# Chapter 10
# Sperm Selection: Effect on Sperm DNA Quality

**Lodovico Parmegiani, Graçiela Estela Cognigni, and Marco Filicori**

**Abstract** The selection of spermatozoa without DNA fragmentation and chromosomal diseases prior to assisted reproductive techniques helps to optimize the outcome of the treatment; in particular, sperm selection prior to in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) is crucial. In fact, although ICSI has been successfully and safely applied worldwide for almost 20 years, at the present time we have no real knowledge regarding the hypothetical long-term side effects on ICSI adults, given the increased likelihood of spermatozoa with defective nuclear content fertilizing oocytes.

In the case of DNA damage, the basal sperm DNA fragmentation rate can be significantly reduced by some sperm processing procedures that improve the percentage of spermatozoa with normal chromatin structure by filtering out DNA-damaged spermatozoa. After this first step, new advances in micromanipulation can be performed to choose the "ideal" mature spermatozoa for ICSI, reducing potential damage to the gametes. In fact, it is possible to prevent fertilization by DNA-damaged and chromosomal-unbalanced spermatozoa by selecting ICSI sperm by maturation markers such as hyaluronic acid or other zona pellucida receptors. Furthermore, novel noninvasive imaging techniques can be valid tools for helping in the morphological selection of ICSI spermatozoa.

**Keywords** Hyaluronic acid • Intracytoplasmic sperm injection • Intracytoplasmic morphologically selected sperm injection • Sperm motility • Physiological ICSI • Physiological IMSI

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## Introduction

Sperm DNA quality plays a major role in male fertility. Many couples failing to achieve pregnancy may be affected by male genomic factors, including sperm DNA damage (Siffroi et al. 1997; Joly-Helas et al. 2007; Kim et al. 2011; Sakkas and Alvarez 2010). It has been demonstrated that when the male partner has a high rate of DNA-damaged spermatozoa, the couple has a high probability of waiting a long time before conceiving (Loft et al. 2003) and should be considered of low potential for natural fertility (Spanò et al. 2000; Zini et al. 2001). Furthermore, if these couples finally achieve pregnancy, they have a higher risk of miscarriage (Spanò et al. 2000). Finally, it should also be pointed out that multiple studies have demonstrated that sperm DNA damage increases with advancing male age (Humm and Sakkas 2013).

For these reasons, the selection of spermatozoa without DNA fragmentation and chromosomal diseases prior to assisted reproductive (AR) techniques helps to optimize the outcome of the treatment, in particular, in the case of in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) (Ebner et al. 2012). In fact, ICSI of aneuploid spermatozoa seems to be the cause of the vast majority of genetic deviations in ICSI newborns (Bonduelle et al. 2002), and the insemination of DNA-damaged spermatozoa seems to be involved in an increased abortion rate (Zini et al. 2008). Furthermore, the injection of DNA-damaged spermatozoa also seems to have long-term side effects in adult animals, such as aberrant growth, premature aging, abnormal behavior, and mesenchymal tumors (Fernandez-Gonzalez et al. 2008). In addition, it should be pointed out that the widespread use of ICSI increases not only the chances of injecting spermatozoa that are defective in their genetic constitution (Sakkas et al. 1997; Marchesi and Feng 2007) but also for centrosome integrity (Schatten and Sun 2009), phospholipase C zeta content (Heytens et al. 2009), and DNA methylation (Navarro-Costa et al. 2010).

For all these reasons sperm selection prior to in vitro insemination by IVF and ICSI is crucial. This selection should be performed keeping to a minimum any additional risk that might increase sperm damage in the laboratory, and – at least for those processes that might be influenced by the embryologist – should be performed in a safe and preferably physiological manner (Parmegiani et al. 2010a, b, c).

In the case of DNA damage, before IVF–ICSI, the basal sperm DNA fragmentation rate can be significantly reduced by some sperm processing procedures such as swim-up (Spanò et al. 2000; Parmegiani et al. 2010a), density gradient (Sakkas et al. 2000), selection by motility without centrifugation (Ebner et al. 2011), and magnetic cell sorting (MACS) with annexin V (Rawe et al. 2010).

These semen selection procedures improve the percentage of spermatozoa with a normal chromatin structure by filtering out DNA-damaged spermatozoa. After this first step, new advances in micromanipulation can be performed to chose the “ideal” mature spermatozoa for ICSI by the restoration of natural fertilization checkpoints such as sperm-hyaluronic acid binding (Parmegiani et al. 2010a) or sperm-zona pellucida binding (Paes de Almeida Ferreira Braga et al. 2009) or by using high magnification microscopy, i.e., intracytoplasmic morphologically selected sperm injection (IMSI) (Bartoov et al. 2003).

## Sperm DNA Damage

DNA fragmentation can be induced by numerous factors, such as apoptosis, oxygen radicals, radiation and chemotherapy, or environmental toxicants. It has been hypothesized that DNA damage in sperm may occur during production or transport by the testes (Sakkas and Alvarez 2010). During spermatogenesis, specific screening mechanisms in the testes mark individual spermatozoa with apoptotic markers, which cause phagocytosis of these cells (Billig et al. 1996). Furthermore, testicular endogenous nuclease activity, assisting protamination, increases the percentage of DNA-damaged spermatozoa (McPherson and Longo 1993). Since testicular spermatozoa show lower levels of DNA damage than epididymal or ejaculated sperm, it could be hypothesized that sperm DNA fragmentation is even more frequently generated during sperm transport through the epididymis (Ollero et al. 2001; Greco et al. 2005). In this scenario, if intratesticular and intraepididymal mechanisms for the removal of defective spermatozoa do not function properly, these gametes will end up in the ejaculate. In fact, sperm DNA fragmentation is high in oligoasthenoteratozoospermic men and correlates with failed fertilization, impaired preimplantation development, and altered pregnancy outcome (Evenson et al. 1999; Carrell et al. 2003; Seli et al. 2004; Borini et al. 2006; Velez de la Calle et al. 2008; Zini et al. 2008). Sperm DNA integrity, and in particular DNA fragmentation, is commonly checked in assisted reproduction (AR) laboratories by specific tests:

- Sperm chromatin structure assay (SCSA) (Evenson et al. 2002),
- TdT-mediated-dUTP nick-end labeling (TUNEL) (Gorczyca et al. 1993)
- Sperm chromatin dispersion (SCD) test (Fernandez et al. 2003),
- Comet assay (Hughes et al. 1996; Enciso et al. 2009).

These assays are based on two different approaches – directly detecting DNA damage (direct tests, e.g., TUNEL) or measuring DNA fragmentation after a rather mild denaturation process (indirect tests, e.g., SCSA, SCD). Both direct and indirect assays seem to be reliable in predicting the levels of DNA fragmentation (Chohan et al. 2006; Zhang et al. 2010). DNA breaks can be single- or double-stranded; single defects are probably easier to repair compared with double breaks (Sakkas and Alvarez 2010), which may generate reciprocal translocations (Richardson and Jasin 2000).

### *DNA Damage and the Outcome of Assisted Reproduction Techniques*

Animal models can be useful in investigating the effect DNA-damaged spermatozoa (Ahmadi and Ng 1999a, b; Fernandez-Gonzales et al. 2008; Upadhya et al. 2010). It is now known that DNA-damaged spermatozoa have the ability to fertilize an

**Table 10.1** Effect of sperm DNA damage on IVF/ICSI outcome[a]

| Author | Patients | Assay | Negatively affected outcome |
|---|---|---|---|
| Morris et al. (2002) | 60 | Comet | Nothing |
| Bungum et al. (2004, 2007, 2008) | 1,296 | SCSA | Nothing |
| Gandini et al. (2004) | 34 | SCSA | Nothing |
| Pregl Breznik et al. (2013) | 133 | SCD | Nothing (ICSI) |
| Lopes et al. (1998) | 131 | TUNEL | Fertilization |
| Huang et al. (2005) | 303 | TUNEL | Fertilization |
| Payne et al. (2005) | 100 | SCSA | Fertilization |
| Sun et al. (1997) | 143 | TUNEL | Fertilization, embryo quality |
| Virant-Klun et al. (2002) | 183 | AO | Fertilization, embryo quality |
| Benchaib et al. (2003) | 104 | TUNEL | Fertilization, pregnancy |
| Muriel et al. (2006) | 85 | SCD | Fertilization, embryo quality |
| Simon et al. (2010) | 360 | Comet | Fertilization, embryo quality |
| Pregl Breznik et al. (2013) | 133 | SCD | Fertilization, embryo quality (IVF) |
| Tomlinson et al. (2001) | 140 | NT | Pregnancy |
| Tomsu et al. (2002) | 40 | Comet | Embryo quality, pregnancy |
| Larson-Cook et al. (2003) | 89 | SCSA | Pregnancy |
| Henkel et al. (2004) | 249 | TUNEL | Pregnancy |
| Seli et al. (2004) | 49 | TUNEL | Embryo quality |
| Virro et al. (2004) | 249 | SCSA | Embryo quality, pregnancy |
| Borini et al. (2006) | 132 | TUNEL | Pregnancy, pregnancy loss |
| Frydman et al. (2008) | 117 | TUNEL | Pregnancy, pregnancy loss |
| Lin et al. (2008) | 223 | SCSA | Pregnancy loss |
| Avendaño et al. (2010) | 36 | TUNEL | Embryo quality, pregnancy |
| Speyer et al. (2010) | 347 | SCSA | Pregnancy |
| Meseguer et al. (2011) | 210 | SCD | Pregnancy |

*AO* acridin orange staining, *NT* in situ nick translation, *SCD* sperm chromatin dispersion test, *SCSA* sperm chromatin structure assay, *TUNEL* TdT-mediated-dUTP nick-end labeling

[a]Slightly modified from Ebner et al. (2012)

oocyte, but embryo development seems to be related to the degree of DNA damage (Upadhya et al. 2010; Ahmadi and Ng 1999a, b). Furthermore, it can be hypothesized that the oocyte can repair the sperm DNA damage. However, this repairing mechanism seems to be efficient only if sperm DNA damage is less than 8 %; damage beyond this level could result in a low rate of embryonic development and high early pregnancy loss (Ahmadi and Ng 1999a).

