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Methods for Sperm Selection for In Vitro Fertilization

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1. Introduction

The outcome of assisted reproductive technologies (ARTs) depends mostly on the quality of input material (oocytes and sperm) used in these procedures. The number of transferable embryos produced in these programs depends on both provision of high quality mature oocytes and adequate numbers of good quality spermatozoa capable of supporting embryo development to term. Semen samples are cellular mixtures composed of: precursor germ cells, subpopulations of viable and nonviable spermatozoa, variable amounts of debris, and multiple leukocyte subtypes, all suspended in seminal plasma (SP). Based on these characteristics and the heterogeneity of the sperm population within the ejaculate, several separation techniques such as swim-up and density gradients (e.g. Percoll®) have been developed. These techniques not only allow for selection of sperm with enhanced motility but may also be used to remove the extender and dead cells (up 50% of total) present in frozen-thawed sperm samples. In addition, selection of normal spermatozoa is of upmost importance in cases of male infertility caused by semen deficiencies characterized by teratospermia, asthenozoospermia and/or oligospermia.

The ideal protocol for enrichment/selection of sperm cells with high fertilizing ability should be: a) non-toxic for spermatozoa, b) easy to perform and inexpensive, c) able to support high-throughput sample processing, d) capable of selecting the best sperm subpopulation for ARTs, leaving behind, seminal plasma, extender (in case of frozen semen) and bioactive substances and cells (leukocytes) that could damage sperm cells. Despite the efforts invested in developing an ideal sperm selection technique by laboratories around the word, to date no single sperm selection protocol meets all desirable characteristics mentioned above. Today, it is recognized that the sperm is more than a mere DNA delivery vehicle to the oocyte; there is evidence that these highly specialized cells play a role far beyond the fertilization process by contributing paternal mRNAs, which it is believed to be crucial for normal early and late embryonic development (Barroso et al., 2009). Therefore, development of systems that allow for identifying the best spermatozoa for fertilization would contribute to improve the currently low live birth rates achieved by ARTs (Wright et al., 2008).

Most sperm selection protocols in use today for ARTs fall in one of the following categories: sperm migration, filtration, density gradient centrifugation or a combination of these methods. During the decision-making process to select a sperm separation protocol is important to consider both the type of infertility and the particular assisted reproductive approach to be used to treat it. For instance, high sperm numbers with vigorous motility are required for successful intrauterine insemination. On the other hand, few motile sperm cells, in the order of thousands, are required for conventional IVF, and even fewer to perform ICSI.

It has been well documented in all mammal species studied so far that ejaculated spermatozoa are subjected to a natural-occurring sperm selection process in the female reproductive tract in order to maximize the chances of successful reproduction. This differential sperm transport favors the ascent of morphologically normal spermatozoa with enhanced fertilizing ability. These natural barriers encountered by spermatozoa in vivo are partially or completely absent when ARTs are applied. Therefore, there is real concern about the possibility of using spermatozoa with suboptimal fertilization and embryo development potential. This is especially true during ICSI in which a single spermatozoon is selected by the technician based solely on motility and morphology parameters. Experimental data indicate that normal sperm morphology is not necessarily associated with DNA integrity (Avendano & Oehninger, 2011) what raises concerns about potential transmission of DNA alterations to next generations.

Knowledge from the natural sperm selection mechanisms that occur in vivo and insights from research in the area of the molecular mechanism that govern sperm physiology will provide basic information for improving current methods of sperm selection and developing novel procedures for accurately select the best spermatozoa for ARTs. In particular, the development of technologies in which a spermatozoon is mechanically introduced by the embryologist into the oocyte, bypassing all natural barriers for sperm selection, emphasize the need for more accurate sperm markers of fertilizing and normal developmental potential.

In the first section of this review we will describe the principles and discuss advantages and disadvantages of established sperm selection methodologies currently in use in the clinical setting. In the second part of this chapter, we will introduce advanced sperm selection techniques and emerging approaches to enrich sperm samples for ARTs.

