MORPHOPATHOLOGY OF SPERM: IT’S IMPACT ON FERTILIZATION

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Abstract
Terato-, astheno- and necrozoospermia negatively influence fertility prognosis in spontaneous conditions or with the use of various assisted reproductive techniques including conventional in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). The correct identification of sperm pathologies will indicate different fertility potentials and outcomes in assisted reproduction technology. Anomalies of only the spermatozoa flagella bear a promising prognosis, but those affecting the sperm chromatin and the neck region entail an increasing chance of failure, which highlights the differential roles played by specific sperm components in fertilization, implantation and early embryonic development. Sperm pathology therefore allows an understanding of abnormal function that goes beyond that provided by classical sperm morphology classifications that are mainly based on descriptions of abnormal sperm shapes with no insight into the mechanisms or the pathological details.

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Introduction
Terato-, astheno- and necrozoospermia negatively influence fertility prognosis in spontaneous conditions or with the use of various assisted reproductive techniques including conventional in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). Because ICSI allows the examination of motility and morphology of the very spermatozoon to be micro-injected, it became clear that abnormal and immotile spermatozoa could successfully fertilize oocytes, and the question was raised about the convenience of using them in assisted reproduction technology procedures (Chemes and Rawe, 2003; Chemes and Rawe, 2007). Among many of the terato-, astheno-, and globozoospermic men, a genetic component is present and the outcome of IVF and ICSI depends mainly on the nature of sperm pathologies (Chemes and Rawe 2003).

During the evaluation of sperm morphology in semen smears, usually the focus is on the morphometric parameters of the normal sperm head, mid-piece and flagellum. Since the publication by Kruger et al. (1986) on the role of sperm morphology during in vitro fertilization treatment, a number of reports debated the validity of the observation (Check et al., 1992; Svalander et al., 1996; Horte et al., 2001).

Evidence-based studies supported the relationship between the percentage of “normal” forms, as defined by strict categorization, with in vitro fertilization rates (Kruger et al., 1986; Kruger et al., 1988; Menkveld et al., 1990) as well as in vivo outcome (Eggert-Kruse et al., 1996; van Waart et al., 2001). The assessment of normal forms on well-prepared and stained slides is the basis of strict morphology criteria. This concept was later advocated by the World Health Organization (WHO, 1999: 2010) as the method of choice to record the percentage normal forms in a given ejaculate. The low cut-off value for sperm morphology of 4% normal spermatozoa, as proposed in the new WHO manual (WHO, 2010), is in agreement with recently published values (Menkveld, 2001; Gunalp et al., 2001; Haugen et al., 2006). In the light of these recent publications, it is clear that strict criteria for normal sperm morphology correlate with the sperm cell’s fertilizing capacity and has prognostic value in assisted reproduction. Yet, the question what is wrong with the abnormal spermatozoa, remains. To
Fig. 1: Light microscopic diagrammatic images of sperm head and midpiece abnormalities

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<th>HEAD ABNORMALITIES</th>
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**HEAD ABNORMALITIES**

**Descriptive morphology**
Abnormal spermatozoa as observed by light microscopy
Abnormal spermatozoa are those that deviate from the defined criteria described for normal. These aberrations are identified due to differences in size, structure, or form (Menkveld 1996), and include immature spermatozoa, sperm precursors, and spermatozoa with midpiece and tail defects. Aberrations are divided into 3 categories namely head (Figure 1), midpiece (Figure 1) and tail (Figure 2) (Menkveld et al., 1990).

**Head aberrations**
Too small or too large: the heads are smaller/larger than the described lower/upper limits of normality. The limits for normality are (WHO 2010):
- Median length: 4.1 μm, (95% CI 3.7 – 4.7);
- Median width: 2.8 μm, (95% CI 2.5 – 3.2);
- Median length-to-width ratio: 1.5 (95% CI 1.3 – 1.8).

