Background: Complete asthenozoospermia, i.e. 100% immotile spermatozoa in the ejaculate, is reported at a frequency of 1 of 5000 men. Its diagnosis implies a poor fertility prognosis even with ICSI. It is extremely important to distinguish between two different groups of patients with complete asthenozoospermia, i.e. virtual or absolute asthenozoospermia. With the former group having some motile spermatozoa after extensive processing of the semen, absolute asthenozoospermia can be associated with metabolic deficiencies, ultrastructural abnormalities of the sperm flagellum, necrozoospermia otherwise it can be idiopathic. In the management of persistent absolute asthenozoospermia, it is very important to elucidate its nature and whenever possible to correct the potential causes.

Methods: We reported data published in the literature on the aetiology of absolute asthenozoospermia and the different techniques to improve ICSI outcome. We propose an algorithm for diagnosis and treatment of this condition.

Results: Different results regarding fertilization, cleavage and pregnancy rate have been published in patients with absolute asthenozoospermia undergoing ICSI. However, the results vary widely depending on the sperm origin and the technique applied for immotile sperm selection. The percentage of viable spermatozoa varies between 0 and 100%.

Conclusions: Absolute immotile spermatozoa is one of the most important causes of reduced fertilization and pregnancy rates after ICSI and different techniques are used to improve ICSI outcomes. However, it still remains unclear which is the best technique to improve the pregnancy outcomes in these couples.

Key words: male infertility / asthenozoospermia / ICSI / immotile spermatozoa / sperm viability testing

Introduction

Asthenozoospermia is a common cause of male infertility. This condition is characterized by reduced motility or absent sperm motility in the fresh ejaculate. Absolute asthenozoospermia, i.e. 100% immotile spermatozoa, is reported at frequency of 1 of 5000 men (Eliasson et al., 1977) and implies a poor fertility prognosis. During natural sperm maturation, motility is acquired during epididymal transit. Sperm motility is important for migration from the vagina to the Fallopian tubes, for penetration of the cumulus oophorus and for processes involved in fertilization. Therefore, a clear association exists between sperm motility and the chance for natural conception (Beauchamp et al., 1984). Since the introduction of ICSI in 1992 by Palermo et al. (1992), couples with severe male-factor infertility, including severe asthenozoospermia, can achieve a pregnancy.
This technique yields both high fertilization and pregnancy rates if motile spermatozoa are used (Van Steirteghem et al., 1993). In some cases, no motile sperm can be found in the ejaculate, a situation which interferes with the success rate after ICSI (Casper et al., 1996; Barros et al., 1997; Vandervorst et al., 1997). Even though the success of ICSI is usually not affected by the overall quality of the basic sperm parameters, the injection of a totally immotile spermatozoon has an overall negative impact on fertilization and pregnancy rates (Liu et al., 1995). A study performed by Nagy et al. on the relationship between the three basic sperm parameters (total sperm count, sperm motility and morphology) and the outcome of ICSI in 996 cycles showed that ‘only one condition had a strongly negative influence on the result of ICSI: where an immotile (presumably dead) spermatozoon was injected into the oocyte’ (Nagy et al., 1995a). In cases of complete asthenozoospermia, the fertilization rate after ICSI with randomly selected immotile spermatozoa is usually very low, especially when using ejaculated spermatozoa (Nagy et al., 1995a, b; Casper et al., 1996; Tournaye et al., 1996). Absence of motile spermatozoa is also observed in epididymal sperm aspirations and in testicular biopsy specimens. Absolute immotile sperm from epididymis should be considered in the same way as ejaculated immotile sperm, however, in the absence of relevant studies, this study will focus on ejaculated sperm.