2. Sperm selection techniques

2.1 Sperm washing

Sperm washing is a simple method which involves the centrifugation of the semen sample once or twice in order to pellet the sperm cells and remove the seminal plasma. The pellet is resuspended in appropriate media and used for ARTs (Bjorndahl et al., 2005; Edwards et al., 1969; Lopata et al., 1978). One of the major disadvantages of this method for sperm preparation is the oxidative cell damage caused by reactive oxygen species (ROS) generated in packaged cells after centrifugation. Sperm plasma membrane contains high amounts of poly-unsaturated fatty acids which are highly susceptible to lipid peroxidation by ROS. Consequently, SP provides ROS metabolizing enzymes and small molecular mass, free radical scavengers such as vitamin C and uric acid to protect germinal cells from damage.

Therefore, sperm function and DNA integrity can be compromised when SP is removed from the ejaculate.

Although centrifugation is useful to remove the SP, the overall sperm quality, in terms of motility remains unaltered or reduced since both motile and immotile fractions of sperm cells are subjected to the gravitational force, with the associated risk of removing part of the motile fraction with the supernatant that is discarded. In addition, centrifugation has been reported to be responsible for chromatin damage in human and stallion spermatozoa (Edwards et al., 1969; Morrell et al., 2011; Mortimer, 2000).

Despite numerous drawbacks associated with this approach, sperm washing is routinely used in the livestock industry to remove most of SP prior to adding the extender for cryopreservation. Extenders for animal semen such as milk- or egg yolk-based extenders, typically contain antioxidants which may counteract oxidant metabolites released during the procedure (Morrell et al., 2011).

2.2 Sperm migration

Mahadevan & Baker (1984) developed the classical washing swim-up (WSU) method, which is easy to perform and very cost effective. It is based on spermatozoa self-propelled active movement from a single centrifuged, pre-washed cell pellet, into an overlaying medium which serves as a hospitable environment for healthy sperm. Normal spermatozoa move away from seminal plasma, but those with tail abnormalities are not capable of migrating into the swim-up medium. Only a small fraction of total motile sperm is recovered by the WSU methodology, therefore its use is mostly restricted to ejaculates with high sperm counts and good motility (Mahadevan & Baker, 1984). The WSU is currently the standard technique used in IVF laboratories for patients with normozoospermia and female infertility. Since WSU includes a centrifugation step, it raises concern about the possibility of sperm plasma membrane and DNA damage due to ROS buildup from pelleted sperm cells, debris and leukocytes (Ford, 1990). To reduce ROS generation and the consequent damage to sperm cells, a modification of the classic WSU procedure was introduced. In this alternative swim-up procedure the liquefied semen sample is directly subjected to swim-up avoiding the initial centrifugation step. Here, sperm population is either underneath, on top or to one side of the migration medium (Mortimer, 2000). Some studies show significantly better midpiece and tail morphology after swim-up than after washing (Hallap et al., 2004).

It has also been shown that swim-up directly from semen into a migration medium supplemented with highly purified hyaluronic acid (HA) favors the selection of motile sperm with intact membranes, resulting in a higher pregnancy rate in clinical IVF programs (Jakab et al., 2005; Wikland et al., 1987).

Side migration is another technique based on the sperm ability to selectively move from one point to another (Hinting & Lunardhi, 2001). Unlike swim-up in which sperm cells move upwards into the medium, in the side migration technique (SMT) sperm move horizontally leaving behind immotile spermatozoa, round cells and debris. According to Hinting & Lunardhi (2001), SMT is an effective and physiological approach to obtain sperm for ICSI from poor-quality semen samples. In their study, semen from men affected with oligozoospermia (sperm concentrations of less than 20 million spermatozoa/ml) were subjected to side migration. The subpopulation of spermatozoa recovered had better

morphology, viability, membrane integrity and nuclear chromatin integrity compared with those selected by traditional WSU and Percoll® gradient columns (PGC) (Hinting & Lunardhi, 2001). However, the number of sperm recovered is low, what limits the use of this technique to select sperm for ICSI procedures.

2.3 Sperm sedimentation

Tea et al. (1984) developed a very gentle separation method which combines swim-up from liquefied semen with a sedimentation step. The sperm selection is accomplished by using a special glass or plastic tube with an inner cone, design that allows for only those spermatozoa capable of swimming out from the liquidized semen to sediment in the inner cone. Since no centrifugation steps are required, generation of ROS is minimized and so is the sperm damage, rendering a very clean fraction of highly motile spermatozoa. The main disadvantage of this method is the very low recovery rate which makes it unpractical for intrauterine insemination or IVF.

