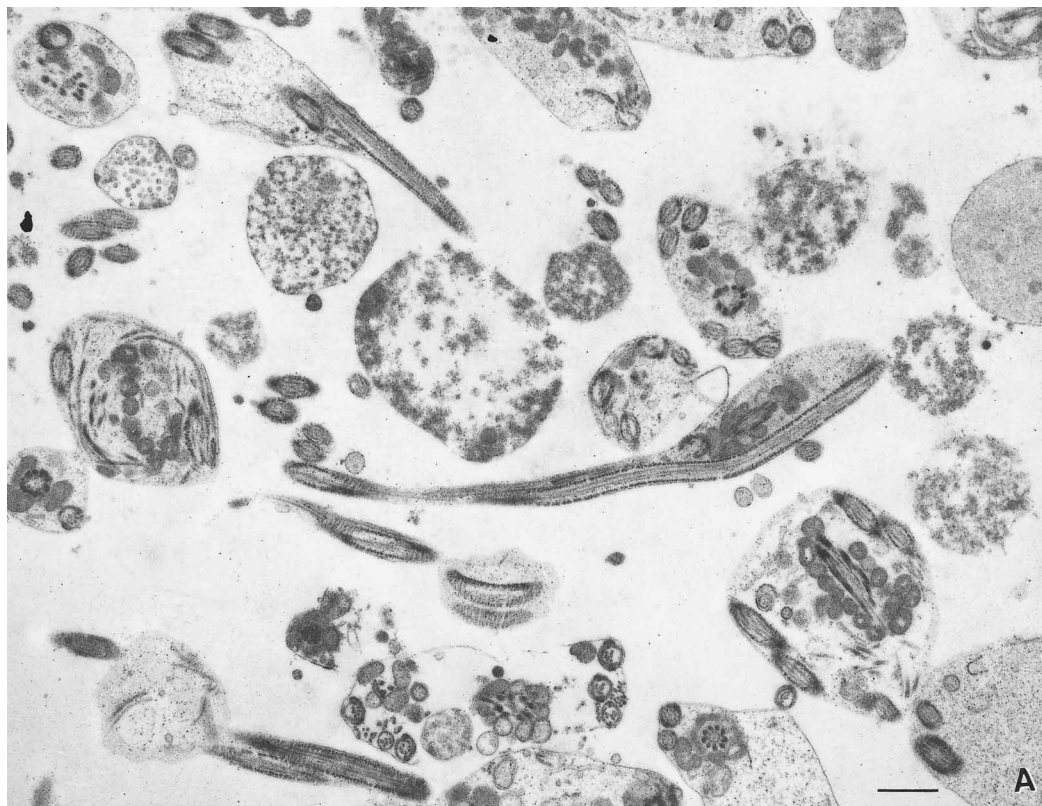
Men who suffer from this syndrome are sterile. No therapeutic measures have been successful in achieving pregnancies. Recent findings that spermatozoa with abnormal head-tail attachments are able to fertilize mature oocytes, which do not proceed to syngamy and cleavage, strongly suggest a defect in the sperm centriole, the organizing center for microtubules in the zygote (Chemes et al, 1999).

The uniform pathologic phenotype, its origin as a consequence of a systematic alteration during spermiogenesis, the fact that seminal characteristics remain constant along clinical evolution even when a pharmacological germ cell depletion-repopulation has been induced, and the familial incidence in men and bulls (Bloom and Birch Andersen, 1970; Baccetti et al, 1989; Chemes et al, 1999) indicate that this characteristic phenotype is likely of genetic origin.

Acephalic or decapitated spermatozoa are primary sperm defects that originate in a failure of migration of the centriole-tail anlage to the caudal pole of the spermatid nucleus during spermiogenesis. As in other systematic sperm defects, the cytologic phenotype is characteristically homogeneous and manifests as headless tails, abnormal head-middle piece attachments, or both. Patients present with primary sterility and no other associated andrological disorders. Familial incidence has been reported, but no genetic abnormality has been demonstrated so far. This condition is stable in time and unresponsive to medical therapies or classical IVF. Even though microinjecting spermatozoa with abnormal head-middle piece attachments has resulted in fertilization, pronuclei do not fuse and zygotes fail to cleave and degenerate.

