

# Use of Assisted Reproduction Techniques in Male Pathologies: Molecule Based Sperm Selection Methods

## Abstract

Infertility is a widespread problem that is found in over 15% of couples. In 50 % of the cases, the male factor is the key. In order to improve pregnancy rates in assisted reproductive techniques (ART), new methods to select sperm subpopulations are required. These methods are under constant evaluation, and their possible genetic and epigenetic effects on the offspring must be considered. New concepts such as DNA fragmentation and sperm apoptosis are being introduced in andrology and are considered to develop new techniques. Recently, molecule-based methods have been developed for the purpose of overcoming deficiencies in classical selection methods that involve sperm morphology and movement. One of these methods is physiologic intracytoplasmic sperm injection (PICS), which selects spermatozoa through their property to bind to hyaluronic acid, a component of the cell-oocyte complexes that acts as a natural selector of spermatozoa. But the top rated method is based on the property of the protein annexin A5 (ANXA V) to bind to phosphatidylserine (PS), a marker of apoptosis. Magnetic-Activated Cell Sorting (MACS) conjugated with ANXA V (MACS-ANXA V) is used to remove apoptotic sperm with exposed PS from a semen sample. The use of MACS-ANXA V seems to produce good quality sperm when it is evaluated by *in vitro* assays. But when MACS-ANXA V is used previous to ART, controversies persist regarding the benefits of sperm selection in reproductive outcomes. In this review, we consider the use of MACS-ANXA V, particularly in cases of male pathologies.

**Keywords:** Annexin V; Hyaluronic acid; DNA sperm fragmentation; Apoptosis; MACS

## Mini Review

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**Abbreviations:** WHO: World Health Organization; ROS: Reactive Oxygen Species; PS: Phosphatidylserine; EPS: Externalization of Phosphatidylserine; ART: Assisted Reproduction Techniques; IUI: Intra-Uterine Insemination; IVF: *In Vitro* Fertilization; ICSI: Intracytoplasmic Sperm Injection; PICS: Physiologic Intracytoplasmic Sperm Injection; SU: Swim-Up; DG: Density Gradient; HA: Hyaluronic Acid; ANX V: annexin A5; MACS: Magnetic-Activated Cell Sorting; MACS-ANX V: Magnetic-Activated Cell Sorting coupled to ANX V; TESA: Testicular Sperm Aspiration

## Introduction

Infertility is a widespread problem that refers to the inability to achieve a clinical pregnancy after a year or more of regular unprotected sexual intercourse [1]. It is found in over 15 % of couples and 50% of the cases are due to male factor. Abnormal semen is responsible for 75% of all cases of male infertility. Semen quality is commonly assayed in andrology laboratories by standard analyses. These analyses should be performed according to the World Health Organization (WHO) guidelines [1] and involve the measures of volume, pH, and sperm concentration, motility and morphology with strict criteria. As WHO guidelines semen analyses sometimes are not able to discriminate between fertile and infertile patients, and have a poor predictive value for the outcome of embryo development, the development of new techniques is required [2]. In recent years, the management

of the male factor has undergone important changes with the introduction of novel concepts such as sperm DNA fragmentation and apoptosis. Therefore, new techniques are being developed in order to analyse these kinds of cell processes.

## Discussion

Mammalian spermatozoa are highly polarized haploid cells that must suffer complex physiological changes before being able to fertilize an oocyte. These changes take place *in vivo* into the Fallopian tube and are collectively known as capacitation [3,4]. Some of the events inherent to capacitation are the production of reactive oxygen species (ROS) and membrane lipids scrambling, with the consequent externalization of phosphatidylserine (EPS) [5]. Apoptosis is a programmed cell death and it is generally accepted that EPS is a marker of apoptosis in most diploid cells. But this phenomenon is not well characterized in spermatozoa.

