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Review Article

Sperm selection techniques in assisted reproduction: a comprehensive review

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ABSTRACT

Sperm selection plays a pivotal role in assisted reproductive technology (ART), directly influencing fertilization rates, embryo development and overall clinical outcomes. While conventional methods such as swim-up and density gradient centrifugation methods are in clinical practice, they offer limited precision in identifying spermatozoa with optimal functional ability and DNA integrity. In response to these limitations, a range of advanced sperm selection techniques has emerged, aiming to improve the selection of high-quality spermatozoa. This review explores the principle, methodologies and clinical evidence behind both established and emerging sperm selection methods. Techniques discussed include magnetic-activated cell sorting (MACS), microfluidic sperm sorting (MSS), hyaluronic acid binding assay/ physiological intracytoplasmic sperm injection (HBA/PICSI) and high-magnification selection methods like intracytoplasmic morphologically selected sperm injection (IMSI). Additionally, novel technologies such as Raman spectroscopy, polarized light microscopy and surface charge-based selection (Zeta potential) are examined for their potential applications in reproductive medicine. While early results suggest these advanced methods may enhance ART outcomes, particularly in cases of male factor infertility, further large-scale randomized controlled trials are needed to confirm their clinical utility and define their optimal use. This review aims to provide ART practitioners with a comprehensive overview of the evolving landscape of sperm selection in ART, highlighting both current practices and future directions.

Keywords: Assisted reproductive technology, ICSI, IMSI, MACS, Microfluidics, Male infertility, PICSI, Sperm selection

INTRODUCTION

The success of assisted reproductive technologies ART is fundamentally influenced by the quality of the gametes used, with both oocytes and spermatozoa playing critical roles in determining fertilization rate, embryo development and clinical pregnancy outcomes.[1] Over the past few decades, significant progress has been made in refining protocols for oocyte retrieval, in vitro fertilization and embryo culture systems. However, sperm selection, a key determinant in ART remains an area with substantial potential for optimization.[2] Conventional semen analysis, as outlined by the World Health Organization (WHO), provides a standardized assessment of sperm concentration, motility and morphology.[3] While useful as a baseline diagnostic tool, these parameters offer only a limited understanding of the functional competence of spermatozoa. Notably, conventional analysis fails to evaluate crucial aspects such as DNA fragmentation, chromatin integrity, mitochondrial function, membrane maturity and capacitation status of the sperm cells and all of these are essential for fertilization, embryo development and successful implantation.[4] In natural conception, spermatozoa are subjected to a complex series of physiological selection barriers within the female reproductive tract. These include cervical mucus penetration, immune system recognition, interaction with the oviductal epithelium, capacitation and the acrosome reaction. Ultimately, only fewer spermatozoa that are functionally competent reach the site of fertilization. In contrast, ART techniques, especially ICSI, bypass these natural barriers. In ICSI, a single sperm is selected and injected directly into the oocyte cytoplasm and sperm selection often is based on gross morphology under standard magnification (200–400X), without assessing the functional competence and DNA integrity.[5] This may inadvertently lead to selection of spermatozoa with subclinical or undetectable defects, potentially impacting fertilisation and embryo quality, affecting pregnancy outcomes.

The importance of refining sperm selection becomes increasingly relevant especially in treating male factor infertility. Male factor infertility is implicated in approximately 50% of all infertility cases, either as a primary or contributing cause, affecting an estimated 7% of men worldwide.[6] Alarmingly, several large scale meta-analyses have documented a global decline in semen quality over the last four decades, characterized by reductions in sperm concentration, total count and motility.[7] These trends highlight the pressing necessity for advanced sperm selection techniques, that are not only biologically relevant but also clinically validated.

