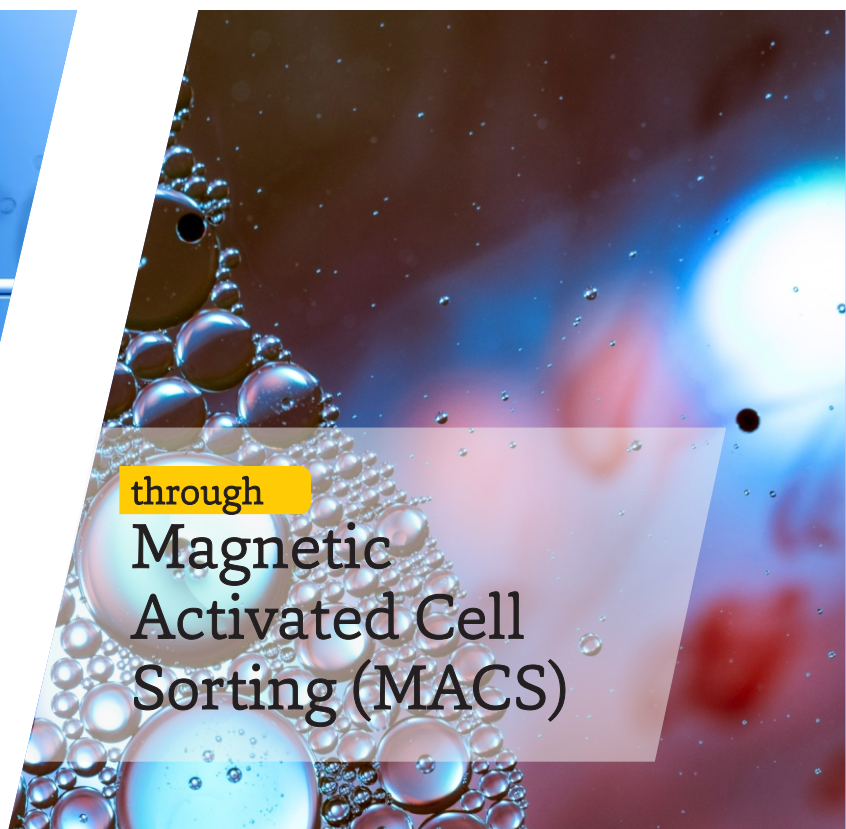




Improving Intracytoplasmic Sperm Injection (ICSI) outcomes

Elnathan Wang



through Magnetic Activated Cell Sorting (MACS)

Background

Sperm quality directly affects fertilization, embryo development, and pregnancy success. Sperm DNA fragmentation and apoptosis can lead to poor embryo quality and implantation failure. Traditional sperm selection methods (Density gradient centrifugation (DGC), swim-up) do not effectively remove apoptotic sperm.

Magnetic activated cell sorting is a sperm selection technique that isolates non-apoptotic sperm using annexin-V paramagnetic microbeads to remove apoptotic (damaged) sperm, ensuring that only viable sperm are used for ICSI. However, this does not replace traditional methods but rather plays an adjunctive role to improving outcomes following ICSI.

Introduction

Creating an euploid embryo depends on normal sperm and oocyte. High sperm DNA fragmentation can negatively impact blastocyst formation rates and can increase rates of embryonic aneuploidy.

While sperm quality does not have a direct relationship with the implantation potential of euploid blastocysts, high sperm DNA fragmentation has been associated with higher rates of early pregnancy loss and increased risk of paternal chromosomal abnormalities in embryos.

Selection of sperm for intracytoplasmic sperm injection based on DNA competence utilizes advanced technologies to identify sperm with higher DNA integrity, which is crucial for successful fertilization and embryo development.

Common methods include: hyaluronan binding assays; physiologic intracytoplasmic sperm injection (PICS); and cumulus oophorus columns which select mature DNA intact sperm. Magnetic activated cell sorting (MACS) targets sperm with low DNA fragmentation or apoptotic markers. These methods complement traditional sperm selection based on morphology and motility, aiming to improve ICSI outcomes by minimizing the use of DNA-damaged sperm.

Magnetic-activated cell sorting (MACS) is an advanced sperm selection technique used to isolate sperm that do not show signs of apoptosis and, therefore, are presumed to have a lower rate of DNA damage. Because the exposure of phosphatidylserine residues on the plasma membrane of sperm cells is an indicator of early apoptosis, MACS uses annexin V-conjugated paramagnetic microbeads that bind to sperm exhibiting externalized PS.

