Chapter 10

Interacytoplasmic Morphologically Selected Sperm Injection: A Tool for Selecting the Best Sperm in Real Time

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.73388

Abstract

Routine sperm parameters are used to evaluate fertility potential of the male partner. Since the introduction of intracytoplasmic sperm injection (ICSI), it seems that the importance of routine parameters of sperm morphology has decreased in the field of assisted reproduction. ICSI has facilitated to achieve fertilization, embryo development, and pregnancies, from the treatment of males with poor-quality spermatozoa. Morphology is the only criteria for sperm aspiration during ICSI. Routine criteria are based on the raw ejaculate-stained sperm cells. Thus, it is important to score and aspirate a good-quality motile spermatozoon, which will contribute to the quality of the developing embryo after ICSI, in real time of the procedure. In ICSI, assessment of sperm morphology is limited due to the low magnification (200 × 400×) and concomitant low resolution. By using intracytoplasmic morphologically selected sperm injection (IMSI), it was demonstrated that a spermatozoon with normal morphology, and more precisely normal nucleus, might affect the incidence of pregnancy. Although the usage of IMSI is currently wider, it is necessary to standardize which sperm to aspirate, due to criteria based on accumulating data. Correlation to DNA integrity, embryo development in vitro, female age, male age, or the routine use of IMSI for all cases are raised in order to maximize the efficiency of IMSI technology.

Keywords: IMSI, high magnification, sperm morphology, ICSI, embryo quality, sperm quality, real time sperm selection, IVF

1. Introduction

Following dedicated research and treatment of Patrick Steptoe and Robert G Edward in Bourn Hall, Cambridge, the first in vitro fertilization (IVF) baby girl Louise Brown was born

in 1978. This revolutionary assisted reproductive technology (ART) attempt brought the fulfillment of a vision to hundreds of thousands of infertile couples to conceive and deliver. The main idea behind the development of the technique was treating women with mechanical factor infertility. Despite the great success of IVF, poor results were obtained in cases of severe male infertility. IVF outcome was satisfactory when oocytes were inseminated with >1.5 million spermatozoa and at least 50% motility could be observed in the specimen. This major limitation of sperm insemination in routine IVF was overcome by intracytoplasmic sperm injection (ICSI), introduced by Palermo in 1992 [1]. The injection of a single sperm into an oocyte, which revealed fertilization and development of viable in vitro embryos, gave a rapid solution to men with severe forms of male factor infertility, allowed application to other forms of infertility. Nowadays, it serves as the main tool for assisting couples to fulfill their wish to conceive [2]. Furthermore, it is also used for various infertility indications as low number and poor morphology of oocytes, thick zona pellucida, pre-implantation genetic diagnosis or screening (PGD, PGS), cases of hepatitis C and human immunodeficiency virus, in vitro maturation, and oocyte cryopreservation. The usage of ICSI enabled to achieve fertilization, embryo development, pregnancies, and deliveries. It has been reported that strict morphologic criteria do not affect ICSI outcomes [3–9]. On the other hand, scientists indicated that the paternal gamete influences the resulting embryo, mainly at the level of blastocyst formation. Several points of negative paternal impact, both genetic and epigenetic, have been identified in embryos following the injection of poor-quality spermatozoa into the oocyte. Using ICSI also clarified some points of the paternal influence on the embryo development, mainly in vitro [10–20]. The specific injected sperm might affect the health of the newborns [21]. Several years ago, a series of reports pointed out that children born as a result of ART were found to have increased frequencies of a number of diseases known to have an epigenetic etiology [22–26], mainly after ICSI [27, 28]. Recently, Santos et al. evaluated the genome-wide DNA methylation together with chromatin organization in human embryos derived by either IVF or ICSI. In this report it was found that infertility per se, rather than ART procedures, may play an important part in predisposition to epimutations that leads to diseases of an epigenetic basis. Interestingly, embryos developing to the blastocyst stage had an apparently normal epigenotype irrespective of the procedure used. These investigators concluded that ICSI per se does not lead to an increased incidence of epigenetic errors [29]. It is accepted that routine morphology characteristics may not necessarily describe the quality of the specific single spermatozoon that was injected into the oocyte. Semen analyses, which describe the general picture of sperm quality of the raw ejaculate, are based on the examination of fixed and stained sperm cells. The latest, obviously, is not suitable for treatment any more. Aspiration of a specific sperm cell, and its injection into the oocyte, during ICSI, regularly takes place under the magnification of 200× or 400×. The sperm cell is randomly chosen when spermatozoa with major morphological abnormalities are generally omitted due to the experienced eyes of the embryologist. It is questionable as to what tool should be used to evaluate morphological criteria in real time during ICSI, which will enable to select a specific spermatozoon, with the highest predictive value for better fertilization, embryo developmental, implantation and pregnancy. De Vos et al. showed a correlation between the gross morphology of the individual injected sperm cell and the formation and development of the resultant embryo. These scientists admitted

