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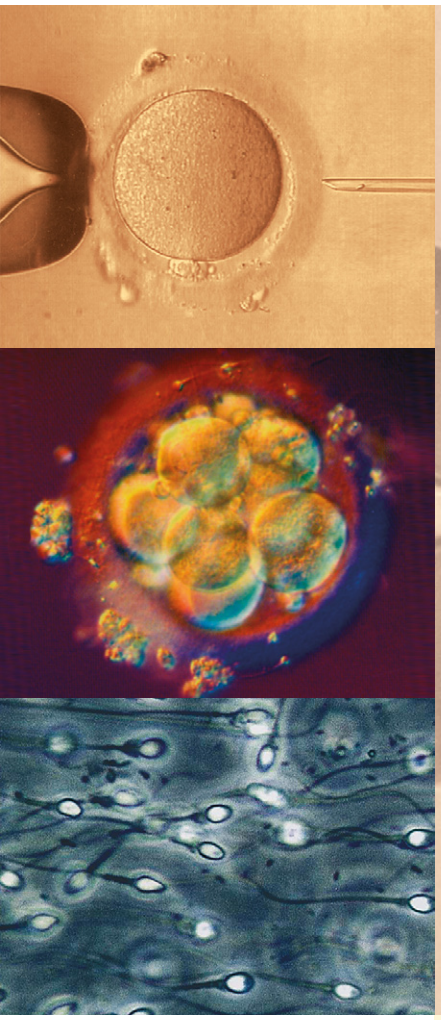

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Sperm Selection in ART

M. Montag, B. Toth, T. Strowitzki

Selection of sperm is a crucial part in assisted reproductive treament (ART). Sperm preparation methods do mainly differentiate according to sperm motility and are indispensable for therapies like intrauterine insemination, in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). Although in the beginning of the era of ICSI andrology was thought to play a minor role, ICSI has offered new options by correlating the treatment outcome to parameters of the individual applied spermatozoon. Hence the possibility for selecting spermatozoa has shifted from parameters which characterize the entire sperm cohort to a single-sperm specific assessment technology. Consequently, sperm selection is a topic which is intensively discussed nowadays. This article gives a comprehensive overview of the technologies which can be applied today and give a prospective on future techniques. **J Reproduktionsmed Endokrinol 2012; 9 (6): 485–9.**

Key words: sperm, morphology, viability, DNA integrity, selection

Introduction

Following the first successful in-vitro fertilization (IVF) in 1978, different possibilities to select and identify the most viable and fertilization-competent spermatozoa were explored. IVF allowed for the first time to determine the efficacy of a biological process which was previously hidden according to the fertilization rate. Sperm preparation and selection was at that time focused on separating the motile spermatozoa from the immotile ones, either by using the swim-up procedure or by preparation with a density gradient.

It was the introduction of intracytoplasmic sperm injection (ICSI) in 1992 [1], which forced the discussion on the role of andrology in assisted reproductive treatment (ART). Initially the success of ICSI provoked questions on the benefit of andrology [2, 3], especially regarding the value of conventionally assessed sperm characteristics like concentration, motility and morphology [4]. However, the technical background of ICSI, namely the injection of a single, selected spermatozoon, soon allowed much deeper insights into the possible benefits of individual sperm parameters for sperm selection. In addition, the improvement in blastocyst culture [5] even allowed to investigate long term effects of sperm characteristics on in-vitro culture and embryo development.

This contribution is focused on the application of recent strategies in sperm selection. The value of these strategies will be discussed and some new, evolving technologies will be presented.

Selection of Viable Spermatozoa

The major sperm selection criterion which is applied by every embryologist during ICSI is sperm viability, which is usually expressed by sperm motility. Only the injection of viable sperm will normally result in the initiation of the fertilization cascade resulting in the embryo development. Immotile spermatozoa can be found in testicular sperm preparations, however, some patients present ejaculates with 100% immotile spermatozoa. Hence a proper strategy is needed in order to identify those which are still viable and can be used for ICSI.

Several possibilities are available nowadays and two major strategies can be distinguished. One aims towards enhancing motility in sperm by activating the mitochondrial energy complex. The other one is focused on detecting spermatozoa with intact membranes as a characteristic of sperm viability.

Motility can be triggered in immotile sperm by the addition of certain substances which interact with the sperm at the mitochondrial level. Initially pentoxifylline was used for this purpose [6]. However, when pentoxifylline is carried over into the oocyte during the injection procedure, it can be detrimental at certain concentrations as suggested by some animal studies [7].

