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Isolated teratozoospermia: a cause of male sterility in the era oF ICSI?

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1. ABSTRACT

Single structural defects involving the totality of ejaculated sperm are among rare cases of untreatable human male infertility. This form of infertility is of genetic origin and is generally transmitted as an autosomal recessive traits. Acrosome agenesis or globozoospermia results from perturbed expression of nuclear proteins or from an altered Golgi-nuclear recognition during spermiogenesis. Failed fertilization after intracytoplasmic sperm injection (ICSI) of acrosomeless sperm is consistent with an inability of sperm to activate oocytes. Acephalic spermatozoa result from a head-neck defect due to a failure of migration of the tail anlagen and related centriole to the caudal pole of spermatids. An abnormal sperm centrosome function may explain the defective embryo cleavage after ICSI with sperm carrying a fragile head-neck junction. Primary cilia dyskinesia (PCD) and dysplasia of the fibrous sheath (DFS) are isolate defects associated with absent or greatly reduced sperm motility due to an abnormal ciliary structure and function (PCD) or to a disorganized fibrous sheath (DFS). Numerous defective genes are potentially involved in human isolated teratozoospermia but such defects have not been defined at the molecular level in most cases. IVF-ICSI is the only available method for obtaining live births with sperm carrying these defects, but the outcome is poor and the genetic risk for the subsequent generation can not be determined.

2. INTRODUCTION

To fulfil its reproductive function *in vivo*, the round spermatid should undergo a complex structural differentiation in the seminiferous tubule, and a functional maturation during the epididymal migration. During spermiogenesis the round spermatid acquires an efficient motor apparatus, an intact acrosome and a condensed nuclear chromatin. The integrity of all these specialised structures are detectable by ultrastructural analysis with transmission electron microscope (TEM) which remains the gold standard for their evaluation (1). The contribution to infertility of an altered structural organization of the different components of ejaculated spermatozoa has been the object of numerous studies extensively reviewed (1-4). Single structural defects that involving the majority or the totality of ejaculated human spermatozoa negate the ability of the male gamete to reach and to fertilize the oocyte will be reviewed. Attention will be focused on animal models engineered in the last two decades to unravel the possible molecular mechanism causing single total structural sperm defects, the genetic origin of which and the modality of transmission are largely demonstrated (v. infra). The outcome of the widespread use of assisted reproductive techniques (ART) proposed to treat infertility due to single total structural sperm defects is also discussed.

In the following sections single structural defects involving the totality of ejaculated sperm will be discussed

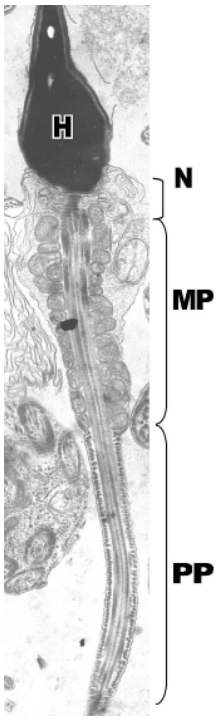

Figure 1. Total view of ejaculated human sperm. H: the sperm head; N: neck or connecting piece, MP: middle piece, PP: principal piece of the flagellum.

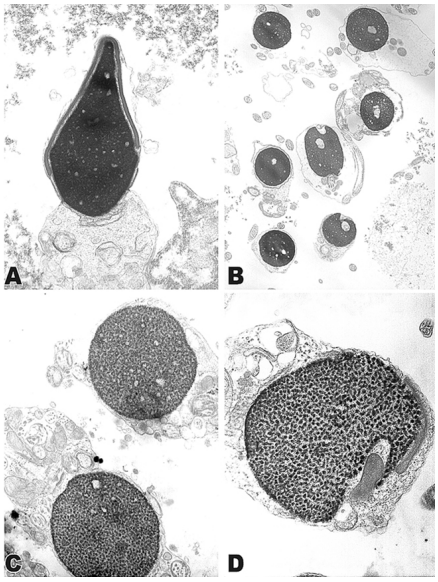

Figure 2. A indicates a normal head covered in the anterior region by a homogeneous electron dense acrosome, while the nucleus shows a condensed chromatin. B-C: Acrosome agenesis. B indicates a low power picture of ejaculated sperm with round heads and no acrosome. C shows round heads embedded in a cytoplasm remnant and a chromatin with a coarse granular texture. D indicates sperm heads with acrosome hypoplasia; an acrosome remnant is present in the most anterior segment of a round head with an incomplete chromatin condensation.

by dissecting the human spermatozoa into four morphofunctional segments: the head, the connecting piece, the neck or middle piece, and the principal piece of the tail (Figure 1).

3. ISOLATE DEFECTS OF THE SPERM HEAD

Two morphofunctional domains form the sperm head: the acrosome and nuclear chromatin (Figure 2A). The acrosome and cell plasmamembrane, are specialized structures involved in gamete recognition and oocyte penetration, while the nuclear chromatin is responsible for the genetic male contribution to the generating embryo (1, 5, 6). Isolate head defect involving all ejaculated sperm are associated with a normal sperm number and to normal sperm motility and include the condition of acrosome agenesis and acrosome hypoplasia

3.1. Acrosome Agenesis/ Hypoplasia

Acrosome agenesis first described by Holstein *et al*, in 1973 (7), is most often associated with a spherical shape of the head so that it is usually defined as "round head defect" or "globozoospermia" (Figures 2B,C). TEM analysis shows a round or oval nucleus embedded in a cytoplasmic remnant without any acrosome or with a thin and short acrosome (Figures 2C, D). The immunhistochemical detection of proacrosin one of the main constituents of the acrosome, confirms either a total absence of the enzyme (8) or the presence of immunoreactive spots in the anterior pole of round-headed sperm (9). Based on the total absence of acrosome, or to the presence of an acrosome remnant, 2 types of round-headed sperm have been proposed (10): type 1 refers to cases with an acrosome agenesis (Figure 2C), while type 2 refers to the acrosome hypoplasia (Figure 2D). The latter condition is indicated also as "miniacrosome" defect (11). Whether or not the two types represent conditions with different fertilization potential is not yet defined. Affected spermatozoa show a lack of post-acrosomal dense lamina (12-14), an incomplete pattern of chromatin condensation (Figures 2C, D) (10, 12, 15,16) and increased rates of DNA fragmentation (17). Absence of acrosome, spherical nucleus and chromatin immaturity, coexist in most reported cases (7, 12, 18- 20). Acrosome agenesis is also associated to an abnormal arrangement of mitochondria in the tail middle piece (10, 21)

3.1.1. Experimental models of human acrosome agenesis

The mechanism that results in the generation of acromeless spermatids during spermiogenesis is not known. Available data suggest that defect results from an altered organization of perinuclear cytoskeleton in the round spermatid; accordingly the Golgi complex prematurely leaves the perinuclear position and fails to generate the acrosome (8). While at the best these observations may help to explain the reason for the lack of a normal acrosome, the concomitant occurrence of a lack of nuclear elongation and of chromatin immaturity suggest that acrosome agenesis results from still unknown defects of genes that control the molecular mechanisms responsible for the morphogenesis of the sperm head. Various animal models suggest that acrosome agenesis and associated

