

Utility of Magnetic Cell Separation as a Molecular Sperm Preparation Technique

Andrology Lab Corner*

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ABSTRACT: Assisted reproductive techniques (ARTs) have become the treatment of choice in many cases of infertility; however, the current success rates of these procedures remain suboptimal. Programmed cell death (apoptosis) most likely contributes to failed ART and to the decrease in sperm quality after cryopreservation. There is a likelihood that some sperm selected for ART will display features of apoptosis despite their normal appearance, which may be partially responsible for the low fertilization and implantation rates seen with ART. One of the features of apoptosis is the externalization of phosphatidylserine (PS) residues, which are normally present on the inner leaflet of the sperm plasma membrane. Colloidal superparamagnetic microbeads (~50 nm in diameter) conjugated with annexin V bind to PS and are used to separate dead and apoptotic spermatozoa by magnetic-activated cell sorting (MACS). Cells with externalized PS will bind to these microbeads, whereas

nonapoptotic cells with intact membranes do not bind and could be used during ARTs. We have conducted a series of experiments to investigate whether the MACS technology could be used to improve ART outcomes. Our results clearly indicate that integrating MACS as a part of sperm preparation techniques will improve semen quality and cryosurvival rates by eliminating apoptotic sperm. Nonapoptotic spermatozoa prepared by MACS display higher quality in terms of routine sperm parameters and apoptosis markers. The higher sperm quality is represented by an increased oocyte penetration potential and cryosurvival rates. Thus, the selection of nonapoptotic spermatozoa by MACS should be considered to enhance ART success rates.

Key words: Annexin V, apoptosis, cryopreservation, human, MACS, male infertility.

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The indications for assisted reproductive techniques (ARTs) have greatly expanded in the last decade and are expected to further increase in the future (Katz et al, 2002). Despite recent advances, the current pregnancy and live birth rates remain to be improved in order to alleviate the socioeconomic burden of failed ART cycles (American Society for Reproductive Medicine and

Society of Assisted Reproductive Techniques, 2004). The increase in ART applications associated with suboptimal success rates has mandated the development of an ideal sperm preparation technique that could be used in these applications.

Various sperm preparation techniques are currently used as the main components of ART procedures (Henkel and Schill, 2003). The main objective for employing these techniques is the selection of a sufficient number of viable motile sperm capable of fertilizing the oocyte(s). Current standard sperm preparation techniques depend on a sedimentation or migration approach to separate spermatozoa based on their motility or density. Nevertheless, molecular events such as sperm apoptosis are overlooked along the course of routine ART, which may negatively impact the final outcomes.

Numerous reports link the presence of apoptosis markers in human sperm with the failure of in vivo and in vitro fertilization (IVF; Host et al, 2000; Tesarik et al, 2001; Henkel et al, 2004; Seli et al, 2004; Barroso et al, 2006). Therefore, the current suboptimal ART success

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rates may be attributed, at least in part, to the inclusion of apoptotic sperm as a result of absent *in vivo* sperm selection barriers. This hypothesis has generated a motivation to develop new protocols for sperm selection based on the presence of apoptosis or apoptosislike markers and manifestations. Such an approach represents the inevitable evolution of sperm preparation techniques that expands to include molecular characteristics in addition to the physical properties.

A new electrophoretic system has been described recently for the rapid isolation of populations of spermatozoa exhibiting high levels of DNA integrity (Ainsworth et al, 2005). The first human pregnancy has been reported in a couple suffering from long-term infertility associated with extensive sperm DNA damage following the application of this electrophoretic system (Ainsworth et al, 2007). Despite the benefits of this technique, the complexity of the separation apparatus used may be a limiting factor against its widespread use in andrology laboratories, specifically those with limited resources.

