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New Advances in Intracytoplasmic Sperm Injection (ICSI)

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1. Introduction

In these last twenty years, intracytoplasmic sperm injection (ICSI) has efficiently permitted the treatment of male factor infertility (Van Steirteghem et al., 1993); the direct injection of spermatozoa into ooplasm has allowed the embryologist to overcome low sperm motility, poor sperm-Zona Pellucida (ZP) binding, and defective acrosome reaction. Although ICSI has been successfully applied worldwide for several years, nevertheless we have no real knowledge regarding the hypothetical long term side effects on ICSI adults. In fact, some doubts about the safety of this technique can arise (Oehninger, 2011) due to the fact that with ICSI some check points of natural fertilization are bypassed and that some steps differ considerably from the physiological process; For instance, the introduction of the sperm tail into the ooplasm may cause sperm nuclear decondensation problems (Dozortsev et al., 1995; Markoulaki et al., 2007). It should be considered that ICSI may increase the risk of injecting spermatozoa with genetic or functional anomalies (Sakkas et al., 1997; Bonduelle et al., 2002; Marchesi & Feng, 2007; Schatten & Sun, 2009; Heytens et al., 2009; Navarro-Costa et al., 2010). For these reasons and to minimize any risk related to ICSI, any new advance in this procedure which can help the operator to restore some of the basic physiological checkpoints and to simulate the natural fertilization process should be welcome (Parmegiani et al., 2010a).

2. Hyaluronic acid (HA) and Zona Pellucida: Two important human fertilization checkpoints

In nature, human oocytes are surrounded by:

- the cumulus oophorus-corona radiata complex (COC), made up of cells and an extracellular matrix of polymerized hyaluronic acid (HA) and proteins
- the Zona Pellucida (ZP), a thick elastic coat of glycoproteins located immediately next to the oocyte (Yanagimachi, 1994)

These layers have to be penetrated by spermatozoa before they fuse with the oolemma.

In the human testis, during spermiogenesis, the elongated spermatids undergo cytoplasmic extrusion and plasma membrane remodelling which determines the formation of the HA and ZP receptors, essential for sperm penetration into oocyte.

At the end of spermiogenesis, different expression levels of two specific proteins seem to be related to sperm maturity, DNA integrity, chromosomal aneuploidy frequency and fertilizing potential. These two proteins are:

- the heat shock protein HspA2 chaperone, involved in meiosis
- creatine kinase (CK), abundant in the sperm cytoplasm (Cayli et al., 2003)

Mature spermatozoa have high HspA2 (Huszar et al., 2000) and low CK (Cayli et al., 2003). In contrast, spermatozoa with arrested maturity have low HspA2 expression, which may cause meiotic defects and probably chromosomal aneuploidies, in fact, mature spermatozoa show a reduction of more than five fold in aneupliody rate than immature ones (Jakab et al., 2005). Immature spermatozoa also have higher levels of CK (Huszar & Vigue, 1993); this high level of CK in immature spermatozoa is due to a sperm defect in terminal spermiogenesis when in normal development the surplus cytoplasm is extruded from the elongating spermatid as 'residual bodies' (Cayli et al., 2003). In contrast, arrested/ diminished maturity spermatozoa show a higher retention of cytoplasm with CK and other cytoplasmic enzymes, increased levels of lipid peroxidation and consequent DNA fragmentation, and abnormal sperm morphology. Due to the lack of their membrane remodelling, these immature spermatozoa have deficiency in the ZP and HA binding sites and for this reason they are not able to fertilize the oocyte naturally.

3. Physiologic HA ICSI

In nature hyaluronic acid (HA), is involved in the mechanism of sperm selection because only mature spermatozoa which have extruded their specific receptors to bind to HA are able to reach the oocyte and fertilize it. The role of HA as "physiological selector" is also well recognized in-vitro. It has been demonstrated that the spermatozoa able to bind to HA in-vitro are those which have completed their plasma membrane remodelling, cytoplasmic extrusion and nuclear maturation (Cayli et al., 2003; Huszar et al., 2003; 2007). Furthermore, HA-bound spermatozoa have a better morphology (Prinosilova et al, 2009; Parmegiani et al., 2010a) and they show a reduced risk of being aneuploid (Jakab et al., 2005) or having fragmented DNA (Parmegiani et al., 2010a). Because of this, selection of spermatozoa by HA prior to ICSI helps to optimize the outcome of the treatment (Parmegiani et al., 2010 a, b) and also has a number of other advantages:

- in practical terms, HA-bound spermatozoa can be easily recovered using an injecting pipette (Balaban et al., 2003)
- HA-containing culture medium have no negative effects on post-injection zygote development (Van den Bergh et al., 2010)
- Because of its natural origin HA can be metabolized by the oocyte (Balaban et al., 2003; Barak et al., 2001; Van den Bergh et al., 2010)

At very least, HA represents a more natural alternative for handling spermatozoa prior to ICSI than the synthetic plastic polyvinylpyrrolidone (PVP), which is routinely used to reduce sperm motility during ICSI procedure in the majority of AR centres and has been hypothesized to have toxic effects on oocytes (Jean et al., 1996; 2001).

A "home made" HA-sperm selection system can be simply produced in any IVF lab (Huszar et al., 2003, Nasr-Esfahani et al. 2008). However at the present time, two ready-to-use systems specially designed for sperm-HA binding selection are currently available:

- a plastic culture dish with microdots of HA hydrogel attached to the bottom of the dish (PICSI® Sperm Selection Device, MidAtlantic Diagnostic - Origio, Måløv, Denmark), Figure 1.
- a viscous medium containing HA (Sperm Slow™, MediCult – Origio)

This new approach to ICSI with HA-bound spermatozoa, when using HA-viscous medium or HA-culture dishes, has been defined as "Physiologic ICSI" (Parmegiani et al., 2010a).

Since both these sperm-HA binding selection systems are easily available, efficient and approved for IVF use (Parmegiani et al., 2010 a; 2010 b; Mènèzo & Nicollet, 2004; Worrilow et al., 2007; 2010) IVF centres can choose the one best suited to their needs. The viscous medium requires a specific procedure of droplet preparation to optimize the selection of HA-bound spermatozoa (Parmegiani et al., 2010b); conversely, it is more versatile than PICSI as it can be used also on a glass-bottom culture dish for high magnification sperm evaluation: "physiologic IMSI" (Parmegiani et al., 2010 a) -*see also paragraph 5, IMSI*. On the other hand, PICSI HA-bound spermatozoa can be easily recognized even by non-trained embryologists.