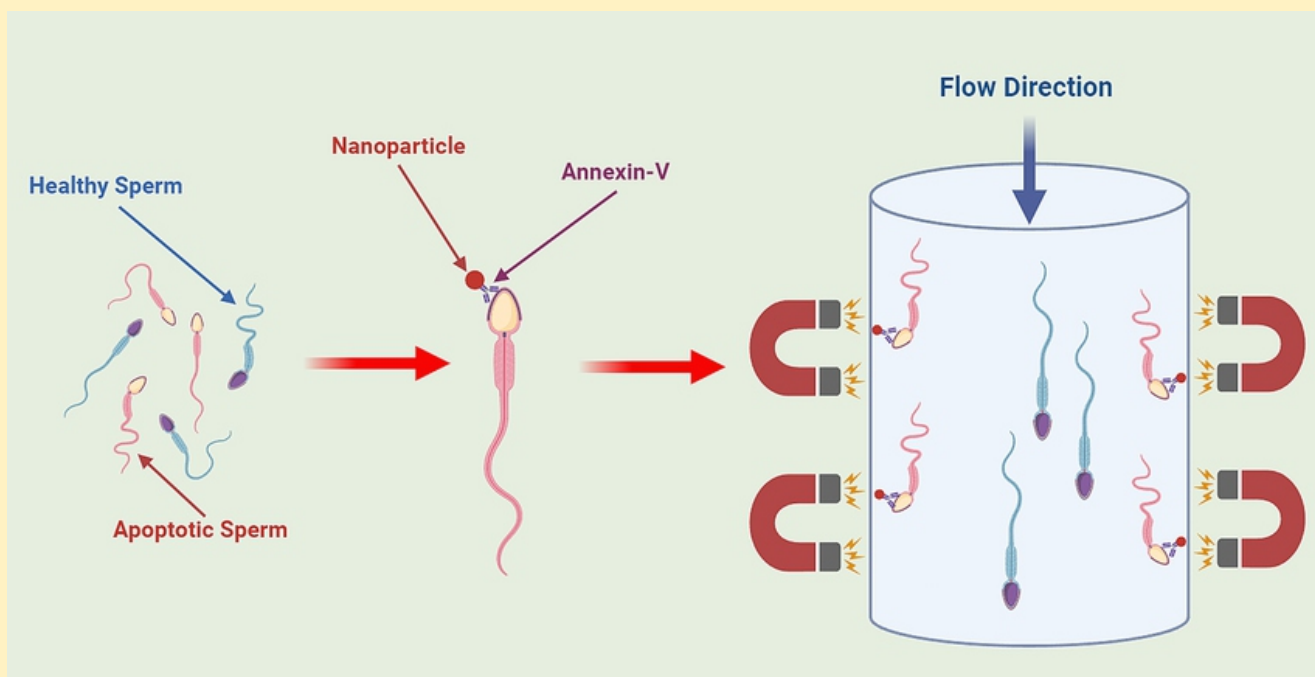
The sperm cell suspension is incubated with annexin V–conjugated microbeads and then allowed to run through the MACS column, which is placed inside a magnet. The sperm that are not bound to the beads elute through the column, leaving the apoptotic sperm behind.

We critically appraise available literature in terms of methodology and outcomes to determine whether MACS should stand alone or be used as an adjunct to traditionally established methods of optimising semen for ICSI, while keeping in view up to date evidence.

Discussion

The ability to isolate cell populations from heterogenous mixtures is a key part of life sciences research. One approach to separating cells is magnetic-activated cell sorting (MACS), which uses magnets to isolate targeted cells from the rest of a biological sample.

MACS, also known as immunomagnetic cell separation, binds magnetic particles to cells through an antibody interaction with surface markers of the targeted cells. Then, those targeted cells are magnetically isolated from the rest of the semen sample.



MACS Step by Step

STEP 1:

Sperm Sample Collection & Preparation

A semen sample is collected from the male partner. Density Gradient Centrifugation (DGC) is used to remove debris and immotile sperm.

STEP 3:

Magnetic Separation

The sample is passed through a magnetic column. The magnet traps apoptotic sperm, while healthy sperm flow through freely.