In our previous research, we adopted a different approach by standardizing magnetic-activated cell sorting (MACS) as a preparation technique that yields motile, viable, morphologically normal spermatozoa that display higher cryosurvival rates as well as higher fertilization potential (Grunewald et al, 2001; Said et al, 2005a; Said et al, 2005b; Grunewald et al, 2006; Said et al, 2006a; Said et al, 2006b; Aziz et al, 2007). The protocol combines 2 different readily available, inexpensive techniques aiming at improving the results of ART. First, double density gradient centrifugation is used to remove seminal plasma and other extraneous constituents of the seminal fluid, leaving only mature viable spermatozoa (Ollero et al, 2001). Second, spermatozoa displaying an apoptosis surface marker are immunolabeled and removed using MACS. Thus, combining the advantages of both standard and advanced molecular sperm preparation techniques would yield spermatozoa with superior quality and higher fertilization potential.

The objective of this review is to present the theory behind the magnetic cell separation technology and the hypothesis of its use as a sperm preparation method. In addition, we review the different applications of MACS in human reproduction and its beneficial impact on normal as well as compromised semen samples. The ramifications of combining density gradient centrifugation with MACS are examined, and the extent of improvement in sperm parameters is outlined. We also highlight the protocol limitations and identify which ART procedures would benefit the most from its application.

Magnetic Cell Separation: Principles and Techniques

In general, cell isolation plays a major role in a variety of biologic and biomedical applications, including disease management. Magnetic cell separation offers advantages of simplicity of operation, low cost, and specificity and sensitivity afforded by use of immunospecific reagents. The outstanding examples of the magnetic cell separation application include the isolation of rare progenitor cells from the human umbilical cord blood and the use of mobilized peripheral blood as a substitute for bone marrow transplantation in patients who underwent irradiation and chemotherapy (Powles et al, 2000; Weissman, 2000).

Typically, this technique employs the use of magnetic particles conjugated to proteins or antibodies to tag cells of interest. To label and separate cells, many types of magnetic microbeads and nanobeads have been developed. The addition of iron oxide compounds (ferromagnetic maghemite $\gamma\text{-Fe}_2\text{O}_3$ and magnetite Fe_3O_4) to monodisperse polystyrene spheres or to a sugar-based skeleton is a typical technique of bead production (McCloskey et al, 2003).

An early type of magnetic separator was developed for use in combination with micrometer-sized Dynabead magnetic particles (Invitrogen, Carlsbad, Calif; Fønnum et al, 2005). Dynabeads were the first magnetic particles used for clinical cell separation applications, particularly bone marrow purging of gliomas prior to transplantation (Kemshead and Ugelstad, 1985). Due to their relatively large size (from 1 μm to 4.5 μm), the preferred application of Dynabeads is negative cell selection, where the cells targeted by the magnetic label are discarded, and only the unlabeled cells are used (Manyonda et al, 1992).

Multiple additional techniques also were introduced as solutions for isolation of a desired cell from a heterogeneous mixture of cells. These techniques include magnetic cell capture from suspensions directly in the field of view of a microscope (Budd et al, 2006) and a specialized system based on bioferrofluids, that is, submicron magnetic particles conjugated to targeting antibodies (Invitrogen; Tibbe et al, 1999, 2002). Recently, the Food and Drug Administration approved the use of CellSearch system for monitoring metastatic breast cancer (Veridex LLC, Warren, NJ).

In comparison to micrometer-sized magnetic particles, the use of colloidal magnetic particles as labeling reagents offers advantages in forming stable suspensions and fast reaction kinetics, similar to immunofluorescence labels. The small size of the particles, in the range of tens to one hundred nanometers, comes with the cost of low magnetic moment, requiring high magnetic fields and gradients, and therefore with the use of specialized,