In response to these challenges, various advanced sperm selection techniques have been developed to improve the identification of spermatozoa with superior genetic and functional integrity. These methods extend beyond conventional analysis and aim to mimic in vivo or enhance natural selection mechanisms.[8] Techniques such as MACS, microfluidic sperm sorting, HBA/PICSI and high-magnification selection techniques (IMSI/MSOME) have demonstrated potential in improving ART outcomes, particularly in cases with male factor infertility or recurrent ART failure.[2,8-10] Additionally, emerging technologies such as Raman spectroscopy, birefringence-based selection, Zeta potential assessment and AI driven sperm selection are currently being explored for their diagnostic and prognostic value.[8,11]

This review provides a comprehensive overview of advanced sperm selection techniques, summarizing their scientific basis, clinical applications and recent advancements. It aims to inform evidence-based practice and guide future research to optimize ART outcomes.

SPERM PREPARATION VERSUS SPERM SELECTION METHODS

Sperm preparation and sperm selection are complementary processes in assisted reproduction aimed at optimizing sperm quality. Sperm preparation focuses on isolating motile and morphologically normal sperm through physical separation techniques, primarily removing debris and non-viable cells, but it does not directly address genetic integrity or functional competence. Conversely, sperm selection targets sperm with superior biological markers of maturity and DNA integrity to enhance fertilization potential and embryo quality, especially in cases of male factor infertility or repeated ART failure. While semen preparation remains a routine clinical step due to its simplicity, sperm selection provides an additional refinement, though its wider clinical adoption requires further validation. Together, these approaches offer a comprehensive strategy for improving reproductive outcomes.

CONVENTIONAL SPERM PREPARATION METHODS

Swim-up procedure

The swim-up technique is a conventional method that selects sperm based on their motility. In this procedure, culture medium is layered over liquefied semen and motile sperm "swim up" into the medium, which is then collected. Various modifications exist, including direct swim-up from semen and swim-up from washed pellets. The swim-up method effectively selects highly motile sperm and can reduce the concentration of leukocytes and debris. Some studies suggest it may result in lower DNA fragmentation rates compared to unprocessed semen.[12] However, swim-up technique typically yields lower sperm recovery compared to other preparations, especially in cases of altered semen parameters.

Simple washing

It is a basic sperm preparation technique commonly used in ART procedures. The method involves diluting liquefied semen with sperm washing media followed by centrifugation to separate motile sperm from seminal plasma, round cells and debris. The resulting sperm pellet is then resuspended in a fresh medium suitable for clinical use. This process helps to remove prostaglandins, non-viable sperm, leukocytes, present in the seminal plasma.[3] While simple and cost-effective, sperm washing does not selectively enrich motile or morphologically normal sperm and is generally less efficient than more advanced sperm preparatory methods.

Density gradient centrifugation

DGC is one of the most commonly employed sperm preparation techniques in ART, designed to separate spermatozoa based on their density. In this method, liquefied semen is layered over a discontinuous gradient, typically 40% and 80% colloidal silica coated with silane and subjected to centrifugation. Mature, motile sperm, which are denser due to nuclear compaction and higher DNA molecular weight, migrate through the gradient and form a soft pellet at the bottom of the tube, which is then washed and resuspended in culture medium. Clinically, DGC is effective in enriching sperm with normal morphology and motility while significantly reducing non-motile sperm, leukocytes, debris and reactive oxygen species.[13] It also shows a reduction in sperm DNA fragmentation compared to unprocessed semen and is particularly recommended for processing semen from viral-positive patients to reduce the risk of transmission.[3] However, DGC has limitations, including the potential for inducing oxidative stress and DNA damage due to mechanical centrifugation and it does not selectively isolate sperm with intact DNA or advanced functional qualities, relying instead on general physical properties like density and motility.[12] Advantages and disadvantages of conventional sperm preparation methods are tabulated in Table 1.

ADVANCED SPERM SELECTION METHODS

Advanced sperm selection based on functional properties

Advanced sperm selection techniques were introduced to overcome the inherent limitations of conventional methods, which primarily rely on physical parameters such as motility and density. While traditional approaches are effective in reducing cellular debris and enriching population of motile spermatozoa, they lack the specificity to distinguish spermatozoa with intact DNA and optimal functional competence. In contrast, advanced methodologies utilize molecular and physiological markers to selectively isolate mature, non-apoptotic spermatozoa with lower levels of DNA fragmentation. These approaches aim to enhance the quality of spermatozoa used in ART procedures, thereby improving clinical outcomes, especially in indicated cases. We have further elaborated on each of these available methods and given a comparative analysis of the same (Table 2).