http://dx.doi.org/10.5772/intechopen.73388

that the low magnification (e.g. magnification of 400× during ICSI) and concomitant low resolution of the morphology of the motile sperm cell have been the limiting factors of their study [30]. It was demonstrated that using a high-power inverted light microscope with a zoom ≥6000× for the examination of motile spermatozoa in real time might positively affect the outcome of ICSI, in terms of fertilization rate, embryo development, and the occurrence of pregnancies [31–35].

2. Intracytoplasmic morphologically selected sperm injection step by step

2.1. The principle of the IMSI microscope

The term IMSI was defined by Bartoov et al. [32, 33]. The idea was based on the magnification up to 6000× and more of the motile sperm cell in real time [33, 35–38]. The sperm analysis is performed using an interference phase contrast inverted microscope with the optics of Nomarski. The magnified image of the sperm cell is displayed on a screen. The final magnification of the "on-screen image" is actually a combination of the magnification of the objective, the camera adapter, ratio between the diagonal screen size in mm, diagonal of the camera chip size in mm, and internal magnification of the microscope. Depending upon these specific characteristics of an IMSI system, the final value of the magnification might vary from 6000× to 6600× as described earlier. It should be clarified that the final various magnifications achieved in IMSI are actually a result of zoom on the image of the sperm cell, which magnifies the structures of its surface and cannot be observed in magnification of 200–400×, due to objective limitations of the human eyes.

2.2. Preparation of the IMSI dish

The high magnification of the objective (100×) with the optics of Nomarski requires a dish with a glass bottom. Droplets of the medium, approximately droplets from 1 to 4 μl, should be located on the dish as described in the diagram of an IMSI dish (**Figure 1**).

In **Figure 1**, the diagram of the IMSI dish describes the arrangement of the observation droplets with spermatozoa, droplets with PVP, droplets of clean medium for the selected spermatozoa, and clean droplets for the injection of the oocytes. The latest should be replaced with fresh droplets of media, prior to the ICSI procedure.

Observation droplets: three droplets of 2 μl are located in the left side of the dish. In these droplets, 1 μl of the processed sperm cells of the sperm ejaculate will be inserted. These droplets of the sperm culture medium might contain, in correlation with the intensity of the sperm motility, between 0 and 10% polyvinylpyrrolidone (PVP) solution [34].

Clean droplets of a clean sperm culture medium: this is required to host the scored sperm cells after the evaluation. In each droplet, aspirated spermatozoa with a distinct morphological score are located.