Table 1. Experimental murine models of human isolated teratozoospermia

Genotype	Deleted factors	Phenotype	References
Csnk2alpha2$^{-/-}$	*Alpha'* catalytic subunit of the casein kinase II (Ck2)	oligozoospermia and globozoospermia	22
H1t2$^{-/-}$	H1T2, an histone H1 variant	Aberrant head elongation; acrosome detachment; poor chromatin condensation	25
Hrb$^{-/-}$	Hrb, an Asn-Pro-Phe (NPF) motif-containing protein	Lack acrosome formation and mitochondrial sheath	27
Gopc$^{-/-}$	GOPC (Golgi-associated PDZ-and coiled-coil motif-containing protein)	Lack acrosome formation and mitochondria disorganization	28
Hook1$^{-/-}$	Hook1, a protein that colocalyzes to the manchette during spermiogenesis	*azh*: abnormal spermatozoon head shape and head decapitation	78, 79
Mdhc7$^{-/-}$	Dynein heavy chain 7 of the inner arm	Defect of sperm motility due to deletion of ATP binding site of *MDHC7;* no gross defect in axoneme structure	138, 139
Mdnah5$^{-/-}$	Dynein heavy chain 5 of the outer arm	Cilia immotility, situs inversus, recurrent respiratory infections	140
tektin-t$^{-/-}$	Component of outer doublet microtubules, near the binding sites for radial spokes, inner dynein arms and nexin links	Partial loss of inner dynein arm in respiratory cilia and in the sperm axoneme. Reduced sperm motility and functionally defective tracheal cilia	147
Spag6$^{-/-}$	Component of the central doublet Spag6	Loss of the central doublet and disorganization of the ODF and of the FS. Reduced sperm motility and functionally defective cilia in the ependymal cells	154
Pol lambda $^{-/-}$	DNA polymerase lambda; disruption of *Dpcd* (for deleted in a mouse model of primary cilia diskinesia)	hydrocephalus, situs inversus totalis and chronic suppurative sinusitis, sperm immotility, and deranged spermatogenesis	155, 157
Akap4$^{-/-}$	Absence of AKAP4, the major protein in the fibrous sheath and reduced expression of AKAP3 and of glyceraldehydes 3-phosphate dehydrogenase-S (GAPDS)	No progressive sperm motility and short flagellum with an incomplete fibrous sheath	113
Hst-6$^{-/-}$ and *Hst-7*$^{-/-}$	defects in hybrid sterility (Hst) loci 6 and 7	Disassembled sperm axoneme microtubules, outer dense fibers and fibrous sheath associated to disorganized mitochondria. Sperm motility undefined	169, 170

defects are related to either a perturbed expression of nuclear proteins during spermiogenesis, or to an alteration in the mechanisms of Golgi-nuclear recognition and docking (Table 1).

Male mice lacking the *alpha'* catalytic subunit of the casein kinase II (Ck2) encoded by the *Csnk2alpha2* gene are infertile and show oligozoospermia and globozoospermia (22). Mutant mice undergo a normal spermiogenesis up to step 8 round spermatids. During following spermatid elongation, acrosomes become detach from the nucleus and appear fragmented. Epididymal spermatozoa are round or ovoid and show a decondensed nuclear chromatin, main features of human globozoospermia. Consistent with a potential role in regulating the development of sperm nuclear structure, Ck2 has been found to be associated with sperm chromatin (23). Abundant expression of Ck2alpha' was found in human spermatozoa (22) suggesting *Csnk2alpha2* as a candidate globozoospermia gene although to date, no mutations in *Csnk2alpha2* gene was identified among six men affected by acrosome agenesis (24). Recently it was described a H1T2, a histone H1 variant selectively and transiently expressed in male haploid germ cells during spermiogenesis, required for proper cell restructuring and DNA condensation during the elongation phase of spermiogenesis (25). Accordingly, male *H1t2*$^{-/-}$ mice show aberrant elongation, a poor chromatin condensation, fragmented DNA, and acrosome detachment (25) all features observed in cases of human acrosome agenesis.

Two experimental models underscore an alteration in the mechanisms of Golgi-nuclear recognition and docking in acrosome agenesis. Hrb is an Asn.Pro-Phe (NPF) motif-containing protein that has been shown to interact with Eps15, a transport vescicle adaptor protein (26). Hrb-deficient mice lack acrosome formation and the primary defect in this model is an arrest of the acrosome vesicles biogenesis at step 2 round spermatids with a failure of proacrosome vesicles to fuse with each other (27). Additional feature of Hrb-deficient mice consists in the development of a tail middle piece lacking the mitochondrial sheath (27). A similar phenotype was observed in Golgi-associated PDZ- and coiled-coil motif-containing protein (GOPC)-deficient mice (28). GOPC is a Golgi-associated protein involved in vesicles transport from the Golgi apparatus (29). Mice bearing a null allele of *Gopc* generated by gene targeting, are infertile and lack acrosomes because of the failure of vesicle transport from the Golgi apparatus to the acrosomal cap at step 2-3 round spermatids (28). Since GOPC is localised at Golgi apparatus of round spermatids (28) while Hrb is localized at the outer membrane of the acrosome (27) where GOPC is not detected, it is assumed that both proteins are differentially involved in vesicle-to-vesicle docking and/or fusion during acrosome biogenesis. In addition to the lack of acrosomes, *GOPC*$^{-/-}$ mice show epididymal spermatozoa with vacuolized ovoid nuclei but a condensed chromatin, and an abnormal arrangement of mitochondria in the middle piece (28), as reported in human globozoospermia (10, 12, 15). These findings raise the possibility that GOPC may be involved in some cases of human acrosome agenesis.

All together, reported experimental models underscore the heterogeneous phenotype of acrosome agenesis and suggest that mutations of different genes differentially implicated in the morphogenesis of sperm nuleous and acrosome may be responsible for this rare defect. The relevance of these genes in human globozoospermia is at present unknown. A polygenic inheritance has been surmised for human acrosome agenesis and the defect has a familial incidence with affected brothers in the same family group (11, 20, 30-32).

Table 2. Reproductive competence after intracytoplasmic sperm injection of sperm from men affected by isolated teratozoospermia

Sperm phenotype	Sperm defect	Fertilization	Live Births	References
Globozoospermia (acrosome agenesis) (n=27)	Precluded interaction with the oocyte; sperm-oocyte-activation deficiency	39% (0% to 100%)	7 in 27 couples	21, 32, 36-43, 48, 49
Head-neck defect (n=22)	Easy separation between head and tail and abnormal centrosome	65% (0% to 100%)	6 in 4 couples	65, 73-75, 80, 83
Primary ciliary dyskinesia/Kartagener's syndrome (n=12)	Sperm immotility	55% (0% to 100%)	8 in 6 couples	172-176, 180, 181, 184, 185
Dysplasia of the fibrous sheath (n=12)	Non-progressive sperm motility	63%±16	14 in 12 couples	4

3.1.2. Reproductive competence of sperm with acrosome agenesis

Functional studies have shown that acrosomeless sperm both in human as well as in experimental models are incompetent for fertilization since they do not interact with the oocyte and are unable to penetrate the zona pellucida and oolemma (15, 27, 33-35). Accordingly, oocyte fertilization could take place provided that the inability to interact and penetrate the female gamete is bypassed by intra cytoplasmic sperm injection (ICSI).