Methods


Diagnosis of absolute asthenozoospermia

From a clinical viewpoint, it is important to distinguish patients in whom some motile spermatozoa can be found after centrifugation and/or selection on a density gradient, ‘the virtual asthenozoospermic’, from patients in whom even after sperm treatment, incubation and extensive search, all spermatozoa observed are 100% immotile, ‘the absolute asthenozoospermic’ (Vandervorst et al., 1997), Nagy et al. (1995b) observed a significant difference in normal fertilization rates between 12 ICSI cycles with ‘absolute asthenozoospermia’ (10.9%) and 54 cycles with ‘virtual asthenozoospermia’ (60.2%). In the ‘absolute asthenozoospermic’ group, a careful differential diagnosis in order to distinguish between conditions with viable or only non-viable spermatozoa (i.e. necrozoospermia) is advocated.

The distinction can easily be performed by assessing sperm viability on a diagnostic occasion. Diagnostic viability tests are mainly based on the functional integrity of the sperm membrane (Jeyendran et al., 1984) and should be performed in samples with <40% progressive motility (World Health Organization, 2010).

The two common diagnostic viability tests based on the sperm membrane integrity are the eosin–nigrosin test and the eosin Y-test. Eosin colours the sperm head depending on vitality: dead spermatozoa are colored red or dark pink and live spermatozoa that exclude the dye, white or light pink (World Health Organization, 2010). Nigrosin increases the contrast between the background and the sperm heads, facilitating discrimination between the two categories. This paper will focus further on the category of ‘patients with absolute asthenozoospermia’.

Aetiology of absolute asthenozoospermia

In general, patients with immotile spermatozoa show significantly higher FSH concentration than those with motile spermatozoa. Elevated FSH may be indicative of a certain degree of testicular failure, leading to the production of dysfunctional spermatozoa (Shulman et al., 1999). The two main causes of absolute asthenozoospermia are ultrastructural defects in the sperm flagellum including genetically inherited and congenital defects, and necrozoospermia including degeneration due to genital infections, oxidative stress, cryopreservation, antisperm antibodies (Lee et al., 2009), metabolic disorders affecting the ATP production (Folgerø et al., 1993), environmental pollutants exposure (Pilieger-Bruss et al., 2004; Hauser and Sokol, 2008), delayed epididymal transport (Wilton et al., 1988) or prolonged periods of anejaculation. However, the aetiology of asthenozoospermia often remains unexplained. In contrast, immotility of testicular spermatozoa is a physiological event resulting from metabolic sperm immaturity or attachment to Sertoli cells (Jow et al., 1993).

Ultrastructural abnormalities of the flagellum

Ultrastructural abnormalities are the result of a defect in spermiogenesis (Zamboni, 1987; Baccetti et al., 1993) with viable but immotile spermatozoa present in the ejaculate. In the mouse, over 200 genes are known to code for proteins involved in microtubule synthesis (Yatsenko et al., 2010). Many ultrastructural defects may thus have a genetic origin and it is thus unlikely that motile spermatozoa are to be found in the testis (Afzelius, 1981). Since the flagella of the spermatozoa and cilia of other cell types share the same ultrastructure, there are three possible combinations of ultrastructural defects resulting in infertility and respiratory airway dysfunction: defects in both flagella and cilia, defects in the flagella only and defects in the cilia.

The immotile-cilia syndrome was described by Afzelius (1976). It is an uncommon autosomal recessive disorder in which the microtubules of ciliated cells and spermatozoa are immotile but have an otherwise normal morphology and viability. The syndrome is characterized by a defective ciliary ultrastructure: dynein arms are absent or axonemal microtubules are abnormal (Fig. 1a and b). Dynein is the motor protein, fixed to the microtubuli, which can transform chemical energy (ATP) into mechanical energy to induce tubular movement. The prevalence of immotile-cilia syndrome is 1:20 000 live births (Cayan et al., 2001). The presence of immotile spermatozoa with normal morphology and viability can be combined with situs inversus in 50% of the cases (Katagener’s syndrome) and with dysfunction of tracheobronchial cilia resulting in recurrent episodes of bronchitis and sinusitis. A variant of the immotile-cilia syndrome is dysplasia of the fibrous sheath (Rawe et al., 2001).
The diagnosis of immotile-cilia syndrome is straightforward: patients have a normal semen volume, normal sperm concentration, a variable morphology but 100% sperm immotility. The patients usually also have a family history of immotile-cilia syndrome. Definitive diagnosis must be confirmed by the use of transmission electron microscopy demonstrating the absence of the dynein arms.