In humans, the correlation between sperm DNA damage and low fertility is evident in natural conception and when performing a "low-technology" AR procedure such as intrauterine insemination (IUI). One prospective study revealed that above a threshold of 12 % sperm having strand breaks, no clinical pregnancy was achieved when performing IUI (Duran et al. 2002). Others studies on IUI confirmed that the best results in terms of pregnancy rate are obtainable when sperm DNA fragmentation index (DFI) is lower than 27–30 % (Bungum et al. 2004, 2007).

When analyzing in vitro insemination techniques, IVF and, particularly, ICSI, the correlation between sperm DNA damage and clinical outcome is more controversial (Table 10.1). Some studies – five original papers (Bungum et al. 2004, 2007, 2008;

Gandini et al. 2004; Lin et al. 2008) and two metaanalyses (Li et al. 2006; Collins et al. 2008) -found no correlation between sperm DNA fragmentation in the neat semen and fertilization rate, embryo quality, or clinical pregnancy. Other studies demonstrated that ICSI allowed a better clinical outcome than IVF in the case of high sperm DNA fragmentation rate (Bungum et al. 2004, 2007; Huang et al. 2005). Furthermore DNA damage is inversely correlated with IVF fertilization rates (Sun et al. 1997; Høst et al. 2000a, b; Huang et al. 2005; Borini et al. 2006). Simon et al. observed that increased sperm DNA fragmentation resulted in lower fertilization rates, poorer embryo quality, and reduced pregnancy rates after IVF and ICSI (2011) and in reduced live birth rates in IVF, but not in ICSI (Simon et al. 2013).

In a very recent study on sibling oocytes, Pregl Breznik et al. (2013) found that sperm DNA damage was inversely related to fertilization and embryo quality in IVF, but the DFI had no relation to these parameters in the case of ICSI. According to these findings, ICSI should be the method of choice when the DFI is near or exceeds the threshold value of 30 % (Bungum et al. 2004, 2008).

### DNA Damage and IVF/ICSI; Fertilization, Embryo Quality-Development Rate

Some authors have found a negative correlation between sperm DNA fragmentation and fertilization rate (Lopes et al. 1998; Benchaib et al. 2003; Huang et al. 2005; Borini et al. 2006) and zygote formation (Muriel et al. 2006) in both IVF and ICSI. However, this seems to be balanced in ICSI by a good subsequent development of embryos (Payne et al. 2005). Other studies reported an inverse correlation between DFI and embryo quality (Tomsu et al. 2002; Virant-Klun et al. 2002; Avendaño et al. 2010; Seli et al. 2004; Virro et al. 2004; Muriel et al. 2006).

### DNA Damage and IVF/ICSI; Pregnancy-Implantation Rate

Meseguer et al. (2011) measured the effect of the DFI on subsequent pregnancy independent of the fertilization procedure (IVF or ICSI) or sperm origin (ejaculate or testicular) and calculated that for every 10 % increase in the DFI, the probability of not achieving pregnancy increased by 1.31. Avendaño et al. (2010) found a 3.5 times higher likelihood of pregnancy when the DFI was less than or equal to 17.6 % after sperm treatment. The DFI threshold in raw semen is still controversial: in a 2003 study, no clinical pregnancy was noted in the case of a DFI greater than or equal to 27 % (Larson-Cook et al. 2003), whereas Henkel et al. (2004) stated that the probability of pregnancy is higher when DFI is less than 36.5 %. Correspondingly, Spanò et al. (2000) observed that under a threshold of 40 % DFI, the pregnancy rate was significantly higher than in patients with a higher DFI. Similarly, a prospective analysis on 360 couples (Simon et al. 2010) showed significantly lower pregnancy rates above a threshold of 44 % DFI.

In terms of implantation, ICSI with a DFI greater than or equal to 19 % was associated with a lower implantation rate (Speyer et al. 2010). It has been hypothesized that "healthy/young" oocytes may compensate for the potential adverse effects on pregnancy and implantation of high sperm DNA fragmentation, but this has been refuted by some studies (Borini et al. 2006; Frydman et al. 2008). Nevertheless, some refinements of ICSI that allow us to minimize the risk of injection of DNA damaged spermatozoa seem to improve the clinical outcome, especially in the case of oocytes of older women (Souza Setti et al. 2013); this may suggest that sperm DNA damage plays a critical role in the case of advanced age of the female partner.

## DNA Damage and IVF/ICSI Miscarriage Rate

It has been hypothesized that, although DNA-damaged spermatozoa can fertilize an oocyte, they are often associated with failed pregnancy or pregnancy loss (Henkel et al. 2004). Borini et al. (2006) observed a higher miscarriage rate in ICSI patients with a DFI greater than 10 %. Kennedy et al. (2011) reported a significant correlation between DFI and spontaneous abortion; furthermore, they observed that couples having triplet pregnancies had a lower DFI than those with pregnancy loss. Conversely, Bellever et al. (2010) found no correlation between a high DFI and spontaneous abortion. In a recent large-scale metaanalysis (1,549 cycles: 640 pregnancies and 122 pregnancy losses) Zini et al. (2008) concluded that sperm DNA damage is associated with a significantly increased risk of pregnancy loss after both IVF and ICSI (2008).

## Remarks

All these studies seem to indicate a correlation between DNA damage and outcome of AR technologies, including IVF and ICSI. Since the outcome of AR treatments depends on many more factors than just sperm quality (above all female factors, but also environmental factors, patient selection, choice of appropriate AR treatment, and others), this makes it extremely difficult to compare the studies and to find a general agreement on the role of sperm DNA damage. Furthermore, it should be pointed out that sperm DNA fragmentation is not a static seminal parameter and the timing for assessing the DNA damage is critical: the in vitro aging and spermatozoa or exposure to nonphysiological conditions before DNA testing have a detrimental effect on DNA (Santiso et al. 2012). Unfortunately, information on the timing of DNA testing and DNA fragmentation dynamics is very often absent in the literature. In conclusion, the various factors influencing treatment outcome, the timing in performing the DNA fragmentation test, and the use of different DNA tests contribute to jeopardizing the reliability of the majority of the studies in the literature.

## Sperm Selection for Assisted Reproduction Techniques: Effect on Sperm DNA Quality

DNA fragmentation rate in the raw semen can be reduced by sperm processing procedures, which improve the percentage of spermatozoa with a normal chromatin structure by filtering out apoptotic spermatozoa. In the case of in vitro insemination techniques (IVF/ICSI) following sperm preparation, high-magnification microscopy and selection by some sperm markers are valid tools in selecting spermatozoa with a reduced risk of being DNA-damaged.

### *Relationship Between Sperm Motility and DNA Integrity*

It is accepted that oligozoospermia (Høst et al. 1999; Burrello et al. 2004), teratozoospermia (Høst et al. 1999; Muratori et al. 2003), and asthenozoospermia (Irvine et al. 2000; Giwercman et al. 2003; Varghese et al. 2009; Mahfouz et al. 2010) are associated with a higher percentage of sperm DNA aberrations.

Conversely, it seems that good sperm motility is related to sperm DNA integrity (Van den Bergh et al. 1998; Ramos and Wetzels 2001; Avendaño and Oehninger 2011). This has been highlighted by Ebner and colleagues (2011), who showed that fast progressive spermatozoa have intact DNA. This is probably due to the fact that not only nuclear but also mitochondrial DNA can be affected by DNA fragmentation (Alvarez 2005); in the latter case, the spermatozoa may have alterations in the production of ATP, which is the "fuel" for sperm motility. This hypothesis is confirmed by various studies. Kasai et al. (2002) observed a direct association between motility, mitochondrial volume, and membrane potential within the sperm midpiece; mutations or deletions within mitochondrial DNA were shown to be associated with reduced sperm motility (Ozmen et al. 2007); and Speyer et al. (2010) noted a positive correlation between DFI and sperm midpiece.

