Effects of magnetic-activated cell sorting on sperm motility and cryosurvival rates

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Objective: To evaluate the effect of magnetic-activated cell sorting in cryopreservation–thawing protocols on sperm motility and cryosurvival rate.
Design: Prospective-controlled study.
Setting: Andrology department at a university-based medical institution.
Patient(s): Ten healthy volunteer sperm donors.
Intervention(s): Sperm populations were separated using annexin-V magnetic-activated cell sorting before and after the cryopreservation–thawing process.
Main Outcome Measure(s): Sperm motility and cryosurvival rate.
Result(s): Annexin-negative sperm separated by magnetic-activated cell sorting had statistically significantly higher motility following cryopreservation–thawing than sperm that were not separated. Similarly, annexin-negative spermatozoa also had higher cryosurvival rate than sperm cryopreserved without magnetic-activated cell sorting and sperm that were annexin-positive.
Conclusion(s): Superparamagnetic annexin V-conjugated microbeads can separate spermatozoa with externalized phosphatidylserine, which is considered one of the early features of late apoptosis. The separation of a distinctive population of nonapoptotic spermatozoa with intact membranes may optimize the cryopreservation–thawing outcome. Magnetic-activated cell sorting using annexin-V microbeads enhances sperm motility and cryosurvival rates following cryopreservation. (Fertil Steril® 2005;83:1442–6. ©2005 by American Society for Reproductive Medicine.)

Key Words: Annexin, cryosurvival rate, magnetic-activated cell sorting, sperm

Cryopreservation of human semen is the most commonly accepted method of preserving male reproductive capacity. Cryopreserved spermatozoa may be used in assisted reproductive techniques (ART) (1), especially in cases where the patient elects to undergo vasectomy for contraception, or, most importantly, when a patient is diagnosed with cancer and the treatment may render him infertile (2). The indications for sperm cryobanking have been greatly expanded by recent breakthroughs in ART, in which immotile but viable sperm can be used successfully for oocyte fertilization through intracytoplasmic sperm injection (ICSI).

Despite recent methodological advances, cryopreservation exerts detrimental effects on spermatozoa that lead to significant decreases in sperm viability and motility and ultimately in decreased cryosurvival rates (CSR) (3). The fertility potential of cryopreserved mammalian spermatozoa is lower than that of fresh sperm. The reduction arises from both a lower post-thaw viability and sublethal dysfunction in a proportion of the surviving subpopulation (4). Programmed cell death (apoptosis) most likely contributes to the decrease in sperm quality after cryopreservation (5).

The sperm plasma membrane is one of the key structures affected by cryopreservation that displays apoptotic features (6). Early phases of disturbed membrane functions are associated with asymmetry of the membrane phospholipids. The phospholipid phosphatidylserine (PS), which is normally present on the inner leaflet of the plasma membrane, becomes externalized to the outer leaflet (7). The externalization of PS is a known early marker for apoptosis (8). Because annexin-V has a high affinity for PS, it cannot pass through an intact sperm membrane. Therefore, when annexin-V binds to spermatozoa, it signifies that the integrity of the membrane has been disturbed (9).

Colloidal super-paramagnetic microbeads (~50 nm in diameter) conjugated with annexin-V may be used to separate dead and apoptotic spermatozoa by magnetic-activated cell sorting (MACS). Cells with PS that has externalized to the outer leaflet will bind to these microbeads. When placed into a column containing iron balls and passed through a strong magnetic field, those cells remain in the separation column. On the other hand, cells with intact membranes remain unlabeled and pass freely through the column (10, 11).

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The process of cryopreservation increases the amount of apoptotic spermatozoa, which in turn decreases the success rate of ART. The binding of superparamagnetic annexin-V microbeads (ANMB) can effectively eliminate spermatozoa in early apoptotic stages from cryopreserved samples (12). Therefore, ANMB-negative spermatozoa may have higher survival potential after cryopreservation. The objective of our study was to determine if the inclusion of MACS in cryopreservation–thawing protocols improves sperm motility and the CSR.

MATERIALS AND METHODS

Sample Preparation

This study was approved by our institutional review board. Semen samples were collected from 10 healthy donors, and semen parameters exceeded the World Health Organization (13) reference ranges for the normal fertile population. To separate the predominantly mature spermatozoa, the liquefied semen was loaded onto a 55% and 80% discontinuous SupraSperm gradient (MediCult, Jyllinge, Denmark) and centrifuged at 500 × *g* for 20 minutes. The resulting 80% pellet (mature spermatozoa) was aspirated and resuspended in human tubal fluid media (HTF; Irvine Scientific, Santa Ana, CA).