In the axonemal 9 + 0 defect, the axoneme is composed of nine outer microtubular doublets equipped with an inner and outer dynein arm arranged in a circle but lacking two central microtubules (Fig. 1c) (Afzelius et al., 1995). An association with autosomal dominant polycystic kidney disease has been reported (Okada et al., 1999).

Necrozoospermia

Necrozoospermia is a rare condition reported in only 0.2–0.5% of infertile males and may have its origin either in the epididymis or in the testis (Ahmadi and Ng, 1999). Necrozoospermia of epididymal origin appears to be caused by either a hostile environment in the epididymis or an inherent structural instability in the spermatozoa (Wilton et al., 1988). Substances like reactive oxygen species can penetrate the cell and induce degenerative processes in the nucleus resulting in DNA damage (Agarwal and Saleh, 2002). Moreover, factors in the cytoplasmic compartments of the spermatozoa may be lost by diffusion through the membrane (Stalf et al., 2005). In cases of necrozoospermia, it is assumed that spermatozoa become apoptotic and lose their viability during their prolonged stay in the male genital tract. Infections of the genital tract, for example male accessory-gland infection (MAGI) and chronic prostatitis, testicular and/or epididymal abnormalities (i.e. hypogonadotropic hypogonadism), antisperm antibodies, prolonged periods of anejaculation, hypertermia, in situ carcinoma of the testis, advanced age, exposure to toxic products or drug addiction may also produce necrozoospermia (Lecomte et al., 1998). It is very important to identify the origin of necrozoospermia and try to correct it. A precise clinical assessment and complementary examinations should be performed, including medical history, urogenital examination, hormonal profile, semen and urine culture after prostatic massage, seminal biochemistry, antibodies analyses, transrectal ultrasonography and testicular biopsy (Lecomte et al., 1998; Tournaye, 1998a). Whenever there is any evidence of MAGI, appropriate antibiotic therapy should be prescribed.

Vandervorst et al. (1997) demonstrated that ‘absolute asthenozoospermia’ in necrozoospermic patients is not always a permanent condition. In some patients with necrozoospermia, ejaculated spermatozoa are non-viable but viable spermatozoa may be retrieved by testicular sperm extraction (TESE) (Devroye et al., 1994; Tournaye et al., 1996). Therefore, it is recommended to perform ICSI in combination with TESE in patients with proven necrozoospermia (Tournaye et al., 1996).

Treatment of infertility because of absolute asthenozoospermia

When absolute asthenozoospermia is diagnosed, a complete andrological work-up is indicated in order to elucidate its origin and define the best treatment option. For all patients, ICSI will be indicated. However, it remains crucial to differentiate conditions with viable from those with only non-viable spermatozoa available.

Perform a correct diagnosis and treatment

An accurate clinical and laboratory evaluation may drastically reduce the incidence of ICSI cycles with only immotile spermatozoa available for ICSI. Causes of sperm immotility and key investigations for their diagnosis are shown in Table I (Tournaye et al., 1998b). The ejaculate should be produced after a standard abstinence period of 3 days (Mansour et al., 1996). Longer abstinence intervals may lead to asthenozoospermia in oligozoospermic patients. Care should be taken to examine the semen samples at 37°C because lower temperatures may affect motility and rarely may cause immobilization. It is extremely important to assess a second semen sample and to confirm the diagnosis as it has been reported that motile spermatozoa can be detected in subsequent ejaculates (Jeyendran et al., 1984). As mentioned above, an accurate medical history (including history of recurrent upper-respiratory tract infections, sexually transmitted diseases and surgery of the genito-urinary tract) and physical examination should be performed. Special attention must be paid to specific signs that may be associated with sperm immotility, such as dextrocardia or wheezing and other symptoms associated with ciliopathies.