Magnetic-activated cell sorting

MACS is an advanced sperm selection technique designed to separate apoptotic from non-apoptotic spermatozoa by exploiting the externalization of phosphatidylserine (PS) on the plasma membrane, a hallmark of early cellular apoptosis. The methodology involves the use of Annexin V-conjugated magnetic microbeads that selectively bind to sperm that expresses PS. When the treated sperm suspension is passed through a magnetic column, apoptotic sperm bound to the microbeads are retained, while non-apoptotic sperms flowing out of the channel are collected.

This approach is grounded in the biological observation that apoptotic sperms not only exhibit PS externalization but are also characterized by increased DNA fragmentation and compromised functional potential. By removing these cells, MACS aims to enrich the sample with viable, functionally competent spermatozoa. Clinical studies have demonstrated the efficacy of MACS in reducing sperm DNA fragmentation and apoptotic markers, with evidence suggesting improved pregnancy outcomes, particularly when used in combination with density gradient centrifugation in couples experiencing recurrent implantation failure or elevated sperm DNA damage.[14] However, despite its benefits, MACS is associated with increased procedural complexity, cost and potential loss of viable sperm, particularly in samples with low sperm count, which may limit its routine application in certain clinical scenarios.[15]

Microfluidic sperm sorting

Microfluidic sperm sorting is an emerging assisted reproductive technology that harnesses the principles of microscale fluid dynamics to isolate high-quality spermatozoa based on their motility and morphology. By mimicking the natural selection processes of the female reproductive tract, these devices guide sperms through microchannels, favouring those capable of directed movement, such as rheotaxis, while excluding immotile sperm and cellular debris.

Scientific studies have shown that this method can effectively select sperms with reduced DNA fragmentation and superior functional characteristics.[16] Early clinical data also indicate potential benefits in improving fertilization outcomes and embryo quality, particularly in cases of high DNA fragmentation or repeated ART failure.[17] Despite the growing commercial availability of these devices, the technique remains in its nascent stages and further large-scale, randomized clinical trials are essential to validate its long-term efficacy and broader application in routine ART procedures.

ADVANCED SPERM SELECTION BASED ON MORPHOLOGICAL ASSESSMENT

Intracytoplasmic morphologically selected sperm injection

IMSI is an advanced sperm selection technique that employs high-magnification microscopy (≥6000X) with differential interference contrast optics to enable detailed assessment of sperm morphology, particularly the presence of nuclear vacuoles. This approach is grounded in the Motile Sperm Organelle Morphology Examination (MSOME), which classifies sperm based on vacuole number, size and location, with Grade I and II sperm (no or minimal vacuoles) considered optimal.

Sperm vacuoles have been linked to chromatin immaturity, DNA fragmentation and aneuploidy and thus their exclusion aims to enhance the genetic quality of sperm selected for ICSI. Early clinical studies by Bartoov et al in 2003 and meta-analyses by Setti et al, in 2011, reported improved pregnancy and reduced miscarriage rates with IMSI, particularly in patients with prior ART failures.[18,19] However, more recent evidence, including systematic reviews by Boitrelle et al presents mixed results regarding its benefit in unselected populations.[20] Despite its potential advantages, the widespread adoption of IMSI and MSOME is limited by the need for specialized equipment, extensive operator training and the time-intensive nature of the technique.

