Removal of annexin V–positive sperm cells for intracytoplasmic sperm injection in ovum donation cycles does not improve reproductive outcome: a controlled and randomized trial in unselected males

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Objective: To determine the effect of removing presumptive apoptotic sperm cells from samples from unselected males by means of magnetic activated cell sorting (MACS) on live-birth delivery rates after intracytoplasmic sperm injection (ICSI) in couples undergoing ovum donation (OD).
Design: Prospective, randomized, triple-blinded, and controlled study.
Setting: Private university-affiliated IVF center.
Patient(s): A total of 237 infertile couples undergoing ICSI as part of an OD program.
Intervention(s): Semen specimens from the control group were prepared by swim-up. Samples from the study group were prepared by swim-up followed by MACS and incubation with annexin V–conjugated microbeads to remove annexin V–positive (AV+) sperm cells.
Main Outcome Measure(s): Fertilization rates, morphological features of early embryo development, implantation rates, ongoing pregnancy rates, and live-birth rates.
Result(s): Similar results were obtained between groups for all the parameters compared: fertilization rates of 75.3% (95% confidence interval [CI], 71.6–78.9) versus 72.1% (95% CI, 68.6–75.7); percentage of good-quality embryos on day 2 of 53.7% (95% CI, 50.3–57.1) versus 51.8% (95% CI, 48.3–55.3) and on day 3 of 54.2% (95% CI, 50.7–57.6) versus 48.9% (95% CI, 45.3–52.4); implantation rates of 42.2% (95% CI, 33.8–48.1) versus 40.1% (95% CI, 34.8–49.6); positive beta-hCG tests of 63.2% (95% CI, 54.7–71.6) versus 68.6% (95% CI, 60.2–76.9), and live-birth rates of 48.4% (95% CI, 39.6–57.1) versus 56.4% (95% CI, 47.3–65.5) in the MACS versus control group. None of the differences reached statistical significance.
Conclusion(s): Applying MACS technology to remove AV+ sperm cells from unselected males does not improve the reproductive outcome of ICSI in OD. (Fertil Steril® 2014;102:1567–75. ©2014 by American Society for Reproductive Medicine.)
Key Words: Magnetic activated cell sorting, MACS, sperm selection, ovum donation, live birth rate, apoptotic sperm

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The continuous development of new assisted reproduction techniques (ART) in recent decades has brought a significant improvement in the overall final outcome of reproductive medicine. However, despite these improvements, there are still a significant number of unsuccessful cases in which repeated attempts are

needed before pregnancy is achieved or in which couples remain childless (1).

The responsibility for the success of ART is shared between male and female gametes. Owing to the low number of oocytes available, the molecular analysis and characterization of female gametes is to be avoided, as it can harm the cell and compromise its viability. Therefore, Spain's national ART regulations dictate that all oocytes should be fertilized without selecting them according to the likelihood of their success.

A different scenario exists regarding spermatozoa, which are usually present in tens of thousands or several million per ejaculate, each one with different molecular characteristics that result from meiosis and genetic recombination during spermatogenesis (2). Moreover, different molecular changes occur in the sperm during their journey through the male reproductive tract, especially within the epididymis. Consequently, the most appropriate sperm can be selected for insemination or microinjection, while surplus spermatozoa of less than optimal quality are discarded.

The main objective of sperm preparation techniques is to obtain a sufficient number of viable, motile, and functional spermatozoa. Currently, the most frequently used of these preparation techniques are swim-up and density gradient centrifugation. These approaches consist of selecting sperm according to motility characteristics without taking into account their molecular features, despite several studies having demonstrated the relevance of certain molecular profiles for reproductive success (3).

Sperm characteristics such as apoptosis and apoptosis-like manifestation, DNA integrity, membrane maturation, and ultrastructure are not directly targeted by routine sperm preparation. The current literature identifies several advanced sperm selection methods on the basis of these characteristics: [1] surface charge (electrophoresis and zeta potential), [2] membrane maturity (hyaluronic acid binding), [3] ultramorphology (high magnification), and [4] apoptosis (magnetic cell sorting) (4). Among the molecular aspects of sperm that are linked to male factor infertility, the process of apoptosis has received special attention in recent years (2, 5). There are different apoptotic markers, but the main ones that are studied are activated caspase-3, integrity of mitochondrial membrane potential, and externalization of phosphatidylserine (PS) residues. Increased levels of sperm cells that show these 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 (6–8).

This hypothesis has generated great interest, and there are numerous groups currently working on the development and evaluation of new protocols for sperm selection based on the detection of apoptosis or apoptosis-like markers. The goal of this work is the establishment of sperm preparation techniques that overcome the aforementioned limitations by including molecular characteristics in addition to the physical, morphological, and kinetic properties of sperm.

Sperm cryopreservation is a widely used procedure in assisted reproduction, but the freezing and thawing that this involves can inflict irreversible injury on the spermatozoa. Indeed, an increase of apoptotic markers in human spermatozoa has been documented after cryopreservation and thawing (9).

One of the earliest stages of apoptosis is plasma membrane disturbance, which is manifested by asymmetry of the lipid bilayer caused by externalization of PS residues normally present on the cell inner leaflet (7, 10).

Annexin V (AV) is a phospholipid-binding protein with a high affinity for PS in the presence of physiological concentrations of Ca^{+2}. AV cannot pass through intact sperm membranes, and, consequently, AV-conjugated superparamagnetic microbeads (AVMB) can be used to effectively separate sperm with externalized PS, a subpopulation of which may be apoptotic, from those that do not (11). This yields two fractions: AV-negative (AV−, sperm presumed to be nonapoptotic with intact membranes) and AV-positive (AV+, sperm presumed to be apoptotic with externalized PS) (12, 13).

Sperm DNA fragmentation takes place later in the apoptotic process, and this parameter should be analyzed to determine which patients need to have their ejaculates processed with MACS. Unfortunately, there is no commercially available kit for measuring sperm apoptosis without the use of complex equipment such as fluorescence microscopes or flow cytometers.

Although generally considered to improve results in infertile couples, there is little clinical data available about the use of these selection techniques in IVF (11) and their impact on morphological features of gametes, embryo development, and final ART outcome (14, 15). In this context, and to avoid oocyte quality bias, we have performed the present prospective, randomized, controlled trial within our ovum donation (OD) program. Our aim was to determine the clinical relevance of using MACS to remove AV+ presumptive apoptotic sperm cells from samples from unselected males (those without previous signs of sperm apoptosis studied) on fertilization rates; early embryo development and morphological features; and implantation, pregnancy, and live-birth rates (16) after intracytoplasmic sperm injection (ICSI) as part of our OD program.

MATERIALS AND METHODS

Design

We conducted a two-arm, unicentric, prospective, randomized, and triple-blinded trial at the Instituto Valenciano de Infertilidad in Valencia, Spain. The primary endpoint of this study was live-birth rate after ICSI, with and without implementation of MACS for sperm selection. Couples attending our center for infertility who had not yet received treatment were enrolled in the study between October 2010 and December 2012. Given that the main intervention of the study involved the handling of sperm samples rather than human beings, clinical trial registration was not required.

Institutional Approval and Informed Consent

This study was approved by the Institutional Review Board of the Instituto Valenciano de Infertilidad, in Valencia, Spain.