**Duplicate**
Sperm with 2 joint heads.

**Elongated**
These aberrations are also known as tapered forms and their heads are typically narrower and longer than the described limits of normality.

**Amorphous**
These abnormalities include all which do fall into one of the above 3 categories. In general, the heads can take any shape or size and are in general non-oval.

**Normal head shape with internal aberrations**
Spermatozoa with a normal oval shape with more than 2 vacuoles, irregular peripheral surface, acrosomal area <40% or >70% of the head area.

**Midpiece aberrations**
The midpiece derives from interaction of centrioles with the spermatid nucleus (Chemes and Rawe, 2007) and includes the following malformations:
- Thick insertion
- Bent
- Asymmetrical insertions
- Thin insertion

**Tail aberrations**
Tail aberrations could be in the following forms: Coiled, Duplicated, Stumped, or bent more than 90°
Sperm pathology and fertility prognosis

According to the location of the aberration, specific defects of specific organelles can be observed that can lead to failure of fertilization or pregnancy. Sperm abnormalities such as terato-, astheno- and necrozoospermia are consistently associated with fertilization disorders in both in-vivo and in-vitro fertilization cases (Kruger et al., 1986; Eggert-Kruse et al., 1996). Thus, normal sperm morphology evaluated after fixation according to strict criteria at 1000-times magnification (Menkveld et al., 1990) is a good diagnostic parameter of fertilization success in vitro (Oehninger & Kruger, 1995). However, for intracytoplasmic sperm injection (ICSI), the apparently most suitable spermatozoon for fertilization is selected by an embryologist using a light microscope at magnifications of x400 in unfixed, unstained, wet sperm preparations. Obviously, the resolution and identification of morphological aberrations is not as good as that in fixed preparation. Nevertheless, success of assisted reproduction is also dependent on the ultramorphology of the spermatozoon that is taken for injection. Hence, discrimination of sperm morphological features by means of light microscopy is not sufficient to detect subtle malformations such as DNA damage or chromosomal aberrations (Garolla et al., 2008; Avendano et al., 2009). As a result, defective spermatozoa might be accepted for normal ICSI procedure (Berkovitz et al., 1999; Wilding et al., 2011). Significantly, higher frequencies of aneuploidy in infertile patients highlight this relationship between normal sperm morphology and genetic abnormalities (Ryu et al., 2001).

Chemes and Rawe (2003) described two main varieties of abnormal spermatozoa. The first was a heterogeneous combination of different alterations that was found randomly distributed in each individual. These alterations could be referred to as nonspecific or non-systematic sperm defects. The second variety involved the vast majority of spermatozoa in a semen sample and might be called systematic in the sense that there was a common sperm phenotype that predominates in a given patient. The first variety was usually secondary to various pathologies that affected the normal function of the testis or the seminal pathway. Systematic alterations generally presented family clustering and had proven or suspected genetic origin. Sperm pathology could therefore be regarded as a step beyond descriptive morphology.

Pathological sperm phenotypes of genetic origin include the following aberrations:

(i) Flagellar abnormalities in motility disorders

These structural abnormalities are responsible for most cases of severe asthenozoospermia. Chemes and Rawe (2003) categorized flagellar anomalies into two groups; the first nonspecific flagellar aberrations group (NSFA), and the second group presented with dysplasia of the fibrous sheath (DFS). Ongoing studies in these men have shown that 33% of the patients with NSFA and 0% of the DFS achieved a pregnancy within 2-6 years with the use of ART or IVF (Chemes and Rawe 2003).

Olmedo et al. (2000) studied retrospectively the efficacy of ICSI among six men diagnosed with DFS. The sperm concentration of these
men was 29x106 spermatozoa/mL with a progressive motility of 0%. The recorded fertilization was 73% with five confirmed pregnancies that resulted in one preclinical abortion, one clinical abortion and three deliveries. However, it is imperative to inform couples of possible transmission risks to offspring.