2.4 Filtration

Glass wool filtration is a very simple, but more expensive procedure, which yields a higher total number of motile spermatozoa compared with those in the swim-up or migration centrifugation protocol, since the whole volume of the ejaculate can be filtrated. This method is also effective at eliminating leukocytes and cell debris, reducing ROS production and ROS-induced sperm damage (Henkel et al., 2005). A major advantage of this approach is that it selects normally chromatin-condensed spermatozoa, a parameter considered as predictive of fertilization ability in vitro.

Motile and viable spermatozoa from poor-quality semen can be recovered using a column of glass beads. This procedure is quick and simple and results in enrichment of the population of spermatozoa of interest. Due to the high sperm recovery, glass filtration method is especially useful for intrauterine insemination. Unluckily, the potential risk of glass bead spilling over into the insemination media has precluded its widespread use in ARTs. An alternative filtration method uses columns of Sephadex beads to produce high yields of morphologically normal sperm cells.

2.5 Density gradient centrifugation

In this procedure, diluted semen is placed on top of a conical centrifuge tube containing increasingly dense layers of a liquid solution called density medium. In the standard procedure for sperm preparation two layers of density medium are used. After centrifuging, highly motile sperm cells are enriched in the soft pellet at the bottom of the tube. The recovered sperm pellet is then washed by centrifugation to remove the density medium. Sperm damage during density gradient selection has been attributed to ROS accumulation associated with multiple centrifugation steps.

Polyvinylpyrrolidone (PVP)-coated silica particles (Percoll®) has been extensively used as a density medium to prepare fresh or frozen sperm specimens for human and animal ARTs. Vast experimental and clinical data support the effectiveness of the Percoll-based gradient methodology to produce viable, highly motile, morphologically normal populations of

spermatozoa for intrauterine insemination, gamete intrafallopian transfer, and conventional IVF and ICSI (Moohan & Lindsay, 1995). Studies that compared WSU with density gradient procedure indicated that the latter is capable of yielding sperm populations with higher percentages of morphologically normal forms and nuclear integrity (Sakkas et al., 2000; Tomlinson et al., 2001). However, exposure of sperm to Percoll® can damage sperm membranes and there exists the risk of contamination of Percoll® with endotoxins, which in turn can cause an inflammatory response in the female reproductive tract. This led to the withdrawal of Percoll® from the market for clinical use in human ARTs in 1996 (Henkel & Schill, 2003).

Alternative commercial density gradients have been developed and are commonly used in human assisted reproduction: Nycodenz® (Nyegaard & Co., Oslo, Norway), PureSperm® (NidaCon Laboratories AB, Gothenburg, Sweden), IxaPrep® (MediCult, Copenhagen, Denmark), SilSelect® (FertiPro N.V., Beernem, Belgium), and ISolate® (Irvine Scientific, Santa Ana, CA, USA). Among these, PureSperm® IxaPrep®, SilSelect®, and ISolate® are silane-coated products which have been promoted as being safer than the PVP-coated particles (Percoll®). These products are non-irritating and have been approved for human in vivo use as they are all bioassay endotoxin-tested and easy to wash out.

2.6 Advanced and emerging sperm separation techniques

In this section we introduce advanced sperm separation/selection procedures. Based on the main criteria used to select a sperm subpopulation, these procedures can be classified as: selection by differential sperm surface charge (electrophoretic separation and Zeta potential), selection of non-apoptotic spermatozoa (magnetic-activated cell sorting), selection based on the sperm membrane maturity (hyialuronic-acid sperm binding) and selection based on ultramorphologic criteria (real-time motile sperm organelle morphology examination –MSOME-, intracytoplasmic morphologically selected sperm injection –IMSI- and ICSI using polarization microscopy). Each advanced methodology has been subjected to prospective studies to determine its ability to affect sperm quality, fertility rate and clinical pregnancy rates. In general, application of these procedures have improved assisted reproductive technology (ART) outcomes, however, to date the number of clinical trials are insufficient to draw definitive conclusions.