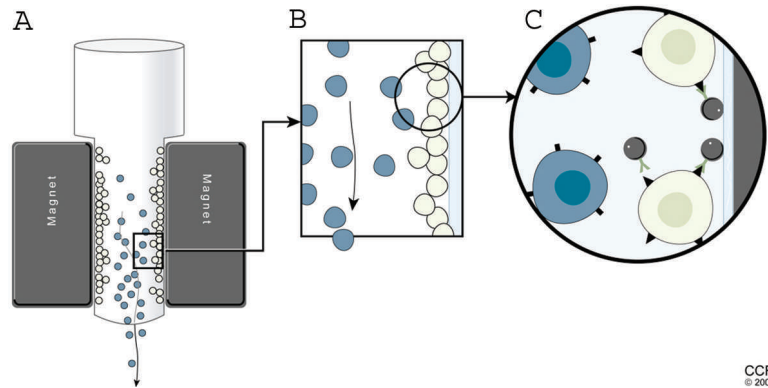
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Figure. Schematic diagram of MiniMACS magnetic cell separation column. **(A)** The solid support consists of closely packed, submillimeter spheres made of soft steel alloy. An external magnetic field magnetizes the solid support, which attracts magnetically labeled cells from a mixture applied to the column. **(B)** A thin slice across the solid support shows interstitial spaces available for flow of the cell mixture. **(C)** The unlabeled cells flow freely through the column, whereas the magnetically labeled cells are retained inside the column and could be recovered by removal of the column from the magnetic field, followed by elution. Reprinted with permission of the Cleveland Clinic, 2007.

magnetic affinity-type columns, typically obtained by soft steel alloy inserts inside the cell suspension container (Figure 1). This concept has been implemented in a particularly successful design, the MACS Microbeads and Columns, developed and commercialized by Miltenyi Biotec GmbH (Bergisch Gladbach, Germany; Miltenyi et al, 1990).

As the colloidal magnetic particles are extremely small (<100 nm), the use of a high-gradient magnetic field is required to retain the labeled cells, approaching 1 tesla (1 tesla = 10,000 gauss) and the local gradients of up to 1000 tesla per meter (Williams et al, 1999; Zhang et al, 2005). The MiniMACS column (Miltenyi Biotec) is specifically designed to generate this strong magnetic field while maintaining optimal cell viability and function. By using a MACS column with a biocompatible coating of ferromagnetic solid support placed in a powerful magnet, the magnetic force is sufficient to retain the target cells labeled with minimum microbeads (Manz et al, 1995). MACS microbeads are superparamagnetic particles that are coupled to highly specific monoclonal antibodies. They are used to magnetically label the target cell population. They are not visible with light microscopy, are biodegradable, and are gentle on cells (Miltenyi et al, 1990). The column schematic is shown in Figure 1. By rinsing the column with buffer, the unlabeled cells are washed out without affecting the labeled cell fraction, thus ensuring optimal recovery. Once the column is removed from the magnet, the labeled fraction can be obtained (Abts et al, 1989).

Magnetic Cell Separation for Preparation of Human Sperm

The inclusion of nonapoptotic spermatozoa is one of the prerequisites for achieving successful fertilization. Apo-

ptosis is an ongoing physiologic phenomenon that maintains the number of germ cells within the supportive capacity of the Sertoli cells (Sinha Hikim and Swerdloff, 1999). Nevertheless, deregulated apoptosis has been associated with the presence of abnormal spermatozoa in semen (Sakkas et al, 1999, 2002; Barroso et al, 2000). As opposed to somatic and testicular germ cells, the significance of apoptosis phenotype in ejaculated spermatozoa remains controversial (Oehninger et al, 2003). Although ejaculated spermatozoa display several apoptosislike characteristics, as in somatic cells, these apoptosis-related features may not indicate the presence of apoptosis death functions (Taylor et al, 2004). The failure to eliminate these abnormal spermatozoa during spermatogenesis—also termed *abortive apoptosis*—may be the reason for their presence in semen (Sakkas et al, 1999, 2002).