### *Sperm Preparation Techniques Before IVF/ICSI*

In the case of DNA damage, prior to IVF–ICSI, basal sperm DNA fragmentation rate can be significantly reduced by some sperm processing procedures. These allow us to select motile spermatozoa, filtering out the low-motile apoptotic ones. During sperm preparation special care should be taken because it should be kept in mind that these procedures themselves might cause DNA damage (Twigg et al. 1998; Donelly et al. 2001; Dalzell et al. 2004; Santiso et al. 2012; Toro et al. 2009). First of all, the sperm must be processed immediately after liquefaction in order to avoid in vitro aging and degeneration of nuclear proteins (Sakkas and Alvarez 2010); then

**Table 10.2** Sperm processing techniques and DNA fragmentation index (DFI) reduction[a]

| | Patients | %DFI raw | % DFI after | % reduction |
|---|---|---|---|---|
| *Swim-up* | | | | |
| Zini et al. (2000a) | 22 | 10.1 | 4.8 | 52.5 |
| Younglai et al. (2001) | 29 | 2.4 | 1.1 | 54.2 |
| Jackson et al. (2010) | 30 | 17.8 | 8.3 | 53.4 |
| Parmegiani et al. (2010a) | 20 | 16.5 | 11.0 | 33.3 |
| *Density gradient centrifugation* | | | | |
| Zini et al. (2000a) | 22 | 10.1 | 13.6 | +34.6 |
| Gandini et al. (2004) | 34 | 12.0 | 5.5 | 54.2 |
| Morrell et al. (2004) | 9 | 20.9 | 12.8 | 38.7 |
| Jackson et al. (2010) | 30 | 17.8 | 7.1 | 60.1 |
| Simon et al. (2010) | 180 | 51.7 | 36.8 | 28.8 |
| Ebner et al. (2011) | 37 | 15.8 | 14.2 | 10.1 |
| Castillo et al. (2011) | 66 | 46.0 | 33.1 | 28.0 |
| *Magnetic-activated cell sorting (MACS)* | | | | |
| Said et al. (2006) | 35 | 14.4 | 9.7 | 32.6 |
| *Hyaluronic acid binding* | | | | |
| Razavi et al. (2010) | 77 | 32.9 | 30.9 | 6.1 |
| *Zeta method* | | | | |
| Razavi et al. (2010) | 77 | 32.9 | 18.2 | 44.7 |
| *Zech selector* | | | | |
| Ebner et al. (2011) | 37 | 15.8 | 0.4 | 97.5 |

[a]Slightly modified from Ebner et al. (2012)

the procedure must be performed so as to avoid spermatozoa exposure to stressful conditions, such as, for example, too high/low temperature and repeated high-speed centrifugation.

Although some articles reported no significant reduction in basal DFI following sperm preparation (Zini et al. 2000a; Gandini et al. 2004; Stevanato et al. 2008; Ebner et al. 2011; Boe-Hansen et al. 2005; Zini et al. 2000b), the vast majority of studies in this field support the fact that the following techniques improve the yield of DNA-intact male gametes:

- "Swim-up" (Zini et al. 2000a; Younglai et al. 2001; Jackson et al. 2010; Marchesi et al. 2010),
- Density gradient centrifugation (Donnelly et al. 2000, 2001; Morrell et al. 2004; Ahmad et al. 2007; Jackson et al. 2010; Marchesi et al. 2010; Castillo et al. 2011),
- Sperm selection based on membrane charge (Chan et al. 2006; Razawi et al. 2010),
- Cell sorting using a membrane-based electrophoretic filtration system (Fleming et al. 2008)
- Magnetic-activated cell sorting (Said et al. 2006; Lee et al. 2010; Polak de Fried and Denaday 2010; Rawe et al. 2010),
- Selection by motility without centrifugation (Ebner et al. 2011).

These studies are summarized in Table 10.2.

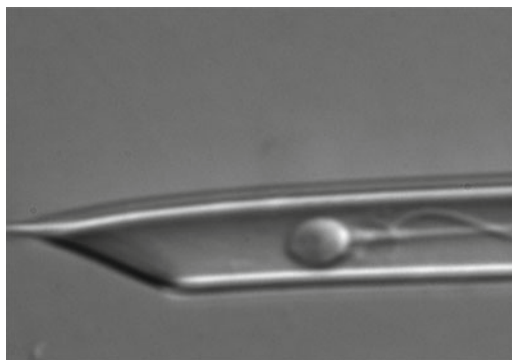

**Fig. 10.1** Spermatozoon at high magnification (>6300×) for IMSI

## *Intracytoplasmic Morphologically Selected Sperm Injection (IMSI)*

The introduction of ICSI (Palermo et al. 1992; Van Steirteghem et al. 1993) made possible pregnancies also in couples affected by severe male factor, in which the male partner had low sperm motility, poor zona binding, poor acrosome reaction, or abnormal sperm morphology. The observation of sperm morphology (Kruger et al. 1986, 1988) at the conventional magnification used for sperm evaluation (400×) has controversial prognostic value in ICSI cycle outcomes (Svalander et al. 1996; De Vos et al. 2003; French et al. 2010) and does not seem to influence embryo development or morphology (French et al. 2010; De Vos et al. 2003). However, at this magnification, sperm dimension and shape are not reliable attributes for predicting chromatin integrity or the presence of numerical chromosomal aberrations (Celik-Ozenci et al. 2004).

When using an unstained, real-time, high-magnification examination of spermatozoa called motile sperm organelle morphology examination (MSOME), it is possible to overcome the limitations of conventional magnification (Bartoov et al. 1994, 2001, 2002). MSOME is performed using an inverted light microscope equipped with high-power Nomarski optic enhanced by digital imaging to achieve a magnification of up to 6300× (Fig. 10.1). "The ICSI procedure using MSOME criteria is defined as IMSI: intracytoplasmic morphologically selected sperm injection" (Bartoov et al. 2003). Some authors have demonstrated that ICSI outcomes can be significantly improved by the exclusive microinjection of spermatozoa with a strictly defined, morphologically normal nucleus, especially in couples with previous ICSI failures (Bartoov et al. 2003; Berkovitz et al. 2005; Hazout et al. 2006; Antinori et al. 2008; Franco et al. 2008; Souza Setti et al. 2010), with severe male factor (Balaban et al. 2011; Souza Setti et al. 2011), or with advanced maternal age (Souza Setti et al. 2013). Conversely, the injection of spermatozoa with abnormal sperm head or with nuclear vacuoles negatively affects embryo development (Vanderzwalmen et al. 2008; Cassuto et al. 2009) and ICSI outcome (Berkovitz et al. 2006a, b;

Cassuto et al. 2009; Nadalini et al. 2009). The positive effect of the injection of spermatozoa without nuclear vacuoles is probably related to their significantly better mitochondrial function and chromatin status, and reduced aneuploidy rate as compared with vacuolized spermatozoa (Garolla et al. 2008; Boitrelle, et al. 2011). Furthermore, spermatozoa free of nuclear morphological malformations were found to be significantly associated with a lower incidence of aneuploidy in derived embryos (Figueira et al. 2011). However, in a few studies ICSI had the same performance as IMSI in terms of fertilization and early embryo development (Nadalini et al. 2009; Mauri et al. 2010; De Vos et al. 2013);

It should be pointed out that the large majority of articles published on IMSI were based on weak study designs or performed on a small number of patients. However, in a recent strict prospective sibling-oocyte study comparing ICSI and IMSI, performed on 350 treatments, De Vos and colleagues (2013) observed comparable results in terms of fertilization, embryo quality, and clinical outcome between the two groups. They concluded that a routine application of IMSI in unselected AR technology patients cannot be advocated. Furthermore, it is true that IMSI is undoubtedly a time-consuming procedure; selecting a normal spermatozoon according to MSOME criteria may require 60–120 min (Antinori et al. 2008). In addition, the process of searching for spermatozoa at high magnification may itself damage sperm cytoplasm: after 2 h on the microscope's heated stage, sperm nucleus vacuolization significantly increases (Peer et al. 2007). Despite these observations, IMSI can be considered a valid tool for safe, noninvasive selection of spermatozoa that have a reduced risk of DNA damage (Garolla et al. 2008; Ebner et al. 2012).

## *Restoration of Natural Fertilization Checkpoints: Hyaluronic Acid and Zona Pellucida Sperm Binding*

In humans during the natural fertilization process, spermatozoa must pass two barriers before fusing with the oocyte membrane. The first is the cumulus oophorus–corona radiata complex, made up of cells and an extracellular matrix of polymerized hyaluronic acid (HA) and proteins. The second is the zona pellucida (ZP), a thick elastic coat of glycoproteins located right next to the oocyte (Yanagimachi 1994). In addition, in the testes during spermiogenesis, still immature spermatozoa (elongated spermatids) undergo cytoplasmic extrusion and plasma membrane remodeling to prepare themselves for oocyte fertilization (Kovanci et al. 2001). This sperm membrane remodeling determines the formation of the HA and ZP receptors. In human spermatozoa, two specific proteins are related to maturity, DNA integrity, chromosomal aneuploidy frequency, and fertilizing potential: the heat shock protein HspA2 chaperone – involved in meiosis – and creatine kinase (CK) – abundant in the sperm cytoplasm (Cayli et al. 2003). Mature spermatozoa have a high expression of HspA2 (Huszar et al. 2000) and a low expression of CK (Cayli et al. 2003). In contrast, spermatozoa with arrested maturity have a low HspA2 and a high CK expression. Low levels of HspA2 can cause meiotic defects and chromosomal

aneuploidies, whereas high retention of CK and other cytoplasmic enzymes can cause lipid peroxidation and, consequently, DNA fragmentation and abnormal sperm morphology (Huszar and Vigue 1993; Cayli et al. 2003).

Due to a lack of or partial membrane remodeling, immature spermatozoa have deficiency in the ZP and HA binding sites, and for this reason they are not able to fertilize oocytes naturally.