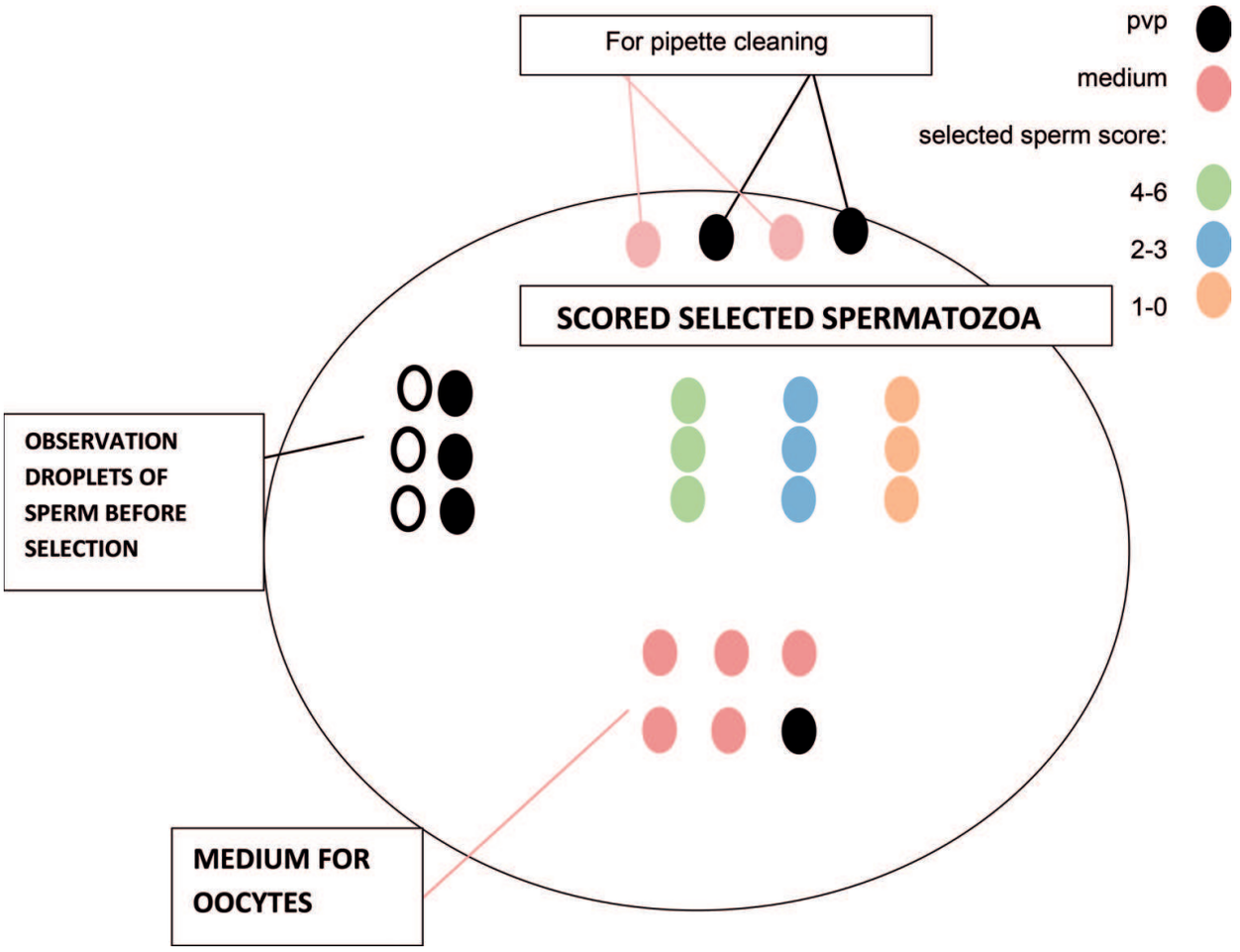


Figure 1. Diagram of an IMSI dish.

Clean droplet of PVP 10%: droplets of 1 μl are located in parallel to the droplets with the spermatozoa. When the intensity of the processed spermatozoa is not high, the authors like to create tiny "bridges" between these two droplets. The tiny bride will allow the passage of spermatozoa to the 10% PVP droplet and it eases the detection on the spermatozoa, as motility of the examined spermatozoa is slowed down. It is recommended to draw tiny extensions from the rim of the PVP droplets to capture the heads of the motile sperm cells. All droplets should be covered by mineral culture oil, which is generally used for the ICSI procedure.

The authors recommend that based on their existing design of the IMSI dish, each laboratory should adapt its special design for the arrangement of the droplets, in accordance with the preference of the laboratory where the ICSI procedure takes place.

3. IMSI in practice

The freshly ejaculated semen is centrifuged at approximately 360 ×*g* for 10 min. In cases when sperm concentration is <5 × 10^6, centrifugation is performed for 1520 min. The pellet is resuspended in 0.5 ml of sperm wash solution (Sperm Preparation Medium, Origio, Denmark). Further assessment is performed by using the bilayer density gradient: 0.5 ml of 50% gradient

solution and 0.5 ml of 80% (Supra Sperm Origio, Denmark) for 15 min. In severe oligospermic cases, 0.3 ml of each of the gradient layers is used and centrifugation in 300 × *g* should last up to 1 h [39, 40]. Final sperm pellet is resuspended in 50–100 μl. Approximately a droplet of 1 μl is placed inside the observation droplet of the pre-prepared IMSI dish. During the observation, motile spermatozoa with rough morphological abnormalities are omitted and not aspirated. Other motile sperm cells are aspirated and evaluated inside the ICSI pipette or in the clean PVP droplet and scored as mentioned above. The individual scored sperm cell is then placed into the appropriate droplet, in the IMSI dish [32, 35]. Aspiration of sperm cells is performed by the means of micromanipulations. There is no preference for a certain micromanipulation system. One should work with a system which is the easiest to handle, with his own hands.