In general, ICSI seems to be the therapy of choice in cases of flagellar pathologies. Chemes and Rawe (2007) reviewed the results on ICSI in 11 patients with primary ciliary dyskinesia (PCD) and 12 with dysplasia of the fibrous sheath (DFS). The reported fertilization rates were between 55-70%. Numerous pregnancies resulting in 21 live births. The abortion rate was 20%. In that report, the authors expressed concern regarding cases presented with 100% immotile spermatozoa. This phenomenon could be misleading since these cases could be complete asthenozoospermia with total necrozoospermia (Chemes and Rawe 2007).

Sperm immotility can have several causes such as structural abnormalities of the axoneme (McClure et al., 1983; Neugebauer et al., 1990), the fibrous sheath (McClure et al., 1983; Eddy et al., 2003; Baccetti et al., 2005; Collodel et al., 2006), and the outer dense fibres (McClure et al., 1983; Haidl & Becker, 1991a; Haidl et al., 1991; Moretti et al., 2008), respectively. The outer dense fibres are essential structures for spermatozoa to generate motility, particularly progressive motility (Henkel et al., 2001; 2003; 2005), and structural shaft defects, or deletions have grave consequences for motility (Haidl et al., 1991a). Among these structural defects, the dysplasia of the fibrous sheath (DFS) is a genetic defect of multigenic nature (for review: Chemes & Rawe, 2003; 2010), and spermatozoa of these patients are not only immotile, but also show distinct morphological abnormalities of the flagellum.

Moreover, abnormalities of the mitochondrial organization are also a cause of asthenotertatozoospermia. In two cases, Rawe et al., (2007) reported successful ICSI with ongoing pregnancies and delivery of a healthy girl in one and a miscarriage in the other case. Such mitochondrial defects are not only responsible for the morphological abnormality, but also low sperm motility. In some patients, in thickened midpieces, supernumerary mitochondria with normal substructure and high mitochondrial membrane potential were identified (Piasecka & Kawiak, 2003). Thus, poor motility may be related to abnormal midpiece morphogenesis where still functional mitochondria are available, which, in turn, might be responsible for signs of apoptosis and DNA fragmentation (Piasecka et al., 2003). On the other hand, disorganized midpieces with abnormal mitochondria are also associated with a CYP17 gene deletion, which is essential for normal mitochondrial architecture and function (Liu et al., 2007). In turn, disturbed mitochondrial membrane potential is directly associated with low sperm motility and fertilizing potential (Kasai et al., 2002; Marchetti et al., 2002).

(ii) Abnormalities of the head/neck attachment and acephalic spermatozoa
The region of head/neck attachment or connecting piece derives from the interaction of the centrioles with the spermatid nucleus. During the early stages of spermiogenesis, the sperm flagellum is constructed from the centriolar complex that approaches the nucleus and attaches to its caudal pole ensuring a linear alignment of the tail with the longitudinal axis of the head (Chemes and Rawe 2003).

Chemes et al. (1999) studied 10 infertile men with acephalic or abnormal head/neck attachments. The ultrastructure analysis showed a total absence of the head while the midpiece section was covered with plasma membrane. However, ICSI pregnancies in two couples with a history of long standing primary infertility in which the spermatozoa of the male partner were either acephalic or had abnormal head and midpiece attachments were reported (Porcu et al., 2003).

(iii) Pathology of the sperm head: acrosome and chromatin anomalies
During spermatogenesis, the acrosome of mature spermatozoa derives from transformations of the Golgi apparatus. In early spermatids, the acrosomal vesicle and granule form inside the Golgi complex that progressively approaches the spermatid nucleus and attaches to it at a site marked by previous changes in the nuclear envelope (Chemes and Rawe 2003). Men with acrosome-less spermatozoa are infertile because the sperm are not able to bind to the zona pellucida and subsequently penetrate the oocytes. Carell et al. (2001) and Vicari et al. (2002) described two types of globozoospermia i.e. Type I completely lack the acrosome and acrosomal enzymes, whereas Type II contains a conical nucleus.
which may be surrounded by large cytoplasmic droplets.