## Hyaluronic Acid Binding

Since in natural fertilization human oocytes are surrounded by HA, this glycoprotein has a pivotal role in the mechanism of sperm selection. In fact, only mature spermatozoa that have extruded their specific receptors to bind to HA are able to reach and fertilize the oocyte. The role of HA as a physiological selector is also well recognized in vitro. In fact, spermatozoa that can bind to HA in vitro have completed their plasma membrane remodeling, cytoplasmic extrusion, and nuclear maturation (Cayli et al. 2003; Huszar et al. 2003, 2007).

Based on this selective ability of HA in binding only to mature sperm (Huszar et al. 2003; Prinosilova et al. 2009), a very simple diagnostic tool for assessing sperm maturity and function, the so-called sperm-hyaluronan-binding assay (HBA), has been developed as a commercial kit (Cayli et al. 2003; Huszar et al. 2003). HBA may be useful in predicting sperm-fertilizing ability in IVF or ICSI. In fact, HBA was found to be correlated with morphology (Ye et al. 2006; Tarozzi et al. 2009) but to a less significant extent than the association between sperm morphology and fertilization rate in IVF (Ye et al. 2006). At present, HBA does not seem to provide information for identifying patients with poor or absent fertilization (Ye et al. 2006; Nijs et al. 2010) or a threshold value for outcome in conventional IVF/ICSI treatment (Nijs et al. 2010; Kovacs et al. 2011). On the other hand, HBA seems to be significantly correlated with good embryo quality, miscarriage rate, and ongoing pregnancy rate after ICSI (Nijs et al. 2010) and with fertilization rate after IVF (Pregl Breznik et al. 2013). Recently, Worrilow et al. (2010, 2013) demonstrated that in patients in whom less than 65 % of total spermatozoa were bound to HA (HBA $\leq$65 %), a modified ICSI procedure with HA-bound spermatozoa – physiological HA-ICSI (see next paragraph) – produced a significant improvement in clinical outcome compared with conventional ICSI.

## Physiological HA-ICSI

The selection of mature spermatozoa by HA represents a straightforward refinement of the ICSI procedure (Parmegiani et al. 2010a, b). It is known that HA-containing products have no negative effects on postinjection zygote development and can be metabolized by the oocyte, and HA-bound spermatozoa can be easily recovered using an injecting pipette (Balaban et al. 2003; Barak et al. 2001; Van den Bergh et al. 2009). A “homemade” HA–sperm selection system can be

produced in any IVF lab; however, at the present time two ready-to-use systems specially designed for sperm–HA binding selection are currently available: (1) PICSI, a plastic culture dish with microdots of HA hydrogel attached to the bottom of the dish, and (2) SpermSlow, a viscous medium containing HA that can be used instead of polyvinylpyrrolidone (PVP), the viscous medium commonly used to slow spermatozoa prior to ICSI.

This new approach to ICSI with HA-bound spermatozoa, when using a HA-viscous medium or HA-culture dishes, has been called physiological ICSI (Parmegiani et al. 2010a). It has been demonstrated that the injection of HA-bound spermatozoa improves embryo quality and development by favoring selection of spermatozoa with normal nucleus and intact DNA (Parmegiani et al. 2010a). Furthermore, improved implantation rates were observed when injecting HA-bound sperm as compared to PVP sperm (Parmegiani et al. 2010b). Nasr-Esfahani et al. (2008) found a higher fertilization rate when injecting oocytes with HA-selected spermatozoa using homemade HA–ICSI dishes. An improvement in fertilization rate and embryo quality and a reduction in the number of miscarriages were found by Worrilow et al. (2007) with PICSI versus conventional ICSI. In subsequent studies, the same authors confirmed that PICSI significantly improved clinical results in ICSI, particularly in patients with an HBA score less than or equal to 65 % (Worrilow et al. 2010, 2013). In contrast, some authors found no differences in ICSI outcome parameters when injecting HA-bound sperm compared with PVP sperm (Mènèzo and Nicollet 2004; Sanchez et al. 2005; Mènèzo et al. 2010; Van den Berg et al. 2009).

At the very least, in none of the studies did physiological HA-ICSI cause a detrimental effect on ICSI outcome parameters. If larger, multicenter, prospective-randomized studies confirm the suggested beneficial effects on ICSI outcome, HA should be considered the first choice for physiologic sperm selection prior to ICSI because of its capacity to reduce genetic complications and for its total lack of toxicity (Parmegiani et al. 2010c). In addition, the use of HA-ICSI avoids PVP, which seems to be toxic and to generate sperm DNA fragmentation (Salian et al. 2012). Since both the sperm–HA binding selection systems (PICSI and SpermSlow) are easily available, efficient, and approved for human IVF use, AR centers can choose the one best suited to their needs (Parmegiani et al. 2012a).

### Zona-Bound Spermatozoa

As well as binding for HA, the spermatozoa–ZP binding process also plays a crucial role in the natural selection of spermatozoa. Immature spermatozoa show a low density of ZP binding sites as well as HA receptors (Huszar et al. 2003). Sperm binding to ZP exhibit attributes similar to those of HA-bound sperm, including minimal DNA fragmentation, normal shape, and low frequency of chromosomal aneuploidies (Yagci et al. 2010). Furthermore, the same sperm membrane protein seems to be involved, firstly, in hyaluronidase activity and, subsequently, in ZP binding (Hunnicutt et al. 1996). The spermatozoa–ZP binding test can be performed by culturing spermatozoa for a short time with immature metaphase I oocytes; in this

way, ZP-bound spermatozoa can be recovered and used for ICSI (Paes de Almeida Ferreira Braga et al. 2009). An increased embryo quality was observed when ZP-bound ICSI was performed compared with conventional ICSI (Paes de Almeida Ferreira Braga et al. 2009; Liu et al. 2011). In a small study, Black et al. (2010) observed a slightly better trend in pregnancy and implantation comparing 39 ZP-ICSI versus 39 conventional ICSI. At the present time, there is little information regarding all the factors involved in sperm-ZP binding and its mechanism, but the spermatozoa–ZP binding test could be an efficient method for identifying competent spermatozoa for ICSI. ZP selection could then be coupled with HA selection in order to replicate the natural path of the spermatozoa toward the oocyte.

## Conclusions

Several factors contribute to the outcome of AR treatments, sperm DNA quality being just one factor. Although the role of sperm DNA damage in the clinical outcome of high-technology AR procedures like ICSI could still be considered debatable, it is undoubtedly wise to use all precautions to limit any potential long-term side effects of these techniques (Ebner et al. 2012).

At present, in the literature, there are still few data on the selection of the ideal sperm for ICSI, perhaps because for many years it was not possible to identify definitively a DNA-intact spermatozoon (Nijs et al. 2009). A potentially worrying aspect of the ICSI of DNA-damaged spermatozoa has been suggested by studies performed on animals that showed not only a negative effect on pregnancy and birth, but also later side effects on the health of adult animals such as aberrant growth, premature aging, abnormal behavior, and mesenchymal tumors (Fernandez-Gonzales et al. 2008). Fortunately, in humans, the risk of injecting DNA-damaged spermatozoa seems to be minimized by conventional sperm preparation techniques prior to ICSI (Zini et al. 2000a; Younglai et al. 2001; Donnelly et al. 2000, 2001; Morrell et al. 2004, Ahmad et al. 2007; Jackson et al. 2010; Marchesi et al. 2010; Castillo et al. 2011), and follow-up studies on ICSI children have demonstrated the basic safety of this revolutionary technique (Van Steirteghem et al. 2002; Leunens et al. 2008; Belva et al. 2011; Woldringh et al. 2011), although a slight increase in chromosome aberration seems to be caused by the injection of aneuploid spermatozoa (Bonduelle et al. 2002).

Given the need to reduce all potential long-term risks on ICSI adults, the search for the ideal spermatozoa is a stimulating challenge for embryologists. A final precise sperm selection is performed at the micromanipulator via IMSI, HA, or ZP sperm binding to minimize the chance of injecting DNA-damaged sperm. There is evidence that HA-bound mature spermatozoa show better morphology than immature sperm, both at conventional (Tygerberg criteria) and at high magnification (MSOME criteria) (Prinosilova et al. 2009; Nasr-Esfahani et al. 2008; Oliveira et al. 2009; Parmegiani et al. 2010a). Furthermore, HA-bound sperm show a low DFI and a reduced incidence of aneuploidies (Nasr-Esfahani et al. 2008; Parmegiani et al. 2010a; Jakab et al. 2005; Sanchez et al. 2005; Yagci et al. 2010). HA-bound mature

spermatozoa showed no cytoplasmic retentions, persistent histones, or DNA fragmentation (Huszar et al. 2007). It should be hypothesized that ZP-bound spermatozoa have characteristics similar to those of HA-bound sperm (Huszar et al. 2007; Yagci et al. 2010; Hunnicutt et al. 1996). In addition, sperm–HA binding seems to be related to DNA methylation, which is important for correct chromatin packaging; in fact, in patients with a total lack of HA binding this feature is restored with supplementation of a methylation effector (Junca et al. 2012). Since during normal spermiogenesis and plasma membrane remodeling, along with formation of the ZP binding site, formation of the HA binding site also occurs, this should form the basis of ideal ICSI sperm selection (Huszar et al. 2007; Ebner et al. 2012).

Regarding sperm morphology, modern technology allows us to obtain very high magnification, and the introduction of IMSI should be considered an exciting chapter in IVF history, whether or not its clinical usefulness is confirmed with studies on a large scale. IMSI technology has enabled exploration of the relationship between the morphology of spermatozoa and their DNA constitution, revealing that vacuole-free spermatozoa have a lower rate of DNA-fragmentation (Franco Jr. et al. 2008), MSOME abnormal spermatozoa show a high level of DNA fragmentation (Wilding et al. 2011), and, in particular, spermatozoa with large vacuoles are characterized by aneuploidies and chromatin condensation defects (Perdrix et al. 2011).