Lack of Acrosome and Acrosomal Hypoplasia

Round-headed, acrosomeless spermatozoa were identified more than 20 years ago (Holstein et al, 1973; Nistal et al, 1978). They display characteristic spherical heads. Upon ultrastructural examination, the acrosome is missing and there is insufficient condensation of the chromatin (Figure 3A and B). Although this defect is recognized on sperm smears, it is advisable to examine the semen of these patients with electron microscopy. This recommendation is based on the existence of round-headed spermatozoa with intact acrosomes (Zamboni, 1987). We have observed acrosomeless spermatozoa of different shapes, including ovoid and amorphous sperm heads. From these considerations it follows that even though spherical heads suggest acrosomeless spermatozoa, not all round heads are devoid of acrosomes, and not all acrosomeless spermatozoa have round nuclei (Figure 3C). Therefore, the



relationship between round shape and lack of acrosome development is not an absolute rule. These considerations are illustrated by 35 patients with acrosomal abnormalities included in our series; in 7 of them the acrosome was missing in all spermatozoa. This alteration was associated with round heads in 4 patients (the classical round head acrosomeless spermatozoa), or to a mixture of round, amorphous, and oval heads (3 cases). The other 28 patients had hypoplastic, small acrosomes that affected 35%–70% of spermatozoa in each sample. This anomaly has been previously reported in the literature as the *mini-acrosome sperm defect* (Baccetti et al, 1991). Acrosomal hypoplasia should be investigated in cases of severe teratozoospermia and can be readily recognized with an electron microscope (Figure 3E and F). According to Zamboni (1992), hypoplasia of the acrosome is frequent in teratozoospermic men, but it is usually overlooked because of its association with other abnormal sperm forms. Patients who suffer from acrosomal agenesis or hypoplasia are infertile because of deficient acrosomal function. However, there is a variable number of spermatozoa with normally formed acrosomes in acrosomal hypoplasia, and the possibility exists that some patients are capable of fertilization. There are reports in the literature of successful microfertilizations using round acrosomeless spermatozoa, and the same may apply to acrosomal hypoplasia, although no such reports are available. A polygenic inheritance has been postulated for the syndrome of acrosomeless spermatozoa (Nistal et al, 1978; Florke-Gerloff et al, 1984), and familial incidence has also been reported in acrosomal hypoplasia (Baccetti et al, 1991).

Acrosomeless spermatozoa and acrosomal hypoplasia are two sperm anomalies involving the development of the acrosome. Spherical sperm heads are characteristic, but there are both spherical heads with acrosomes and nonspherical acrosomeless spermatozoa. No medical therapies are available. Classical IVF is not effective because of acrosomal deficiency, but the use of ICSI has been successful. Acrosomal hypoplasia has been associated with severe teratozoospermia, but it is not known how frequently this occurs. There are various reports of familial incidence in patients with acrosomeless spermatozoa or acrosomal hypoplasia, but the last form is sometimes considered as an acquired sperm anomaly.

Defects in Chromatin Condensation and Compaction

During spermiogenesis, the chromatin undergoes a complex process of changes in its chemical composition and

macromolecular organization. In many rodents, chromatin condensation proceeds until the nucleus assumes a homogeneous, dense appearance. This is not the case in humans, in whom normally small hypodense areas in the sperm nucleus can be observed. Zamboni (1987) described deficiencies in the process of chromatin maturation that resulted in big “lacunar” defects in which the compact arrangement of the chromatin was replaced by granulo-fibrillar or “empty” areas that occupy as much as 20%–50% of the nucleus (Figure 3D and F). These defects frequently coexist with granular immature chromatin. The presence of these defects anticipates a grim fertility prognosis. We have studied a large number of men with teratozoospermia with predominant amorphous head defects and less than 14% normal forms (strict morphology). Some of these patients were infertile; others were able to establish pregnancy, but spontaneous abortion occurred during the first trimester. Ultrastructural examination revealed lacunar defects of the chromatin in as many as 50% to 80% of spermatozoa. There are reports of abnormal DNA organization, chromosomal abnormalities, and DNA breaks in spermatozoa from patients with chromatin abnormalities (Kjessler, 1974; Evenson et al, 1980; Abramsson, 1982; Sakkas et al, 1999a). These abnormalities may be associated with apoptotic sperm, or with sperm cultured in vitro for prolonged periods, which may have implications for their use in assisted reproductive techniques, fertilization, and early embryo development (Baccetti et al, 1996; Sakkas et al, 1998, 1999b; Blanc-Layrac et al, 2000).