Clinical reports noted that the sperm of globozoospermic men cases lack the ability to activate the oocyte (Chemes and Rawe 2007), thus leading to fertilization failure. Although globozoospermia was previously thought to be a sterilizing pathology of the human male, the advances ICSI made it possible to achieve a few successful fertilizations or with some pregnancies (Liu et al., 1995; Stone et al., 2000; Coetzee et al., 2001).

It is possible that fertilization in these cases was achieved with the Type II globozoospermia. Nevertheless, one has to bear in mind that sperm from patients with Type II globozoospermia might carry damaged DNA because cytoplasmic droplets contain cytoplasmic enzymes that fuel oxidative stress (Gomez et al., 1996).

Discussion
As early as 1916 the importance of normal sperm morphology has been noted (Cary 1916). However, the manner in which the normality or abnormality of the spermatozoa should be evaluated has been a controversial field of continuous debate. Despite the arguments for and against the presence of normal sperm in an ejaculate and its association with fertilization, some of the latest sperm selection techniques still rely on the morphological configuration of the sperm or the nucleus (Bartoov et al., 2002).

The introduction of modern morphological, biochemical and molecular techniques together with recent advancements in reproductive medicine resulted in the identification and characterization of various distinct morphological forms (normal and abnormal) of males. Researchers soon realized that there were variable amounts of abnormal, immotile and dead spermatozoa in the ejaculates of fertile individuals and that these percentages were pathologically increased in numerous cases of male infertility. MacLeod and Gold (1951) first introduced a classification of sperm based on size and shape and then Freund (1966) described and classified the different sperm forms in 6 categories. In 1971, Eliasson rejected the counting of only one defect and published sperm morphology criteria based on actual measurements for head, mid-piece and tail (Eliasson 1971). David’s laboratory (David et al., 1975) followed by introducing a multiple entry system for morphology taking into account all abnormalities and their combinations. In 1980, 1987 and 1992 the World Health Organization manuals introduced the concept of normality and cut off value for fertility. These WHO value were not evidence-based and were basically ignored by the clinicians, since there was no solid relevance to infertility or to assisted reproduction. It was only in 1984 that the morphology debate started. That was with the advent of the Tygerberg strict criteria that focussed on the importance of the so-called ideal sperm (Kruger et al., 1986, Kruger et al., 1988).

One of the most severe abnormalities of sperm structure is the dysplasia of the fibrous Sheath. As a consequence, sever asthenozoospermia or even total sperm immotility which is caused by serious disturbances in the organization of the sperm fibrous sheath is present (Olmedo et al., 2000). Before the advent of ICSI, asthenozoospermia was one of the major causes of infertility because immotile spermatozoa were unable to reach the oocyte and penetrate normally. The descriptive studies by Chemes and colleagues set the ground for a correct diagnosis of sperm abnormalities that identifies defective mechanisms involved, and allowed for an evidence-based treatment of severe male factor infertility (Chemes and Sedo 2012).

In conclusion, knowledge of the exact cause for the infertility problem will empower clinicians to guide the consulted couple to reach their reproductive goal. A correct identification of sperm pathologies indicates different fertility potentials and outcomes in assisted reproduction technology. For example, anomalies of only the spermatozoa flagella bear a promising prognosis, but those affecting the sperm chromatin and the neck region entail an increasing chance of failure, which highlights the differential roles played by specific sperm components in fertilization, implantation and early embryonic development. Sperm pathology therefore allows an understanding of abnormal function that goes beyond that provided by classical sperm morphology classifications that are mainly based on descriptions of abnormal sperm shapes with no insight into the mechanisms or the pathological details.

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