IMSI's lengthy procedure can be circumvented by preselecting HA sperm merging high-magnification microscopy with HA-sperm selection. This procedure has been called physiological IMSI (Parmegiani et al 2012b). In fact, HA helps to select a subpopulation of spermatozoa with normal nuclei according to MSOME criteria (Parmegiani et al. 2010a).

In conclusion, most of the alternative possible refinements of the ICSI procedure described in this chapter are easily reproducible and straightforward; for this reason, they could be offered to ICSI patients not only to optimize clinical results but, at the very least, to prevent fertilization by DNA-damaged and chromosomally unbalanced spermatozoa.

## References


Ahmad L, Jalali S, Shami SA et al (2007) Sperm preparation: DNA damage by comet assay in normo- and teratozoospermics. Arch Androl 53:325–338

Ahmadi A, Ng SC (1999a) Fertilizing ability of DNA-damaged spermatozoa. J Exp Zool 284: 696–704

Ahmadi A, Ng SC (1999b) Developmental capacity of damaged spermatozoa. Hum Reprod 14: 2279–2284

Alvarez JG (2005) The predictive value of sperm chromatin structure assay. Hum Reprod 20: 2365–2367

Antinori M, Licata E, Dani G et al (2008) Intracytoplasmic morphologically selected sperm injection: a prospective randomized trial. Reprod Biomed Online 16:835–841

Avendaño C, Oehninger S (2011) DNA fragmentation in morphologically normal spermatozoa: how much should we be concerned in the ICSI era? J Androl 32:356–363

Avendaño C, Franchi A, Duran H et al (2010) DNA fragmentation of normal spermatozoa negatively impacts embryo quality and intracytoplasmic sperm injection outcome. Fertil Steril 94:549–557

Balaban B, Lundin K, Morrell JM et al (2003) An alternative to PVP for slowing sperm prior to ICSI. Hum Reprod 18:1887–1889
Balaban B, Yakin K, Alatas C et al (2011) Clinical outcome of intracytoplasmic injection of spermatozoa morphologically selected under high magnification: a prospective randomized study. Reprod Biomed Online 22:472–476
Barak Y, Menezo Y, Veiga A et al (2001) A physiological replacement for polyvinylpyrrolidone (PVP) in assisted reproductive technology. Hum Fertil 4:99–103
Bartoov B, Eltes F, Pansky M et al (1994) Improved diagnosis of male fertility potential via a combination of quantitative ultramorphology and routine semen analyses. Hum Reprod 9: 2069–2075
Bartoov B, Berkovitz A, Eltes F (2001) Selection of spermatozoa with normal nuclei to improve the pregnancy rate with intracytoplasmic sperm injection. N Engl J Med 345:1067–1068
Bartoov B, Berkovitz A, Eltes F et al (2002) Real-time fine morphology of motile human sperm cells is associated with IVF-ICSI outcome. J Androl 23:1–8
Bartoov B, Berkovitz A, Eltes F et al (2003) Pregnancy rates are higher with intracytoplasmic morphologically selected sperm injection than with conventional intracytoplasmic injection. Fertil Steril 80:1413–1419
Bellver J, Meseguer M, Muriel L et al (2010) Chromosome microdeletions, sperm DNA fragmentation and sperm oxidative stress as causes of recurrent spontaneous abortion of unknown etiology. Hum Reprod 25:1713–1721
Belva F, De Schrijver F, Tournaye H et al (2011) Neonatal outcome of 724 children born after ICSI using non-ejaculated sperm. Hum Reprod 7:1752–1758
Benchaib M, Braun V, Lornage J et al (2003) Sperm DNA fragmentation decreases the pregnancy rate in an assisted reproductive technique. Hum Reprod 18:1023–1028
Berkovitz A, Eltes F, Yaari S et al (2005) The morphological normalcy of the sperm nucleus and pregnancy rate of intracytoplasmic injection with morphologically selected sperm. Hum Reprod 20:185–190
Berkovitz A, Eltes F, Ellenbogen A et al (2006a) Does the presence of nuclear vacuoles in human sperm selected for ICSI affect pregnancy outcome? Hum Reprod 21:1787–1790
Berkovitz A, Eltes F, Lederman H et al (2006b) How to improve IVF-ICSI outcome by sperm selection. Reprod Biomed Online 12:634–638
Billig H, Chun SY, Eisenhauer K et al (1996) Gonadal cell apoptosis: hormone-regulated cell demise. Hum Reprod Update 2:103–117
Black M, de Liu Y, Bourne H H et al (2010) Comparison of outcomes of conventional intracytoplasmic sperm injection and intracytoplasmic sperm injection using sperm bound to the zona pellucida of immature oocytes. Fertil Steril 93:672–674
Boe-Hansen GB, Ersboll AK, Christensen P (2005) Variability and laboratory factors affecting the sperm chromatin structure assay in human semen. J Androl 26:360–368
Boitrelle F, Ferfouri F, Petit JM et al (2011) Large human sperm vacuoles observed in motile spermatozoa under high magnification: nuclear thumbprints linked to failure of chromatin condensation. Hum Reprod 7:1650–1658
Bonduelle M, Van Assche E, Joris H et al (2002) Prenatal testing in ICSI pregnancies: incidence of chromosomal anomalies in 1586 karyotypes and relation to sperm parameters. Hum Reprod 17:2600–2614
Borini A, Tarozzi N, Bizzaro D et al (2006) Sperm DNA fragmentation: paternal effect on early post-implantation embryo development in ART. Hum Reprod 21:2876–2881
Bungum M, Humaidan P, Spanò M et al (2004) The predictive value of sperm chromatin structure assay (SCSA) parameters for the outcome of intrauterine insemination, IVF and ICSI. Hum Reprod 19:1401–1408
Bungum M, Humaidan P, Axmon A et al (2007) Sperm DNA integrity assessment in prediction of assisted reproduction technology outcome. Hum Reprod 22:174–179
Bungum M, Spanò M, Humaidan P et al (2008) Sperm chromatin structure assay parameters measured after density gradient centrifugation are not predictive for the outcome of ART. Hum Reprod 23:4–10

Burrello N, Arcidiacono G, Vicari E et al (2004) Morphologically normal spermatozoa of patients with secretory oligo-astheno-teratozoospermia have an increased aneuploidy rate. Hum Reprod 19:2298–2302

Carrell DT, Liu L, Peterson CM et al (2003) Sperm DNA fragmentation is increased in couples with unexplained recurrent pregnancy loss. Arch Androl 49:49–55

Cassuto NG, Bouret D, Plouchart JM et al (2009) A new real-time morphology classification for human spermatozoa: a link for fertilization and improved embryo quality. Fertil Steril 92:1616–1625

Castillo J, Simon L, de Mateo S et al (2011) Protamine/DNA ratios and DNA damage in native and density gradient centrifugated sperm from infertile patients. J Androl 32:324–332

Cayli S, Jakab A, Ovari L et al (2003) Biochemical markers of sperm function: male fertility and sperm selection for ICSI. Reprod Biomed Online 7:462–468

Celik-Ozenci C, Jakab A, Kovacs T et al (2004) Sperm selection for ICSI: shape properties do not predict the absence or presence of numerical chromosomal aberrations. Hum Reprod 19:2052–2059

Chan PJ, Jacobson JD, Corselli JU et al (2006) A simple zeta method for sperm selection based on membrane charge. Fertil Steril 85:481–486

Chohan KR, Griffin JT, Lafromboise M et al (2006) Comparison of chromatin assays for DNA fragmentation evaluation in human sperm. J Androl 27:53–59

Collins JA, Barnhart KT, Schlegel PN (2008) Do sperm DNA integrity tests predict pregnancy with in vitro fertilization. Fertil Steril 89:823–831

Dalzell LH, McVicar CM, McClure N et al (2004) Effects of short and long incubations on DNA fragmentation of testicular sperm. Fertil Steril 82:1443–1445

de Figueira RC, Braga DP, Setti AS et al (2011) Morphological nuclear integrity of sperm cells is associated with preimplantation genetic aneuploidy screening cycle outcomes. Fertil Steril 95:990–993

de Paes Almeida Ferreira BD, Iaconelli A Jr, de Cassia Savio FR et al (2009) Outcome of ICSI using zona pellucida-bound spermatozoa and conventionally selected spermatozoa. Reprod Biomed Online 19:802–807

De Vos A, Van De Velde H, Joris H et al (2003) Influence of individual sperm morphology on fertilization, embryo morphology, and pregnancy outcome of intracytoplasmic sperm injection. Fertil Steril 79(1):42–48

De Vos A, Van de Velde H, Bocken G et al (2013) Does intracytoplasmic morphologically selected sperm injection improve embryo development? A randomized sibling-oocyte study. Hum Reprod 28:617–626

Donnelly ET, O'Connell M, McClure N et al (2000) Differences in nuclear DNA fragmentation and mitochondrial integrity of semen and prepared human spermatozoa. Hum Reprod 15:1552–1561

Donnelly ET, Steele EK, McClure N et al (2001) Assessment of DNA integrity and morphology of ejaculated spermatozoa from fertile and infertile men before and after cryopreservation. Hum Reprod 16:1191–1199