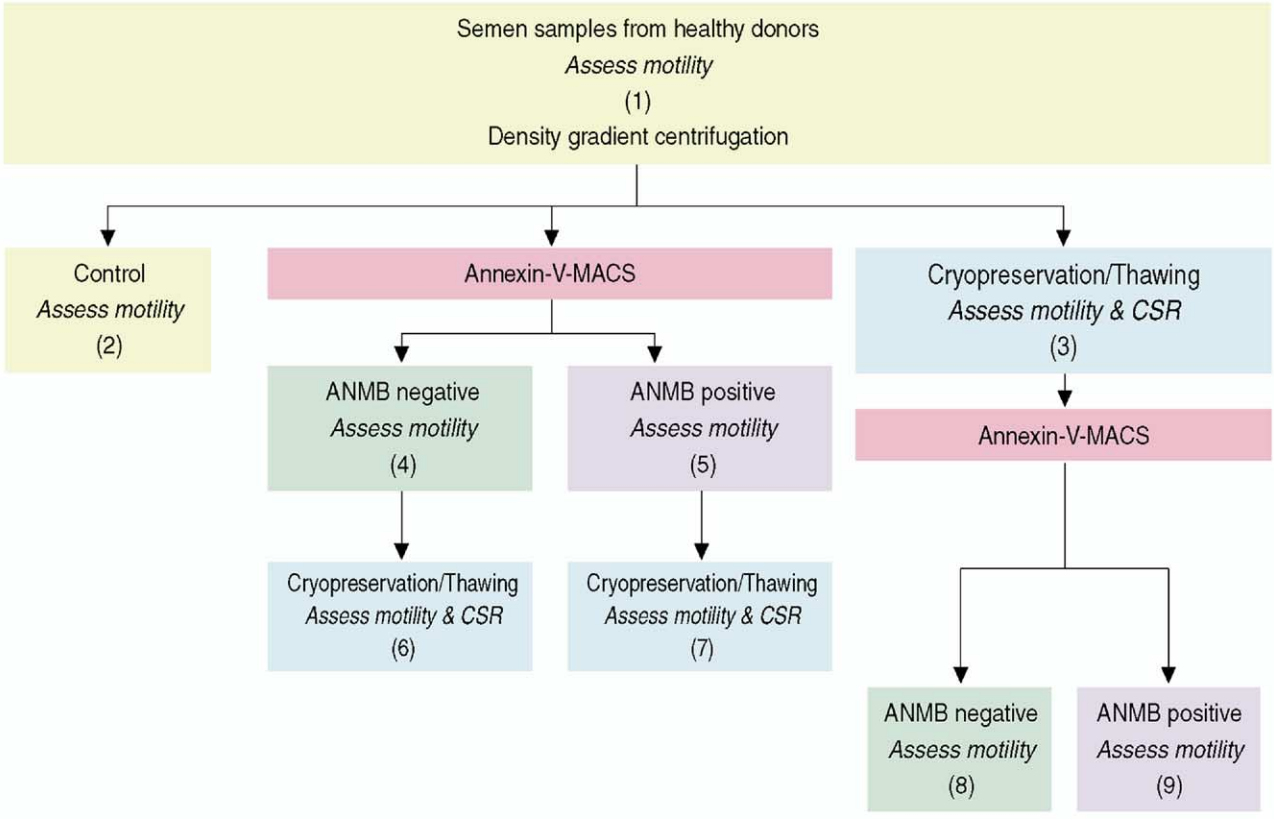
The sperm cell suspension was divided into two separate fractions. The first was subjected to MACS followed by cryopreservation and thawing. The second was cryopreserved-thawed first and then subjected to MACS. Sperm motility was assessed in all fractions at each step of the experiment. The different steps of our experiment design are illustrated in Figure 1.

Isolation of Spermatozoa with Deteriorated Membranes by MACS

The sperm suspensions were passed through a magnetic field (MiniMACS; Miltenyi Biotec, Bergisch Gladbach, Germany), and the spermatozoa were classified as either ANMB-positive or ANMB-negative based on the binding of the microbeads to their outer surface (14).

FIGURE 1

Flow diagram of overall experiment design. Spermatozoa from same sample were subjected to cryopreservation–thawing before and after MACS. Number in parenthesis represents the aliquot number. MACS = magnetic activated cell separation; ANMB = annexin-V magnetic microbead; CSR = cryosurvival rate.



Said. Effect of MACS on sperm cryopreservation. Fertil Steril 2005.