Since the first ICSI-baby obtained with acrosomeless sperm in 1995 (36), ICSI has been used to treat few other cases (Table 2). Available data show that the fertilization rate following ICSI with acrosoless sperm is low both in human and in experimental models (21, 28, 32, 37-41) and livebirths were seldomly reported (38, 40, 42, 43). This suggests that beside the inability to interact and penetrate the female gamete, acrosomeless sperm do show additional defects that limit their fertilization ability and competence for embryonic development also when submitted to ICSI. The low ability to activate oocytes, that therefore do not complete meiosis after fertilization (21, 38, 40), results in a low fertilisation rate by round-headed sperm after ICSI.

Oocyte activation is characterized by a two-step pattern of rises in intracellular Ca2+ concentrations. A first Ca2+ rise originates from the oocyte cortex after sperm-oocyte membrane interaction and is followed 30 min later by a series of shorter Ca2+ transients of high amplitude that continue for 3-4h (oscillator) (44). The oscillator function is dependent on the release of a sperm-associated oocyte activation factor that conditions the oocyte to sustain repetitive Ca2+ releases from intracellular stores (45). Tesarik *et al*, (2002)(46) have shown that also an oocyte factor is involved in activating the oscillator mechanism. A heterologous ICSI model of human sperm into mouse oocytes (mouse oocyte activation test, MOAT) confirmed a sperm-oocyte-activation deficiency in men affected by acrosome agenesis (47, 48).

Successful assisted oocyte activation has been obtained with calcium ionophore treatment in isolate cases of ICSI with round-head spermatozoa (38, 49). Recently this treatment in a group of six men affected by globozoospermia and a sperm-oocyte-activation deficiencies documented by use of MOAT, restored fertilization and embryo development rates to a normal level and allowed to obtain pregnancies in 5 out of 6 cases (48). The clinical use of calcium ionophores in assisted reproduction is however limited by insufficient knowledge of their potential toxic effect on oocytes and embryos. Alternative techniques of assisted oocyte activation include a vigorous aspiration of oocyte cytoplasm (Tesarik *et al*, 2002), or electrical oocyte activation (50). Worth mentioning is the observation that electrical oocyte activation restored fertilization and embryo development after ICSI with spermatozoa from GOPC-deficient mice that lacked acrosomes and failed to activate oocyte after ICSI (28). This underscores the relevance of experimental models of globozoospermia to explore the effectiveness and potential hazards of methods of sperm-oocyte manipulation to be offered to couples with man affected by deficient oocyte-activation ability after ICSI.

The low competence for embryo development of acrosomeless sperm submitted to ICSI might be related also to their chromatin immaturity. It has been shown that DNA of spermatozoa with only partial condensation of chromatin is single-stranded rather than double-stranded (51, 52) and consequently more prone to undergo spontaneous denaturation. Ejaculated or testicular sperm carrying diffuse chromatin defects show a low oocyte fertilisation rate (53) and a low ability to support embryonic development when microinjected into the oocyte (54, 55). In the case of acrosome agenesis this does not seem to be related to the presence of karyotype abnormalities in affected spermatozoa, according to some authors (39, 47, 56), while a high level of sperm aneuploidy was reported by others (17, 31,57-60).

In conclusion acrosome agenesis involving all ejaculated spermatozoa represents a structural defect associated to an altered moulding of the head and to an altered chromatin condensation that underlies a still poorly defined defective spermiogenesis at molecular level. Acrosome agenesis is recognised already at light microscopy although TEM analysis is the gold standard to correctly define all coexisting defects, namely the head shape, the absence or acrosome hypoplasia and the chromatin immaturity (1). ICSI techniques can be offered in these cases although a low fertilization rate and a low pregnancy rate are expected due to the low ability of these microinjected sperm to activate oocytes. The future development of safe methods of assisted oocyte activation during ICSI is expected to improve or restore the fertility potential of men affected by acrosome agenesis.

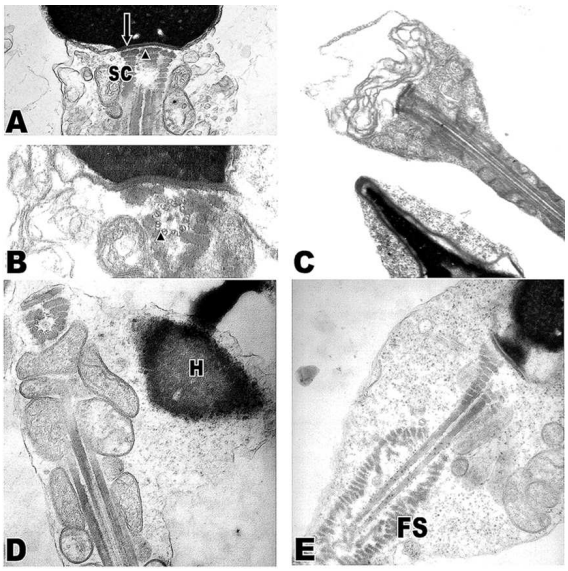

Figure 3. A: shows the connecting piece of a normal sperm; the segmented columns (sc) are adjacent to the basal plate (arrow) which lines the posterior surface of the nucleus; the arrowhead indicates the capitulum. B shows a transverse section view of the proximal centriole (arrow head) embedded between the segmented columns. C shows a headless tail in an ejaculate of a men affected by a head-neck defect; the uppermost segment of a tail with a normal organization of the middle piece is embedded in a voluminous cytoplasm remnants containing whorled membranes. D indicates an abnormal alignment of the head (H) with the mid-piece resulting in a sperm with bent head. The connecting piece shows a normal organization of capitulum, segmented columns and of the proximal centriole. E shows a sperm tail of a man affected by dysplasia of the fibrous sheath; the middle piece is almost absent and few mitochondria are scattered in the cytoplasm remnant, while the fibrous sheath (FS) shows disorganized ribs.

4. ISOLATE DEFECTS OF THE SPERM TAIL

Four distinct segments form the sperm tail: the connecting piece, the middle piece, the principal piece and the terminal piece (see 1 for a detailed description) (Figure 1). Structural abnormalities of the different components of the tail are responsible for altered/absent sperm motility.

4.1. Defects of the connecting piece: the head-neck defect

The connecting piece constitutes the most cranial segment of the sperm tail and ensures the connection between the head and the flagellum. During spermiogenesis the two centrioles migrate to a pole of the spermatid nucleus and directly contribute to the assembly of the connecting piece and axonemal microtubules. Both centrioles participate to the morphogenesis of the capitulum and the segmented columns (Figures 3A, B), while the distal centriole is the template of the doublets microtubules of the sperm axoneme (61, 62). The proximal centriole establishes a contact with the nucleus, which shows a concavity – the implantation fossa- where the nuclear membrane forms on the cytoplasmic side an electron dense layer- the basal plate- (Figures 3A, B).