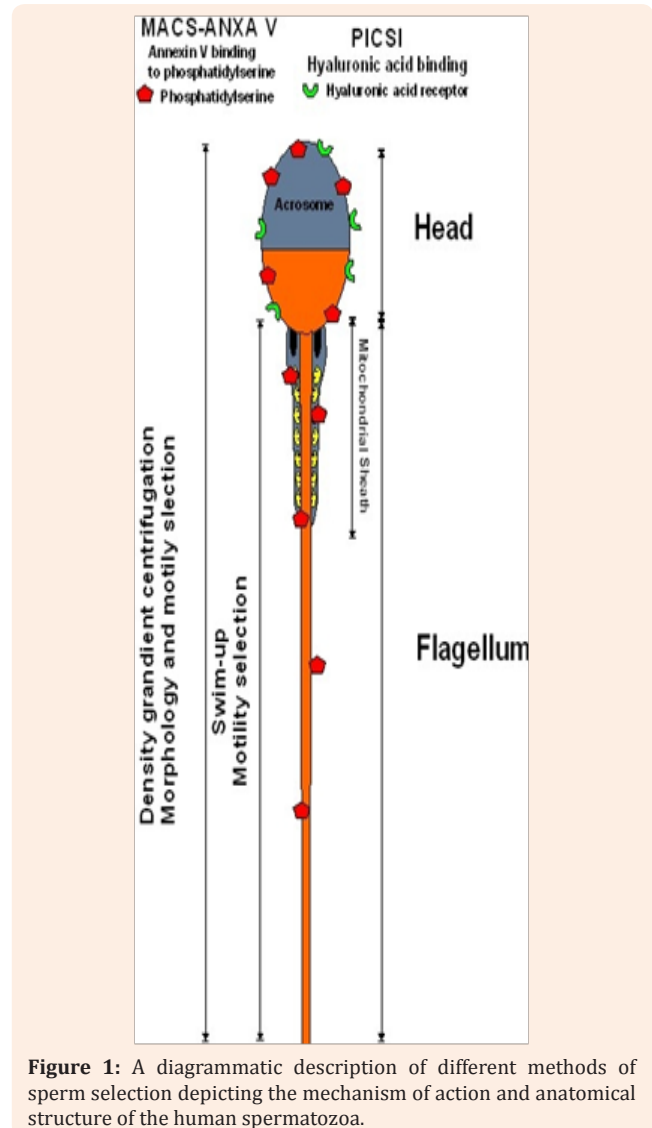
As mentioned before, sperm DNA fragmentation analysis is a novel concept introduced in the management of male infertility and it has been related to the presence of ROS. Irreversible DNA fragmentation may be produced during spermatogenesis, during sperm's journey through the male tract, by incorrect chromatin packaging, during apoptosis, or it may be caused *in vitro* by manipulation in laboratories. It is also enhanced by external factors such as environmental hazards, smoking and increased testicular temperature. Sperm DNA damage has also been related

to pathologies such as cryptorchidism, varicocele, inflammatory processes, infections, cancer, chemotherapy, radiotherapy and stress [6]. Infertility is currently treated worldwide by assisted reproduction techniques (ART) which still show suboptimal efficiency. ART includes: intra-uterine insemination (IUI), *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI), followed by embryo transfer. In IVF the gametes are placed together in artificial media for fertilization to occur. In ICSI instead, a spermatozoon is chosen and injected directly into the oocyte's cytoplasm. Thus, in this last procedure spermatozoa bypass most physiological processes. As a result, sperm with low quality such as damaged DNA and other non-noticeable defects might fertilize an oocyte producing an embryo with developmental problems. Despite this fact, ICSI is the treatment of choice when a severe male factor is present.

In ART, an initial step is used to render sperm with higher fertilizing potential. This step is based on the premise that cervical mucus selectively allows only progressively motile sperm of normal shape to penetrate and migrate through the cervix [7]. This selection step is performed by conventional techniques such as glass wool filtration, swim-up (SU), and density gradient (DG). All these methods select a subpopulation of sperm with higher morphological and motility qualities. Among these spermatozoa, one spermatozoon is selected based only on its morphology and motility for ICSI. New methods are constantly being thought in order to optimize sperm selection for ICSI and recently biological and molecular approaches have been conveyed to this regard. Human oocytes are surrounded by hyaluronic acid (HA) that acts as a natural selector of spermatozoa. Based on this phenomenon, a biological test using HA-coated slides has been developed [8]. In this method, spermatozoa that bind to HA-coated slides are selected and used for ICSI. The procedure is called "physiologic ICSI", PICS [9]. Although some reports show that PICS has a considerably higher probability ( $\approx 5$  fold) to achieve pregnancy than ICSI, a meta-analysis of all available studies concluded that although an improvement in embryo quality is provided by PICS over ICSI, the evidence is not enough to justify the routine use of PICS [10].