STEP 2:

Annexin V Binding

Apoptotic sperm externalize phosphatidylserine (PS) on their membrane. Annexin V-coated magnetic beads bind to PS, marking apoptotic sperm for removal.

STEP 4:

Sperm Collection & ICSI Use

Non-apoptotic sperm is collected and analyzed. Sperm is injected into eggs during ICSI.

MAGNETIC ACTIVATED CELL SORTING (MACS)

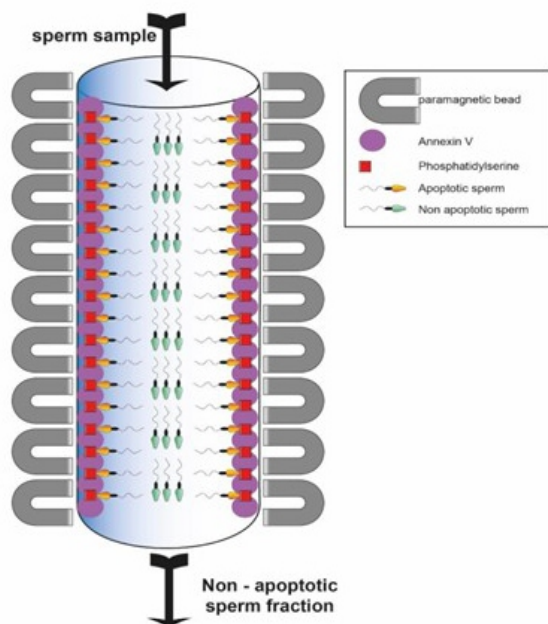


Figure 2: Sperm cell passing through column with annexin V. The sperm cells able to pass through the column represent the viable one (green heads) while the apoptotic fraction remain trapped inside the column (yellow heads).

human reproduction



Article Navigation

JOURNAL ARTICLE
O-301 Can advanced sperm selection techniques improve blastocyst euploidy rate of abnormal sperm DNA fragmentation cases to the level of normal ones; prospective randomized controlled trial
E Hasanen, M Hozayen, A Elshimy, Y Hazem, H Alkhader, H Zaki
Human Reproduction, Volume 38, Issue Supplement_1, June 2023, dead093.365,
<https://doi.org/10.1093/humrep/dead093.365>
Published: 22 June 2023

RCT 2 on Advanced Sperm Selection (MACS vs. PICSI) in ICSI

Objective:

To directly compare MACS vs. PICSI to see which one works better for ICSI patients.

Methodology:

The Participants were couples undergoing ICSI who were randomly assigned to two groups. The MACS group used magnetic selection to remove bad sperm while the PICSI group selected sperm based on hyaluronic acid binding.

Outcomes Measured:

Fertilization rates, cleavage rates, and blastocyst formation.

Randomized Controlled Trial > Reprod Sci. 2022 Jan;29(1):220-228.
doi: 10.1007/s43032-021-00642-y. Epub 2021 Jun 2.

Reproductive Outcomes of Different Sperm Selection Techniques for ICSI Patients with Abnormal Sperm DNA Fragmentation: a Randomized Controlled Trial

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Affiliations + expand
PMID: 34076869 DOI: [10.1007/s43032-021-00642-y](https://doi.org/10.1007/s43032-021-00642-y)

RCT 1: Comparing MACS, PICSI, Testicular Sperm and DGC for ICSI

Objective:

To compare different sperm selection techniques for men with high sperm DNA fragmentation (SDF) to see which method improves ICSI outcomes the most.

Methodology:

- 302 couples undergoing ICSI were randomly assigned to four groups:
- The Control group (DGC) used the standard sperm preparation method.
- The MACS group used magnetic sorting to remove apoptotic (dying) sperm.
- The PICSI group used selected sperm based on their ability to bind hyaluronic acid (mimicking natural sperm selection).
- Testicular sperm extraction (Testi) retrieved sperm directly from the testicles instead of the ejaculate.

Key outcomes measured were fertilization rate, embryo quality, clinical pregnancy rate, and live birth rate.