Activation of caspases, disruption of mitochondrial membrane potential, and increased DNA fragmentation are some of the apoptotic features that have been identified in ejaculated spermatozoa (Gorczyca et al, 1993; Kasai et al, 2002; Weng et al, 2002; Taylor et al, 2004). Another specific apoptotic event reported in human spermatozoa is the externalization of the phospholipid phosphatidylserine (PS) from its normal location in the inner leaflet of the sperm plasma membrane to the outer surface (Vermees et al, 1995; Oosterhuis et al, 2000). Annexin V is a phospholipids-binding protein that has high affinity for PS but lacks the ability to pass through an intact sperm membrane (van Heerde et al, 1995). Therefore, any binding between annexin V and PS needs to occur on the outer surface of the sperm plasma membrane, indicating that the membrane integrity has been compromised (Glander and Schaller, 1999).

We have used superparamagnetic microbeads conjugated with annexin V to label spermatozoa with

externalized PS. MACS using annexin V–conjugated microbeads proved to be an effective method for separating nonapoptotic spermatozoa from those with deteriorated plasma membranes based on the externalization of PS. MACS separation of sperm yields 2 fractions: annexin negative (intact membranes, non-apoptotic) and annexin positive (externalized PS, apoptotic; Grunewald et al, 2001; Glander et al, 2002).

The labeling strategy employed in our protocol is based on considering PS externalization in human sperm as an apoptotic manifestation. However, alterations in the sperm phospholipid bilayer were also reported to be a part of the normal sperm physiology. During sperm capacitation, protein kinases have been identified as mediators for signaling pathways that lead to externalization of PS and phosphatidylethanolamine (PE; Gaddella and Harrison, 2002; Kotwicka et al, 2002; de Vries et al, 2003). These findings may not be a direct representation of deregulated apoptosis in human sperm due to species-specific differences in sperm capacitation as well as different methodologies used for capacitation induction (Muratori et al, 2004). Moreover, the extent and site of PS externalization may be a determining factor in indicating whether spermatozoa are undergoing apoptosis or capacitation. In support of the association of PS externalization and annexin V binding with sperm apoptosis, motility, morphology, and viability were significantly reduced in the annexin-positive fraction (Said et al, 2005a,b; Said et al, 2006a; Aziz et al, 2007). The strong correlation between PS externalization, caspase 3 activation, and mitochondrial changes typical of apoptosis further supports this observation (Grunewald et al, 2001, 2006; Paasch et al, 2004, 2005).

Role of Magnetic Cell Separation in Male Reproduction

MACS Applications—Superparamagnetic microbeads coupled with the use of specific antibodies can effectively separate cells. Based on the antibody used, there are multiple applications for MACS technology in the field of male reproduction. Initially, magnetic beads were used to select and characterize acrosome-reacted sperm as well as to evaluate its ability to penetrate zona-free hamster oocytes (Okabe et al, 1992; Moutaffian and Parinaud, 1995). However, since the acrosome reaction of the fertilizing spermatozoon must be exactly synchronized with its penetration through the egg vestments (Tesarik, 1989), selecting acrosome-reacted sperm would be of little value in the context of *in vitro* human fertilization.

MACS combined with anti-CD45 microbeads has been used repeatedly with great success to eliminate leukocytes from seminal fluid (Krausz et al, 1992; Hipler

et al, 1998). In our protocol, MACS is used to isolate nonapoptotic spermatozoa after conducting double density gradient centrifugation. Following the centrifugation, the sperm pellet below the high-density layer is usually devoid of immature sperm as well as leukocytes (Kessopoulou et al, 1992). Thus, our approach abolishes the need for anti-CD45 MACS.

MACS could be used to isolate antibody-labeled from antibody-free spermatozoa in males with autoimmune infertility. These patients could benefit from this protocol, since it avoids potential damage to fragile sperm and does not seem to induce any modification of semen qualities. Subsequently, the concentration of sperm after MACS separation might be useful for insemination in infertile couples with autoimmune male infertility (Kiser et al, 1987; Foresta et al, 1990; Vigano et al, 1991).