Finally, we will comment on emerging approaches for sperm selection which are currently in a developmental phase. These include: Raman spectroscopy, confocal light absorption and scattering microscopy –CLASS- and sperm chemotactic-based methods.

2.6.1 Sperm surface charge for sperm selection

There are two different approaches to select sperm based on the differential net electric charge on the sperm plasma membrane: electrophoretic system (SpermSep® CS-10, NuSep Ltd., Frenchs Forest, Australia) (Ainsworth et al., 2005) and zeta potential method (Chan et al., 2006).

2.6.1.1 Electrophoretic system

The electrophoresis-based technology was developed at Dr. Aitken´s laboratory in Australia (Ainsworth et al., 2005) and later commercialized by NuSep Ltd. as Microflow® CS-10

(renamed to SpermSep® CS-10). This device uses an electric field to separate sperm cells based on size and electronegative charge. It is composed of four chambers: two outer chambers and two inner chambers (incubation and collection). The outer chambers (filled with buffer) house the platinum-coated titanium mesh electrodes. A polyacrilamyne membrane separates the outer chambers from the inner chambers allowing for the movement of small molecules, water and ions between them. The inner chambers comprise the inoculation compartment and the collection compartment separated by a polycarbonate separation membrane which pore size excludes leukocytes and precursor germ cells that normally contaminate semen samples. The semen specimen is loaded into the incubation chamber and allowed to equilibrate for 5 min before applying a current of 75 mA and variable voltage (18-21 V). The selected sperm subpopulation is recovered from the collection chamber after 5 min of application of the electric field and it is ready for ARTs.

There is evidence that the electronegativity on the sperm surface indicates normal differentiation and is associated with CD52 expression on sperm membrane (Schroter et al., 1999) and other glycoproteins (Ainsworth et al., 2011). These observations and the fact that CD52 is correlated with normal sperm morphology and capacitation (Giuliani et al., 2004), may account for the ability of the electrophoresis separation method to select sperm with significantly improved morphology with low levels of DNA damage (Ainsworth et al., 2005). Key futures of the electrophoresis system that make it attractive for ART laboratories are: the whole process of selection can take only a few minutes and the generation of ROS is minimized because of lack of centrifugation steps. On the other hand, the cost associated with acquisition of the electrophoresis separation device may be prohibitive for andrology laboratories with limited resources.

The first live birth from an embryo conceived with a spermatozoon selected by the novel electrophoretic approach was reported in 2007 (Ainsworth et al., 2007). The study involved a couple with long-term infertility associated with extensive sperm DNA damage. Later, a prospective controlled trial was performed to demonstrate that the membrane-based electrophoresis system is as effective as and considerably faster than the DGC to prepare spermatozoa for both IVF and ICSI (Fleming et al., 2008).

2.6.1.2 Zeta potential method

Sperm cells can be selected based on their negative zeta electrokinetic potential (Chan et al., 2006) which is the overall charge a particle, in this particular case a spermatozoon, acquires in a specific medium. A mature sperm cell has a negative zeta potential of -16 to -20 mV (differential potential between the sperm membrane and its surroundings) (Ishijima et al., 1991). The zeta potential method is very simple to perform and it does not require special equipment, therefore it is inexpensive. Briefly, washed sperm in serum-free medium is introduced in a conical tube which has been positively charged by rubbing or rotating the tube on a latex glove. Electronegatively charged sperm (mature) attach to the walls of the tube by electrostatic forces and the non-adherent sperm fraction and other contaminants are removed by inverting the tube. Selected adherent sperm cells are recovered by rinsing the tube with serum-supplemented medium.

Regarding the morphology and functional characteristics of sperm selected by zeta potential, experimental data indicated that this method advantages the conventional DGC in terms of percentage of morphologically normal sperm, hyperactivation, DNA integrity and

maturity, but not motility (Chan et al., 2006). Results from a randomized prospective study with sperm selected with a combination of DGC/zeta potential or DGC alone previous to ICSI indicated that the combination may increase fertilization rates and possibly pregnancy rates in infertile couples associated with male factor infertility (Kheirollahi-Kouhestani et al., 2009). However, definitive data that demonstrate the benefits of applying the zeta potential approach to select sperm for human assisted conception is still missing.