Recently another substance with a similar mode of action was introduced: theophylline. This molecule has been tested in human IVF and is nowadays widely used to stimulate immotile sperm prepared from ejaculates or testicular biopsies [8]. Usually sperm motility starts within 5–15 minutes after addition of theophylline, however, the effect may only last for a limited time period.

In case that theophylline may not result in revitalizing spermatozoa, another option is to investigate membrane integrity by using the so-called hypoosmotic swelling (HOS) test [9] which reflects damage of the sperm tail membrane. Changing the osmotic environment of the sperm suspension, forces the sperm to adapt to this change. In case of a sperm with a damaged membrane, the osmotic equilibration does occur very fast and without any notice. However, in spermatozoa with an intact membrane the difference in osmolarity results in the release of water from the sperm cytoplasm and these spermatozoa show an osmotic reaction which is chracterized by a curling of the sperm tail. Several publications reported on the possibility

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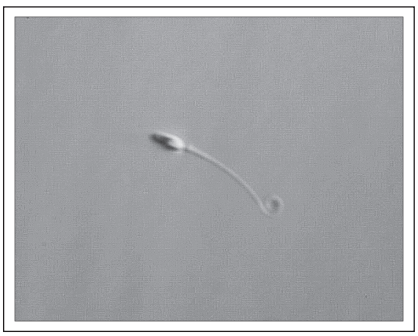

Figure 1. Sperm viability as proven by laser. A viable sperm is shown after the application of a single laser shot. The laser shot caused a curling of the end of the sperm tail which is indicative for sperm viability. Non-viable sperm show no reaction of the sperm tail. Despite the curling of the sperm tail these sperm can be used for ICSI without any further treatment as the laser causes also a permeabilization of the sperm plasma membrane [15].

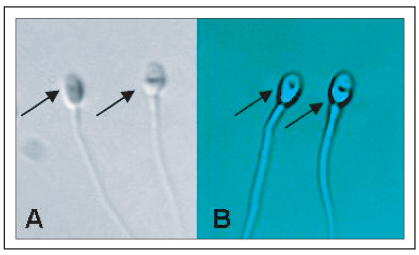

Figure 2. Comparison of digital enhanced high magnification image versus conventional Hoffman modulation contrast. This figure shows a picture of two sperm taken with a standard Hofmann 40× objective **(A)** and a 63× objective with a high numerical aperture and using Cytoscreen **(B)** a digital image enhancement software (Octax, Bruckberg, Germany). The presence of a small vacuole in the head of the right sperm as visualized by digital enhanced high magnification can be seen in the standard Hofmann contrast picture as a irregularity in the sperm head. Reprinted from [23].

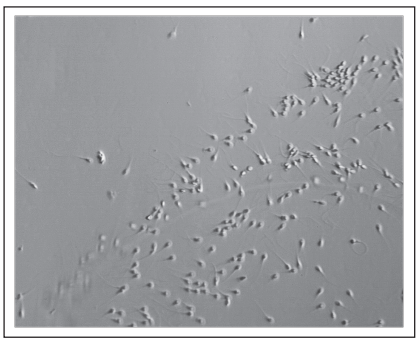

Figure 3. Sperm selection by hyaluronic acid binding. The figure shows a dish where the lower right part is coated with hyaluronic acid. Sperm with hyaluronic acid binding sites attach to the coated part and are expected to be mature and suitable for ICSI. Spermatozoa at the upper left corner have not bound and are still motile. These sperm are immature and should not be used for ICSI.

using the HOS test to identify viable sperm in testicular [10] as well as ejaculated preparations [11–12] and to use these selected spermatozoa for ICSI resulting in live birth.

Another test has proven to be effective which uses a laser to identify immotile but viable spermatozoa for subsequent ICSI [13]. This test applies a single laser shot to the far end of the sperm tail which causes a curling of the sperm tail in viable sperm only (Fig. 1). This curling reaction is similar to the one observed in the HOS test. The laser method for sperm selection prior to ICSI has resulted in high fertilization and cleavage rates in cases with immotile sperm in fresh testicular biopsy material as well in cases with immotile sperms [13, 14]. Laser viability testing has been developed from another method which was used for immobilization of spermatozoa prior ICSI instead of using capillaries [15].