ADVANCED SPERM SELECTION BASED ON SURFACE PROPERTIES

Hyaluronic acid binding assay/ physiological intracytoplasmic sperm injection

HBA, utilized in PICSI, offers a biomimetic approach to sperm selection utilising the natural affinity between mature spermatozoa and HA, a key component of the cumulus oophorus surrounding the oocyte. Mature spermatozoa express specific HA receptors following spermiogenesis and plasma membrane remodelling, making HA binding a functional marker of sperm maturity and genomic integrity. In PICSI, sperms that bind to HA-coated microdots are selected for injection, as they are mature and more likely to exhibit lower rates of DNA fragmentation.[21] Clinical studies, including a meta-analysis by Beck-Fruchter et al in 2016 and the HAB select trial by Miller et al in 2019 have shown that PICSI may reduce miscarriage rates, although improvements in live birth rates remain inconclusive.[22,23] This technique is particularly advantageous for patients with high sperm DNA fragmentation or recurrent ART failures. However, its clinical utility can be limited in cases of severe oligozoospermia due to insufficient HA-binding sperm and the technical expertise required for implementation.

Sperm selection based on surface charge (Zeta potential method)

Sperm selection based on surface charge, also known as the zeta potential method, leverages the net negative charge of mature spermatozoa resulting from sialic acid residues acquired during epididymal maturation. This negative surface charge reflects proper membrane remodelling, a key marker of sperm maturity and genomic integrity. In this technique, sperm with a high negative charge adhere to positively charged surfaces, such as the inner wall of a specially treated test tube, allowing for selective retrieval of functionally competent sperm.

Scientific studies have shown that sperm selected via this method exhibit lower levels of DNA fragmentation and chromosomal abnormalities.[24] Early clinical findings, including those by Chan et al, in 2006, suggest potential improvements in fertilization rates and embryo quality.[25] However, despite its scientific rationale, the zeta potential method is not widely adopted in routine clinical practice due to limited large-scale validation and the presence of more established sperm selection techniques.

EMERGING SPERM SELECTION TECHNOLOGIES

Raman spectroscopy and confocal RAMAN micro spectroscopy

These non-invasive techniques use laser light to assess the biochemical composition of sperm without labelling or damaging the cells, based on molecular vibrations. These methods can detect differences in DNA integrity and membrane structure, with studies like Huser et al, in 2009 showing their ability to distinguish sperm with high DNA fragmentation.[26] While promising for real-time and label-free sperm quality assessment, their clinical use remains limited due to the need for specialized equipment and expertise, keeping them primarily within the research domain.

Polarized light microscopy

Polarized light microscopy is a non-invasive technique used to assess sperm chromatin integrity by evaluating the birefringence of the sperm head, which reflects the degree of DNA packaging. Under polarized light, mature sperm with well condensed chromatin exhibit symmetrical birefringent patterns, while abnormal patterns are linked to DNA damage and chromosomal anomalies. Clinical studies, though limited, suggest that selecting birefringent sperm may enhance fertilization outcomes and embryo quality.[27] However, the need for specialized equipment and expertise, along with limited large-scale validation, currently restricts its routine clinical application.

Thermotaxis and chemotaxis-based selection

Thermotaxis and chemotaxis-based sperm selection techniques aim to replicate the natural guidance mechanisms of the female reproductive tract by isolating sperm that respond to temperature gradients or chemical molecules such as progesterone, mimicking in vivo environment.[28] These methods utilize specialized chambers to attract functionally competent sperm capable of navigating these gradients, reflecting their enhanced fertilization potential. Although promising in theory and supported by preclinical data, these approaches remain largely experimental, with limited translation into clinical practice due to challenges in standardization and reproducibility. Further research is needed to validate their efficacy and feasibility in assisted reproductive technologies.

GUIDELINE PERSPECTIVES ON ADVANCED SPERM SELECTION TECHNIQUES IN ART

Current professional guidelines from European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) provide limited formal endorsement of advanced sperm selection techniques in routine clinical practice. ESHRE guidelines primarily emphasize standard laboratory methods such as swim-up and density gradient centrifugation and do not currently recommend techniques like IMSI, physiological ICSI (PICSI), MACS or microfluidic sorting as standard approaches in assisted reproduction.[29] Similarly, ASRM guidance focuses on validated procedures for semen analysis and sperm handling and while acknowledging the growing interest in advanced selection methods, these techniques are routinely not recommended due to limited high-quality evidence.[30] Both ESHRE and ASRM highlight the importance of individualized treatment strategies, advocating for the selective use of advanced sperm selection methods tailored to specific patient characteristics, particularly in cases of poor ART outcomes or notable sperm abnormalities, while recognizing that further large-scale randomized controlled trials are required to confirm their clinical effectiveness.