2.6.2 Selection of non-apoptotic spermatozoa

2.6.2.1 Magnetic-activated cell sorting (MACS)

The externalization of the phospholipid phosphatidylserine (PS) to the sperm plasma membrane is a characteristic feature of the apoptotic phenomenon that occurs early during the process of sperm cell death. This basic knowledge has prompted investigators to develop a magnetic-based selection system for sperm cells that can separate early apoptotic from non-apoptotic germ cells (MACS, Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). Since externalized PS has high affinity to Annexin V, apoptotic sperm cells bind to Annexin V-conjugated paramagnetic microbeads. The magnetically labeled sample is passed through a magnetic column where magnetically labeled apoptotic or dead spermatozoa are retained in the column while the unlabeled non-apoptotic spermatozoa are collected in the flow-through for further processing for ARTs (Grunewald et al., 2001; Manz et al., 1995).

Despite magnetic cell sorting method is highly effective at removing apoptotic sperm cells, unfortunately it is not able to eliminate leukocytes, immature germ cells, seminal plasma and other contaminants from the semen sample. This is the reason why MACS separation is normally performed in conjunction with DGC (Said et al., 2005a; Said et al., 2005b). Repeated centrifugations and resuspensions associated with DGC can cause sperm loses imposing a limitation for semen samples characterized by limited sperm counts (Said et al., 2008).

Non-apoptotic markers in the MACS-selected population such as high mitochondrial potential and low caspase activation are consistent with the known selection criterion of this methodology. In addition, sperm sample parameters that are improved in the subpopulation selected by MACS include: sperm motility and morphology, sperm DNA damage and hamster oocyte penetration potential of spermatozoa (Lee et al., 2010; Said et al., 2006a; Said et al., 2006b). Clinical pregnancy data collected so far indicate that the use of MACS may be of especial value for cases of male infertility associated with high incidence of apoptotic and DNA damaged sperm (Dirican et al., 2008).

2.6.3 Selection based on sperm membrane maturity

2.6.3.1 Hyaluronic acid sperm binding

The presence of HA binding sites on sperm outer membrane is regarded as a sign of sperm maturity, and constitutes the basic principle for a sperm binding assay (Jakab et al., 2005). In this assay, HA is immobilized on a solid surface (polystyrene culture dish) and the washed sperm sample is allowed to interact with the HA coated surface for 15 min. An individual sperm attached to the dish is picked up with the ICSI pipette and used for oocyte injection.

As HA is a natural occurring compound present in cervical mucus, cumulus cells and follicular fluid, the binding method is considered to have minimal biosafety risks for both the embryo and the patient.

The device called PICSI® (preselected intracytoplasmatical sperm injection), commercialized by ORIGIO MidAtlantic Devices Inc. (Mt Laurel, NJ, USA), uses a conventional polystyrene culture dish enhanced with tree microdots of hyaluronan where the sperm suspension is added.

Sperm maturity has been associated with certain desirable sperm traits such as: improved viability and motility, intact acrosomes, lower caspase-3 activation and lower frequency of chromosomal aneuploidies (Huszar et al., 2007; Huszar et al., 2003). Studies documenting the use of sperm selected by HA method in the clinical ART setting are still scarce and somehow contradictory. While one study reported significantly increased fertilization rate of oocytes injected with HA-selected sperm and only a marginal effect on pregnancy rate (Nasr-Esfahani et al., 2008), in other studies by Permegiani et al. (2010a; 2010b) oocytes injected with sperm selected by the binding method originated better quality embryos but no effect was detected on fertilization and pregnancy rates.

2.6.4 Selection based on live sperm morphology

It has been long recognized that sperm morphology is one of the major determinants of male fertility both in vitro and in vivo. ICSI is an assisted reproductive technique that is gaining acceptance for treatment of different forms of male infertility. In this procedure, a sperm cell is selected by the embryologist based on sperm morphology and motility and introduced into the mature oocyte, bypassing all natural selection barriers at fertilization. However, sperm evaluation at x 400 magnification (which is the standard magnification used to select sperm for ICSI) is unable to provide enough resolution for an accurate sperm morphological assessment.