In a few cases, the presence of immotile spermatozoa can be due to treatable conditions, e.g. antisperm antibodies, infections of genital tract (i.e. Escherichia coli), latex condom exposure or exposure to

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**Figure 1** Electron microscopy of sperm axonemes. (a) A normal axoneme. (b) Electron microscopy showing absence of outer and inner dynein arms in a patient with 100% immotile spermatozoa. (c) Electron microscopy showing a 9 + 0 syndrome.
other toxic compounds in the collection device and prolonged periods of anejaculation.

Figure 2 shows the proposed diagnostic and therapeutic flow chart. As can be seen, in many occasions no specific curative treatment is available or sometimes no specific diagnosis can be made.

**No cause found or no corrective measure available**

If no cause is found or no treatment is available, the only option is to carefully select the sperm that can be used for ICSI.

In necrozoospermia, all the spermatozoa in the ejaculate are non-viable, and hence TESE is the only possible option, but there is no guarantee of retrieving motile or viable spermatozoa for ICSI (Tournaye et al., 1996). There are reports of clinical success with ICSI using sperm of testicular origin that started moving after prolonged, overnight incubation in culture medium (Emiliani et al., 2000; Wood et al., 2003). It has been shown in patients with obstructive azoospermia that the percentage of viable testicular spermatozoa is higher than the percentage of motile spermatozoa (Verheyen et al., 1995). Unfortunately, often testicular sperm are immotile immediately after biopsy retrieval and frozen-thawed testicular samples show additional loss of motility (Bachtell et al., 1999). Therefore, especially in non-obstructive azoospermia, it is preferable to consider a fresh biopsy retrieval if the embryologist is confronted with the presence of only real immotile sperm after thawing (Verheyen et al., 2004).

However, although the fertilization rate is higher when using motile testicular spermatozoa for ICSI, the number of embryos available for transfer, the implantation potential and the pregnancy rate are not significantly different between motile and immotile testicular spermatozoa (Nagy et al., 1998; Shulman et al., 1999) due to careful selection of the immotile testicular spermatozoa for ICSI.

The methods in order to select spermatozoa for ICSI from samples with absolute asthenozoospermia are reviewed in the following paragraphs (see flow chart, Fig. 2). Although the algorithm presented in Fig. 2 proposes to perform TESE in cases with 100% necrozoospermia, TESE may also be considered in men with a normal testicular function whenever only a limited proportion of ejaculated sperm are alive because TESE will yield more viable sperm.

**The hypo-osmotic swelling test (HOST)**

First described by Jeyendran et al. in 1984, this test evaluates the functional integrity of the sperm membrane (Liu et al., 1997). The World Health Organization recommends to use the hypo-osmotic swelling test as a vitality test alternative to dye exclusion. HOST is useful when staining of spermatozoa is avoided (World Health Organization, 2010). Although the percentage of sperm survival and motility may be affected by exposure to hypo-osmotic solutions, this does not necessarily imply a decreased functionality of the surviving sperm in ICSI (Verheyen et al., 1997).