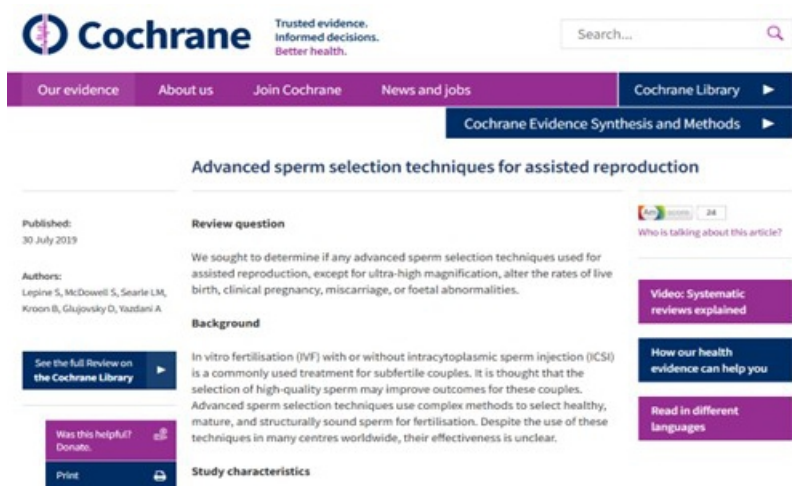
Key Findings established that MACS & PICSI improved fertilization rates and embryo quality compared to DGC.

Key Findings:

Both MACS and PICSI improved fertilization and embryo cleavage rates. MACS had a slight advantage in embryo quality. MACS also had a slight advantage in embryo quality.

Conclusion:

Both MACS and PICSI are useful sperm selection methods, but neither showed a major advantage in terms of pregnancy or live birth rates. Using MACS might improve embryo quality, but more studies are needed to see if it will translate into higher live birth rates.



RCT 3 Cochrane Review of 8 RCTs on MACS & Other Sperm Selection Methods

Objective:

To systematically analyze all RCTs available on MACS and other advanced sperm selection techniques to determine their effect on live birth rates.

Methodology:

The study reviewed 8 RCTs with a total of 4,147 women undergoing IVF/ICSI. It compared MACS, PICSI, and other sperm selection techniques to standard sperm preparation methods.

Outcomes Measured:

The outcomes measured live birth rates (the most important measure), fertilization rates, embryo quality, and miscarriage rates.

Key Findings:

MACS may improve fertilization and embryo development but there is no strong evidence that MACS improves live birth rates. Data on miscarriage rates was limited, so it is unclear if MACS helps reduce pregnancy loss.

RCT 1	302 couples with high sperm DNA fragmentation	MACS vs. PICSI vs. Testicular Sperm vs. DGC	MACS & PICSI improved fertilization & embryo quality but no major impact on live birth rates	MACS may help men with high DNA fragmentation but isn't a guaranteed solution
RCT 2	ICSI patients	MACS vs. PICSI	Both methods improved embryo quality; no significant difference in pregnancy/live birth rates	MACS is beneficial, but not clearly better than PICSI
Cochrane Review	8 RCTs (4,147 women)	MACS vs. Standard Methods	MACS improves sperm selection & embryo quality, but no confirmed increase in live birth rates	More research is needed to prove MACS increases take-home baby rates

Figure 3: Summary of randomised controlled trials and cochrane review.

Conclusion

MACS may improve sperm selection and embryo quality, but more studies are needed to confirm if it truly increases live birth rates. There is still not enough high-quality evidence to recommend MACS for all patients. More large-scale, well-controlled RCTs are needed before MACS becomes a routine sperm selection method.

Recommendations

- Selective Use of MACS in ICSI: MACS is a promising technique for specific male factor infertility cases. It should be used selectively, not as a routine sperm selection method.
- Combining MACS with other ART strategies (e.g., embryo screening, endometrial receptivity testing) may further improve success rates.
- MACS should be used for men with high sperm DNA fragmentation (> 30%), confirmed by TUNEL (Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling), SCSA (Sperm Chromatin Structure Assay), or Comet (single cell gel electrophoresis) assays, in cases of recurrent implantation failure, and unexplained infertility.

References

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2. Hozyen, M., et al. (2022). "Reproductive Outcomes of Different Sperm Selection Techniques for ICSI Patients with Abnormal Sperm DNA Fragmentation: a Randomized Controlled Trial." Reproductive Sciences, 29(1), 220-228. <https://pubmed.ncbi.nlm.nih.gov/34076869/>
3. Pacheco, A., et al. (2021). "Magnetic-Activated Cell Sorting (MACS): A Useful Sperm-Selection Technique in Cases of High Levels of Sperm DNA Fragmentation." Biology, 10(12), 1175. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7763893/>