CLINICAL APPLICATIONS AND FUTURE DIRECTIONS IN ADVANCED SPERM SELECTION

Advanced sperm selection techniques offer targeted benefits in specific clinical scenarios. In male factor infertility, methods such as microfluidic sorting or MACS may be useful in patients with elevated DNA fragmentation, while IMSI/MSOME is particularly suited to cases of severe teratozoospermia.[31-33] For unexplained infertility and recurrent ART failure, approaches like PICSI, MACS and IMSI have shown potential to improve implantation and reduce miscarriage rates.[22,23] In the context of advanced maternal age, reducing sperm DNA damage via MACS or microfluidics may mitigate the compounded risk of poor embryo development.[16] However, the clinical utility of these techniques must be balanced against cost and complexity, with conventional methods are often sufficient for patients without prior ART failures.

Advancements in sperm selection are shifting towards automated, artificial intelligence driven systems and multi-parameter platforms that assess morphology, motility and DNA integrity simultaneously. Emerging omics based approaches, including metabolomics, proteomics and epigenetics, offer non-invasive insights into sperm function, aiming to improve ART outcomes by mimicking natural selection processes.

Table 1: Advantages and disadvantages of conventional sperm preparation methods.

Technique	Advantages	Disadvantages
Swim-up procedure	Simple and cost effective, selects highly motile sperm, low ROS (reactive oxygen species) production	Yields low post-wash sperm concentration, not suitable for samples with reduced sperm motility, time-consuming
Simple washing	Quick and easy, less laborious, preferrable method when sperm quality is good	Poor separation of motile from immotile sperm and debris, centrifugation may lead to ROS production
Density gradient centrifugation	Removes debris, immotile sperm and round cells, high recovery of motile and morphologically normal sperm	Can cause ROS generation due to multiple centrifugations, involves the use of silica coated silane particles, expensive

Table 2: Comparative analysis of advanced sperm selection techniques.

Sperm selection based on	Technique	Advantages	Disadvantages
Functional properties	Microfluidic techniques	Mimics natural sperm selection, minimal ROS, as no centrifugation is involved, quick and easy to use	Not suitable for samples with lower sperm count, cannot process large volume samples, expensive
	MACS	Selects non-apoptotic sperm cells, may eliminate sperm with DNA damage, non-invasive	Requires specialized equipment, involves prior preparation, especially by DGC, expensive and limited data on long term clinical outcome
Morphological assessment	IMSI	Selects sperms with better morphology, eliminating vacuoles in head	Morphological selection is subjective and doesn't involve functional components, requires specialized equipment, time consuming and expensive
Surface properties	HBA/PICSI	Selects mature sperm, non-invasive, easy to perform, may enable sperm selection with intact DNA	Cannot be employed in cases of altered sperm parameters and in testicular retrieved sperms, no significant improvement in clinical outcome, expensive
	Zeta potential method	Selects mature sperm, may enable sperm selection with intact DNA	Not suitable when the sperm number is low, requires specialized equipment, effectiveness is still under research

CONCLUSION

Advancements in sperm selection have expanded the possibilities for optimizing outcomes in assisted reproductive technology programs. While conventional methods remain widely used, emerging techniques assessing functional and molecular aspects of spermatozoa attributes to offer valuable tools, particularly for patients with male factor infertility or previous ART failures. Although clinical evidence supports their use in selected populations, further validation through large-scale trials are needed. In future, the integration of automation, artificial intelligence and omics-based profiling will offer significant potential to enhance sperm selection strategies, ultimately improving clinical outcomes.

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