The original HOST solution consists of a mixture of equal parts of fructose and sodium citrate (150 mOsm) with a calculated ionic strength of 0.15. With this solution, a maximal number of clearly swollen spermatozoa are identifiable (Jeyendran et al., 1984). Live spermatozoa with normal membrane function show swelling of the cytoplasm and curling of the tail due to water influx when exposed to hypo-osmotic conditions. These changes are visualized easily under light microscopy. But there is no information regarding the possible detrimental effects of the non-physiological solution on sperm prior to ICSI. The use of HOST in distinguishing viable from non-viable immotile spermatozoa for ICSI was first reported by Desmet et al. (1994) who obtained a fertilization rate of 3% with the injection of HOST-selected spermatozoa into 1 day-old unfertilized oocytes. Casper et al. (1996) used the HOST to select viable but immotile spermatozoa for ICSI and they reported a fertilization and cleavage rate of 43 and 39%, respectively, compared with 26 and 23% when spermatozoa were randomly selected in eight ICSI cycles. In 1997, Tsai et al. compared four different hypo-osmotic solutions. They concluded that 150 mOsm NaCl is the optimal HOST solution for selecting viable spermatozoa for ICSI and that the incubation period of sperm in this solution should not exceed 1 min (Tsai et al., 1997). Trying to find the best hypo-osmotic solution, Verheyen et al. (1997) compared three solutions (Jeyendran solution, a mixture of 50% of isotonic Earle’s medium and 50% milli-Q water and milli-Q water alone) and concluded that the solution composed of 50% isotonic medium and 50% distilled water preferable for selecting viable spermatozoa for ICSI in cases of complete asthenozoospermia (Verheyen et al., 1997). Liu and coworkers used this solution for the selection of immotile ejaculated spermatozoa for ICSI and reported one pregnancy of three (Liu et al., 1997). Barros et al. (1997) reported a fertilization rate of 41.9% using a modified HOST and described two clinical pregnancies. Lin et al. (1998) showed that the HOST was not able to predict the viability of spermatozoa after freezing/thawing procedure.

**Mechanical touch technique**

Testing the flexibility of the sperm tail is an alternative but subjective method to select viable immotile spermatozoa. The principle of this technique is to evaluate the tail flexibility by touching it with the ICSI pipette (Soares et al., 2003). Some authors consider the test positive if the tail bends and recovers its original position. Sperm rigidity and incapacity to recover to the initial tail position is considered a sign of non-viability (Marques de Oliveira et al., 2004). Others consider the test

<table>
<thead>
<tr>
<th>Table 1 Cause of absolute asthenozoospermia and key investigations for diagnosis.</th>
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</thead>
<tbody>
<tr>
<td>Immobile-cilia syndromes</td>
</tr>
<tr>
<td>Ultrastructural axonemal defects</td>
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<tr>
<td>Periaxonemal ultrastructural defects</td>
</tr>
<tr>
<td>Necrozoospermia</td>
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<tr>
<td>Enzymatic deficiencies</td>
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<tr>
<td>Antisperm antibodies</td>
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<tr>
<td>Infections (chronic prostatitis)</td>
</tr>
<tr>
<td>Exposure of ejaculate to cold, spermicides</td>
</tr>
<tr>
<td>Prolonged abstinence</td>
</tr>
</tbody>
</table>

Reprinted with permission from the author (Tournaye, 1998b).
positive if the tail moves up and down independently of the head movement and negative when head and tail move together when touched with the ICSI pipette (Soares et al., 2003). Using this technique, Soares et al. (2003) reported a fertilization rate of 30.3% with the immotile ejaculated sperm, while Marques de Oliveira et al. (2004) reported a fertilization rate of 73.4% with fresh immotile testicular spermatozoa. However, the reliability of this technique depends largely on the expertise of the embryologist performing the assessment.

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**Exposure to pentoxifylline**