An altered morphogenesis of connecting piece results in a rare condition of isolated teratozoospermia: the head-neck defect. This consists in ejaculation of headless motile tails and tailless heads. The uppermost segment of the tail in the head-neck defect is embedded in a voluminous cytoplasm droplet (Figure 3C) that is miss-interpreted at light microscopy as a small head – hence the wrong definition of "pinheads" (63). The defect is suggested to be due to a failure of migration of the tail anlagen and related centriole to the caudal pole of the spermatids (64, 65). As a result the tail and the head develop independently. Sertoli cells eliminate by phagocytosis decapitated tails and decaudated heads during spermiogenesis (65, 66). The presence of headless tails and tailless heads in the lumen of seminiferous tubule (66) as well as in the ejaculate, suggests that the physical separation between head and neck is also traced at spermiation or during epididymal transit of the spermiated spermatozoa.

The analysis of almost 23 cases so far documented in eleven reports (64-74) demonstrates that this defect is extremely heterogeneous. In most cases isolate motile tails and very few or no isolated heads are observed at light microscopy in the ejaculate. A variant of this defect is the "easily decapitated" sperm defect in which minimal manipulation of apparently normal ejaculated sperm results in a widespread sperm decapitation (75, 65). According to ultrastructural analysis various defects are described. The most common configuration of head-neck defect in ejaculated spermatozoa includes absence of the implantation fossa and of the basal plate on the nucleus of decaudated heads, while decapitated tails show one or two centrioles embedded in a cytoplasmic droplet containing few scattered mitochondria not assembled in a middle piece. This main defect may coexist with the occurrence of headless tail containing a normal organization of the mid piece (Figure 3C) (65). Few cases are also described showing an abnormal alignment of the head with the mid-piece resulting in sperm with bent heads (Figure 3D). Sperm with bent heads coexist with headless tails suggesting the existence in a same case of different degree of abnormality of the head-neck junction (65).

4.1.1. Experimental models of human spermatozoa with head-neck defect

Originally described by De Lannou (1979)(67) and by Perotti *et al*, (1981) (68) a genetic origin of the defect in the head-neck junction is suggested by its occurrence in different components of the same family (65, 70, 74), and by its persistence over time (65). The condition of ejaculated acephalic spermatozoa is not restricted to human being, indeed it is repeatedly reported in bulls (76 as a review), and occasionally in boars (77). The nature of the primary defect of this syndrome is unknown. Mice carrying the autosomal recessive mutation "abnormal spermatozoon head shape" (*azh*) display an abnormal head morphology associated to sperm decapitation (78) (Table 1). A

mutation of *Hook1* gene has been shown to be responsible for *azh* mice, suggesting that HOOK1 is involved in the attachment of the flagellum to the spermatid head (79). Therefore human *HOOK1* gene could serve as a candidate gene for mutational analysis in infertile men with sperm head-neck defects.

4.1.2. Reproductive competence of sperm with head-neck defect

The fragility of the head-neck junction resulting in a separation between head and tail precludes a spontaneous fertilization in couples in which the men is affected by defects of the sperm connecting piece. Assisted fertilization by ICSI is also precluded in the most severe form of head-neck defect resulting in ejaculation of headless spermatozoa. The reproductive potential after ICSI of testicular sperm in this case is unknown although the presence of headless tails in the lumen of seminiferous tubules in case of a severe form of head-neck defect (66) suggests that testicular sperm carry the same fragility of head-neck region resulting in decapitation during manipulation of testicular tissue. Indeed sperm extracted from testicular biopsy in a case of head-neck defect failed to show a better quality compared to ejaculated sperm (80). Assisted fertilization by ICSI with bent sperm obtained in cases of head-neck defect resulted in normal fertilization rates but no evolutive pregnancies (65, 73, 80).

The lack of sperm aster formation when sperm with head-neck defect were injected into bovine oocytes, suggests that the lack of syngamy and cleavage after ICSI were probably a consequence of an abnormal centrosome (80), the organizer of the sperm aster at fertilization (81, 82). More recently, fertilization by ICSI with bent head sperm in two brothers carrying a severe defect of head-neck junction, resulted in both cases in two pregnancies and birth of apparently healthy four babies (74) (Table 2). This suggests that selected cases of defect in the connecting piece are compatible with the formation of a normal sperm aster and normal embryo development (74). Fertilization by ICSI in the variant form of defect of connecting piece resulting in a “easily decapitated” spermatozoa (75), resulted in occasional pregnancies and live births after microinjection of intact sperms or separated sperm head and tail (75, 83).

In conclusion abnormalities of the sperm head-neck junction is likely caused by a failure in the caudal migration of the centrioles during spermiogenesis. This is usually associated to a dysfunction of the centrosome, leading to an altered formation of sperm aster at fertilization and consequent retardation or arrest of embryo development, when affected sperm are microinjected in the oocyte (80). Occasional pregnancies obtained in selected cases after fertilization by ICSI however suggest that defects of sperm head-neck junction cannot be viewed as conditions of absolute sterility. Genes mutated in decapitated spermatozoa as well as the nature of the primary defect of this syndrome are still unknown. Concern is moreover raised by the potential consequence of assisted fertilization by using sperm with an altered aster formation. The sperm aster is essential in human for pronuclear movement towards the union of male and female genomes resulting in normal syngamy and early embryonic development (84). An altered aster may result in chromosome mosaicism in the formed embryos due to the abnormal distribution of the chromosomes between the blastomeres with resultant precocious embryo degeneration or development of embryos with chromosomal number abnormalities (85). A preimplantation diagnosis is therefore recommended in case of successful ICSI with sperm carrying head-neck defects.

4.2. Defects of the mid-piece

Mitochondria within the sperm mid-piece deliver adenosine triphosphate (ATP), necessary to provide energy for sperm movement (86). Human sperm mitochondrial DNAs is eliminated after fertilization (87), so that human embryonal mitochondrial DNA is inherited exclusively from the mother (88). In the human sperm middle piece mitochondria are arranged in a helix of 11-13 gyri, with two mitocohondria per girus (Figure 1).

A disorganization associated to reduced number of mitochondria involving the whole sperm population has been reported (2) but it is not clear whether it represents an isolate defect or rather a condition associated to other tail defects as will be discussed (Figure 3E). According to Zamboni (1992) (89), rare cases of severe asthenozoospermia are related to the absence of mitochondria in the middle piece. In a group of 10 men affected by isolate asthenozoospermia but otherwise normal semen parameters, sperm mitochondria showed a reduced number of gyres or a disordered arrangement compared to a group of 10 men with normal sperm motility (90). In a patient with inherited mitochondrial disease, it has been reported a severe asthenozoospermia with extensive defects of the sperm middle piece (91). Mitochondria were regularly arranged but showed an increased matrix, thickening of the membranes, parallelization of the cristae and lipid inclusions as reported in other tissues in mitochondrial disorders (92). These few observations suggest that isolate defects of mitochondria in the sperm mid-piece may be responsible for asthenozoospermia in selected cases. Studies on sperm mitochondria genome have shown that large deletions (93-95) or point mutations in the ATPase gene (96-98) are associated to infertility and reduced sperm motility. Neither the prevalence of structural defects of the sperm middle piece among infertile men nor their possible genetic origin is at present known since no ultrastructural sperm analysis was provided in these studies.

