Sperm Selection in Assisted Reproduction Techniques: Short Review

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ABSTRACT
Quality of sperm is a critical factor to success in terms of pregnancy rates in assisted reproduction treatments (ART). There is an increasing interest in improving procedures of sperm selection before in vitro fertilization with or without intracytoplasmic sperm injection. And since DNA abnormalities are known to have negative correlation with ART outcomes, novel approaches in sperm laboratory techniques are involved in select cells with normal DNA.

Keywords: Sperm Selection; Assisted Reproduction Techniques; DNA Damage; DNA Fragmentation; Apoptosis; in Vitro Fertilization

Introduction
In the last two decades, assisted reproductive technologies (ART) have become the treatment of choice in many cases of infertility and, although the continuous development of these techniques has brought a significant improvement in the overall final outcome of reproductive medicine, there is still a significant number of unsuccessful cases in which repeated attempts are needed before pregnancy is achieved, or in which couples remain childless [1]. Studies of infertile couples have demonstrated that male factor plays a major role and that is the reason why the basic semen analysis is one of the most relevant test used in this evaluation. The quality of semen is mainly based on the assessment of sperm concentration, motility and morphology, according to World Health Organization (WHO) [2]. Nevertheless, WHO values for semen evaluation are just a reference and may fail, and this is the reason why a significant proportion of patients exhibiting normal semen parameters values have unexplained infertility.

Procedures that could improve sperm selection are needed.

Discussion
The main objective of laboratory techniques for sperm preparation is to obtain a suitable number of viable, motile, and functional spermatozoa. Currently, the most frequently used are swim-up and density gradient centrifugation. All of these techniques select sperm according to motility characteristics without taking into account their molecular features, despite several studies have demonstrated the relevance of certain molecular profiles for reproductive success [3]. Novel approaches such as electrophoretic separation and magnetic cell separation have demonstrated encouraging results for the isolation of spermatozoa with superior quality [4], although its mode of action does not include the identification of apoptotic markers in spermatozoa.

Another essential aspect in the study of sperm quality is evaluation of the spermatozoa DNA integrity. Intact human sperm DNA is necessary for successful fertilization, embryo development, full expression of male fertility potential and probability to achieve pregnancy in natural conception, as well as in ART [5]. It has not been easy to evaluate DNA integrity. For example, detection of DNA strand breaks or the level of protamination and compaction of the chromatin [6] are methods that destroy the sperm. Nevertheless, recent noninvasive methods have been developed to select sperm free of DNA damage for clinical use.

One example is the application of morphologically selected sperm (IMSI), which uses motile sperm organelle morphological examination (MSOME), and it has shown its effectiveness only in increasing the clinical pregnancy and live birth rates in cases of recurrent implantation failure [7]. Another technique described is isolation of mature sperm by detecting hyaluronic acid (HA) in sperm surface binding sites but this method has provided no clinical or embryological successes [8].

A new method for selection of spermatozoa has been proposed, Magnetic Activated Cell Sorting (MACS). This technique employs several types of nanobeads particles and magnetic microbeads conjugated to proteins or antibodies to tag cells of interest.

Key features of activated apoptosis are activation of caspase-3, increase abnormalities such as sperm DNA
fragmentation [9,10], disruption of the mitochondrial transmembrane potential, loss of membrane integrity and, finally, phospholipid phosphatidylserine (PS) externalization, all events directly linked to failure of fertilization, clinical pregnancy and pregnancy loss during assisted reproduction treatments.

Increased levels of sperm cells that show apoptotic markers are detected in the ejaculate of infertile males, and suboptimal ART success rates have been attributed, at least in part, to the lack of in vivo sperm selection criteria that avoid or eliminate apoptotic spermatozoa [11, 12].

**Conclusion**

Unlike what happens with oocytes, the spermatozoa are usually present in tens of thousands or several millions per ejaculate, each one with different molecular characteristics that result from a complex process which involves a series of meiosis and mitosis, changes in cytoplasmic architecture, replacement of somatic cell histones with transition proteins, and the final addition of protamines. Alterations in the spermatogenic events result in the release of immature, abnormal spermatozoa in the ejaculate that can affect the outcomes in ART.

Accurate sperm selection should be considered as one of the essential steps to guarantee success in infertile couple’s treatments.

**References**