Pentoxifylline (PTX) is a 3′,5′-nucleotidase phosphodiesterase inhibitor of the methylxantine group that enhances sperm motility by increasing intracellular cAMP. It is known that the intracellular cAMP concentration plays a role in sperm motility (Tash and Means, 1983). The beneficial use of PTX on improving sperm motility has been published by several groups (Yovich et al., 1988;
Fountain et al., 1995; Rizk et al., 1995; Tarlatzis et al., 1995; Negri et al., 1996). In 1988, Yovich et al. reported the first results using PTX for conventional IVF in cases of severe male-factor infertility and obtained five pregnancies in a pronuclear-stage embryo transfer programme (Yovich et al., 1988). Concerning the embryotoxicity, Tournaye et al. (1993a); Tournaye et al. (1993b); Tournaye et al. (1994) observed several adverse effects of PTX on mouse embryos, but these effects could be avoided by washing spermatozoa after PTX exposure. Testing the embryotoxicity of PTX in mouse IVF and embryo transfer models, Yovich (1993) showed that the fertilization rate and the blastocyst formation rate were not lower if only sperm were exposed to PTX. However, the results were lower if also the oocytes were exposed to PTX. Terriou et al. (2000) demonstrated that short exposure of sperm to PTX followed by sperm washing does not affect early embryo development after ICSI with a cleavage rate, pregnancy rate and the implantation rates of 95.4, 30.6 and 12.3%, respectively.

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients (n)</th>
<th>Sperm origin</th>
<th>Technique applied</th>
<th>Viable spermatozoa (%)</th>
<th>Fertilization rate (2PN) (%)</th>
<th>Clinical pregnancies (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casper et al. (1996)</td>
<td>8 patients</td>
<td>Epidymal (4 cases)</td>
<td>HOST</td>
<td>31.1 ± 5.8</td>
<td>43</td>
<td>3</td>
</tr>
<tr>
<td>Liu et al. (1997)</td>
<td>3 patients</td>
<td>Ejaculated and F-TESE</td>
<td>HOST</td>
<td>NM</td>
<td>76.4</td>
<td>1</td>
</tr>
<tr>
<td>Vandervorst et al.</td>
<td>11 patients</td>
<td>Ejaculated</td>
<td>Eosin Y stain*</td>
<td>0–34</td>
<td>12.4</td>
<td>0</td>
</tr>
<tr>
<td>Barros et al. (1997)</td>
<td>6 cycles</td>
<td>Ejaculated</td>
<td>HOST</td>
<td>5</td>
<td>41.9</td>
<td>2 (1 twin)</td>
</tr>
<tr>
<td>Nagy et al. (1998)</td>
<td>14 cycles</td>
<td>F-TESE</td>
<td>HOST</td>
<td>NM</td>
<td>46</td>
<td>4</td>
</tr>
<tr>
<td>Ron-El et al. (1998)</td>
<td>3 initial cycles</td>
<td>Ejaculated</td>
<td>Eosin Y stain*</td>
<td>41 ± 7.4</td>
<td>3</td>
<td>1 (twin)</td>
</tr>
<tr>
<td></td>
<td>6 repeated cycles</td>
<td>Ejaculated</td>
<td>Eosin Y stain*</td>
<td>71 ± 6.9</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Okada et al. (1999)</td>
<td>16 patients</td>
<td>Ejaculated</td>
<td>Eosin Y stain*</td>
<td>15–80</td>
<td>38.6</td>
<td>0</td>
</tr>
<tr>
<td>Shulman et al. (1999)</td>
<td>19 cycles</td>
<td>TESE</td>
<td>NM</td>
<td>NM</td>
<td>51</td>
<td>3</td>
</tr>
<tr>
<td>Terriou et al. (2000)</td>
<td>20 cycles</td>
<td>MESA, F-TESE, Fr-Th TESE, Fr Ep</td>
<td>HOST</td>
<td>6–60</td>
<td>45.2</td>
<td>6</td>
</tr>
<tr>
<td>El-Nour et al. (2001)</td>
<td>4 patients</td>
<td>Ejaculated</td>
<td>HOST</td>
<td>15–46</td>
<td>47</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>10 patients</td>
<td>TESE</td>
<td>HOST</td>
<td>20–100</td>
<td>43</td>
<td>4 (1 twin)</td>
</tr>
<tr>
<td></td>
<td>1 patient</td>
<td>Electroejaculated</td>
<td>HOST</td>
<td>18</td>
<td>60</td>
<td>1 (twin)</td>
</tr>
<tr>
<td>Salam et al. (2001)</td>
<td>15 patients</td>
<td>Ejaculated</td>
<td>HOST mod.</td>
<td>NM</td>
<td>42.7</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>12 patients</td>
<td>F-TESE</td>
<td>HOST mod.</td>
<td>NM</td>
<td>30.1</td>
<td>2</td>
</tr>
<tr>
<td>Soares et al. (2003)</td>
<td>10 cycles</td>
<td>F-TESE, TESA, PESA</td>
<td>MTT</td>
<td>NM</td>
<td>30.3</td>
<td>1</td>
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<td>Aktan et al. (2004)</td>
<td>10 patients</td>
<td>Ejaculated, F-TESE</td>
<td>HOST versus LAISS</td>
<td>21.5 versus 22</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>21 patients</td>
<td>F-TESE</td>
<td>LAISS</td>
<td>NM</td>
<td>45.4</td>
<td>5</td>
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<td></td>
<td>24 patients</td>
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<td>LAISS</td>
<td>NM</td>
<td>64.2</td>
<td>8</td>
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<td>Marques de Oliveira et al. (2004)</td>
<td>6 patients</td>
<td>Fr-Th TESE</td>
<td>MTT</td>
<td>NM</td>
<td>65.7</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>10 patients</td>
<td>F-TESE</td>
<td>MTT</td>
<td>NM</td>
<td>73.4</td>
<td>3</td>
</tr>
<tr>
<td>Salam et al. (2005)</td>
<td>25 patients</td>
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<td>HOST</td>
<td>NM</td>
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<td>7</td>
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<tr>
<td></td>
<td>19 patients</td>
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<td>HOST</td>
<td>NM</td>
<td>42.7</td>
<td>5</td>
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<td>Kovacic et al. (2006)</td>
<td>47 cycles</td>
<td>TESA, TESE</td>
<td>PTX</td>
<td>NM</td>
<td>66</td>
<td>18</td>
</tr>
</tbody>
</table>