Alterations in chromatin maturation and compaction have been observed in maturing spermatids, which indicates that they are of testicular origin. This diagnosis identifies a specific pathology of the chromatin in many cases of teratozoospermia, and provides an understanding of its etiology that is deeper than that derived from the classification of spermatozoa according to head shapes (tapering, amorphous, etc), which adds little to our understanding of abnormal sperm function. In view of these observations it is advisable to look for structural alterations of the chromatin in all individuals with teratozoospermia in whom acrosomal hypoplasia is sometimes associated (Figure 3F).

Longitudinal observations have revealed that the incidence of chromatin abnormalities varies along clinical evolution (in a particular case from 35% to 80%) and are spontaneously reversible in some patients. This probably

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Figure 2. Acephalic or decapitated spermatozoa. (A) Panoramic view of the sperm pellet. Note absence of sperm heads. The cranial ends of spermatozoa show a cytoplasmic thickening surrounding the midpiece. (B) Acephalic spermatozoon with normal configuration of the midpiece. The neck region is directly covered by the plasma membrane (asterisks). (C) Maturing spermatid in a testicular biopsy. There is complete disruption of the head-flagellum attachment. The flagellum that normally lodges in the caudal pole of the nucleus is absent. The perinuclear cistern (arrowheads) covers this region. Acrosomal development, nuclear elongation, and chromatin condensation is normal. Bars represent 1 μm (A) and 0.5 μm (B, C).