With the objective of improving accuracy of sperm selection based on morphological features, Bartoov et al. (2002) developed a method for real-time sperm evaluation known as motile sperm organelle morphology examination (MSOME). MSOME sperm evaluation is performed in an inverted light microscope equipped with high-power differential interference contrast optics (Nomarski/DIC; magnification x150) enhanced by digital imaging (magnification, ×44) to achieve a total magnification of over 6000. At this magnification, it is possible to define the morphological normalcy of five sperm organelles (acrosome, postacrosomal lamina, neck, tail and nucleus) as observed at high magnification. Among these organelles, evaluation of sperm nucleus (shape and chromatin content) by MSOME appears be the most important feature conditioning ICSI outcome (Bartoov et al., 2003). Intracytoplasmic morphologically selected sperm injection (IMSI) is a modification of ICSI, in which the injected spermatozoon is selected by the technician at high magnification using MSOME normalcy criteria. When these techniques are used correctly by trained personnel, a significant correlation between morphology and fertilization rate was demonstrated (Bartoov et al., 2002). In addition, pregnancy and live birth rates were significantly higher in the IMSI group compared with that in the conventional ICSI group, but IMSI failed to boost fertilization and cleavage rates and did not improve embryo

morphology. Similarly, Souza Setti et al. (2010) reported that IMSI improved pregnancy and abortion rates, but not fertilization rate. In conjunction, these results suggest that the sperm morphology traits that guide sperm selection during IMSI will have repercussions in late ART outcomes as evidenced by increased pregnancy and birth rates and diminished abortion rates.

Another optical system used to select live sperm for ICSI is based on birefringency (Gianaroli et al., 2008) generated by the incidence of polarized light on longitudinally oriented protein filaments on the postacrosomal region of the sperm (Baccetti, 2004). Sperm birefringency is evaluated with an inverted microscope equipped with polarizing and analyzing lenses. The proportion of birefringent sperm in a sample is correlated positively with sperm concentration, motility and viability (Gianaroli et al., 2008). In addition, using this optical system, it is possible to differentiate acrosome-reacted from acrosome-intact sperm before microinjection. It has been hypothesized that microinjection of acrosome reacted sperm during ICSI would improve the outcomes of this technique since it mimics more closely the natural phenomenon of fertilization. Clinical data collected so far support this hypothesis, as pregnancy rates originated from embryos produced with acrosome-reacted spermatozoa were significantly higher compared with those in the control group (ICSI with non-reacted spermatozoa) (Gianaroli et al., 2010).

2.6.5 Emerging methods for sperm selection

2.6.5.1 Raman spectroscopy

It has been documented that sperm cells with apparently normal morphology may have DNA fragmentation and other types of DNA damage (Angelopoulos et al., 1998; Avendano et al., 2009), which can affect embryo quality and pregnancy outcome if ICSI is performed with such defective sperm cells (Avendano et al., 2010). In light of the worldwide use of ICSI as major tool to treat infertility, the development of a technique that can non-invasively provide information about sperm chromatin packaging and nuclear normalcy before sperm injection would impact positively on ART outcomes. Micro-Raman spectroscopy holds promise to provide information about packaging of nuclear DNA in individual living sperm cells. Raman spectroscopy is a spectroscopic technique that examines the inelastic scattering of photons (a change in frequency of photons) caused by molecular bonds. The photons originated from a laser source are absorbed by the sample and then reemitted with a frequency different to that in the original source what is called Raman effect. Photons can lose part of the energy and are red-shifted or gain energy and are blue-shifted. In biological specimens, photon shifting provides information about conformation, composition and intermolecular interaction in macromolecules (e.g. DNA-protein). There are a few reports on application of this technique to study molecular interactions in individual sperm cells (Huser et al., 2009; Mallidis et al., 2011; Meister et al., 2010). Huser et al. (2009) used Raman spectroscopy to obtain spectra from individual human sperm and reported that there are vibrational marker modes that can be valuable to assess sperm chromatin packaging. Results from this study also indicate that the DNA packaging in sperm with abnormal shape differs from that in normal sperm. In other study (Mallidis et al., 2011), Raman spectra were obtained from individual sperm cells before and after exposition to UVB radiation. Through the analysis of the spectra it was possible to detect the sites and location of UVB-induced

sperm DNA damage (Mallidis et al., 2011). Further studies are warranted in order to establish a possible relationship between sperm DNA packaging/damage (as detected by Raman spectrometry) and sperm function at fertilization and beyond.