Another method promotes the removal of sperm that have begun apoptosis from the sample. Sperm particularities such as scarce cytoplasm and organelles and transcriptional inactivity rise doubts about the similarities of its apoptosis with that of other cells. However, EPS is considered to occur in apoptotic sperm [11]. Thus, a novel method based on binding of the protein annexin A5 (ANX V) to EPS has been developed. In this method Magnetic-Activated Cell Sorting (MACS) is performed with colloidal superparamagnetic microbeads (50 nm diameter) coupled to ANX V (MACS-ANXA V). A diagrammatic description of different methods of sperm selection depicting the mechanism of action and anatomical structure of the human spermatozoa is shown in Figure 1. The sperm/micro-beads suspension is then loaded on a separation column, which is placed in a fitted magnet. Sperm bound to ANX V by EPS stay trapped in the matrix, and the separation process gives two sperm populations: EPS-negative (vital, non-apoptotic sperm with intact membranes) and EPS-positive (apoptotic cells) [12,13]. As it was mentioned before,

sperm that have begun capacitation expose PS on their membrane surface, therefore, capacitated sperm may also be trapped into the column. MACS-ANX V was developed and commercialized by Miltenyi Biotec GmbH (Bergisch Gladbach, Germany) and is frequently used in combination with SU, DG or both.



**Figure 1:** A diagrammatic description of different methods of sperm selection depicting the mechanism of action and anatomical structure of the human spermatozoa.

*In vitro* analysis of sperm populations obtained after MACS-ANXA V selection of semen from men exposed to environmental hazards as manganese and phthalates, results in a selected population with decreased apoptosis and DNA damage rates [14,15]. A study using DG-MACS-ANXA V, applied to semen from men with varicocele, showed no negative effects on sperm motility and isolation of a population of sperm with decreased DNA fragmentation rate [16]. Moreover, a study was performed to compare DG-MACS-ANXA V versus DG using samples from astheno teratozoospermic (n=17) and teratozoospermic (n=12) patients [17]. In this study a population of sperm with better chromatin quality was obtained after DG-MACS-ANXA V than upon DG alone.

On the other hand, a randomized trial using donated oocytes and semen from unselected men, showed no differences in fertilization, implantation, pregnancy, and live-birth rates after selection of sperm with MACS-ANXA V compared with washed semen [18]. However, the born of a healthy baby was recorded using MACS-ANXA V for sperm selection from semen of a patient with bilateral varicocele, surgically treated, and with high rate of sperm DNA fragmentation [19]. Another study resulted in a single and a twin pregnancies after MACS-ANXA V sperm selection from semen of a man with asthenoteratozoospermia and another man with surgically treated varicocele and high rate of sperm DNA fragmentation [20]. In other study that comprised oligozoospermic, asthenozoospermic, oligoasthenospermic and/or teratozoospermic patients, improved sperm morphology and slightly higher implantation rates were noted in favour of MACS-ANX V over DG sperm selection [21]. According to a case report, MACS-ANXA V was used to select sperm obtained by testicular sperm aspiration (TESA) before ICSI in a man who had a traffic accident that resulted in paraplegia, without any chance of intercourse. In such case report, the born child was examined at the age of 4 finding normal development [22]. Another case report showed that the use of MACS-ANXA V on cryo-preserved spermatozoa with high DNA damage from a patient with non-Hodgkin's lymphoma, resulted in the birth of healthy twins [23].

## Conclusion

MACS-ANXA V is a promising new technology for sperm selection but its improvement in ART success is still under debate. Most of the studies described here have different experimental design, inclusion criteria and population size. To confirm or refute the use of this technique in clinical practice, further controlled and randomized studies are required. Moreover, oocytes coming from young women show a capability to repair sperm DNA damage. Thus, in order to test the efficiency of a new technique, standardized studies with the same oocytes quality (i.e. donated from women in programs of IVF), should be performed. While an improvement in the quality of sperm populations recovered by the new presented methods is evident by *in vitro* sperm analyses, the *in vivo* results need more, conclusive and evidence. It appears that when the damage in sperm DNA is not severe, oocytes of good quality can repair it and result in successful pregnancies, but when sperm DNA damage is severe, such as in patients with varicocele, cancer or men exposed to environmental hazards, MACS-ANXA V seems to be an option to obtain clinical pregnancies and healthy born children. Regarding apoptosis, taking into account that such phenomenon begins in the testicles it would be important to consider the use of MACS-ANXA V in combination with TESA.

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