3.1. Choosing the sperm

The group of Bartoov classified the selected spermatozoa morphology as "normal" and "second" choice, based on the data collected by scanning and transmission electron microscopy. Some characteristics of the morphology of the head of the sperm cell such as smoothness, symmetry, oval configuration, average length and width (4.75 ± 0.28 and 3.28 ± 0.20 mm, respectively), and nuclear chromatin mass containing no more than one vacuole were defined for normalcy of the sperm nucleus [32, 33]. The "second-choice" motile sperm was not clearly depicted. One might only speculate that during IMSI, when no normal head spermatozoa can be found, the alternative then is to select motile sperm cells that are morphologically the second-best choice.

The Cassuto and Barak Score was developed in order to define more precisely the preferable spermatozoon that should be injected into the oocyte, in real time [35]. This scoring system was established after checking and taking into consideration the sperm defects, which negatively affect the development of the embryo in vitro and had the name of HAVBIC: **H**ead: normalcy and shape, **A**crosome: presence or absence, **V**acuoles: presence or absence, **B**asis of the sperm head, **I**nsertion: the axial position of the sperm, and **C**ytoplasmic droplet: presence or absence. Logistic regression was used for fertilization and embryo development as dependent variables. Coefficients were calculated and tested by comparing receiver operating characteristic (ROC) curves. The best results were achieved with an area under the curve of 0.618, deriving the following formula: Score of spermatozoa = 2 × Head + 3 × Vacuole + 1 × Base. Following these findings, this classification system took into consideration three major parameters of the sperm nucleus: normalcy of shape and size of sperm head, lack of vacuoles and normalcy of the base of the head of the specific spermatozoon. Each of the normal parameter received a value = 1 when normal or = 0 when abnormal. Classification of the individual sperm cell, with a maximum of score 6, is calculated by the embryologist, who performs the IMSI, with this friendly formula, described above, and distinguishes between three classes:

Class 1: spermatozoa of highest quality, score 4–6; class 2: spermatozoa, score 1–3; class 3: low-quality spermatozoa, score 0. In their study a difference was noted between the fertilization rate of oocytes with regard to the classification of the injected spermatozoa ($P < 0.04$; chi square = 6.31). A pair-wise comparison showed a higher fertilization rate in oocytes injected with class 1 spermatozoa in comparison to class 3 ($P < 0.01$; chi square = 6.3). A difference was noted in the development rate into expanded blastocyst ($P < 0.03$; chi square = 6.71), no expanded blastocyst was observed in embryos resulted from injection of class 3 spermatozoa (score of 0) [35].

4. IMSI in patients with a high rate of sperm DNA fragmentation

DNA integrity was assessed by Hazout et al. in 72 patients, referred to IMSI and ICSI. DNA fragmentation rate was evaluated by TUNEL assay. Improvement of clinical outcomes was evident both in patients with an elevated degree of sperm DNA fragmentation and in those with normal sperm DNA status [36]. Similar results were obtained by de Almeida Ferreira Braga et al. and Setti et al., who showed that fertilization and high-quality embryo rates were similar in patients with a high incidence of sperm DNA fragmentation tested in sibling oocytes split into ICSI and IMSI. Their observation suggested that IMSI, but not DNA sperm fragmentation assay per se, could be a beneficial tool in improving IVF-ICSI results [41, 42]. In another independent study no correlation was found between abnormal sperm head morphology as assessed by high magnification (score 0) and DNA fragmentation. However, the rate of chromatin decondensation of their score 0 spermatozoa was twice as high as the spermatozoa that scored 4–6 (19.5% vs. 10.1%; $P < 0.0001$) [43]. This finding might explain the former observation of these researchers that no expanded blastocyst was developed following the injection of spermatozoa with the lowest morphology score [35].