Morphological Sperm Selection for Assisted Reproduction: MSOME/IMSI

ICSI allowed for the first time to inject spermatozoa with known morphological characteristics. This possibility was first applied by Bartoov and colleagues [16]. They created the term morphological sperm organelle examination (MSOME) which simply characterizes sperm morphology investigated at a high magnification using 100× oil immersion objectives and the classic differential interference contrast (DIC) in combination with glass bottom dishes. The most prominent morphological feature which they described were sperm head vacuoles. The application of MSOME to ICSI resulted in the name IMSI, intracytoplasmic morphologically-selected sperm injection.

Prior the introduction of IMSI, large retrospective studies on the possible correlation between sperm morphology and IVF outcome concluded, that morphology plays no role for ICSI [17] giving the idea that sperm selection based on morphology is of no benefit. This study, like many others investigating the effect of sperm morphology, used mean values of normal sperm for the correlation which was calculated during sperm analysis. However, IMSI enabled for the first time a closer look on the individual sperm cell used for injection by high magnification light microscopy.

Several studies showed a benefit of IMSI for different male factors in regard to higher fertilization, implantation and pregnancy rates as well as to lower miscarriage rates (retrospective studies [18, 19]; prospective study [20]). One study reported better embryo development and blastocyst formation following IMSI [21]. However, a more recent prospective randomized trial (RCT) found no benefit at all for unselected ICSI patients [22] and one further RCT was finished preterm due to non-inferiority of the study group receiving IMSI [Rienzi et al., personal communication]. Overall, a benefit by applying IMSI was not achieved by numerous investigators. A possible explanation for these diverging results could be the way in which conventional ICSI is performed in an individual laboratory and how experienced the embryologists are in performing the ICSI procedure and in particular the selection of spermatozoa for injection. Collecting sperm using a 20× objective is quite common and at that low magnification any morphological defects of the sperm head are difficult to detect. On the other hand the use of a well aligned microscope equipped with 40× Hoffman contrast optics and optimal adjustment of the optical beam does allow for detection of morphological alterations. Although these cannot be visualized in detail, the presence of defects at the sperm head as well as sperm head vacuoles can usually be seen to a certain extent. This is illustrated in a comparative analysis in Figure 2, where it is shown, that IMSI does not require 100× oil immersion objectives and Nomarski differential contrast.

Despite these controversial discussions, IMSI has sharpened the view of embryologists while selecting sperm for ICSI and definetly highlights the important role of andrology.

Sperm Selection by Hyaluronic Acid Binding

The use of hyaluronic acid as a sperm surface marker for sperm selection has a long standing background and can be attributed to the work of the group of Gabor Huszar and colleagues (reviewed by [24]). The ratio behind this approach

is that mature sperm has a different composition of the sperm plasma membrane in regard to some surface markers compared to immature spermatozoa. The clue to these findings was attributed to creatine phosphokinase which is present at increased levels in immature spermatozoa [25]. As it was found that sperm binding to zona pellucida in sperm binding assays contained lower amounts of creatine phosphokinase, a link was established between the remodelling of sperm plasma membrane and sperm maturation. This eventually led to the discovery of hyaluronic-acid binding sites on the surface of mature spermatozoa [26] and consequently to the development of diagnostic test procedures for identifying mature sperm. The principal background of these tests is, that those sperms which show binding to hyaluronic acid are the ones which should subsequently be used for ICSI (Fig. 3). To date the most common applied therapeutical options are dishes or slides coated with hyaluronic-acid or a ready-to-use solution which contains hyaluronic-acid and can be applied instead of a viscous (PVP-containing) medium for the direct isolation of sperm prior to ICSI.

Interestingly, the remodelling of the sperm plasma membrane seems also to correlate with some other prognostic factors like DNA integrity [27] and sperm aneuploidy [28]. However, at present the clinical benefit of sperm selection by hyaluronic acid binding is only available in abstract form [29] and further data are still awaited.

Sperm Selection by Magnetic-Activated Cell Sorting

Besides the selection of spermatozoa for binding sites to hyaluronic-acid, another surface marker allows selection of non-apoptotic spermatozoa. The presence of phospatidylserine residues at the outer sperm membrane is an early marker of sperm apoptosis [30]. Annexin-V protein has the ability to bind to phosphatidylserine and this has led to the development of a sperm selection methodology which uses Annexin-V coated microbeads. The principle behind this procedure is that coated microbeads are mixed with a sperm sample and apoptotic sperm will bind to the beads. Using a magnetic-activated cell sorting column the beads with the bound dead or apoptotic sperm will be retained whereas the nonapoptotic Annexin-V negative sperm will flow through the column. It was found, that spermatozoa isolated by this technology show a higher morphological grade and integrity [31]. This selection procedure might also enrich sperm, which are capacitated and have undergone the acrosome-reaction [32].