3.1 PICSI procedure

PICSI dishes are conventional plastic culture dishes pre-prepared with 3 microdots of powdered HA. The powdered HA is re-hydrated by adding a 5 µL droplets of fresh culture medium to each of the three microdots. A 2 µL droplet with suspension of treated spermatozoa is then connected with a pipette tip to these culture medium droplets. The PICSI dish is incubated under oil; within 5 minutes the bound spermatozoa are attached by their head to the surface of the HA-microdots and are spinning around their head (Figure 1).

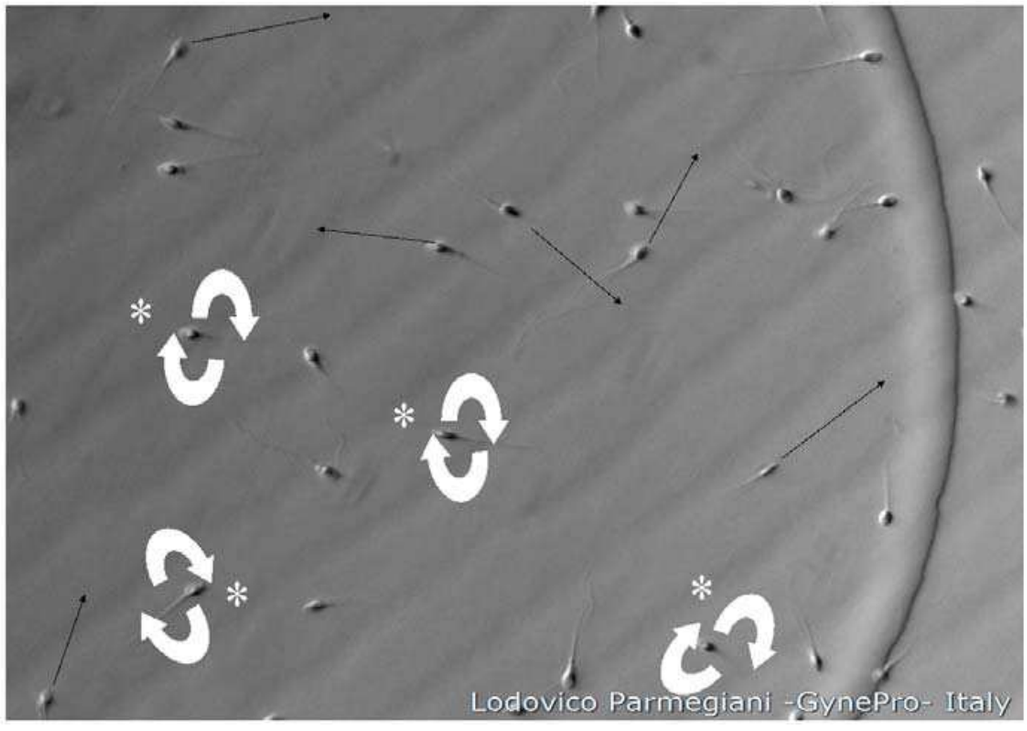

Fig. 1. Spermatozoa in PICSI dish (magnification 400 X)

An ICSI injecting pipette is used to pick the best motile HA-bound sperm up and inject them one by one into an oocyte. The ICSI injecting pipette can be previously loaded with viscous medium (PVP or Sperm Slow) to facilitate sperm micromanipulation.

In PICSI, HA-sperm (*) are bound by the head to the bottom of the dish and have vigorous motility with the tail spinning around their head. HA-unbound spermatozoa, in contrast, swim free all around the droplet of culture medium with varied motility.

3.2 Sperm slow procedure (*Parmegiani et al., 2010b*)

On a culture dish (plastic or glass bottomed), a 2 µL droplet with suspension of treated spermatozoa is connected with a pipette tip to a 5 µL droplet of fresh culture medium. Simultaneously, a 5 µL droplet of Sperm Slow is connected with a pipette tip to the 5 µL droplet of fresh culture medium (Figure 2). The spermatozoa on this culture dish are incubated for 5 min at 37°C under oil. Spermatozoa bound to HA are slowed (as if trapped in a net) in the junction zone of the 2 droplets, these spermatozoa are selected and detached by injecting pipette and subsequently injected into oocytes. In Sperm Slow, HA-bound sperm tail appears stretched, its motility is dramatically slowed and its beats have narrow amplitude. HA non-bound spermatozoa swim all around the medium droplet, they are less slowed by the viscosity of the medium and their tail-beats have wider amplitude.

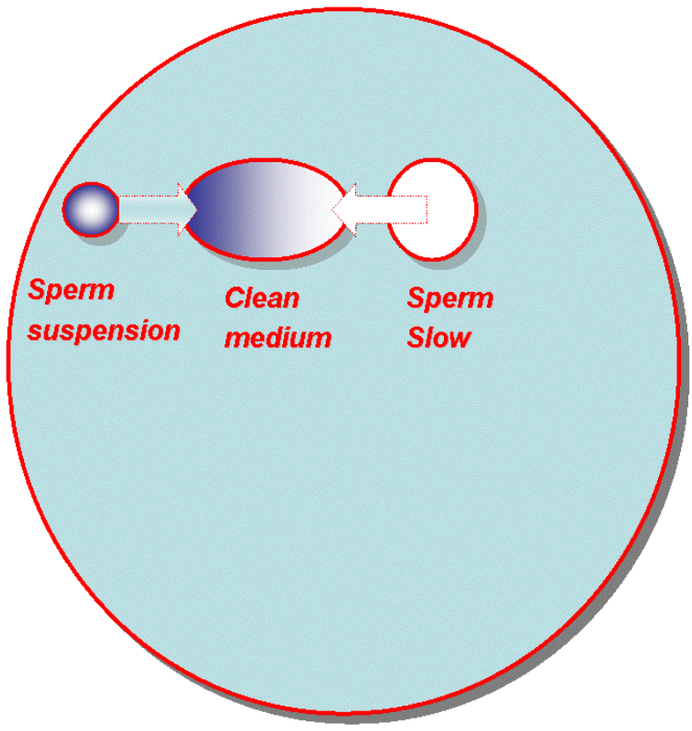

Fig. 2. Sperm Slow droplet preparation

A 2 µL droplet with suspension of treated spermatozoa is connected with a pipette tip to a 5 µL droplet of fresh culture medium. Simultaneously, a 5 µL droplet of Sperm Slow is connected with a pipette tip to the 5 µL droplet of fresh culture medium.