Fr-Ep, frozen epidymal; F-TESE, FRESH TESE; Fr-Th TESE, frozen-thawed TESE; HOST, Hypo-osmotic swelling test; HOST mod, modified hypo-osmotic swelling test; LAISS, laser-assisted immotile sperm selection; MESA, microsurgical epididymal sperm aspiration; MTT, mechanical touch technique; PESA, percutaneous epididymal sperm aspiration; PTX, pentoxifylline; TESA, testicular sperm aspiration; TESE, testicular sperm extraction; NM, not mentioned.

*aTo detect viable spermatozoa.

**Laser-assisted immotile sperm selection**

In 2004, Aktan et al. published a novel method to identify viable but immotile spermatozoa by applying a single laser shot close to the tip of the sperm tail using a non-contact 1.48 μm diode laser system. With this technique, they found two possible reactions: either the spermatozoa show no reaction at all, or the tail of the spermatozoa starts curling within a few seconds after the laser shot. Spermatozoa showing a curling reaction were considered to be viable and were selected for injection. The procedure was carried out in normal culture medium and spermatozoa were immediately used for ICSI. They reported that patients with complete asthenozoospermia had higher fertilization and cleavage rate with Laser-assisted immotile sperm selection (LAISS) than with HOST (64.2 versus 46.5% P < 0.003 and 79.6 versus 51.4% P < 0.0001, respectively; Aktan et al., 2004). Regarding the safety aspects of the procedure, the laser energy applied does not permeabilize or damage the sperm...
membrane (Montag et al., 1999). No increased DNA fragmentation was found, even after application of multiple shots directly on the sperm head, probably due to the minimal damage zone (1 mm) of the diode laser beam (Montag and Rink, 2001). Gerber et al. (2008) reported a singleton pregnancy after using immotile but viable spermatozoa selected by a combination of HOST test and single laser shots for ICSI. However, this technique is expensive if to be purchased for this purpose only and is not available in all IVF centres.