4.3. Defects of the principal piece

The human sperm tail is composed by the axoneme, a microtubule-based structure present in all cilia and flagella of eukaryotic cells that has been well conserved through evolution. In the middle piece of the human sperm tail, the axoneme is surrounded by the outer dense fibers (ODF) and by the mitochondrial sheath (Figure 4C), while in the principal piece, the axoneme is surrounded by the fibrous sheath (FS) and ODF are only present in the most cranial segment (Figure 4A) (see 1 for a detailed description).

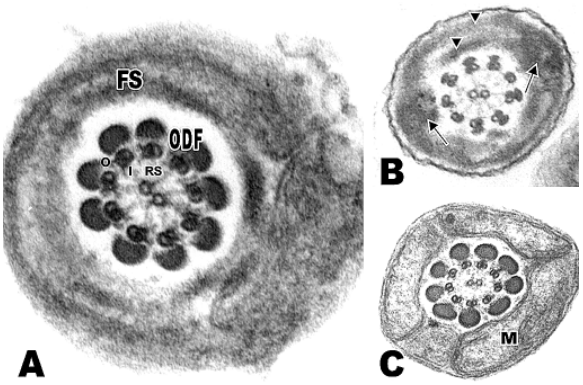

Figure 4. A indicates a cross section of a normal sperm axoneme at level of the principal piece, showing the 9 peripheral microtubule doublets with the inner (I) and outer (O) dynein arms joined to the central microtubule doublet by the radial spoke (RS). The axoneme is surrounded by outer dense fibers (ODF) and than by the ribs of the fibrous sheath (FS). B and C show the axoneme in two different forms of primary cilia diskinesia. B shows an axoneme with the absence of the inner and outer dynein arms; note the normal organization of the fibrous sheath formed by two longitudinal columns (arrows) joined by transverse semicircular ribs (arrow heads). C shows an axoneme with the absence of the inner dynein arm and of the radial spoke; the axoneme is surrounded by normal ODF and regularly arranged mitochondria (M).

The axoneme generates the motility resulting in flagellar beating. Studies in the *Chlamydomonas reinhardtii*, a unicellular biflagellate green alga in the order Volvocales with flagella amazingly similar in structure and function to mammalian cilia and flagella, have provided significant progress in our underestanding molecular mechanisms for assembly and function of different components of the axoneme in the eukariotes (99-101). Dyneins, molecular motor proteins, bind the A tubule of each of 9 outer doublets of the axoneme (Figure 4A). In the presence of ATP, the dyneins bind the B tubule of the adjacent outer doublet and move along the B tubule toward its minus end, resulting in a sliding between pairs of outer doublets while interdoublet sliding is constrained by the structural links (nexin) between the doublets and this results in flagellar bending. Axonemal dyneins are arranged in inner and outer arms different in function and composition. Mutations that selectively affected genes coding components of outer or inner dyneins in the *Chlamydomonas reinhardtii* demonstrate that the outer arms add power and adjust beat frequencies while the inner arms generate the axonemal waveform (102). The inner arms are organized into seven distinct isoforms and are formed by 11 heavy chains and numerous intermediate and light chains and contain among protein components, actin and the calcium-binding protein, centrin (99 as a review). The outer arms are organized as a single isoform with three catalytic heavy chains, two intermediate chains and eight light chains involved in dynein activity regulation. A docking complex formed by three polypeptides is responsible for the assembly of the outer arm onto the A tubule of the peripheral doublet (103 as a review). The central part of the heavy chain contains nucleotide binding motifs with ATP hydrolytic site (104), its N-terminal part is required for assembly of the dynein complexes (105), while the C-terminal segment contains the microtubule-binding region (106). Radial spokes (RS) connect each peripheral doublet with the central pair of microtubules (CP) (Figure 4A) and fulfil with the latter the role of key regulators of dynein activity, by altering the phosphorylation state of the different dynein isoforms (99 as reviews, 107). Accordingly, mutants lacking components of RS/CP in the *Chlamydomonas reinhardtii* have paralyzed flagella (108).

The axoneme organization and function unravelled in recent years in the model of *Chlamydomonas reinhardtii* is substantially shared by the axonemal system of mammal cilia and flagella including the sperm tail, the cilia of respiratory, oviductal, and endometrial epithelia, and some of flagellar proteins in *Chlamydomonas reinhardtii* show more than 75% identity and similarity to proteins with similar function in human sperm (100). Whereas the axonemal system of the cilia is surrounded only by the cell membrane, in the spermatozoa the ODF, the FS and the cell membrane surround the axoneme. The ODF are nine cylindrical elements of different lengths associated with the corresponding peripheral doublet of the axoneme (Figure 4A), and are composed of several cysteine- and proline-rich, intermediate filament-like proteins (101 as a review). At least 14 polypeptides are identified in ODF of rat sperm and are believed to play a role in maintenance of its passive elastic structure (109).

The FS is a flagellar exoskeleton organized into two longitudinal columns that run along the length of the principal piece, regularly joined by transverse semicircular ribs (Figures 4B, 5B). The protein composition of FS includes at least 18 polypeptides (109) that seem to serve as a scaffold for several enzymes of energy metabolism and as signalling molecules for sperm motility (110 as a review). Three members of A-kinase anchor proteins (AKAP) family were characterized in spermatozoa FS (AKAP 4, AKAP3, and TAKAP-80), along with hexokinase (HK1-S), Rho-binding protein, rhophillin and its binding protein ropporin (reviewed in 110). AKAP3 and –4, the most abundant structural proteins of the FS bind to one another and both anchor adenosine monophosphate cyclic (cAMP)-dependent protein kinase A (PKA) via the regulatory subunit of the kinase. Genes coding for both AKAP were sequenced and the regions of the respective binding sites between the two AKAPs and for PKA were identified (111, 112). Targeted disruption of the *AKAP4* gene in mice results in sperm immotility and abnormal short tails (113) underscoring a role of AKAP proteins in the acquisition of sperm tail motility.