3.3 Clinical efficiency of "physiologic ICSI"

It has been demonstrated that the injection of HA-bound spermatozoa improves embryo quality and development by favouring selection of spermatozoa with normal nucleus and intact DNA: in fact, top-quality embryo rate is higher in HA–ICSI than in conventional PVP-ICSI and embryo development rate has also been found to be significantly increased (Parmegiani et al., 2010 a). Furthermore, HA-ICSI may speed up the time-consuming IMSI (Parmegiani et al., 2010 a). The largest study published to date as full article (428 patients) comparing physiologic HA-ICSI to conventional PVP-ICSI (Parmegiani et al., 2010 b) revealed that injection of HA-bound spermatozoa determines a statistically significant improvement in embryo quality and implantation.

A positive trend in fertilization and pregnancy rates - when injecting HA-bound spermatozoa – has been reported (Mènèzo & Nicollet, 2004). Nasr-Esfahani et al. (2008) have also published a study showing a higher fertilization rate when injecting oocytes with HA-selected spermatozoa.

A statistically significant improvement in fertilization rate and embryo quality and a reduction in the number of miscarriages were found by Worrilow et al. (2007) performing PICSI versus conventional ICSI. In a subsequent study, the same authors demonstrated that PICSI significantly improves embryo quality, significantly reducing embryo fragmentation rate on day 3 and favours good blastocyst formation and clinical pregnancy rate (Worrilow et al., 2010).

In contrast, one report found no differences in fertilization, pregnancy and implantation rates (Sanchez et al., 2005); this lack of significant clinical improvements after the injection of HA-bound spermatozoa may be due to the small number of patients studied (18). Recently, a historical comparison between 2014 HA-ICSI and 1920 PVP-ICSI showed no statistically significant increase in embryo quality and pregnancy rate for physiologic ICSI (Mènèzo et al., 2010).

Van den Berg et al., (2010) found no difference in zygote score when injecting, in a prospective randomized way, 407 sibling metaphase II oocytes, with either HA bound (HA+) or non-bound (HA-) spermatozoa. Our group (Parmegiani et al., 2010 c) questioned the ethical aspect of this study, which was based on the injection of HA non–bound spermatozoa, due to the risk of transmission of chromosomal anomalies.

In conclusion, most of the studies cited above showed an improved clinical outcome of physiologic ICSI using HA-viscous medium or HA-dish (Parmegiani et al., 2010 a; 2010b; Worrilow et al., 2007; 2010; Nasr-Esfahani et al., 2008). At the very least, in all the studies physiologic ICSI never caused a detrimental effect on ICSI outcome parameters (Table 1). If larger multi-centre 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., 2010 c).

FR: fertilization rate; EQ: embryo quality; PR: pregnancy rate; IR: implantation rate; MR: miscarriage rate; ND: not described.

Authors	*HA-System*	*N° of treatments or patients*	*HA-ICSI determines :*
Menezo et Nicollet, 2004	Sperm Slow	92 HA-ICSI vs 110 PVP-ICSI	No differences
Sanchez et al, 2005	N.D.	18 HA-ICSI versus control group	No differences
Worrilow et al, 2007	PICSI	240 couples: PICSI vs PVP-ICSI	Improvement in FR, EQ, MR
Nasr-Esfahani et al, 2008	home-made	50 couples: sibling oocytes; HA-ICSI vs PVP-ICSI	Improvement FR
Van Den Berg et al, 2009	Sperm Slow	44 couples: sibling oocytes; HA+ vs HA- sperms	No differences
Parmegiani et al, 2010 a	Sperm Slow	125 HA-ICSI vs 107 PVP-ICSI	Improvement in EQ
Parmegiani et al, 2010 b	Sperm Slow	331 HA-ICSI vs 97 PVP-ICSI	Improvement in EQ, IR
Worrilow et al, 2010	PICSI	215 couples: PICSI vs PVP-ICSI	Improvement in EQ
Menezo et al, 2010	Sperm Slow	2014 HA-ICSI vs 1920 PVP-ICSI	No differences

Table 1. Studies on injection of HA-bound spermatozoa

4. Zona - Bound spermatozoa

Immature spermatozoa have a low density of ZP binding sites as well as HA receptors (Huszar et al., 2003). Human sperm bound to ZP exhibit attributes similar to those of HA-bound sperm, including minimal DNA fragmentation, normal shape, and low frequency of chromosomal aneuploidies (Yagcy et al., 2010). Furthermore, in some mammals, the same sperm membrane protein is involved firstly in hyaluronidase activity and subsequently in ZP binding (Hunnicutt et al., 1996). These findings suggest that the spermatozoa–ZP binding process plays an important role in the natural selection of spermatozoa as well as HA.

A spermatozoa-ZP binding test can be performed by culturing spermatozoa for a couple of hours with immature metaphase I oocytes; the spermatozoa bound to ZP can be recovered with an injecting pipette and used for ICSI: when using this system Paes de Almeida Ferreira Braga et al. (2009) found that the injection of ZP-bound spermatozoa increases embryo quality. Black et al. (2010) observed a trend in implantation and clinical pregnancy rates when injecting ZP-bound spermatozoa in a study on ZP-ICSI versus conventional ICSI. Liu et al. (2011) observed a significant improvement in top embryo quality rate comparing ZP-ICSI with conventional ICSI.

Even though at the present time there is little information regarding all the factors involved in sperm-ZP binding and its mechanism, these last studies suggest that the spermatozoa–ZP binding test may be an efficient method for identifying competent spermatozoa for ICSI. ZP selection could then be coupled to HA selection in order to replicate the natural path of the spermatozoa towards the oocyte.