Birefringence-polarization microscopy

This technique assumes that polarization microscopy of a sperm with birefringent head and midpiece (reacted sperm) implies healthy mature sperm. As reported by Baccetti (2004), viable human spermatozoa are naturally birefringent, whereas in pathological conditions, dead, necrotic spermatozoa are devoid of birefringence due to the absence of conventional sperm texture. Birefringence might identify different sperm regions due to their different response to polarized light (i.e. a normal progressive spermatozoa show a non-luminous acrosome and a luminous, normal-sized compact nucleus and flagellum) (Collodel et al., 2010). In 2009, Chattopadhyay et al. reported a pregnancy rate of 46% when ICSI was performed by injecting a sperm with birefringent head. Moreover, Crippa et al. (2009) concluded that the selection of birefringent spermatozoa increases the chances of identifying a vital sperm cell having intact DNA. However, more comparative studies should be performed because this technique is expensive.

Results of ICSI with immotile spermatozoa

Different results regarding fertilization, cleavage and pregnancy rate have been published in patients with absolute asthenozoospermia undergoing ICSI. However, the results vary widely depending on the sperm origin and the technique applied for immotile sperm selection (Table II). The percentage of viable spermatozoa varies between 0 and 100%. Fertilization and clinical pregnancy rates range from 3 to 100%. Fertilization and clinical pregnancy rates range from 3 to 100% (Liu et al., 1997; Ron-El et al., 1998) and 0 to 38.3%, respectively (Vandervorst et al., 1997; Kovacic et al., 2006). To our knowledge, Kovacic et al., (2006) reported the largest series (47 cycles) using testicular sperm, with a fertilization rate of 66% and a pregnancy rate of 38.3%. Kahraman et al. (1997) reported the first pregnancy and delivery of a healthy child after ICSI treatment with immotile testicular spermatozoa in a patient with absolute asthenozoospermia.

Conclusion

ICSI is nowadays widely used in couples with severe male-factor infertility and it is extensively demonstrated that it improves the fertilization and pregnancy rates when motile spermatozoa are used (de Mendoza et al., 2000; Nagy et al., 2000). However, for patients with complete asthenozoospermia, although reported in only 1 of 5000 men, fertility prognosis is very poor, even with ICSI. Absolute immotile spermatozoa is one of the most important causes of reduced fertilization and pregnancy rates after ICSI (Liu et al., 1995b; Nagy et al., 1995b; Liu et al., 1997). Impaired results have been obtained with ejaculated spermatozoa (Nagy et al., 1995b; Casper et al., 1996; Tournaye et al., 1996) due to the difficulty of distinguishing viable from non-viable immotile spermatozoa. Differentiation between the two categories is very important as sperm vitality is one of the main conditions in order to achieve pregnancy. Several methods have been described to detect viable spermatozoa that can be injected and improve the outcome of ICSI in couples with complete asthenozoospermia. While some tests are purely diagnostic (eosin-Y and eosin–nigrosin test), others such as the mechanical touch technique or HOST can be applied without specific equipment in order to select immotile sperm for ICSI treatment. New techniques, LAISS or birefringence testing for example, require expensive equipment and are therefore not accessible in all IVF centres. However, it remains unclear which is the best technique to improve pregnancy outcomes in couples with total immotile spermatozoa. To the best of our knowledge, there are no comparative studies showing which viability test is preferentially applied. Therefore, there is a need to perform properly designed clinical studies to compare the different techniques. As the prevalence of absolute asthenozoospermia is rare, a multicentre study is advocated.

Authors’ roles

C.O. involved in acquisition, analysis and interpretation of data, drafting the paper and final approval; G. V. in analysis and interpretation of data, revising the paper and final approval; D.R. in acquisition, analysis and interpretation of data, revising the paper and final approval; M.C. and P.D. in interpretation of data, revising the paper and final approval; T.H. in conception and design, analysis and interpretation of data, revising the paper and final approval.

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