MACS has been employed to facilitate the analysis of distinctive homogeneous spermatogenic cell populations by overcoming the heterogeneity of somatic and germ cells within the testicular tissue (Pesce and De Felici, 1995; von Schonfeldt et al, 1999; van der Wee et al, 2001). Immunoglobulin G was applied against the c-kit receptor protein for the immunomagnetic isolation of spermatogonia and spermatocytes. The use of the c-kit receptor as a marker for cell sorting of immature germ cells is supported by multiple studies that document its presence on the outer membrane of spermatogonia (Yoshinaga et al, 1991; Dym et al, 1995; Loveland and Schlatt, 1997). Therefore, it is evidently clear that the successful application of MACS relies mainly on the selection of specific antibody and an appropriate labeling strategy.

In a recent study, MACS did not eliminate all tumor cells from murine testicular cell suspensions (Geens et al, 2007). EL-4 tumor cells were labeled using R-phycoerythrin conjugated rat anti-CD49f monoclonal antibody. Following MACS with antiphycoerythrin microbeads, the number of spermatogonial stem cells (CD49f⁺) increased significantly from 3.94% to 40.46%, whereas the number of H-2Kb⁺ (EL-4) cells decreased from 10.35% to 3.54%. However, spermatogenesis was observed in only a limited number of testes that received purified cell suspension transplantations, and malignancy developed in 1 of 20 mice. The lack of absolute MACS efficiency in this study may be attributed to the nonspecificity of the antibodies used. Similarly, MACS did not prove to be an effective sex selection method for the isolation of Y-bearing sperm, since the H-Y antigen used as a surface marker is considerably expressed among X chromosome-bearing sperm as well (Sills et al, 1998).

Sensitivity and Specificity of Magnetic Beads for Extraction of Apoptotic Spermatozoa—We evaluated the sensitivity and specificity of annexin V–MACS beads for the separation of apoptotic spermatozoa in order to

advocate their use in sperm preparation. Sperm samples from 30 men (15 infertile patients and 15 fertile donors) were fractionated by equilibrating with magnetic beads carrying annexin V antibodies. To show if this technique was effective, the bound fraction from each sperm sample was released from the beads, labeled with fluorescein isothiocyanate-conjugated anti-annexin V antibodies, and re-separated by fluorescence-activated cell sorting. On average, 72.2% of these sperm were recognized by the cell sorter as annexin V positive (Paasch et al, 2003).

In a complementary experiment, the fraction of each sperm sample that did not bind to the beads was similarly labeled and sorted. In this case, 5.2% of the sperm were recognized as annexin V positive by the cell sorter. Electron microscopy revealed microbead binding on membranes at the acrosomal and postacrosomal regions only in the annexin-positive fraction. The antibody binding in only 72% of annexin-positive cells could be due to failure of detection by the antibodies as a result of ongoing membrane degradation. In addition, although 5% of annexin-negative sperm showed binding of the anti-annexin V antibody, no beads were found in that group by electron microscopy. Unspecific binding most probably contributed to the 5% figure. Overall, these results confirm that MACS effectively removes spermatozoa with PS bound to annexin V (Paasch et al, 2003).

Advantage of Combining MACS With Routine Sperm Preparation Techniques—MACS employing annexin V microbeads can effectively remove apoptotic sperm; however, there are other components in an ejaculate, (eg, leukocytes, debris, plasma) that should be removed as well. Therefore, there is a need to combine MACS with other sperm preparation techniques. We have optimized MACS by examining its combination with either double density gradient centrifugation or by 1-step sperm wash. Our results showed that the combination of double density gradient centrifugation and annexin V MACS was superior to all other preparation methods combined or separate in providing spermatozoa with highest quality in terms of motility, viability, and apoptosis markers (caspase 3 and mitochondrial membrane potential; Said et al, 2005a). Similarly, annexin V-negative sperm following MACS showed a lower incidence of DNA fragmentation (Said et al, 2006a).