5. Intracytoplasmic morphologically selected sperm injection (IMSI)

The conventional magnification for sperm evaluation at ICSI is a maximum 400 X. Some studies demonstrate that sperm morphology according to strict criteria (Kruger et al., 1986; 1988):

- has little prognostic value in ICSI cycle outcomes (Svalander et al., 1996; French et al., 2010)
- does not influence embryo development or morphology (French et al., 2010)

But, it seem logical that the goal of obtaining the most viable embryo and reducing diseases in newborns is dependent on the selection of ideal gametes, both oocytes and spermatozoa (Parmegiani et al., 2010 c). Unfortunately, when observed at 400-1000 magnification, sperm dimension and shape are no reliable attributes for predicting chromatin integrity or presence of numerical chromosomal aberrations (Celik-Ozenci et al., 2004). To improve "imaging" sperm selection, the group of Bartoov (1994, 2001, 2002) developed a method of unstained, real-time, high magnification evaluation of spermatozoa (MSOME: motile sperm organelle morphology examination). 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 X (Figure 3). Application of MSOME selection in patients undergoing ICSI demonstrated that morphological integrity of the human sperm nucleus is an important parameter associated with pregnancy rate (Bartoov et al., 2003, Berkovitz et al., 2005). The modified ICSI procedure based on MSOME criteria was defined as IMSI: intracytoplasmic morphologically selected sperm injection (Bartoov et al., 2003). A matched study (Bartoov et al., 2003) revealed that pregnancy rate was significantly increased in IMSI as compared with routine ICSI, and implantation rate was even the 3-fold higher. Berkovitz et al., 2005 found an increase in abortion rate from 10% (no spermatozoa with normal nuclei) to 57% if no normal sperm for ICSI was available. In fact, ICSI outcome is significantly improved by the exclusive microinjection into the oocyte of spermatozoa with a strictly defined, morphologically normal nucleus, 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; Mauri et al., 2010; Souza Setti et al., 2010) or with severe male factor (Balaban et al., 2011, Souza Setti et al., 2011). IMSI positive effect is not evident on day 2 embryos (Mauri et al., 2010) but, conversely, the injection of spermatozoa with abnormal sperm head or with nuclear vacuoles negatively affects embryo development in day 5-6 (Vanderzwalmen et al., 2008) and ICSI outcome (Berkovitz et al., 2006a; 2006b; Cassuto et al., 2009; Nadalini et al., 2009). The positive effect on ICSI outcome given by the injection may be due to the significantly better mitochondrial function, chromatin status and reduced aneuploidy rate of spermatozoa without nuclear vacuoles when compared with vacuolized spermatozoa (Garolla et al., 2008; Boitrelle, et al., 2011). In addition, spermatozoa free of nuclear morphological malformations are related with lower incidence of aneuploidy in derived embryos (Figueira et al., 2011).

It should be mentioned that IMSI is a time-consuming procedure: selecting a "normal" MSOME spermatozoon requires 60-120 minutes (Antinori et al., 2008). Furthermore, the process of searching for spermatozoa at high magnification may itself damage sperm cytoplasm: sperm nucleus vacuolization significantly increases after 2 hours on the microscope's heated stage (Peer et al., 2007). IMSI procedure can be speeded up by merging

of high magnification microscopy together with HA-sperm selection. In fact, a HA-medium may help to select a sub-population of spermatozoa with normal nucleus according to MSOME criteria: Parmegiani et al (2010a) found that nucleus normalcy rate was significantly higher in HA-bound spermatozoa than in spermatozoa in PVP.

It can be concluded that, despite the time consuming procedure and the cost of the instrument for high magnification microscopy, IMSI has proved itself a valid tool for safe, non-invasive sperm selection and it can be widely applied in the near future.

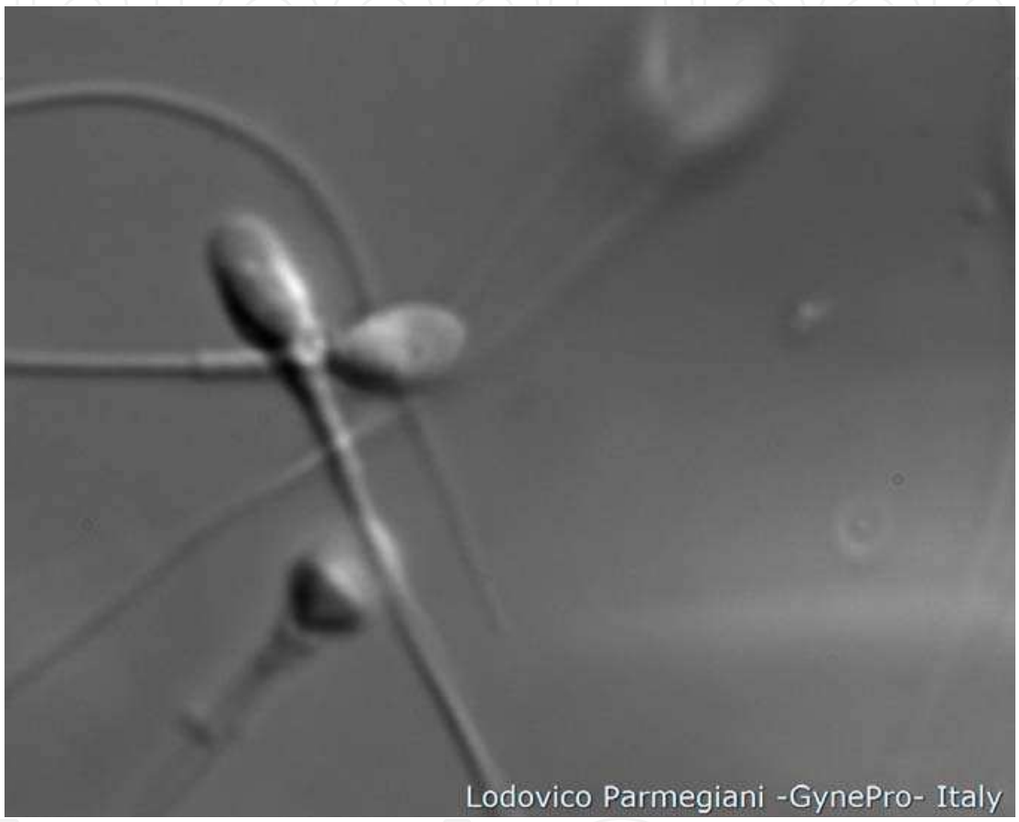

Fig. 3. Human Spermatozoa (magnification >6300 X)