Duran EH, Morshedi M, Taylor S et al (2002) Sperm DNA quality predicts intrauterine insemination outcome: a prospective cohort study. Hum Reprod 17:3122–3128

Ebner T, Shebl O, Moser M et al (2011) Easy sperm processing technique allowing exclusive accumulation and later usage of DNA-strandbreak-free spermatozoa. Reprod Biomed Online 22:37–43

Ebner T, Filicori M, Tews G, Parmegiani L (2012) A plea for a more physiological ICSI. Andrologia 44(1):2–19

Enciso M, Sarasa J, Agarwal A et al (2009) A two-tailed Comet assay for assessing DNA damage in spermatozoa. Reprod Biomed Online 18:609–616

Evenson DP, Jost LK, Marshall D et al (1999) Utility of the sperm chromatin structure assay as a diagnostic and prognostic tool in the human fertility clinic. Hum Reprod 14:1039–1049

Evenson DP, Larson KL, Jost LK (2002) Sperm chromatin structure assay: its clinical use for detecting sperm DNA fragmentation in male infertility and comparisons with other techniques. J Androl 23:25–43

Fernandez JL, Muriel L, Rivero MT et al (2003) The sperm chromatin dispersion test: a simple method for the determination of sperm DNA fragmentation. J Androl 24:59–66
Fernandez-Gonzalez R, Moreira PN, Perez-Crespo M (2008) Long-term effects of mouse intracytoplasmic sperm injection with DNA-fragmented sperm on health and behavior of adult offspring. Biol Reprod 78:761–772
Fleming SD, Ilad RS, Griffin AM et al (2008) Prospective controlled trial of an electrophoretic method of sperm preparation for assisted reproduction: comparison with density gradient centrifugation. Hum Reprod 23:2646–2651
Franco JG Jr, Baruffi RL, Mauri AL et al (2008) Significance of large nuclear vacuoles in human spermatozoa: implications for ICSI. Reprod Biomed Online 17:42–45
French DB, Sabanegh ES Jr, Goldfarb J et al (2010) Does severe teratozoospermia affect blastocyst formation, live birth rate, and other clinical outcome parameters in ICSI cycles? Fertil Steril 93:1097–1103
Frydman N, Prisant N, Hesters L et al (2008) Adequate ovarian follicular status does not prevent the decrease in pregnancy rates associated with high sperm DNA fragmentation. Fertil Steril 89:92–97
Gandini L, Lombardo F, Paoli D et al (2004) Full-term pregnancies achieved with ICSI despite high levels of sperm chromatin damage. Hum Reprod 19:1409–1417
Garolla A, Fortini D, Menegazzo M et al (2008) High-power microscopy for selecting spermatozoa for ICSI by physiological status. Reprod Biomed Online 17:610–616
Giwercman A, Richthoff J, Hjøllund H et al (2003) Correlation between sperm motility and sperm chromatin structure assay parameters. Fertil Steril 80:1404–1412
Gorczyca W, Traganos F, Jesionowska H et al (1993) Presence of DNA strand breaks and increased sensitivity of DNA in situ to denaturation in abnormal human sperm cells: analogy to apoptosis of somatic cells. Exp Cell Res 207:202–205
Greco E, Scarselli F, Iacobelli M et al (2005) Efficient treatment of infertility due to sperm DNA damage by ICSI with testicular spermatozoa. Hum Reprod 20:226–230
Hazout A, Dumont-Hassan M, Junca AM et al (2006) High-magnification ICSI overcomes paternal effect resistant to conventional ICSI. Reprod Biomed Online 12:19–25
Henkel R, Hajimohammad M, Stalf T et al (2004) Influence of deoxyribonucleic acid damage on fertilization and pregnancy. Fertil Steril 81:965–972
Heytens E, Parrington J, Coward K et al (2009) Reduced amounts and abnormal forms of phospholipase C zeta (PLCζ) in spermatozoa from infertile men. Hum Reprod 24:2417–2428
Høst E, Lindenberg S, Kahn JA et al (1999) DNA strand breaks in human sperm cells: a comparison between men with normal and oligozoospermic sperm samples. Acta Obstet Gynecol Scand 78:336–339
Høst E, Lindenberg S, Smidt-Jensen S (2000a) DNA strand breaks in human spermatozoa: correlation with fertilization in vitro in oligozoospermic men and in men with unexplained infertility. Acta Obstet Gynecol Scand 79:189–193
Høst E, Lindenberg S, Smidt-Jensen S (2000b) The role of DNA strand breaks in human spermatozoa used for IVF and ICSI. Acta Obstet Gynecol Scand 79:559–563
Huang CC, Lin DP, Tsao HM et al (2005) Sperm DNA fragmentation negatively correlates with velocity and fertilization rates but might not affect pregnancy rates. Fertil Steril 84:130–140
Hughes CM, Lewis SE, McKelvey-Martin VJ et al (1996) A comparison of baseline and induced DNA damage in human spermatozoa from fertile and infertile men, using a modified comet assay. Mol Hum Reprod 2:613–619
Humm CK, Sakkas D (2013) Role of increased male age in IVF and egg donation: is sperm DNA fragmentation responsible? Fertil Steril 99(1):30–36
Hunnicutt GR, Primakoff P, Myles DG (1996) Sperm surface protein PH-20 is bifunctional: one activity is a hyaluronidase and a second, distinct activity is required in secondary sperm-zona binding. Biol Reprod 55:80–86
Huszar G, Vigue L (1993) Incomplete development of human spermatozoa is associated with increased creatine phosphokinase concentration and abnormal head morphology. Mol Reprod Dev 34:292–298

Huszar G, Stone K, Dix D et al (2000) Putative creatine kinase M-isoform in human sperm is identified as the 70-kilodalton heat shock protein HspA2. Biol Reprod 63:925–932
Huszar G, Ozenci CC, Cayli S et al (2003) Hyaluronic acid binding by human sperm indicates cellular maturity, viability, and unreacted acrosomal status. Fertil Steril 79(3):1616–1624
Huszar G, Jakab A, Sakkas D et al (2007) Fertility testing and ICSI sperm selection by hyaluronic acid binding: clinical and genetic aspects. Reprod Biomed Online 14:650–663
Irvine DS, Twigg JP, Gordon EL, Fulton N, Milne PA, Aitken RJ (2000) DNA integrity in human spermatozoa: relationships with semen quality. J Androl 21:33–44
Jackson RE, Bormann CL, Hassun PA et al (2010) Effects of semen storage and separation techniques on sperm DNA fragmentation. Fertil Steril 94:2626–2630
Jakab A, Sakkas D, Delpiano E et al (2005) Intracytoplasmic sperm injection: a novel selection method for sperm with normal frequency of chromosomal aneuploidies. Fertil Steril 84: 1665–1673
Joly- Helas G, de La Rochebrochard C, Mousset-Siméon N et al (2007) Complex chromosomal rearrangement and intracytoplasmic sperm injection: a case report. Hum Reprod 22:1292–1297
Junca A, Marti BG, Tosti E et al (2012) Sperm nucleus decondensation, hyaluronic acid (HA) binding and oocyte activation capacity: different markers of sperm immaturity? Case reports. J Assist Reprod Genet 29:353–365
Kasai T, Ogawa K, Mizuno K et al (2002) Relationship between sperm mitochondrial membrane potential, sperm motility, and fertility potential. Asian J Androl 4:97–103
Kennedy C, Ahlering P, Rodriguez H et al (2011) Sperm chromatin structure correlates with spontaneous abortion and multiple pregnancy rates in assisted reproduction. Reprod Biomed Online 22:272–276
Kim JW, Chang EM, Song SH et al (2011) Complex chromosomal rearrangements in infertile males: complexity of rearrangement affects spermatogenesis. Fertil Steril 95:349–352
Kovacs P, Kovats T, Sajgo A et al (2011) The role of hyaluronic acid binding assay in choosing the fertilization method for patients undergoing IVF for unexplained infertility. J Assist Reprod Genet 28:49–54
Kovanci E, Kovacs T, Moretti E et al (2001) FISH assessment of aneuploidy frequencies in mature and immature human spermatozoa classified by the absence or presence of cytoplasmic retention. Hum Reprod 16:1209–1217
Kruger TF, Menkveld R, Stander FS et al (1986) Sperm morphologic features as a prognostic factor in in vitro fertilization. Fertil Steril 46:1118–1123
Kruger TF, Acosta AA, Simmons KF et al (1988) Predictive value of abnormal sperm morphology in in vitro fertilization. Fertil Steril 49:112–117
Larson-Cook KL, Brannian JD, Hansen KA et al (2003) Relationship between the outcomes of assisted reproductive techniques and sperm DNA fragmentation as measured by the sperm chromatin structure assay. Fertil Steril 80:895–902
Lee TH, Liu CH, Shih YT et al (2010) Magnetic-activated cell sorting for sperm preparation reduces spermatozoa with apoptotic markers and improves the acrosome reaction in couples with unexplained infertility. Hum Reprod 25:839–846
Leunens L, Celestin-Westreich S, Bonduelle M et al (2008) Follow-up of cognitive and motor development of 10-year-old singleton children born after ICSI compared with spontaneously conceived children. Hum Reprod 23:105–111
Li Z, Wang L, Cai J et al (2006) Correlation of sperm DNA damage with IVF and ICSI outcomes: a systematic review and meta-analysis. J Assist Reprod Genet 23:367–376
Lin MH, Kuo-Kuang Lee R et al (2008) Sperm chromatin structure assay parameters are not related to fertilization rates, embryo quality, and pregnancy rates in in vitro fertilization and intracytoplasmic sperm injection, but might be related to spontaneous abortion rates. Fertil Steril 90:352–359
Liu F, Qiu Y, Zou Y et al (2011) Use of zona pellucida-bound sperm for intracytoplasmic sperm injection produces higher embryo quality and implantation than conventional intracytoplasmic sperm injection. Fertil Steril 95:815–818
Loft S, Kold-Jensen T, Hjollund NH et al (2003) Oxidative DNA damage in human sperm influences time to pregnancy. Hum Reprod 18:1265–1272