The system of flagellar motility involve more than 400 proteins in the sperm tail, less than half of which have been characterized (101). Defects of this complex molecular system is expected to be involved in an altered sperm motility causing infertility, but at present the molecular defect causing human asthenozoospermia is recognized in a small number of cases. Isolate defects of

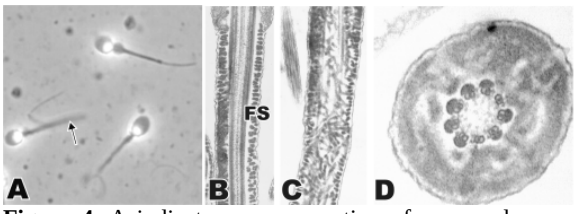

Figure 4. A indicates a cross section of a normal sperm axoneme at level of the principal piece, showing the 9 peripheral microtubule doublets with the inner (I) and outer (O) dynein arms joined to the central microtubule doublet by the radial spoke (RS). The axoneme is surrounded by outer dense fibers (ODF) and than by the ribs of the fibrous sheath (FS). B and C show the axoneme in two different forms of primary cilia diskinesia. B shows an axoneme with the absence of the inner and outer dynein arms; note the normal organization of the fibrous sheath formed by two longitudinal columns (arrows) joined by transverse semicircular ribs (arrow heads). C shows an axoneme with the absence of the inner dynein arm and of the radial spoke; the axoneme is surrounded by normal ODF and regularly arranged mitochondria (M).

the principal piece causing an absent or greatly reduce motility in human sperm include primary cilia diskinesia/immotile cilia syndrome and dysplasia of the fibrous sheath.

4.3.1. Primary cilia diskinesia/Immotile cilia syndrome

Immotile cilia syndrome (ICS) indicate a condition associated to sperm immotility and recurrent respiratory tract infections when all cilia and flagella functions are involved (114-116). In about 50% of patients respiratory tract infections due to altered mucociliary clearance, are associated to bronchiectasis and situs viscerum inversus thus constituting the autosomal recessive disorder known as Kartagener's syndrome (117).

The impaired motility in ICS is due to defects of the axoneme. The lack of inner and/or outer dynein arms (Figures 4B,C) constitutes the main and first described defect in sperm and respiratory cilia axonemes (114, 118-120). Several other ultrastructural defects have been noted including lack of radial spoke (Figure 4C) (121), absent central pair of microtubules (Figure 5D) (122-124), transposition of microtubules (125) and dysplasia of the fibrous sheath (126) (Figure 5D). Immotile cilia syndrome was renamed primary ciliary dyskinesia (PCD) or dyskinetic cilia syndrome by Rossmann *et al*, (127, 128) after observing that ciliar motion in affected cilia of the respiratory tract is deranged but not absent. Accordingly, residual sperm motility is occasionally present in some affected infertile men with spermatozoa selectively lacking the outer dynein arm (116, 129-131). The structural and clinical heterogeneity of flagella and cilia defects is farther underscored by the reports of patients with Kartagener's syndrome with normal cilia organization (120, 132-135,) or normal ultrastructure of sperm tail (134, 135).

4.3.1.1. Experimental models of human PCD

Linkage studies have revealed extensive locus heterogeneity in human PCD (136, 137). This reconciles with the observation that affected patients display different axoneme defects. The classic form with lack of dynein arms supports the hypothesis that mutations in dynein genes are involved. Different mice lines deficient in axoneme dynein chains have been proposed as models for PCD (Table 1). Reduced but not abolished sperm and tracheal cilia motility is present in mice lacking the dynein heavy chain 7 (*Mdhc7*) gene (138), which encodes a putative heavy chain of the inner arm (139). This is a single copy gene in the murine genome assigned to chromosome 14 (139). *Mdhc7*$^{-/-}$ mice are infertile due to reduced sperm motility but lack gross defects in the axoneme structure (138). The targeted mutation was generated by deleting the ATP binding site of the *Mdhc7*, suggesting that mutations which affect the central part of a dynein heavy chain are responsible for a reduced motor activity but not for structural abnormalities of the axoneme (138). The human homologous gene *HDHC7* (renamed as *DNAH1*) has been identified and maps to 3p21.3 (139), a potential human PCD locus (136), suggesting that HDHC7 (*DNAH1*) is a candidate gene for some cases of PCD (138) not associated to structural defects of the axoneme. A mouse model that reproduces most of the classical features of human PCD was generated by an insertional mutation in axoneme dynein heavy chain 5 (*Mdnah5)* gene (140). *Mdnah5*$^{-/-}$ mice lack outer dynein arms and exhibit cilia immotility, situs inversus and recurrent respiratory infections. Unknown is the phenotype of the sperm axoneme since most *Mdnah5*$^{-/-}$ mice die during the first 2-3 weeks of age (140). Of interest is the finding that 10 spontaneous mutations in *DNAH5*, the human homolog of mouse *Mdnah5* gene, have been reported in eight families affected by PCD (141) indicating *DNAH5* as a gene for human PCD. Spontaneous mutations have been also reported in genes encoding for heavy dynein chain 11 (*DNAH11*) gene (142), and for intermediate type 1 chain (*DNAI1*) gene (143-145) in human PCD. Mutations of *DNAH5* and of *DNAI1* are mostly associated to the absence of outer dynein arms (ODA) in respiratory cilia while mutations of *DNAH11* only, are associated to a normal ultrastructure of respiratory cilia. Unfortunately no data are reported on the ultrastructure of sperm axoneme in affected patients. Immunofluorecence imaging and use of specific antibodies directed against ODA heavy chain DNAH5, recently documented an absent or miss-localization of the protein in respiratory cilia of PCD patients with *DNAH5* mutations while a normal distribution of ODA heavy chain was observed in the sperm tail of a patient with *DNAH5* mutation (146). This suggests different modes of ODA generation in respiratory cilia and in the sperm tail (146) and underscores the need of extensive genetic and ultrastructural studies on respiratory cilia and on sperm axoneme in PCD patients to unravel the genotype/phenotype relationship in human PCD.

Reported mutations of dynein genes are associated to less than 30% of cases of human PCD, confirming that this is a genetically heterogeneous condition and indicating that the causative mutation/s in most cases are unknown. Recent experimental models demonstrate that a partial or total loss of cilia inner dynein arm and a clinical PCD may result from mutations of genes different from those coding for dyneins (Table 1). A partial

loss of inner dynein arm was reported in respiratory cilia and in the sperm axoneme of *tektin-t* mutant mice (147). Tektin is a component of the flagella outer doublet microtubules localised near the binding sites for radial spokes, inner dynein arms and nexin links (148). Homozigous mutant male mice for *tektin-t*, a component of *tektins* genes expressed in mammals sperm tail (149-151), are infertile due to a reduced but not absent sperm motility, and have functionally defective tracheal cilia. These findings prompted authors to surmise that tektin-t is involved in the assembly or attachment of the inner dynein arm to microtubules of flagella axonemes and that the *tektin-t* gene may be one of the potential causal genes for human PCD (147).