5.1 IMSI procedure

Spermatozoa are generally first treated with a density gradient system. Then, the prepared sperm suspension is put into a PVP (or Sperm Slow in case of "Physiologic IMSI", *Parmegiani et al., 2010 a*) droplet, on a glass-bottom culture dish under oil. In order to choose the best spermatozoa to inject, sperm "nucleus normalcy" is evaluated. The nucleus normalcy is assessed in real time according to Motile Sperm Organelle Morphology Examination (MSOME) criteria. According to MSOME criteria, normally-shaped sperm nucleus is smooth, symmetric, and with oval configuration. Average lengths and widths (± Standard Deviation) must be 4.75±0.28 μm and 3.28 ±0.20 μm, respectively. Nuclear chromatin content is considered abnormal if sperm head contains one or more vacuoles (diameter of 0.78±0.18) that occupy more than 4% of the normal nuclear area. To be considered morphologically normal, a sperm nucleus has to have both

normal shape and normal chromatin content. For rapid evaluation of nuclear normalcy, a fixed, transparent, celluloid form of a sperm nucleus fitting MSOME criteria for length and widths can be superimposed on the examined cell on the screen: the nuclear shape is considered abnormal if it differs in length or width by 2 standard deviations from the normal mean axes values; vacuoles can be examined using a similar celluloid form (Figure 4). Alternatively, spermatozoa can be measured for nuclear length, width and vacuoles with specific digital imaging softwares

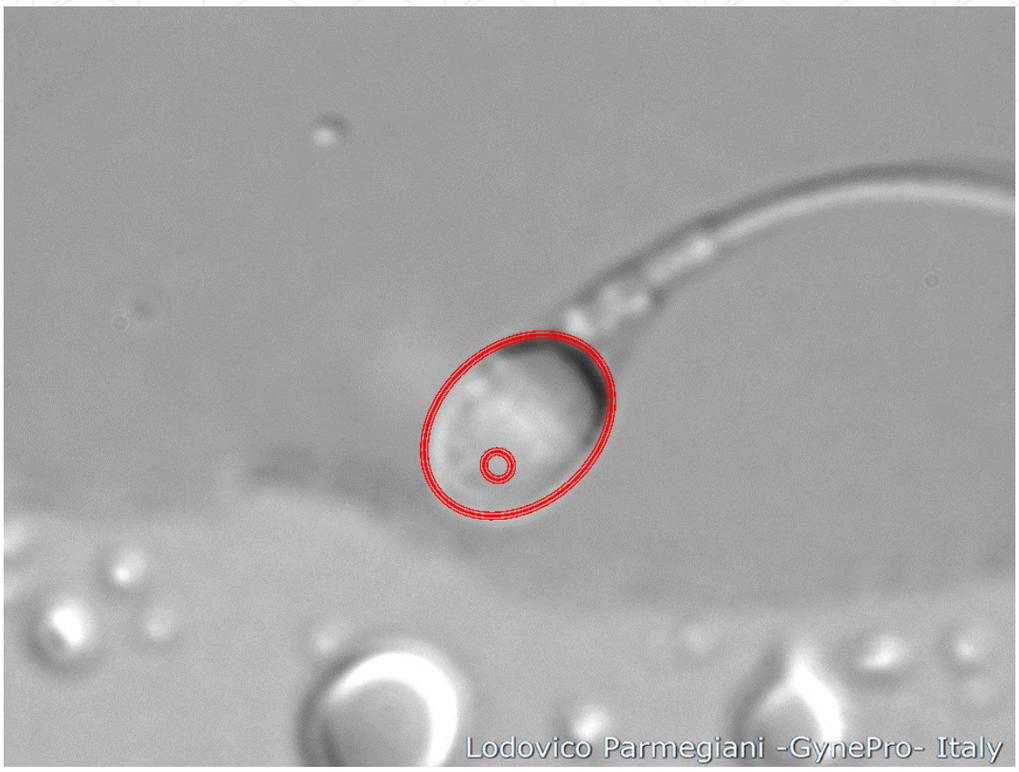

Fig. 4. IMSI procedure. Human Spermatozoa (magnification >10'000 X)

For evaluation of nuclear normalcy, a fixed, transparent, celluloid form of a sperm nucleus fitting MSOME criteria for length and widths is superimposed on the examined cell on the screen: the nuclear shape is considered abnormal if it differs in length or width by 2 standard deviations from the normal mean axes values; vacuoles are examined using a similar celluloid form (Bartoov et al., 2003).

6. Sperm head birefringence

A new tool for sperm selection is the application of polarization microscopy to ICSI (Baccetti, 2004). This method is based on the birefringence characteristics of the sperm protoplasmic texture. In the mature sperm nucleus, there is a strong intrinsic birefringence associated with nucleoprotein filaments that are ordered in rods and longitudinally oriented. An inverted microscope specifically equipped with polarizing lenses allows for the real-time selection of birefringent spermatozoa for ICSI. The localization of the birefringence in the postacrosomial region indicates that the acrosomial reaction has already occurred; the

injection of acrosome reacted spermatozoa seems to favour the development of viable ICSI embryos (Gianaroli et al., 2010). The injection of birefringent spermatozoa seems to be useful, especially in cases of oligoasthenoteratozoospermia or testicular spermatozoa (Gianaroli et al., 2008; 2010).

7. Conclusions

The introduction of ICSI (Palermo et al., 1992) has changed in a revolutionary way the world of assisted reproduction technology allowing us to efficiently treat patients with:

- oligoasthenoteratozoospermia
- testicular spermatozoa
- limited number of oocytes
- previous IVF failures

In these situations, suboptimal spermatozoa could by-pass the physiological check-points of natural fertilization and generate embryos, and subsequently babies. Conventional ICSI has the hypothetical risk of injecting immature, DNA damaged, aneuploid, low motile, morphologically abnormal, zona binding deficient, poor acrosome reacted, spermatozoa. Nowadays, we have no real knowledge of the effects of suboptimal sperm selection on ICSI adults in the long term, at least for humans. A potentially worrying aspect of the injection of DNA damaged spermatozoa for example, has been suggested by studies performed on animals which 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 ageing, abnormal behaviour, and mesenchymal tumours (Fernandez-Gonzales et al., 2008).