Lopes S, Sun JG, Jurisicova A et al (1998) Sperm deoxyribonucleic acid fragmentation is increased in poor-quality semen samples and correlates with failed fertilization in intracytoplasmic sperm injection. Fertil Steril 69:528–532

Mahfouz R, Sharma R, Thiyagarajan A et al (2010) Semen characteristics and sperm DNA fragmentation in infertile men with low and high levels of seminal reactive oxygen species. Fertil Steril 94:2141–2146

Marchesi DE, Feng HL (2007) Sperm DNA integrity from sperm to egg. J Androl 28:481–489

Marchesi DE, Biederman H, Ferrara S et al (2010) The effect of semen processing on sperm DNA integrity: comparison of two techniques using the novel Toluidine Blue Assay. Eur J Obstet Gynecol Reprod Biol 151:176–180

Mauri AL, Petersen CG, Oliveire JB et al (2010) Comparison of day 2 embryo quality after conventional ICSI versus intracytoplasmic morphologically selected sperm injection (IMSI) using sibling oocytes. Eur J Obstet Gynecol Reprod Biol 150:42–46

Morrell JM, Moffatt O, Sakkas D et al (2004) Reduced senescence and retained nuclear DNA integrity in human spermatozoa prepared by density gradient centrifugation. J Assist Reprod Genet 21:217–222

McPherson S, Longo FJ (1993) Chromatin structure-function alterations during mammalian spermatogenesis: DNA nicking and repair in elongating spermatids. Eur J Histochem 37:109–128

Menezo Y, Nicollet B (2004) Replacement of PVP by hyaluronate (SpermSlow™) in ICSI – impact on outcome. Abstract of 18th world congress on fertility and sterility IFFS

Menezo Y, Junca AM, Dumont-Hassan M et al (2010) "Physiologic"' (hyaluronic acid-carried) ICSI results in the same embryo quality and pregnancy rates as with the use of potentially toxic polyvinylpyrrolidone (PVP). Fertil Steril 94(1):232

Meseguer M, Santiso R, Garrido N et al (2011) Effect of sperm DNA fragmentation on pregnancy outcome depends on oocyte quality. Fertil Steril 95:124–128

Morris ID, Ilott S, Dixon L et al (2002) The spectrum of DNA damage in human sperm assessed by single cell gel electrophoresis (Comet assay) and its relationship to fertilization and embryo development. Hum Reprod 17:990–998

Muratori M, Maggi M, Spinelli S et al (2003) Spontaneous DNA fragmentation in swim-up selected human spermatozoa during long term incubation. J Androl 24:253–262

Muriel L, Garrido N, Fernández JL et al (2006) Value of the sperm deoxyribonucleic acid fragmentation level, as measured by the sperm chromatin dispersion test, in the outcome of in vitro fertilization and intracytoplasmic sperm injection. Fertil Steril 85:371–383

Nadalini M, Tarozzi N, Distratis V et al (2009) Impact of intracytoplasmic morphologically selected sperm injection on assisted reproduction outcome a review. Reprod Biomed Online 19(l3):45–55

Nasr-Esfahani MH, Razavi S, Vahdati AA et al (2008) Evaluation of sperm selection procedure based on hyaluronic acid binding ability on ICSI outcome. J Assist Reprod Genet 25:197–203

Navarro-Costa P, Nogueira P, Carvalho M et al (2010) Incorrect DNA methylation of the DAZL promoter CpG island associates with defective human sperm. Hum Reprod 25:2647–2654

Nijs M, Creemers E, Cox A et al (2009) Chromomycin A3 staining, sperm chromatin structure assay and hyaluronic acid binding assay as predictors for assisted reproductive outcome. Reprod Biomed Online 19:671–684

Nijs M, Creemers E, Cox A et al (2010) Relationship between hyaluronic acid binding assay and outcome in ART: a pilot study. Andrologia 42:291–296

Oliveira JB, Massaro FC, Mauri AL et al (2009) Motile sperm organelle morphology examination is stricter than Tygerberg criteria. Reprod Biomed Online 18:320–326

Ollero M, Gil-Guzman E, Lopez MC et al (2001) Characterization of subsets of human spermatozoa at different stages of maturation: implications in the diagnosis and treatment of male infertility. Hum Reprod 16:1912–1921

Ozmen B, Caglar GS, Koster F et al (2007) Relationship between sperm DNA damage, induced acrosome reaction and viability in ICSI patients. Reprod Biomed Online 15:208–214

Palermo G, Joris H, Devroey P et al (1992) Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. Lancet 340(8810):17–18

Parmegiani L, Cognigni GE, Bernardi S et al (2010a) "Physiologic ICSI": hyaluronic acid (HA) favors selection of spermatozoa without DNA fragmentation and with normal nucleus, resulting in improvement of embryo quality. Fertil Steril 93:598–604

Parmegiani L, Cognigni GE, Ciampaglia W et al (2010b) Efficiency of hyaluronic acid (HA) sperm selection. J Assist Reprod Genet 27:13–16

Parmegiani L, Cognigni GE, Filicori M (2010c) Risks in injecting hyaluronic acid non-bound spermatozoa. Reprod Biomed Online 20:437–438

Parmegiani L, Cognigni GE, Bernardi S et al (2012a) Comparison of two ready-to-use systems designed for sperm-hyaluronic acid binding selection before intracytoplasmic sperm injection: PICSI vs. sperm slow: a prospective, randomized trial. Fertil Steril 98:632–637

Parmegiani L, Cognigni GE, Filicori M (2012b) New advances in intracytoplasmic sperm injection (ICSI). In: Bin W (ed) Advances in embryo transfer. InTech, New York, pp 99–115. doi:10.5772/39227

Payne JF, Raburn DJ, Couchman GM et al (2005) Redefining the relationship between sperm deoxyribonucleic acid fragmentation as measured by the sperm chromatin structure assay and outcomes of assisted reproductive techniques. Fertil Steril 84:356–364

Peer S, Eltes F, Berkovitz A, Yehuda R et al (2007) Is fine morphology of the human sperm nuclei affected by in vitro incubation at 37 degrees C? Fertil Steril 88:1589–1594

Perdrix A, Travers A, Chelli MH et al (2011) Assessment of acrosome and nuclear abnormalities in human spermatozoa with large vacuoles. Hum Reprod 26:47–58

Polak de Fried E, Denaday F (2010) Single and twin ongoing pregnancies in two cases of previous ART failure after ICSI performed with sperm sorted using annexin V microbeads. Fertil Steril 94(351):e16

Pregl Breznik B, Kovačič B, Vlaisavljević V (2013) Are sperm DNA fragmentation, hyperactivation, and hyaluronan-binding ability predictive for fertilization and embryo development in in vitro fertilization and intracytoplasmic sperm injection? Fertil Steril 99:1233–1241

Prinosilova P, Kruger T, Sati L et al (2009) Selectivity of hyaluronic acid binding for spermatozoa with normal Tygerberg strict morphology. Reprod Biomed Online 18:177–183

Ramos L, Wetzels AM (2001) Low rates of DNA fragmentation in selected motile human spermatozoa assessed by the TUNEL assay. Hum Reprod 16:1703–1707

Rawe VY, Boudri HU, Alvarez Sedó C et al (2010) Healthy baby born after reduction of sperm DNA fragmentation using cell sorting before ICSI. Reprod Biomed Online 20:320–323

Razavi SH, Nasr-Esfahani MH, Deemeh MR et al (2010) Evaluation of zeta and HA-binding methods for selection of spermatozoa with normal morphology, protamine content and DNA integrity. Andrologia 42:13–19

Richardson C, Jasin M (2000) Frequent chromosomal translocations induced by DNA double-strand breaks. Nature 405:697–700

Said T, Agarwal A, Grunewald S et al (2006) Selection of nonapoptotic spermatozoa as a new tool for enhancing assisted reproduction outcomes: an in vitro model. Biol Reprod 74:530–537

Sakkas D, Alvarez JG (2010) Sperm DNA fragmentation: mechanisms of origin, impact on reproductive outcome, and analysis. Fertil Steril 93:1027–1036

Sakkas D, Bianchi PG, Manicardi GC (1997) Chromatin packaging anomalies and DNA damage in human sperm: their possible implications in the treatment of male factor infertility. In: Barratt C, De Jonge C, Mortimer D, Parinaud J (eds) Genetics of human male infertility. Editions EDK, Paris, pp 205–221

Sakkas D, Manicardi G, Bizzaro D et al (2000) Possible consequences of performing intracytoplasmic sperm Injection (ICSI) with sperm possessing nuclear DNA damage. Hum Fertil 3:26–30