Table 1. Sperm Selection Technologies and their Predictive Relationship

Sperm Selection Method	Sperm Function/Characteristics
Morphology (MSOME)	Sperm aneuploidy [44] DNA integrity [44]
DNA integrity	Morphology [38, 45]
Hyaluronic binding	Morphology [46]; MSOME [47] DNA integrity [27] Sperm aneuploidy [28]
Sperm birefringence	Morphology [34, 48] DNA integrity [49]
Electrophoretic separation	Morphology [36] DNA integrity [36]
Magnetic activated cell sorting (MACS)	Motility [50] DNA integrity [51]

Sperm Selection by Polarization Microscopy

Sperm birefringence has recently been used as a new selection method for spermatozoa. Using a special type of polarization microscopic set-up, different birefringence properties were described in human spermatozoa [33]. Interestingly, sperm birefringence was found to be linked to the acrosomal status where the more beneficial birefringence patterns were characteristic for spermatozoa which had undergone the acrosome reaction [34]. According to the data of a prospective randomized study, birefringence based sperm selection resulted in embryos which were highly viable and implantation-competent [35].

Sperm Selection by Electrophoresis

Electrophoresis is another new technology which does allow separation of mature from immature sperm cells, where mature sperm show a negative charge of the sperm plasmalemma. The degree of maturity and net charge has been correlated with the presence of a protein called CD52 on the surface of spermatozoa. This approach has been already used in a clinical setting [36] and further investigations showed that sperm prepared by this methodology are equivalent to those isolated by density gradient centrifugation [37]. However, further trials are still indicated in order to identify patients which benefit.

Sperm Selection for DNA Integrity

The importance of sperm DNA integrity has been discussed for several years [38] as DNA damage, either single stranded or double stranded bears the risk of inducing apoptotic reactions and hence cell death. Therefore direct selection for DNA integrity would be of great interest (reviewed by [39]). Numerous tests have been developed and applied to investigate DNA damage in human spermatozoa. Most of the exisiting methods assessing DNA damage have the great disadvantage that they only give a measure of a sperm sample but not of individual sperm. Moreover, most sperms are either dead or contaminated with a dye after assessment. However, there is no diagnostic test in place which allows selecting a single individual sperm immediately and under constant visualization by microscopy.

At present there are two projects which aim to solve this problem. One approach uses a peptide from the terminal end of the P53 protein recognizing damaged DNA even in living intact cells. Such a test would allow for coupling diagnostics and subsequent therapeutic intervention in one step and could probably be the base for proper studies to identify patients which benefit of DNA integrity testing [Wells, personal comunication]. Another project is based on Raman spec-

troscopy which has been recently proposed as a non-invasive method to look at spermatozoa [40, 41] and to investigate damage in sperm DNA [42]. Both projects are ongoing and for now we have to rely on those tests which, as a side effect, seem to correlate with DNA integrity like hyaluronic-acid binding, polarization microscopy and IMSI.

Summary and Conclusions

The success rates in ART reached a plateau somewhere around 25–30% according to recent international registries [43]. In order to enhance the overall success rates, further efforts have to focus on how to select the best gametes and embryos. Therefore the selection of the "one and only proper sperm cell" is a major task for the near future.

Although numerous selection methods are available already, the scientific background of some methods is poorly understood. There are exciting technologies which work with a sperm sample but not with individual sperm cells. Other technologies are letal to the sperm cell or cannot yet be applied to live spermatozoa. In addition, several other functional sperm parameters are known and can be assessed by diagnostic investigations like sperm oocyte activation capacity, but corresponding selection methods are still missing.

Interestingly, some of the selection methods which are in place are based on one special aspect but show a high correlation with other tests as well (Tab. 1). Thus one of the major requirements would be to develop a selection method which is based on a sound diagnostic background, preferably covers various aspects of sperm function and allows for an immediate use of the selected sperm cell. Naturally, such an approach can only be realized in combination with ICSI.

In summary, we will need further research which is focused on diagnostic as well as therapeutic tools to overcome the problems we may encounter in certain ART treatment cycles without forgetting the patient and the possible options which we may have for treatment.

Conflict of Interest

All authors report no conflict of interest.

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