5. IMSI and head-sperm vacuoles

Vanderzwalmen et al., classified the spermatozoa according to the presence and size of vacuoles into four groups: Grade I: normal shape and no vacuoles; Grade II: normal shape and maximum of two small vacuoles; Grade III: normal shape and more than two small vacuoles or one large vacuole; and Grade IV: large vacuoles in conjunction with abnormal head shapes or other abnormalities at the level of the base of the sperm head. The outcome of embryo development in a group of 25 patients after sibling oocyte injection with the four different grades of spermatozoa showed no significant difference in embryo quality up to day 3. However, the occurrence of blastocyst formation was 56.3 and 61.4% with grade I and II spermatozoa, respectively, compared with 5.1% with grade III and 0% with grade IV, respectively ($P < 0.001$) [44]. It is not clear yet why presence of vacuoles in the sperm head is such an important parameter of sperm quality. Some reports showed no correlation between the appearances of vacuoles to male infertility [45–47]. One of these studies was an unpowered investigation [47] and another evaluated the sperm under magnification of 1000× [45]. Many others reported that vacuoles might negatively be associated with male fertility potential [32–37, 44, 48–54]. Moreover, investigation of the relation between sperm vacuoles and acrosome reaction suggested that there might be a negative link between presence of vacuoles and acrosome reaction of the sperm [55, 56]. Consequently, IMSI could be a method for assisting the removal of the acrosome reaction-resistant spermatozoa.

The personal attitude of the authors of the current dissertation is that majority of abnormalities observed under the high magnification of IMSI in real time are probably not visible while using routine conditions with lower magnifications. It is likely, though, that the benefit of scoring scale of the sperm cell is a kind of “fine tuning”; IMSI therefore is more beneficial for motile spermatozoa which have normal morphological appearance under magnification 200–400×.

6. IMSI should not be used for all

It seems that IMSI was a promising revolutionary technique in terms of improving the outcome of ICSI treatments. One might agree that high magnification achieved by the technique contributes with a better evaluation of the aspirated sperm cell for the injection providing encouraging results. On the other hand, prolonged sperm manipulation, special instrumentation, additional number of embryologists who should be trained and expertly perform the technology, and the additional cost for the patients might increase the cost effectiveness of the procedure. Taking all the above into consideration, patients should be given counseling to undergo IMSI, for a better chance to conceive.

It appears that there is no advantage or benefit over standard ICSI in terms of clinical outcome in an unselected infertile population. Although there were trends for higher implantation, clinical pregnancies, and live birth rates in the IMSI group, using the technique did not reveal an improvement in the clinical outcome compared with ICSI [57, 58]. The authors of the current publication, therefore, will try to discuss the benefits of IMSI in cases of patients with repeated implantation failures, severe male factor infertility, and advanced paternal and maternal age.

6.1. Patients with repeated implantation failures

Sixty-two couples with at least two previous consequent pregnancy failures after routine ICSI cycles underwent IMSI in the following cycle. The matched control group comprised 50 couples, who underwent routine ICSI treatment and previously experienced the same number of ICSI failures in the same center. Fertilization and top-quality embryo rates were similar in both groups. A higher pregnancy rate with a lower miscarriage rate were achieved in the IMSI group, in comparison to the control group (66.0% vs. 30.0%; $P < 0.01$; 33.0% vs. 9.0%; $P < 0.01$, respectively) [33]. Following that study, this new concept of sperm selection prior to ICSI was undertaken in additional centers, with encouraging results. Efficacy of IMSI was examined, for instance, in 12 couples with two or more repeated conventional ICSI failures, who underwent an additional conventional ICSI attempt, followed by a high magnification IMSI attempt. Fertilization and cleavage rates and embryo morphology were similar when we compared the two sequential attempts (ICSI attempt vs. the following IMSI cycle). However, improved clinical outcomes such as implantation, pregnancy, delivery, and birth rates were observed in IMSI attempts when compared with ICSI (20.3% vs. 0.8%, 37.6% vs. 2.4%, 33.6% vs. 0.0%, 17.6% vs. 0.0%, respectively; $P < 0.001$) [36]. Another meta-analysis compared the outcomes of conventional ICSI vs. IMSI cycles. It was concluded that IMSI not only improves the percentage of top-quality embryos, implantation, and pregnancy rates but also reduces miscarriage rates as compared with ICSI [59]. Findings of a retrospective study in 42 couples supported the former as well. These scientists examined the efficiency of the IMSI technique in patients with at least three repeated IVF-ICSI failure. The investigators demonstrated superior implantation, clinical pregnancy, and live birth rates in the IMSI group, moreover a lower miscarriage rate [60]. These data, in addition to the abovementioned, pointed toward IMSI as an important tool for the selection of the best spermatozoon for the injection of oocytes in cases of repeated IVF treatment failure.