Salian SR, Kalthur G, Uppangala S et al (2012) Frozen-thawed spermatozoa from oligozoospermic ejaculates are susceptible to in situ DNA fragmentation in polyvinylpyrrolidone-based sperm-immobilization medium. Fertil Steril 98:321–325

Sanchez M, Aran B, Blanco J et al (2005) Preliminary clinical and FISH results on hyaluronic acid sperm selection to improve ICSI. Hum Reprod 20(Suppl 1):i200

Santiso R, Tamayo M, Gosálvez J et al (2012) DNA fragmentation dynamics allows the assessment of cryptic sperm damage in human: evaluation of exposure to ionizing radiation, hyperthermia, acidic pH and nitric oxide. Mutat Res 734:41–49

Schatten H, Sun QY (2009) The role of centrosomes in mammalian fertilization and its significance for ICSI. Mol Hum Reprod 15:531–538
Seli E, Gardner DK, Schoolcraft WB et al (2004) Extent of nuclear DNA damage in ejaculated spermatozoa impacts on blastocyst development after in vitro fertilization. Fertil Steril 82:378–383
Siffroi JP, Benzacken B, Straub B et al (1997) Assisted reproductive technology and complex chromosomal rearrangements: the limits of ICSI. Mol Hum Reprod 3:847–851
Simon L, Brunborg G, Stevenson M et al (2010) Clinical significance of sperm DNA damage in assisted reproduction outcome. Hum Reprod 25:1594–1608
Simon L, Castillo J, Oliva R et al (2011) Relationships between human sperm protamines, DNA damage and assisted reproduction outcomes. Reprod Biomed Online 23:724–734
Simon L, Proutski I, Stevenson M et al (2013) Sperm DNA damage has a negative association with live-birth rates after IVF. Reprod Biomed Online 26:68–78
Souza Setti A, Ferreira RC, de Almeida P, Ferreira Braga D et al (2010) Intracytoplasmic sperm injection outcome versus intracytoplasmic morphologically selected sperm injection outcome: a meta-analysis. Reprod Biomed Online 21:450–455
Souza Setti A, Figueira RD, de Almeida P, Ferreira Braga D et al (2011) Intracytoplasmic morphologically selected sperm injection benefits for patients with oligoasthenozoospermia according to the 2010 World Health Organization reference values. Fertil Steril 95:2711–2714
Souza Setti A, de Cássia Sávio Figueira R, Paes de Almeida Ferreira Braga D et al (2013) IMSI is beneficial in cases of advanced maternal age: a prospective randomized study. Reprod Biomed Online. In Press
Spanò M, Bonde JP, Hjøllund HI et al (2000) Sperm chromatin damage impairs human fertility. The Danish first pregnancy planner study team. Fertil Steril 73:43–50
Speyer BE, Pizzey AR, Ranieri M et al (2010) Fall in implantation rates following ICSI with sperm with high DNA fragmentation. Hum Reprod 25:1609–1618
Stevanato J, Bertolla RP, Barradas V et al (2008) Semen processing by density gradient centrifugation does not improve sperm apoptotic deoxyribonucleic acid fragmentation rates. Fertil Steril 90:899–890
Sun JG, Jurisicova A, Casper RF (1997) Detection of deoxyribonucleic acid fragmentation in human sperm: correlation with fertilization in vitro. Biol Reprod 56:602–607
Svalander P, Jakobsson AH, Forsberg AS et al (1996) The outcome of intracytoplasmic sperm injection is unrelated to 'strict criteria' sperm morphology. Hum Reprod 11:1019–1022
Tarozzi N, Nadalini M, Bizzaro D et al (2009) Sperm-hyaluronan-binding assay: clinical value in conventional IVF under Italian law. Reprod Biomed Online 19(Suppl 3):35–43
Tomlinson MJ, Moffatt O, Manicardi GC et al (2001) Interrelationships between seminal parameters and sperm nuclear DNA damage before and after density gradient centrifugation: implications for assisted conception. Hum Reprod 16:2160–2165
Tomsu M, Sharma V, Miller D (2002) Embryo quality and IVF treatment outcomes may correlate with different sperm comet assay parameters. Hum Reprod 17:1856–1862
Toro E, Fernández S, Colomar A et al (2009) Processing of semen can result in increased sperm DNA fragmentation. Fertil Steril 92:2109–2112
Twigg JP, Irvine DS, Aitken RJ (1998) Oxidative damage to DNA in human spermatozoa does not preclude pronucleus formation at intracytoplasmic sperm injection. Hum Reprod 13: 1864–1871
Upadhya D, Kalthur G, Kumar P et al (2010) Association between the extent of DNA damage in the spermatozoa, fertilization and developmental competence in preimplantation stage embryos. J Turkish-German Gynecol Assoc 11:182–186
Van den Bergh M, Emiliani S, Biramane J et al (1998) A first prospective study of the individual straight line velocity of the spermatozoon and its influences on the fertilization rate after intracytoplasmic sperm injection. Hum Reprod 13:3103–3107
Van Den Bergh M, Fahy-Deshe M, Hohl MK (2009) Pronuclear zygote score following intracytoplasmic injection of hyaluronan-bound spermatozoa: a prospective randomized study. Reprod Biomed Online 19:796–801
Van Steirteghem AC, Nagy Z, Joris H et al (1993) High fertilization and implantation rates after intracytoplasmic sperm injection. Hum Reprod 8:1061–1066

Van Steirteghem AC, Bonduelle M, Devroey P et al (2002) Follow-up of children born after ICSI. Hum Reprod Update 8:111–116
Vanderzwalmen P, Hiemer A, Rubner P et al (2008) Blastocyst development after sperm selection at high magnification is associated with size and number of nuclear vacuoles. Reprod Biomed Online 17:617–627
Varghese AC, Bragais FM, Mukhopadhyay D et al (2009) Human sperm DNA integrity in normal and abnormal semen samples and its correlation with sperm characteristics. Andrologia 41:207–215
Velez de la Calle JF, Muller A, Walschaerts M et al (2008) Sperm deoxyribonucleic acid fragmentation as assessed by the sperm chromatin dispersion test in assisted reproductive technology programs: results of a large prospective multicenter study. Fertil Steril 90:1792–1799
Virant-Klun I, Tomazevic T, Meden-Vrtovec H (2002) Sperm single-stranded DNA, detected by acridine orange staining, reduces fertilization and quality of ICSI-derived embryos. J Assist Reprod Genet 19:319–328
Virro MR, Larson-Cook KL, Evenson DP (2004) Sperm chromatin structure assay (SCSA) parameters are related to fertilization, blastocyst development, and ongoing pregnancy in in vitro fertilization and intracytoplasmic sperm injection cycles. Fertil Steril 81:1289–1295
Wilding M, Coppola G, di Matteo L et al (2011) Intracytoplasmic injection of morphologically selected spermatozoa (IMSI) improves outcome after assisted reproduction by deselecting physiologically poor quality spermatozoa. J Assist Reprod Genet 28:253–262
Woldringh GH, Horvers M, Janssen AJ et al (2011) Follow-up of children born after ICSI with epididymal spermatozoa. Hum Reprod 7:1759–1767
Worrilow KC, Huynh HT, Bower JB et al (2007) PICSI vs. ICSI: statistically significant improvement in clinical outcomes in 240 in vitro fertilization (IVF) patients. Fertil Steril 88(1):s37
Worrilow KC, Eid S, Matthews J et al (2010) Multi-site clinical trial evaluating PICSI, a method for selection of hyaluronan bound sperm (HBS) for use in ICSI: improved clinical outcomes. Hum Reprod 25(1):i7
Worrilow KC, Eid S, Woodhouse D et al (2013) Use of hyaluronan in the selection of sperm for intracytoplasmic sperm injection (ICSI): significant improvement in clinical outcomes–multicenter, double-blinded and randomized controlled trial. Hum Reprod 28:306–314
Yagci A, Murk W, Stronk J et al (2010) Spermatozoa bound to solid state hyaluronic acid show chromatin structure with high DNA chain integrity: an acridine orange fluorescence study. J Androl 31:566–572
Yanagimachi R (1994) Mammalian fertilization. In: Knobil E, Neill JD (eds) The physiology of reproduction. Raven Press Ltd, New York, pp 189–317
Ye H, Huang GN, Gao Y, de Liu Y (2006) Relationship between human sperm-hyaluronan binding assay and fertilization rate in conventional in vitro fertilization. Hum Reprod 21:1545–1550
Younglai EV, Holt D, Brown P et al (2001) Sperm swim-up techniques and DNA fragmentation. Hum Reprod 16:1950–1953
Zhang LH, Qiu Y, Wang KH et al (2010) Measurement of sperm DNA fragmentation using bright-field microscopy: comparison between sperm chromatin dispersion test and terminal uridine nick-end labeling assay. Fertil Steril 94:1027–1032
Zini A, Finelli A, Phang D et al (2000a) Influence of semen processing technique on human sperm DNA integrity. Urology 56:1081–1084
Zini A, Nam RK, Mak V et al (2000b) Influence of initial semen quality on the integrity of human sperm DNA following semen processing. Fertil Steril 74:824–827
Zini A, Bielecki R, Phang D et al (2001) Correlations between two markers of sperm DNA integrity, DNA denaturation and DNA fragmentation, in fertile and infertile men. Fertil Steril 75: 674–677
Zini A, Boman JM, Belzile E et al (2008) Sperm DNA damage is associated with an increased risk of pregnancy loss after IVF and ICSI: systematic review and meta-analysis. Hum Reprod 23:2663–2668