We have also characterized the morphology of spermatozoa selected using our MACS protocol. Non-apoptotic sperm subpopulations separated by MACS had an improved sperm morphology profile, as demonstrated by significantly higher proportions of sperm with normal morphology and significantly lower sperm deformity index scores and percentages of sperm with

acrosomal defects, midpiece defects, cytoplasmic droplet, and tail defects (Aziz et al, 2007). Therefore, data clearly indicate the advantage of integrating MACS as a part of sperm preparation protocols.

Evaluation of Sperm Recovery Following Cell Sorting Using Magnetic Beads—Our novel protocol for sperm preparation is a 2-step procedure that combines double density gradient centrifugation with MACS. Subjecting a semen sample to multiple manipulations could result in substantial sperm loss. Therefore, we have undertaken the evaluation of the extent of cell loss during the procedure. Following density gradient centrifugation combined with MACS, the average number of cells lost during preparation was limited to only 15%. The number of total motile sperm showed a significantly positive correlation with the concentrations of spermatozoa and volumes of microbeads used ($r = 0.81$, $P < .0001$ and $r = 0.76$, $P < .0001$, respectively), which indicates that our protocol results in high sperm yield and minimal cell loss (Said et al, 2006b).

Implementation of MACS in Cryopreservation Protocols—Despite recent methodologic advances, the detrimental effects of cryopreservation on human spermatozoa are still markedly noted. Apoptosis very likely contributes to the decrease of sperm quality after cryopreservation, as evidenced by the impairment of the membrane integrity, including the translocation of PS from the inner to the outer leaflet of the sperm plasma membrane (Duru et al, 2001).

We have evaluated the effects of integrating MACS in cryopreservation-thawing protocols on sperm motility and cryosurvival rates. Nonapoptotic sperm separated by MACS prior to cryopreservation had significantly higher motility following cryopreservation thawing than sperm that were not separated by MACS. Similarly, nonapoptotic spermatozoa also had higher cryosurvival rates than sperm cryopreserved without MACS (Said et al, 2005b). The higher cryosurvival in MACS-separated sperm may be a direct result of excluding those that display manifestations of apoptosis. Nonapoptotic sperm separated by MACS showed significantly higher levels of intact mitochondria and lower pan-caspase activation following cryopreservation thawing compared with sperm that were not separated (Paasch et al, 2005; Grunewald et al, 2006). Therefore, data indicate that separating a distinctive population of nonapoptotic spermatozoa with intact membranes may optimize cryopreservation thawing outcome.

Enhanced Fertilization Potential of Spermatozoa Prepared by MACS—Despite the proven higher quality of sperm prepared by MACS in terms of routine parameters and molecular markers, the fertilization potential remains the ultimate sperm function. The zona-free hamster oocyte sperm penetration assay was used to

assess the sperm-oocyte penetration capacity. Nonapoptotic sperm exhibited significantly higher oocyte penetration potential compared with annexin-positive sperm and controls not separated by MACS. In support of the correlation between sperm apoptosis and fertilization, the sperm-oocyte penetrating capacity showed a significant positive correlation with motility ($r = 0.7$) and mitochondrial membrane integrity ($r = 0.7$), as well as a significant negative correlation with the percentage of active caspase 3 ($r = -0.6$) and binding to externalized PS ($r = -0.5$; Said et al, 2006a).