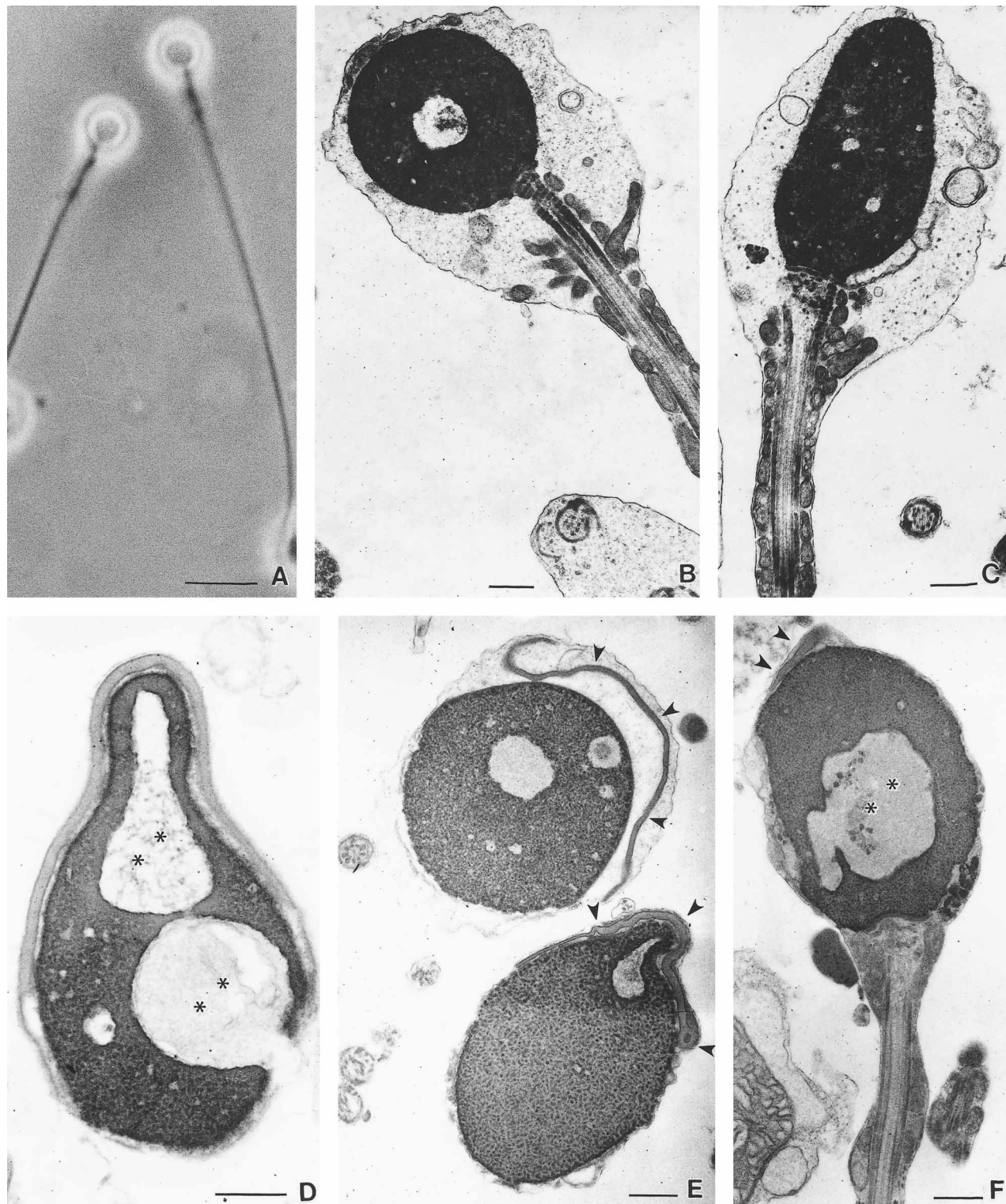


Figure 3. (A, B) Round-headed acrosomeless spermatozoa. In (C), the absence of acrosome is associated with an elongated (not round) head. (D) Conspicuous lacunar defects on chromatin condensation (asterisks). (E) Small detached acrosomes (arrowheads) in acrosomal hypoplasia. (F) Combination of lacunar defect in chromatin condensation (asterisks) and hypoplastic acrosome (arrowheads). Bars represent 10 μm (A) and 0.5 μm (B-F).

indicates that a genetic component is not involved in its pathogenesis. Moreover, they have also been observed in association with inflammatory bowel disease and may be caused by environmental or pharmacological agents (Zamboni, 1987). We have been unable to find consistent exposures to toxicants in this population. Recently, Baccetti et al (1997) reported that treatment with follicle-stimulating hormone (FSH) can improve some sperm defects, which may be related to the improvement in fertility rates secondary to FSH treatment of men in assisted fertility programs (Acosta et al, 1992). In view of the demonstrated involvement of FSH in spermiogenesis, it would be worthwhile to test the possible therapeutic effect of FSH on chromatin abnormalities.

Defects of chromatin maturation and compaction are characterized by lacunar and immature chromatin. They present usually in severe teratozoospermia, and are sometimes associated with acrosomal hypoplasia. There is no familial incidence. Inflammatory bowel disease and sulphamide administration have been linked to chromatin abnormalities. Their incidence in spermatozoa fluctuates along clinical evolution or diminishes after sulphamide withdrawal. Infertility or abortions during the first trimester have been reported in these patients.

Final Remarks

In the previous pages, I have attempted to summarize the available literature on sperm pathology and our personal experience with the ultrastructural study of more than 1000 semen samples from sterile individuals. This experience leads us to believe that the use of electron microscopy is a valuable diagnostic tool in clinical andrology because it can refine the diagnosis of abnormal spermatozoa, establish reliable prognostic-therapeutic indicators, and act as an effective partner of routine semen analysis and functional tests of sperm competence.

The use of ultrastructural examination of spermatozoa does not require setting up this technology in all andrology laboratories. The initial processing can be easily performed in any laboratory and samples can be submitted to a specialized laboratory for diagnosis. We have successfully developed this organizational approach and routinely use it as a significant assistance in the diagnosis of infertile patients. These methodologies have allowed us to move from an empirical diagnosis of sperm anomalies to a comprehensive approach to sperm pathology that derives useful information from different sources: routine semen analysis, functional sperm tests, and ultrastructural examination of spermatozoa. They do not compete with each other, but cooperate in providing a correct diagnosis, a prognostic tool, and a deeper understanding of the mechanisms of abnormal reproduction in the sterile male.

A final word about genetic counseling in men with

sperm pathologies of genetic origin. Patients should be informed of the possible genetic risks involved in using spermatozoa that may carry mutated genes, and the likelihood of transmission of infertility to male offspring. In these cases, infertility seems to be the entire risk. Most couples would accept this risk with the understanding that treatments are already available and may become more effective in the future. A word of caution seems appropriate when sperm defects are linked to other nonreproductive pathologies such as chronic respiratory disease in cases of ICS or DFS. The fact that no respiratory symptoms have been detected in any newborns whose fathers have DFS opens a door of hope. In all cases, informed consent should be obtained from couples before proceeding to ICSI. Definitive counseling will not be possible until the gene or genes that are responsible and the mode of transmission of these phenotypes are known in detail.