6.2. IMSI in cases of severe male factor infertility

Usage of IMSI had a significant contribution to the accumulated knowledge of male infertility. At present, few randomized controlled trials are available assessing the advantages of IMSI over the conventional ICSI procedure. Antinori et al. assessed 446 couples, randomly referred to ICSI or IMSI, with at least 2 previous diagnoses of male factors due to severe oligoasthenoteratozoospermia [53]. Despite their initial poor reproductive prognosis, patients with two or more previous failed attempts benefited the most from IMSI not only in terms of increased pregnancy rate (29.8% vs. 12.9%; $P = 0.017$) but also lower miscarriage rates. Patients diagnosed with poor reproductive prognosis with two or more previous failed attempts benefited the most from IMSI. Study of patients with motile sperm less than 0.1×10^6/ml after the swim-up technique showed a positive influence of IMSI on fertilization, implantation, and pregnancy rates [42, 61]. More reports regarding patients with isolated teratozoospermia or severe oligospermia pointed to the benefits following the selection of injected sperm cell using IMSI. Higher clinical pregnancy and higher implantation rates were observed, in comparison to the conventional aspiration of spermatozoa in ICSI [62, 63].

7. IMSI: maternal age and pre-implantation genetic screening

Age-related decline in the quality of the oocytes, which affects the ICSI outcome, is a known phenomenon. Cassuto et al. distinguished between the quality of embryos resulting from oocytes of women younger than 30 years and those from women of 30 years and older, following the injection of spermatozoa selected using IMSI. When class 2 or 3 spermatozoa (moderate and bad morphological score) was injected, a lower rate of best and good embryos developed in the group of the older female patients (>30 years old) in comparison with the rate in the younger group (maternal age ≤ 30). Conversely, when a high-quality spermatozoon (score 4–6) was injected, the age-related quality of the oocyte is negligible; no difference was detected when the ratio of high-quality embryos was compared in young and older women. This is logical because these "top spermatozoa" do not need any repair. According to their findings, these researchers have pointed at the important contribution of a high-quality spermatozoon, scored in a high magnification microscope, for the injection of oocytes aspirated from women of 30 years and older [35]. This outcome is not surprising because younger oocytes are capable of "repairing" the DNA of the injected spermatozoon. In ICSI, the direct sperm deposition probably does not have a delay in the cell cycle, as it might happen due to insemination in vitro of the oocytes. The extra time helps to save maternal ribonucleic acid (mRNA), which partially might overcome epigenetic defects [11, 16, 19, 64].

Embryo chromosomal status was examined in couples who underwent their first IVF-PGS cycle for aneuploidy due to advanced maternal age [65]. Couples were randomly addressed to routine ICSI or IMSI (n = 60). All cases of sperm concentration less than 1×10^6/ml and sperm motility less than 20% were excluded from the study to minimize the influence of male factor infertility.

There was an increased incidence for sex chromosome aneuploidy in ICSI embryos when compared with IMSI (23.5% vs. 15.0%, respectively). IMSI was associated with a lower risk of sex chromosome abnormalities (odds ratio 0.57; confidence interval 0.37–0.90). The incidence of chaotic embryos was also higher with the ICSI procedure in comparison to IMSI (27.5% vs. 18.8%). An unexpected difference in gender incidence rates of euploid embryos was detected. The latest was supported by Setti et al., when a higher incidence of XX embryos derived from IMSI cycles in comparison with ICSI was noticed (66.9% vs. 52.5%, respectively) [42]. It is possible that IMSI-selected "normal" spermatozoa may carry a higher proportion of the X chromosome, which might lead to such findings.

Data also demonstrated a consistent decline in semen quality, as reflected by morphological evaluation by high-power microscope magnification, with increased age, suggesting the use of IMSI as routine in the older group of patients [66, 67].