Fortunately, in humans, the risk of injecting DNA damaged spermatozoa seems to be minimized by classical sperm preparation techniques prior to ICSI (Zini et al. 2000; Younglai et al., 2001; Donnelly et al., 2000; Ahmad et al., 2007; Jackson et al., 2010; Marchesi et al., 2010; Castillo et al., 2011: Ebner et al, 2011) and follow-up studies on ICSI children have demonstrated the safety of this technique (Van Steirteghem et al., 2002, Leunens et al., 2008; Belva et al., 2011; Woldringh et al., 2011) although a slight increase of chromosome aberration seems to be caused by the injection of aneuploid spermatozoa (Bonduelle et al., 2002).

The recent refinements of the ICSI procedure described in this chapter, are reliable, easy-to-do, non-invasive and in some cases "closest to the nature" than the conventional procedure. For example, selecting spermatozoa prior to ICSI by their maturation markers such as HA-ZP receptors (Huszar et al., 2003; Paes de Almeida Ferreira Braga et al., 2009) it is possible at very least to mimic nature in order to restore physiological selection and prevent hypothetical fertilization by DNA damaged and chromosomal unbalanced spermatozoa. In addition, non-invasive imaging sperm selection techniques such as IMSI (Bartoov et al., 2003) or sperm head birefringence (Gianaroli et al., 2008) can be valid tools for helping in selection of the ideal spermatozoa.

In fact, sperm selection based on non invasive morphology or maturity markers helps the embryologist in selection of the "ideal" spermatozoa to inject. These new advances in ICSI may allow the selection of the spermatozoa contributing to the improve:

- fertilization
- embryo quality
- blastocyst formation
- pregnancy
- reduction in abortion.

Furthermore, some of these new technologies also help the standardization of ICSI, reducing intra-operator and inter-operator variability in choosing the spermatozoon to inject. For example, HA-ICSI offers to the embryologist the possibility to recognize the spermatozoa which have completed the maturation process. On the other hand, IMSI allows a precise sperm evaluation and measurement. In particular, these two techniques may also be merged together, pre-selecting HA-bound spermatozoa before High –magnification evaluation. This combined procedure (Physiologic IMSI) speeds up the "time consuming" sperm selection according to MSOME criteria (Parmegiani et al, 2010 a)

The easy reproducibility of these new advances in ICSI should encourage the embryologists and clinicians to automatically offer these technical improvements to all ICSI patients, not only to optimize clinical results but most of all to restore some basic check-points of natural fertilization which are bypassed in the conventional ICSI.

8. Acknowledgment

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9. References

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

Antinori M, Licata E, Dani G, Cerusico F, Versaci C, D'angelo D, Antinori S. (2008) Intracytoplasmic morphologically selected sperm injection: a prospective randomized trial. *Reprod Biomed Online* 16, 835-841.

Baccetti B. (2004) Microscopical advances in assisted reproduction. *J Submicrosc Cytol Pathol* 36, 333-339.

Balaban B, Lundin K, Morrell JM, Tjellström H, Urman B, Holmes PV. (2003) An alternative to PVP for slowing sperm prior to ICSI. *Hum Reprod* 18, 1887-1889.

Balaban B, Yakin K, Alatas C, Oktem O, Isiklar A, Urman B. (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, Elder K. (2001) A physiological replacement for polyvinylpyrrolidone (PVP) in assisted reproductive technology. *Hum Fert* 4, 99-103.

Bartoov B, Eltes F, Pansky M, Langzam J, Reichart M, Soffer Y. (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, Kogosowski A, Menezo Y, Barak Y. (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, Kogosovsky A, Yagoda A, Lederman H, Artzi S, Gross M, Barak Y. (2003) Pregnancy rates are higher with intracytoplasmic morphologically selected sperm injection than with conventional intracytoplasmic injection. *Fertil Steril* 80, 1413-1419

Belva F, De Schrijver F, Tournaye H, Liebaers I, Devroey P, Haentjens P, Bonduelle M. (2011) Neonatal outcome of 724 children born after ICSI using non-ejaculated sperm. *Hum Reprod* 7, 1752-1758.

Berkovitz A, Eltes F, Yaari S, Katz N, Barr I, Fishman A, Bartoov B. (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, Peer S, Feldberg D, Bartoov B. (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, Peer S, Ellenbogen A, Feldberg B, Bartoov B. (2006b) How to improve IVF-ICSI outcome by sperm selection. *Reprod Biomed Online* 12, 634-638.

Black M, Liu de Y, Bourne H, Baker HW. (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.

Boitrelle F, Ferfouri F, Petit JM, Segretain D, Tourain C, Bergere M, Bailly M, Vialard F. Albert M. Selva J. (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, Keymolen K, Devroey P, Van Steriteghem A, Liebaers I. (2002) Prenatal testing in ICSI pregnancies: incidence of chromosomal anomalies in 1586 karyotypes and relation to sperm parameters. *Hum Reprod* 17, 2600-2614.

Castillo J, Simon L, de Mateo S, Lewis S, Oliva R.(2011) Protamine/DNA ratios and DNA damage in native and density gradient centrifugated sperm from infertile patients. J Androl 32, 324-32.

Cassuto NG, Bouret D, Plouchart JM, Jellad S, Vanderzwalmen P, Balet R, Larue L, Barak Y. (2009) A new real-time morphology classification for human spermatozoa: a link for fertilization and improved embryo quality. *Fertil Steril* 92, 1616-1625.

Cayli S, Jakab A, Ovari L, Delpiano E, Celik-Ozenci C, Sakkas D, Ward D, Huszar G. (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, Catalanotti J, Demir R, Bray-Ward P, Ward D, Huszar G. (2004) Sperm selection for ICSI: shape properties do not predict the absence or presence of numerical chromosomal aberrations. *Hum Reprod* 19, 2052-2059.

Donnelly ET, O'Connell M, McClure N, Lewis SE. (2000) Differences in nuclear DNA fragmentation and mitochondrial integrity of semen and prepared human spermatozoa. *Hum Reprod* 15, 1552-1561.

Dozortsev D, Rybouchkin A, De Sutter P, Dhont M. (1995) Sperm plasma membrane damage prior to intracytoplasmic sperm injection: a necessary condition for sperm nucleus decondensation. *Hum Reprod* 10, 2960-2964.