2.6.5.2 Confocal light absorption and scattering microscopy (CLASS)

Confocal light absorption and scattering microscopy (CLASS) is an optical system that combines confocal microscopy, a well-established high magnification microscopic technique, with light-scattering spectroscopy (Fang et al., 2007). This combination allows for observation of submicrometer structures in viable cells attaining the spatial resolution of electron microscopy without the need of contrasting agents which are required for conventional optical microscopy. Results from studies in biological systems demonstrated that through the use of CLASS technique it is feasible to monitor individual organelles, such as mitochondria, lysosomes and microsomes in living cells (Itzkan et al., 2007). To our knowledge there is no published work regarding the use of CLASS microscopy to study sperm ultrastructure.

2.6.5.3 Sperm chemotaxis

Mammalian spermatozoa have the ability to be actively guided to the egg (that resides at fertilization site) by mechanisms known as chemotaxis and thermotaxis (Eisenbach & Giojalas, 2006). Chemotaxis is the movement of cells following a concentration gradient of chemoattractans whereas thermotaxis is the movement of cells along a temperature gradient. Experimental data support the hypothesis that progesterone (at pM concentrations), secreted by oocyte cumulus cells, is the major chemoattractant for human (Teves et al., 2006) and rabbit spermatozoa (Guidobaldi et al., 2008). Since only a small fraction of capacitated spermatozoa are chemotactically responsive in in vitro assays, it is tempting to hypothesize that the population with enhanced ability to migrate to the chemoattractant source is endowed with superior morphologic/functional features. Based on this principle, a microchannel-based device to assess sperm motility and chemotxis has been recently developed (Xie et al., 2010). However, the impact of using chemotaxis-selected sperm on ART outcomes is currently unknown.

3. Conclusion

Since the world's first "test-tube" baby was born in Great Britain in 1978 (Steptoe & Edwards, 1978), we have witnessed a tremendous progress in the field of human ART which is reflected in the high rates of success accomplished in infertility treatments. Despite these advances, live birth rates achieved by assisted conception remain relatively low and could be improved (Wright et al., 2008). In light of the known influence of the fertilizing spermatozoon not only on early but also on late embryonic development, selection of the best sperm from heterogeneous sperm samples would impact positively on the outcomes of human ARTs. Accurate identification of normal/healthy spermatozoa is of especial importance during ICSI, in which a sperm cell is deliberately injected into the mature oocyte by the technician bypassing all natural barriers. There is great concern about the risk of using sperm with chromosomal abnormalities and/or damaged DNA what can lead to inadvertently transmission of genetic diseases to the offspring. Therefore, improvements of the available sperm selection techniques and/or development of new methods for precise sperm selection are highly desirable. Despite encouraging preliminary results obtained with

advanced sperm selection techniques, more research is warranted to address safety issues before widespread application of these methods. In this regard, animal models can provide answers to important safety concerns related to the introduction of advanced and emerging methods for sperm selection into human ART.

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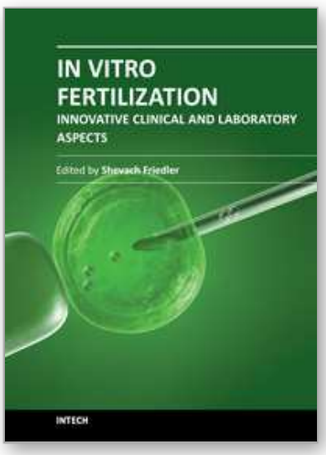

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The field of In Vitro Fertilization is a relatively new field in medicine, constantly on the move. This field is an exquisite example of the vast power in the complementary use of basic research with clinical practice and opened a new route of great basic and clinical research possibilities. The knowledge base that allowed the accomplishment of the idea of in vitro fertilization and embryo transfer has much developed since. The vast body of research pertaining to this field allowed deepening our understanding in the processes related to reproduction. In this book on in vitro fertilization we present new and interesting updated information in various aspects of this field. This work is a result of collaborative work of an international group of professionals dedicated to contribute to the advancement of our knowledge.

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