

Combining Magnetic Cell Sorting Technique with Sperm Preparation for Assisted Reproduction

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The low fertilization and implantation rates in assisted reproduction may be related to programmed cell death (apoptosis), which decreases sperm quality after cryopreservation. A classic feature of apoptosis is the externalization of phospholipid phosphatidylserine (PS) residues, normally present on the inner leaflet of the sperm plasma membrane. Annexin-V has a high affinity for PS but 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.

Colloidal super-paramagnetic microbeads (~50 nm in diameter) conjugated with annexin-V may be used to separate dead and apoptotic spermatozoa by magnetic cell sorting (MACS). Cells with externalized PS and deteriorated plasma membranes will bind to these microbeads. When placed into a separation column containing super-paramagnetic annexin V conjugated microbeads and passed through a strong magnetic field, the apoptotic and dead spermatozoa (annexin-positive) are retained in the column. On the other hand, non-apoptotic cells with intact membranes (annexin-negative) do not attach to super-paramagnetic annexin V conjugated microbeads and pass freely through the column (Figure 1).

To determine if MACS technology can improve rates

of ART, we conducted a study to evaluate the quality of the non-apoptotic sperm prepared by MACS while examining its tolerance to cryopreservation-thawing (cryosurvival rate) as well as its oocyte penetration potential.

Semen specimens collected from healthy donors were prepared by double density gradient centrifugation (DGC) followed by MACS or DGC only (controls). The sperm quality was monitored in terms of motility and the presence of apoptotic markers: caspase-3 activation (CP-3), disruption of mitochondrial membrane potential (MMP) and externalized phosphatidylserine residues. The percentage of sperm that survived the cryopreservation process (percentage CSR) was calculated using the following formula: 100 X post-thaw total motile sperm/pre-freeze total motile sperm. The sperm-oocyte penetration potential was assessed using the zona-free hamster oocyte penetration assay (SPA). Results of the SPA were evaluated as the percentage of oocytes penetrated by sperm (percentage penetrated) and the average number of sperm per oocytes penetrated (sperm capacitation index, SCI). For results of the study, see Table 1.

Our results clearly indicate that the selection of non-apoptotic spermatozoa by MACS should be considered to enhance success rates of assisted reproduction.

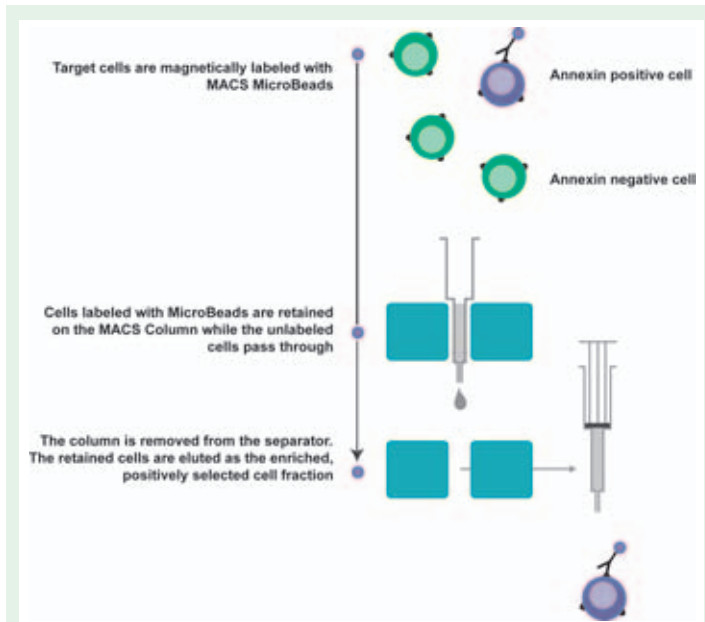


Figure 1

Table 1. Outcome of different parameters in controls, annexin-negative and annexin-positive sperm.

Parameter	Controls	MACS separation	
		Annexin-negative	Annexin-positive
Motility (%)	76.2 ± 8.6	83.2 ± 8.1**	19.2 ± 9.7**
Caspase-3 (% active)	8.9 ± 8.1	3.7 ± 1.2*	57.6 ± 16.1**
MMP (% intact)	87.9 ± 11.1	92.2 ± 8.4**	39.1 ± 15.7**
Externalized PS (% positive)	5.8 ± 3.2	3.4 ± 1.7	54.9 ± 18.1**
Percentage penetrated oocytes	33.8 ± 6.9	44.5 ± 12.6**	20.8 ± 5.3**
SCI	1.5 ± 0.6	1.8 ± 0.3*	1.2 ± 0.4*

Values are expressed as mean ± standard deviation. **p <0.01; *p <0.05 in comparison to controls. Controls = separation by double density gradient; MACS = magnetic cell sorting; MMP = mitochondrial membrane potential; PS = phosphatidylserine; SCI = sperm capacitation index, average number of sperm penetrated per oocytes.

The cryosurvival rate (%CSR) was highest in the annexin-negative spermatozoa that were separated by MACS prior to freezing when compared with the annexin-positive spermatozoa.