Four genes encoding for structural components of the central doublet of the ciliar axoneme in *Chlamydomonas reinhardtii*, PF15, PF16, PF20, and KLP1, were cloned and their crucial involvement in flagella motility was demonstrated in mutant's strains showing paralysed flagella (107). Murine and human genes orthologous to *Chlamydomonas reinhardtii pf20* were cloned (152, 153). PF20 co localized with Spag6, the mammalian orthologue of *Chlamydomonas reinhardtii* PF16, to the central doublets of mouse sperm axoneme (153). Mutant mice lacking sperm-associated antigen 6 (Spag6) are infertile and show marked reduced sperm motility associated to a disorganization of flagella structure including loss of the central doublet, and disorganization of ODF and of FS (154). The presence of hydrocephalus in mutant mice demonstrates the presence of the defect also in ependymal cilia and suggests that Spag6 is essential for mammalian cilia and flagella motility (154). The implication of *SPAG6* and of *PF20* as potential candidate genes for human PCD is undetermined although the analysis of five patients in whom cilia and flagella showed abnormal central doublets, failed to detect mutations of *PF20* in 3 patients, while intragenic polymorphisms were found in two patients (152).

A total loss of inner dynein arms in cilia from the respiratory epithelium and ependymal cell layer was also observed in mice defective in DNA polymerase lambda (*Pol lambda* $^{-/-}$) (155) that is involved in maintenance of genomic integrity (156). *Pol lambda*$^{-/-}$ mice develop hydrocephalus, situs inversus totalis and chronic suppurative sinusitis. The few surviving males are sterile as a result of sperm immobility and deranged spermatogenesis. Recently it was shown that in this mouse model of PCD, a novel gene named *Dpcd* (for deleted in a mouse model of primary cilia diskinesia) was likely disrupted (157). *DPCD*, the human homolog of *Dpcd* is expressed in human testis and its expression increases during *in vitro* human ciliogenesis. However no disease-causing mutations in the coding sequence of the gene was observed in 51 PCD patients (157).

In conclusion reported data show that PCD is a rare disease due to an abnormal cilia structure and function. PCD is classically transmitted as an autosomal recessive trait although a familiar case of complex X-linked phenotype, combining primary PCD and retinitis pigmentosa due to mutated retinitis pigemntosa GTPase regulator gene (*RPGR*) was recently reported (158). PCD is a genetically and clinically heterogeneous disorder resulting from a variety of molecular defects largely undefined and not necessarily restricted to dynein genes of the axoneme. Future multidisciplinary genetic, ultrastructural, and clinical studies will help to clarify the genotype/phenotype of PCD.

4.3.2. Dysplasia of the fibrous sheath

Dysplasia of the fibrous sheath (DFS) refers to a condition of absent or severely reduced sperm motility associated to modification of the fibrous sheath involving the totality of ejaculated sperm (3, 159-161). At light microscopy sperm show short and irregularly thick tails (Figure 5A) so that this condition is also indicated as "stump tail" or "short tail" (162). Ultrastructural analysis documents disorganized constituents of fibrous sheath (Figures 5C, D), associated to an absence of the mid-piece with scattered mitochondria randomly assembled (Figure 3E). These defects may coexist with missing axoneme central microtubule pairs (Figure 5D). The absence of dynein arms in selected cases is associated with clinical findings of PCD due to the absence of dynein in bronchial cilia and indicates a variant form of PCD associated to DFS (126).

DFS arises from an altered organization of the fibrous sheath during spermiogenesis (159,160) and shows a familial incidence in $\geq$ 20% of patiens (3, 4, 160, 162) thus underscoring its genetic origin. Analysis of the family trees seems to indicate an autosomic recessive inheritance (4).

4.3.2.1. Experimental models of human DFS

To date the molecular defect in human DFS is undefined. Several proteins have been identified on isolated human sperm fibrous sheath (163), and two of them only, AKAP4 (originally called hAKAP82) (111), and AKAP3 (originally called FSP95 as fibrous sheath protein of 95 kDa) (112) were characterized at molecular level. Targeted disruption of *Akap4*, an X-linked gene that code for AKAP4, the major protein in the fibrous sheath (164) results in male infertile mice with no progressive motility and short flagellum (113) (Table 1). Mutant mice show an incomplete fibrous sheath somehow reminiscent of human DFS while all other components of the tail develop normally. Mice lacking AKAP4 show also a greatly reduced sperm tail expression of AKAP3 and of glyceraldehydes 3-phosphate dehydrogenase-S (GAPDS), a germ cell specific glicolytic enzyme bound to the fibrous sheath (165, 166). These observations suggest that AKAP4 associates with a pre-existing template to complete formation of the fibrous sheath; its absence results in a loss of other proteins of the fibrous sheath including enzymes in the glycolytic pathway required for sperm functions (113).

The analysis of 9 patients affected by DFS showed normal immunohistochemical localization of AKAP4 and of AKAP 3 in the disorganised fibrous sheath and sequence analysis of the AKAP4 and AKAP3 binding sites did not reveal mutations (167). This was confirmed by

a recent study where AKAP4 protein was detected in the disorganised fibrous sheath of a patient with a DFS while it was absent in a patient with a total absence of fibrous sheath (168). In this latter case AKAP4 was however normally expressed in testicular spermatids. Available data support the hypothesis that human DFS might be caused by a mutation in a gene (s) affecting formation of the fibrous sheath template during spermiogenesis rather than in the *AKAP4* gene (167). Intriguing to this is the sperm phenotype of mice with defects in hybrid sterility (Hst) loci 6 and 7 (169, 170). Affected mice show singlet microtubules, outer dense fibres and components of fibrous sheath not assembled in a regular tail, associated to a disorganization of the mitochondria in the middle piece, suggesting that *Hst*-6 and *Hst*-7 gene products are involved in sperm tail assembly by a still undefined mechanism.

In conclusion available data underscore the concept that sperm tail defects may be caused by alterations of a variety of gene products still largely unknown that under normal conditions are co-ordinately involved in the synthesis, assembly, stability and function of different components of the sperm tail.

4.3.3. Reproductive competence of sperm with PCD or DFS

Isolate defects of the sperm tails are usually associated with normal head forms suggesting that sperm, although unable to fertilize oocyte *in vivo* or *in vitro* because of their immotility, might be able to fertilize oocyte by ICSI technique. However even after ICSI, fertilization rate is low using immotile ejaculated sperm (171-173) (Table 2). The major technical problem with the use of immotile sperm is their differentiation between live and dead sperm. This may explain the rare reports of live births (174) and the low pregnancy rate (175) with immotile ejaculated sperm from patients affected by PCD and total sperm immotility. Indeed a successful IVF after ICSI with a live male birth was reported in a case of a man affected by PCD/Kartagener's syndrome, with use of ejaculated sperm showing non-progressive motility (176).