We also have evaluated the fertilization potential of spermatozoa prepared by MACS following intracytoplasmic sperm injections (ICSI) using an animal model. Nonapoptotic sperm from 18 fertile donors had sperm chromatin decondensation (SCD) values following ICSI that were comparable to controls not prepared by MACS. We also found a weak correlation between apoptosis markers (caspase 3 activation, mitochondrial membrane potential, and DNA damage) with the results of SCD, which may indicate a weak impact of apoptosis on early fertilization stages (Said et al, 2006a). Later stages, such as embryo development, blastocyst development rate, and clinical pregnancy rates, may show such a correlation (Tesarik et al, 2004; Virro et al, 2004). On the other hand, our recent study using semen samples collected from 21 infertile men showed significantly higher SCD following ICSI in sperm prepared by MACS ($44.2\% \pm 15.8\%$ vs $31.3\% \pm 13.1\%$, $P < .01$; unpublished data). Therefore, it appears that employing MACS as a sperm preparation technique prior to ICSI could be of benefit for compromised semen samples from infertile men, which may result in increased success rates, since ICSI is mainly indicated in the presence of a male factor.

Results showing that MACS separation enhances the sperm-oocyte penetration potential indicate its potential benefit during ART procedures, such as intrauterine insemination (IUI) or IVF. On the other hand, the value of using MACS prior to ICSI requires further investigations. Other published reports document the association of apoptosis and DNA damage with the fertilization rates following IUI and IVF but not with ICSI (Sun et al, 1997; Host et al, 2000; Bungum et al, 2004). The discrepancy may be due to the technical nature of ICSI that plays an important role in minimizing the impact of sperm preparation methods. During ICSI, a motile and, as far as possible, morphologically normal sperm is selected for injection. Therefore, there is an increased chance of using a sperm with intact DNA. On the other hand, during IUI and IVF, a larger number of spermatozoa are used, and a natural selection process takes place to control the sperm penetration.

Future Directions

The application of MACS allows for sperm selection based on sperm apoptosis in addition to routine parameters, such as motility and morphology. Data generated from previous studies serve as a basis for projects that evaluate magnetic cell sorting safety and efficiency in clinical settings for patients undergoing IVF. Transfer of our new approach to clinical IVF laboratories may lead to significant improvement in ART success rates and subsequently reduce the physical, psychologic, and financial burdens for patients.

The continuing development of magnetic cell separation techniques offers opportunities for improved sperm cell sorting. A particularly interesting approach is based on cell magnetophoretic mobility. Magnetophoretic mobility is analogous to the electrophoretic and sedimentation mobilities encountered in electrical and sedimentation separations, respectively (McCloskey et al, 2003). The magnitude of the mobility of a magnetically tagged cell distinguishes these cells from unlabeled ones and allows effective cell sorting in continuously flowing cell suspensions (Chalmers et al, 1998; Jing et al, 2007). This approach may lead to further improvement in the quality of the sorted sperm cells by selection of spermatozoa with higher fertilization potential and elimination of the apoptotic cells.

Conclusions

MACS is considered a flexible, fast, and simple cell sorting system for separation of large numbers of cells according to specific cell surface markers. Most importantly, the procedure employs a relatively inexpensive technology that could be easily applied in andrology laboratories. The combination of MACS with double density gradient centrifugation yields a clean sperm population characterized by higher motility, viability, and morphology. Moreover, sperm prepared by MACS display reduced apoptosis manifestations, including DNA fragmentation. In this context, MACS may be considered a unique molecular preparation technique that complements conventional sperm preparation protocols.

The successful application of MACS mainly depends on the employment of an efficient and specific immunolabeling strategy. It is evidently clear that the separation of a nonapoptotic fraction using MACS not only enhances the quality of retrieved sperm quality but also improves sperm fertilization potential, as documented by higher oocyte penetration capacity. Therefore, including an additional step of sperm preparation involving the isolation of apoptotic sperm using MACS may significantly enhance the outcome of some ARTs, such as IUI or IVF. The potential clinical application of MACS as a sperm preparation technique during IUI

and IVF warrants the validation of these findings in infertile men. MACS could also be used to isolate spermatozoa with compromised genomic integrity. Nevertheless, the value of integrating MACS in sperm preparation prior to ICSI requires further investigation in a clinical ART program.

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