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References

- Abramsson L, Beckman G, Duchek M, Nordenson I. Chromosomal aberrations and male infertility. *J Urol*. 1982;128:52–53.
- Acosta AA, Khalifa E, Oehninger S. Pure human follicle stimulating hormone has a role in the treatment of severe male infertility by assisted reproduction: Norfolk's total experience. *Hum Reprod*. 1992;7:1067–1072.
- Afzelius BA, Eliasson R. Flagellar mutants in man: on the heterogeneity of the immotile-cilia syndrome. *J Ultrastruct Res*. 1979;69:43–52.
- Afzelius BA, Eliasson R, Johansen D, Lindholmer C. Lack of dynein arms in immotile human spermatozoa. *J Cell Biol*. 1975;66:225–232.
- Baccetti B, Burrini AG, Capitani S, Collodel G, Costantino-Ceccarini E, Di Cairano G, Estenoz M, Martini L, Moretti G, Piomboni P. The

- “short tail” and “stump” defect in human spermatozoa (notulae seminologicae 2). *Andrologia*. 1993;25:331–335.
- Baccetti B, Burrini AG, Collodel G, Magnano AR, Piomboni P, Renieri T, Sensini C. Morphogenesis of the decapitated and decaudated sperm defect in two brothers. *Gamete Res*. 1989;23:181–188.
- Baccetti B, Burrini AG, Collodel G, Piomboni P. Apoptosis in human ejaculated sperm cells (notulae seminologicae 9). *J Submicrosc Cytol Pathol*. 1996;4:587–596.
- Baccetti B, Burrini AG, Collodel G, Piomboni P, Renieri T. A “mini-acrosome” sperm defect causing infertility in two brothers. *J Androl*. 1991;12:104–111.
- Baccetti B, Strehler E, Capitani S, Collodel G, De Santo M, Moretti E, Piomboni P, Wiedeman R, Sterzik K. The effect of follicle stimulating hormone therapy on human sperm structure (notulae seminologicae 11). *Hum Reprod*. 1997;12:1955–1968.
- Barthelemy C, Tharanne MJ, Lebos C, Lecomte P, Lansac J. Tail stump spermatozoa: morphogenesis of the defect. An ultrastructural study of sperm and testicular biopsy. *Andrologia*. 1990;22:417–425.
- Bisioli C, Nodar F, Rolla ED, Papier S, Ruhlman C, Chemes HE. Performance reproductiva mediante ICSI en hermanos no gemelos con displasia completa de la vaina fibrosa espermática. (Reproductive outcome in two brothers (not twins) with dysplasia of the fibrous sheath). In: *Proceedings of the Annual Meeting of the Argentine Society of Andrology*. Buenos Aires: Argentine Society of Andrology; 1999; 8(2):22–23.
- Bisson JP, Leonard C, David G. Caractere familial de certaines perturbations morphologiques des spermatozoides. *Arch Anat Cytol Pathol*. 1979;27:230–233.
- Blanc-Layrac G, Bringuier AF, Guillot R, Feldmann G. Morphological and biochemical analysis of cell death in human ejaculated spermatozoa. *Cell Mol Biol (Noisy le Grand)*. 2000;46:187–197.
- Bloom E, Birch Andersen A. Ultrastructure of the “decapitated sperm defect” in Guernsey bulls. *J Reprod Fertil*. 1970;23:67–72.
- Brugo Olmedo S, Nodar F, Chillik C, Chemes H. Successful ICSI with sperm from a patient with dysplasia of the fibrous sheath and chronic respiratory disease. *Hum Reprod*. 1997;12:1497–1499.
- Brugo Olmedo S, Rawe V, Nodar F, Galaverna G, Acosta A, Chemes HE. Pregnancies established through intracytoplasmic sperm injection (ICSI) using spermatozoa with dysplasia of the fibrous sheath. *Asian J Androl*. 2000;2:125–130.
- Carrera A, Gerton GL, Moss SB. The major fibrous sheath polypeptide of mouse sperm: structural and functional similarities to the A-kinase anchoring proteins. *Dev Biol*. 1994;165:272–284.
- Chemes H. The significance of flagellar pathology in the evaluation of asthenozoospermia. In: Baccetti B, ed. *Comparative Spermatology 20 Years Later*. Sero Symposium Publications, vol 75. New York: Raven Press; 1991:815–819.
- Chemes H, Brugo Olmedo S, Carrere C, Oses R, Carizza C, Leisner M, Blaquier J. Ultrastructural pathology of the sperm flagellum. Association between flagellar pathology and fertility prognosis in severely asthenozoospermic men. *Hum Reprod*. 1998;13:2521–2526.