Ebner T, Shebl O, Moser M, Mayer RB, Arzt W, Tews G. (2011) Easy sperm processing technique allowing exclusive accumulation and later usage of DNA-strandbreak-free spermatozoa. *Reprod Biomed Online* 22, 37-43.

Fernandez-Gonzalez R, Moreira PN, Perez-Crespo M, Sanchez-Martin M, Ramirez MA, Pericuesta E, Bilbao A, Bermejo-Alvarez P, de Dios HJ, De Fonseca FR, Gutiérrez-Adán A. (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.

Figueira R de C, Braga DP, Setti AS, Iaconelli A Jr, Borges E Jr. (2011). Morphological nuclear integrity of sperm cells is associated with preimplantation genetic aneuploidy screening cycle outcomes. *Fertil Steril* 95, 990-993.

Franco JG Jr, Baruffi RL, Mauri AL, Petersen CG, Oliveira JB, Vagnini L. (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, Desai N. (2010) Does severe teratozoospermia affect blastocyst formation, live birth rate, and other clinical outcome parameters in ICSI cycles? *Fertil Steril* 93, 1097-1103.

Garolla A, Fortini D, Menegazzo M, De Toni L, Nicoletti V, Moretti A, Selice R, Engl B, Foresta C. (2008) High-power microscopy for selecting spermatozoa for ICSI by physiological status. *Reprod Biomed Online* 17, 610-616.

Gianaroli L, Magli MC, Collodel G, Moretti E, Ferraretti AP, Baccetti B. (2008) Sperm head's birefringence: a new criterion for sperm selection. *Fertil Steril* 90, 104-112.

Gianaroli L, Magli MC, Ferraretti AP, Crippa A, Lappi M, Capitani S, Baccetti B. (2010) Birefringence characteristics in sperm heads allow for the selection of reacted spermatozoa for intracytoplasmic sperm injection. *Fertil Steril* 93, 807-813.

Hazout A, Dumont-Hassan M, Junca AM, Cohen BP, Tesarik J. (2006) High-magnification ICSI overcomes paternal effect resistant to conventional ICSI. *Reprod Biomed Online* 12, 19-25.

Heytens E, Parrington J, Coward K, Young C, Lambrecht S, Yoon SY, Fissore RA, Hamer R, Deane CM, Ruas M, Grasa P, Soleimani R, Cuvelier CA, Gerris J, Dhont M, Deforce D, Leybaert L, De Sutter P. (2009) Reduced amounts and abnormal forms of phospholipase C zeta (PLCζ) in spermatozoa from infertile men. *Hum Reprod* 24, 2417-2428.

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, Vigue L. (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, Zavaczki Z, Hansch E, Vigue L. (2003) Hyaluronic acid binding by human sperm indicates cellular maturity, viability, and unreacted acrosomal status. *Fertil Steril* 79 (Suppl 3), 1616-1624.

Huszar G, Jakab A, Sakkas D, Ozenci CC, Cayli S, Delpiano E, Ozkavukcu S. (2007). Fertility testing and ICSI sperm selection by hyaluronic acid binding: clinical and genetic aspects. *Reprod Biomed Online* 14, 650-663.

Jackson RE, Bormann CL, Hassun PA, Rocha AM, Motta EL, Serafini PC, Smith GD.(2010) Effects of semen storage and separation techniques on sperm DNA fragmentation. *Fertil Steril* 94, 2626-2630.

Jakab A, Sakkas D, Delpiano E, Cayli S, Kovanci E, Ward D, Revelli A, Huszar G. (2005) Intracytoplasmic sperm injection: a novel selection method for sperm with normal frequency of chromosomal aneuploidies. *Fertil Steril* 84, 1665-1673.

Jean M, Barriere P and Mirallie S. (1996) Intracytoplasmic sperm injection without polyvinylpyrrolidone: an essential precaution? *Hum Reprod* 11,2332.

Jean M, Mirallie S, Boudineau M, Tatin C and Barriere P. (2001) Intracytoplasmic sperm injection with polyvinylpyrrolidone: a potential risk. Fertil Steril, 76,419-420.

Kruger TF, Menkveld R, Stander FS, Lombard CJ, Van der Merwe JP, van Zyl JA, Smith K. (1986) Sperm morphologic features as a prognostic factor in in vitro fertilization. *Fertil Steril* 46, 1118-1123.

Kruger TF, Acosta AA, Simmons KF, Swanson RJ, Matta JF, Oehninger S. (1988) Predictive value of abnormal sperm morphology in in vitro fertilization. *Fertil Steril* 49, 112-117.

Leunens L, Celestin-Westreich S, Bonduelle M, Liebaers I, Ponjaert-Kristoffersen I. (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.

Liu F, Qiu Y, Zou Y, Deng ZH, Yang H, Liu DY. (2010). 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.

Marchesi DE, Biederman H, Ferrara S, Hershlag A, Feng HL. (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.

Markoulaki S, Kurokawa M, Yoon SY, Matson S, Ducibella T, Fissore R (2007) Comparison of Ca2+ and CaMKII responses in IVF and ICSI in the mouse. *Mol Hum Reprod* 13, 265-272.

Mauri AL, Petersen CG, Oliveire JB, Massaro FC, Baruffi LR, Franco JG Jr. (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.

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, De Mouzon J, Cohen-Bacrie P, Ben Khalifa M. (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 (Supp 1): 232.

Navarro-Costa P, Nogueira P, Carvalho M, Leal F, Cordeiro I, Calhaz-Jorge C, Gonçalves J, Plancha CE (2010) Incorrect DNA methylation of the DAZL promoter CpG island associates with defective human sperm. *Hum Reprod* 25, 2647-2654.

Nadalini M, Tarozzi N, Distratis V, Scaravelli G, Borini A (2009) Impact of intracytoplasmic morphologically selected sperm injection on assisted reproduction outcome: a review. *Reprod Biomed Online* 19 (Supp l3), 45-55

Nasr-Esfahani MH, Razavi S, Vahdati AA, Fathi F, Tavalaee M (2008) Evaluation of sperm selection procedure based on hyaluronic acid binding ability on ICSI outcome. *J Assist Reprod Genet* 25, 197-203.