7.1. IMSI and paternal age

Regarding the question of sperm quality in correlation to male age, it was described that increased male age is associated with a decrease in semen volume of 3–22%, a decrease in sperm motility of 3–37%, and a decrease in percentage of normal sperm of 4–18%, when comparing 30-year-old men with 50-year-old men, with no consistent effect on sperm concentration. Moreover, with control for a female partner, a relative decrease in pregnancy rates of 23 and 38%, increased risks for subfecundity ranging from 11 to 25%, and relative increase in months to achieve pregnancy up to 20% were found, comparing men <30 years old with men >50 years old, respectively [66]. Recently, IMSI provided remarkable information. Considering assessment of semen samples from 975 men who underwent IMSI, two forms of spermatozoa were considered: normal spermatozoa and spermatozoa with large nuclear vacuoles (LNV) [67]. At least 200 spermatozoa per sample were evaluated and the percentages of normal and LNV spermatozoa were determined. The subjects were divided into three groups according to age: Group I ≤ 35 years old; Group II: 3640 years; and Group III ≥ 41 years. Ratio of normal sperm cells in the older group (Group III) was lower than in the younger groups (I and II; $P < 0.05$). Percentage of LNV spermatozoa was higher in the older group (III) than in the younger (I and II) groups ($P < 0.05$). Regression analysis demonstrated a decrease in the incidence of normal sperm with increasing age ($P < 0.05$; $r = -0.10$). There was a positive correlation between the percentage of spermatozoa with LNV and male age ($P < 0.05$, $r = 0.10$).

These results demonstrated a consistent decline in semen quality, as reflected by morphological evaluation following IMSI with increased age, and support the routine use of IMSI for ICSI as a criterion for semen analysis in older group of patients.

8. Conclusions

The introduction to IMSI enabled to morphologically evaluate the individual motile sperm cell prior to its injection into the oocyte. The possibility to correlate each injected spermatozoon

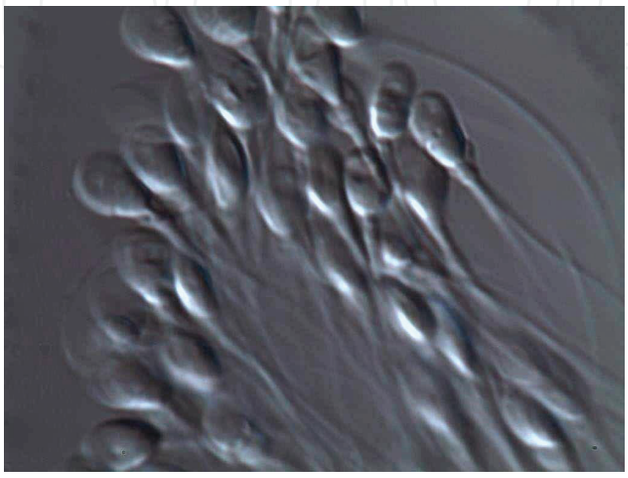
to the specific in vitro developing embryo has led to a better understanding of which sperm characteristics should be examined. Selecting a good-quality spermatozoon with normal morphology by using IMSI might be beneficial to embryonic development and to increased implantation and pregnancy rates. Taking into consideration the vast amount of knowledge accumulated, it seems that there is no advantage to recommend IMSI as a routine procedure for the entire populations referred to IVF-ICSI. In regard to the aforementioned studies and the experience accumulated, the authors pointed out that the following populations will have higher chances to conceive, while addressed to IMSI are couples with repeated implantation failures, cases of severe male factor infertility, advanced male age (>41 years old), and advanced maternal age (>30 years old). However, the latest should be considered in combination with deteriorated sperm quality.

It is also suggested to perform an independent observational diagnosis, using high magnification for the motile spermatozoa on a processed specimen prior to the time of the ICSI/IMSI procedure. This gives the laboratory an idea about the percentage of high-quality spermatozoa. In normospermia cases, according to routine sperm analyses, it is recommended to refer the couples to IMSI only when IMSI pre-analysis demonstrated less than 7% of high-scored (score 4–6) motile spermatozoa (Barak and Ellenbogen, personal communication). Moreover, according to the current knowledge no prenatal or postnatal complications in the mothers and offspring were observed following the IMSI procedure. The effectiveness of IMSI is still controversial mainly due to variations in inclusion criteria, stimulation protocols, sperm and oocyte qualities, and many additional confounding variables frequent in the IVF cycles.

However, there is no doubt that usage of the IMSI technique definitely opened a wider door for the hope of couples in their journey to fulfill their wish for a child. Further investigations should take place, to improve our knowledge in using this technique.

A. Appendix

General view of spermatozoa during IMSI.



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