- Chemes H, Brugo Olmedo S, Zanchetti F, Carrere C, Lavieri JC. Dysplasia of the fibrous sheath. An ultrastructural defect of human spermatozoa associated with sperm immotility and primary sterility. *Fertil Steril*. 1987a;48:664–669.
- Chemes HE, Carizza C, Scarinci F, Brugo S, Neuspiller N, Schwarzsztain L. Lack of a head in human spermatozoa from sterile patients: a syndrome associated with impaired fertilization. *Fertil Steril*. 1987b; 47:310–316.
- Chemes H, Morero JL, Lavieri JC. Extreme asthenozoospermia and chronic respiratory disease. A new variant of the immotile cilia syndrome. *Int J Androl*. 1990;13:216–222.
- Chemes HE, Puigdomenech ET, Carizza C, Brugo Olmedo S, Zanchetti F, Hermes R. Acephalic spermatozoa and abnormal development of the head-neck attachment. A human syndrome of genetic origin. *Hum Reprod*. 1999;14:1811–1818.
- Escalier D, David G. Pathology of the cytoskeleton of the human sperm flagellum: axonemal and periaxonemal anomalies. *Biol Cell*. 1984;50: 37–52.
- Evenson DP, Darzynkiewicz Z, Melamed MR. Relation of mammalian sperm heterogeneity to infertility. *Science*. 1980;210:1131–1133.
- Florke-Gerloff S, Topfer-Petersen E, Muller-Sterl W, Mansouri A, Schatz R, Schirren C, Schill W, Engel W. Biochemical and genetic investigation of round headed spermatozoa in infertile men including two brothers and their father. *Andrologia*. 1984;16:187–202.
- Gallo A, Ripoli C, Chemes HE, Coco R. Posibilidad de fertilidad en el Síndrome de Kartagener con microfertilización asistida (Fertility by ICSI in Kartagener's Syndrome). In: *Proceedings of the Annual Meeting of the Argentine Society of Andrology*. Buenos Aires: Argentine Society of Andrology; 1999;8(2):34–35.
- Holstein AF, Schirren C, Schirren CG. Human spermatids and spermatozoa lacking acrosomes. *J Reprod Fertil*. 1973;35:489–491.
- Kjessler B. Chromosomal constitution and male reproductive failure. In: Mancini RE, Martini L, eds. *Male Fertility and Sterility*. London: Academic Press; 1974:231–247.
- Nistal M, Herruzo A, Sanchez Corral F. Teratozoospermia absoluta de presentación familiar: espermatozoides microcéfalos irregulares sin acrosoma. *Andrologia*. 1978;10:234–240.
- Pedersen H, Rebbe H. Absence of arms in the axoneme of immotile human spermatozoa. *Biol Reprod*. 1975;12:541–544.
- Perotti ME, Giarola A, Gloria M. Ultrastructural study of the decapitated sperm defect in an infertile man. *J Reprod Fertil*. 1981;63:543–549.
- Pilder SH, Olds-Clarke P, Orth J, Jester WF, Dugan L. HSt7: a male sterility mutation perturbing sperm motility, flagellar assembly and mitochondrial sheath differentiation. *J Androl*. 1997;18:663–671.
- Ross A, Christie S, Edmond D. Ultrastructural tail defects in the spermatozoa from men attending a subfertility clinic. *J Reprod Fertil*. 1973;32:243–251.
- Sakkas D, Mariethoz E, Manicardi G, Bizzaro D, Bianchi PG, Bianchi U. Origin of DNA damage in ejaculated human spermatozoa. *Rev Reprod*. 1999a;1:31–37.
- Sakkas D, Mariethoz E, St John JC. Abnormal sperm parameters in humans are indicative of an abortive apoptotic mechanism linked to the Fas-mediated pathway. *Exp Cell Res*. 1999b;251:350–355.
- Sakkas D, Urner F, Bizzaro D, Manicardi G, Bianchi PG, Shoukir Y, Campana A. Sperm nuclear DNA damage and altered chromatin structure: effect on fertilization and embryo development. *Hum Reprod*. 1998;4:11–19.
- Stalf T, Sanchez R, Kohn FM, Schalles U, Kleinstein J, Hinz V, Tielsch J, Khanaga O, Tjurlley H, Gips H. Pregnancy and birth after intracytoplasmic sperm injection with spermatozoa from a patient with tail stump syndrome. *Hum Reprod*. 1995;10:21.
- Zamboni L. The ultrastructural pathology of the spermatozoon as a cause of infertility: the role of electron microscopy in the evaluation of semen quality. *Fertil Steril*. 1987;48:711–734.
- Zamboni L. Sperm structure and its relevance to infertility. *Arch Pathol Lab Med*. 1992;116:325–344.