Oehninger S. (2011) Clinical management of male infertility in assisted reproduction: ICSI and beyond. Int J Androl, 34:e319-329

Paes Almeida Ferreira de Braga D, Iaconelli A Jr, Cassia Savio de FR, Madaschi C, Semiao-Francisco L, Borges E Jr. (2009) Outcome of ICSI using zona pellucida-bound spermatozoa and conventionally selected spermatozoa. *Reprod Biomed Online* 19, 802-807.

Palermo G, Joris H, Devroey P, Van Steirteghem AC. (1992) Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet* 340(8810),17-18.

Parmegiani L, Cognigni GE, Bernardi S, Troilo E, Ciampaglia W, Filicori M (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, Pocognoli P, Marchi F, Filicori M. (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.

Peer S, Eltes F, Berkovitz A, Yehuda R, Itsykson P, Bartoov B. (2007) Is fine morphology of the human sperm nuclei affected by in vitro incubation at 37 degrees C? *Fertil Steril* 88, 1589-1594.

Prinosilova P, Kruger T, Sati L, Ozkavukcu S, Vigue L, Kovanci E, Huszar G. (2009) Selectivity of hyaluronic acid binding for spermatozoa with normal Tygerberg strict morphology. *Reprod Biomed Online* 18, 177-183.

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: *Genetics of human male infertility* (eds Barratt C, De Jonge C, Mortimer D, Parinaud J), pp 205-221. Editions EDK, Paris, UK.

Sanchez M, Aran B, Blanco J, Vidal F, Veiga A, Barri PN, Huszar G. (2005) Preliminary clinical and FISH results on hyaluronic acid sperm selection to improve ICSI. *Hum Reprod* 20 (Supp1), i200.

Schatten H & Sun QY. (2007) The role of centrosomes in mammalian fertilization and its significance for ICSI. *Mol Hum Reprod* 15, 531-538.

Souza Setti A, Ferreira RC, Paes de Almeida Ferreira Braga D, de Cássia Sávio Figueira R, Iaconelli A Jr, Borges E J. (2010) Intracytoplasmic sperm injection outcome versus intracytoplasmic morphologically selected sperm injection outcome: a meta-analysis. *Reprod Biomed Online* 21, 450-455

Sousa Setti A, Figueira RD, Paes de Almeida Ferreira Braga D, Iaconelli A Jr, Borges E Jr. (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.

Svalander P, Jakobsson AH, Forsberg AS, Bengtsson AC, Wikland M. (1996) The outcome of intracytoplasmic sperm injection is unrelated to 'strict criteria' sperm morphology. *Hum Reprod* 11, 1019-1022.

Van Den Bergh M. Fahy-Deshe M. Hohl M.K. (2009) Pronuclear zygote score following intracytoplasmic injection of hyaluronan-bound spermatozoa: a prospective randomized study. *Reprod Biomed Online* 19, 796-801.

Vanderzwalmen P, Hiemer A, Rubner P, Bach M, Neyer A, Stecher A, Uher P, Zintz M, Lejeune B, Vanderzwalmen S, Cassuto C, Zech NH. (2008) Blastocyst development after sperm selection at high magnification is associated with size and number of nuclear vacuoles. *Reprod Biomed Online* 17, 617-627.

Van Steirteghem AC, Nagy Z, Joris H, Liu J, Staessen C, Smitz J, Wisanto A, Devroey P. (1993) High fertilization and implantation rates after intracytoplasmic sperm injection. *Hum Reprod* 8, 1061-1066.

Van Steirteghem AC, Bonduelle M, Devroey P, Liebaers I. (2002) Follow-up of children born after ICSI. *Hum Reprod Update* 8, 111-116.

Wilding M, Coppola G, di Matteo L, Palagiano A, Fusco E, Dale B. (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, Reuser JJ, de Groot SA, Steiner K, D'Hauwers KW, Wetzels AM, Kremer JA (2011) Follow-up of children born after ICSI with epididymal spermatozoa. Hum Reprod 7: 1759-1767.

Worrilow KC, Huynh HT, Bower JB, Anderson AR, Schillings W, Crain JL. (2007) PICSI vs. ICSI: statistically significant improvement in clinical outcomes in 240 in vitro fertilization (IVF) patients. *Fertil Steril* 88 (Supp1), s37.

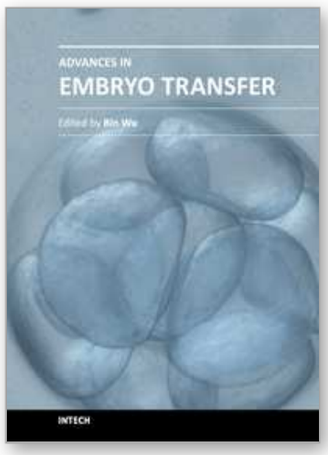
Worrilow KC, Eid S, Matthews J, Pelts E, Khoury C, Liebermann J. (2010) Multi-site clinical trial evaluating PICSI, a method for selection of hyaluronan bound sperm (HBS) for use in ICSI: improved clinical outcomes. *Hum Reprod* 25 (Supp1), i7.

Yagci A, Murk W, Stronk J, Huszar G. (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: *The Physiology of Reproduction*.(eds: Knobil E & Neill JD), pp. 189-317. Raven Press Ltd, New York,.

Younglai EV, Holt D, Brown P, Jurisicova A, Casper RF. (2001) Sperm swim-up techniques and DNA fragmentation. *Hum Reprod* 16, 1950-1953.

Zini A, Finelli A, Phang D, Jarvi K. (2000) Influence of semen processing technique on human sperm DNA integrity. *Urology* 56, 1081-1084.



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Embryo transfer has become one of the prominent high businesses worldwide. This book updates and reviews some new developed theories and technologies in the human embryo transfer and mainly focus on discussing some encountered problems during embryo transfer, which gives some examples how to improve pregnancy rate by innovated techniques so that readers, especially embryologists and physicians for human IVF programs, may acquire some new and usable information as well as some key practice techniques. Major contents include the optimal stimulation scheme for ovaries, advance in insemination technology, improved embryo transfer technology and endometrial receptivity and embryo implantation mechanism. Thus, this book will greatly add new information for readers to improve human embryo transfer pregnancy rate.

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