Casper *et al*, (1996) (177) have proposed the use of the hypo-osmotic swelling (HOS) test to select immotile, alive sperm for ICSI. This test has been developed to investigate the ability of the sperm membrane to transport fluids (178). Sperm with a functional membrane undergo swelling of the cytoplasm space and sperm tail fibres curl when exposed to a hypo-osmolar medium. Sperm with damaged plasma membrane will not have cytoplasm swelling and the tails will remain uncurled. By microinjecting immotile but curled ejaculated sperm in 13 couples, fertilization rate was greatly improved compared to that obtained with immotile sperm not selected with HOS test, and one patient delivered twin boys (177). A pregnancy with birth of twin healthy babies was also reported with the same procedure in a case of man affected by isolate total sperm immotility (179). By using ejaculated immotile sperm selected with HOS test for ICSI, Peeraer *et al*, (2004) (180) obtained a 66% fertilization rate and one singleton pregnancy in a couple with man affected by PCD. To increase the chance to obtain immotile, alive sperm suitable for ICSI in three cases with man affected by PCD, Ron-El *et al*, (1998) (181) proposed the use of immotile sperm obtained after repeated ejaculations on the oocyte retrieval day. The pregnancy rate was greatly increased compared to that obtained in a previous attempt without repeated ejaculations (48% and 3% respectively) and one couple delivered twins babies.

A significantly improved fertilization and pregnancy rate has been also obtained in cases with men affected by total sperm immotility by using testicular sperm for ICSI (171, 172, 182, 183). ICSI with immotile testicular sperm selected by HOS test was applied in four couples with men affected by PCD/Kartagener's syndrome resulting in a normal fertilization rate and pregnancies in 3 of 4 cases, with birth of healthy twin children and of a healthy girl (184, 185). IVF with ICSI has been also used to treat couples with men affected by DFS. The residual non-progressive motility usually observed in this tail defect allows an easy selection for ICSI of live sperm that probably explain the fairly good fertilization rate (63%) and the birth of 14 healthy babies in 12 treated couples (reviewed by 4).

Taken together reported data demonstrate that advanced technologies of assisted reproduction, allow to obtain pregnancies in cases of isolate sperm tail defects resulting in immotility or residual non-progressive motility. It remains unknown whether ICSI with testicular or ejaculated sperm carrying genetic defects, certainly not competent for natural conception, is associated with the risk of transmission of these defects to the next generation. To date few babies born after ICSI with sperm of men affected by Kartagener's syndrome did not carry a situs inversus (174,176, 181). However since the nature of the genetic defect is still uncertain and its phenotype is heterogeneous, it is not excluded the possibility that medical problems will manifest later along with fertility problems. A proper counselling is therefore mandatory in handling couples in which man is affected by isolated teratozoospermia leading to sperm immotility.

5. THE ULTRASTRUCTURAL SPERM ANALYSIS IN SEVERE ASTHENOZOOSPERMIA

Defects of the sperm tail leading to severe asthenozoospermia or total immotility such as PCD or DFS involve sub microscopic components of the tail; hence the diagnosis of PCD relies only on an ultrastructural analysis by TEM. DFS is suspected already after light microscopy examination but TEM analysis is required for an appropriate diagnosis due to the possible association of DFS with PCD. The identification by TEM analysis of isolate sperm defects of genetic origin leading to sperm immotility or severe asthenozoospermia is needed in the era of assisted reproductive technology considering the dramatic effect of the female age on outcome of IVF (186, 187), and on outcome of IVF-ICSI with male factor infertility (188, 189). A sound and straightforward diagnosis of isolate sperm defects of genetic origin while avoiding time-wasting and expensive empiric treatments, may thus improve the probability of pregnancy and live

birth after IVF-ICSI of the affected couple. The genetic defect in most cases of isolate sperm defects leading to reduced or absent motility is still uncertain. A diagnosis of these defects, presently based on ultrastructural analysis only, is therefore unavoidable to make patients aware of the potential genetic risk for the next generation in using for IVF-ICSI sperm carrying unknown genetic defects that make a natural conception unlikely or impossible.

The elevated cost of TEM analysis and the available scarce experienced centres require that this test is reserved to very selected cases among the huge number of 30% of infertile couples which seeks consultation because of sperm defects or dysfunctions (190). Low sperm motility - the laboratory category which should include isolate defects of the tail involving the majority of ejaculated sperm - accounts for approximately 33.6% of patients attending an infertility clinic because of abnormal semen (191). An extended TEM analysis of 400 unselected ejaculates with <30% motile sperm has shown that isolate sperm tail defects was present in 11% of cases with total immotility mostly of which had a complete absence of dynein arms (192). In the same study, a partial absence of dynein arms or an absence of central doublet of the axoneme in all sperm was observed in some ejaculates with a residual progressive motility. This reconciles with the report of a reduced but not absent motility in occasional men affected by PCD selectively lacking the outer dynein arm only (116, 129-131) and in men affected by DFS (3, 160, 161).

In a recent quantitative ultrastructural evaluation of asthenozoospermic ejaculates with a sperm vitality of more than 50% assessed by dye-exclusion test (193), PCD was found in 33% and DFS in 13% of 30 men affected by persistent total sperm immotility, while two cases of PCD and one case of DFS was found in a group of 43 men with a residual motility (< 10%) (194). Based on these data, TEM analysis is indicated in cases of total sperm immotility, or long-standing severe asthenozoospermia associated to normal sperm vitality in order to identify conditions of deranged sperm motility of genetic origin to be treated with IVF-ICSI.

6. PERSPECTIVE

The prevalence of isolate structural defects involving the totality of ejaculated sperm among infertile patients is not known probably due to the limited although unavoidable use of the ultrastructural analysis for their correct identification. Isolated teratozoospermia of the head can be suspected by light microscopy on stained sperm smears but TEM analysis remains the gold standard for an appropriate diagnosis. Defects of the sperm tail require an ultrastructural analysis with TEM due to their submicroscopic nature. This expensive and time-consuming test is mandatory to cases of total, long-standing immotility or severe asthenozoospermia associated to normal sperm vitality.

The genetic origin of these defects is indicated by their persistence over time and by the familiar incidence but the genetic defects at molecular level are still undefined in most cases. Numerous experimental mouse models in the last decade have indicated a variety of defective genes as potential models of human isolated teratozoospermia. Defects involve either the head or the tail of the sperm and are responsible for infertility due in the former case to the inability to penetrate the egg investment and to activate the oocyte and in the latter case to the inability to reach the oocyte. Multidisciplinary genetic, ultrastructural, and clinical studies will help to clarify the genotype/phenotype of isolate structural defects involving the totality of ejaculated sperm.

IVF-ICSI techniques are the only available method to obtain live births with sperm carrying these defects. A poor outcome of ART should be expected in most of reviewed conditions. Advanced techniques such as experimental oocyte activation after ICSI in case of acrosome agenesis, a condition associated to a deficiency of oocyte activation, or the use for ICSI of live sperm collected from testicular tissue in case of PCD, have improved the outcome of IVF-ICSI. The use of experimental murine models for head sperm defect should improve our knowledge on the possible human application of *in vitro* oocyte activation after ICSI with sperm carrying head defects associated to a deficiency of oocyte activation.

The genetic hazard associated to IVF-ICSI with sperm carrying genetic defects and a phenotype non-compatible for a natural conception is undetermined yet. An ultrastructural analysis of the ejaculate is recommended in long-standing sperm immotility or severe asthenozoospermia to avoid time-wasting treatments and to make patients aware of the potential genetic risk for the next generation in using for IVF-ICSI sperm carrying unknown genetic defects that make a natural conception unlikely or impossible.

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