Review

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Review

Sperm Selection Methods in ART: Out with the New, the Old Is Better?

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Abstract: Male infertility, accounting for nearly half of infertility cases worldwide, has spurred significant research into its causes, diagnosis and treatment strategies. Genetic abnormalities, social causes, environmental exposures, lifestyle and further health conditions are key contributors. Essential to improving ART outcomes is among other, the selection of high-quality sperm, which requires methods that assess sperm motility, morphology, DNA integrity and oxidative stress levels. Traditional techniques such as semen analysis, Swim-Up, and density gradient centrifugation (DGC) are still widely used but disscussion takes place regarding the limitation in detecting DNA damage and oxidative stress. Advanced methods like magnetic-activated cell sorting (MACS) and microfluidic sorting have emerged as more precise tools for selecting sperm with better genetic integrity, although they face challenges in standardization, cost, and clinical adoption. Emerging technologies such as artificial intelligence (AI) and Raman spectroscopy offer the potential for more automated, accurate sperm selection, minimizing human error and variability. However, the integration of these methods into clinical practice requires further validation through large-scale studies, including assessments of their long-term safety and cost-effectiveness. Future research should focus on refining sperm selection techniques, tailoring them to personalized infertility confrontation and addressing gaps in evidence to improve ART outcomes and patient care.

Keywords: swim-up; DGC; MACS; microfluidics; sperm sorting; assisted reproduction

1. Introduction

Male infertility accounts for almost half of the burden of infertility in couples from various parts of the globe and affects a significant percentage of the world population. This condition has influenced much interest in research on male fertility factors, diagnostic approaches, and intervention strategies (Agarwal et al., 2022; Krausz & Casamonti, 2017). Among various causes, genetic anomalies, living habits, environmental exposure, and health conditions such as varicocele and hormonal imbalance have been identified as the reasons for the prevalence of male infertility (Sharma et al., 2021; Krausz & Riera-Escamilla, 2018; Salas-Huetos et al., 2019). These can be hard to identify since male factor infertility usually is asymptomatic. As such, most cases can only be diagnosed when couples seek reproductive support on account of child conception difficulties (Esteves et al., 2020; Leaver et al., 2022; Glazer et al., 2017). To many such couples, Assisted Reproductive Technology (ART) has offered promising solutions to their infertility problems, especially methods such as in vitro fertilization (IVF) and intracytoplasmic sperm injection, with ICSI being exceptionally helpful in cases where infertility is due to severe male factor (Barratt et al., 2017; Vander Borght & Wyns, 2018; Palermo et al., 2015). For attempts to optimize ART outcome, the selection of high-quality sperm is obviously an important aspect. The techniques of sperm selection attempt to isolate the healthiest sperm based on motility, morphology, and genetic integrity parameters in order to increase fertilization rate and optimize embryo development (Agarwal et al., 2022; Simon et al., 2017; Cho et al., 2017). Traditional semen analysis assesses sperm for motility, concentration, vitality and

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morphology, and these parameters remain critical to initial diagnostic evaluations (Esteves et al., 2020; Barazani et al., 2014; Tomlinson et al., 2016). However, conventional semen analysis cannot reflect finer details of sperm quality, especially with respect to DNA integrity and other molecular biomarkers, recently recognized as influential in fertilization success and embryo quality (Esteves et al., 2020; Agarwal & Majzoub, 2017; Evenson & Wixon, 2006). For instance, today, oxidative stress markers and DNA fragmentation indices are known to be strong predictors of sperm health. High DFI values are associated with poor outcomes of even ICSI, as reported by Zhao et al. 2018 and Zini & Agarwal 2018. All this emergent awareness has thus inspired a plea for improved techniques of sperm selection based on the underlying problems in quality issues, hence affording much more refined selections than allowed by conventional assessments (Dada et al., 2018).

One major area of inquiry into male factor infertility is that of oxidative stress and how it affects sperm quality. Oxidative stress is a result of the imbalance between ROS and antioxidants. It acts as a source of sperm DNA, protein, and cell membrane damage, which consequently affects motility and morphology and hence fertilization capability (Agarwal et al., 2015; Aitken & Curry, 2012; Showell et al., 2014) While these levels of ROS are naturally occurring, they can increase with environmental influences like infection or lifestyle factors, including smoking or poor diet (Aitken et al., 2014; Shiraishi & Matsuyama, 2018; Sharma & Agarwal, 2018). Since the state of oxidative stress affects sperm function and integrity directly, sperm selection techniques may become of primary importance in attempts to improve the outcomes of assisted reproductive technology (Sharma et al., 2021; Esteves et al., 2015; Said et al., 2005). Techniques like magnetic-activated cell sorting (MACS), which eliminate apoptotic sperm with deteriorated membranes, have been promising in reducing ROS-induced impairment and thereby improving pregnancy rates with the use of ART, as evident by Esteves et al. (2015) and Dirican et al. (2019).

DNA fragmentation, as quantified through the DNA Fragmentation Index, DFI, represents another critical biomarker in assessing sperm quality for ART. High DFI is constantly associated with reduced pregnancy rates and an increased risk of miscarriage due to the importance of DNA integrity in embryo development (Barratt et al., 2017; Sakkas & Alvarez, 2010; Muriel et al., 2006). Advanced testing methods such as the TUNEL assay and Sperm Chromatin Structure Assay (SCSA) have, over time, made it possible to detect sperm with impaired DNA integrity more precisely and systematically, thereby helping in the more effective selection of sperm (Ramasamy et al., 2015; Esteves et al., 2019; Erenpreiss et al., 2006). Indeed, novel approaches to sperm selection, such as microfluidic sorting, which selects out sperm with low DFI, have demonstrated the potential to improve the success rates of ART by isolating sperm with superior genetic integrity (Zhao et al., 2018; Quinn et al., 2018; Raheem et al., 2013). Microfluidics itself is an emerging technique that effectively models the natural processes of sperm selection by using a physical barrier through which only the most motile of sperm could pass. It is also expected to enhance clinical outcomes since it selects sperm having a lower DFI and a better morphology (Esteves et al., 2020; Schuster et al., 2021; Shirota et al., 2016). Proteomic and molecular profiling provide further insight into sperm selection and the outcome of ART. Thus, proteomics can be defined as a subdiscipline that studies the protein composition of sperm cells, which can identify unique biomarkers associated with fertility potential, hence allowing more personalized treatment options for ART (Ramasamy et al., 2015; Nixon et al., 2019; Intasqui et al., 2018). By using proteomics, various other proteins responsible for sperm motility, vitality, and DNA integrity are identified, which probably further presents additional selection methods. For instance, the acrosome reaction is related to proteins enabling sperm to penetrate the egg; thus, successful fertilization relies on such proteins, which become markers that have to be present in functional sperm selection (Zhao et al., 2018; Castillo et al., 2021; Chiu et al., 2016). This has been informed by advances in epigenetic profiling, which looks at modifications of DNA without actually changing the DNA sequence, and demonstrates the environmental and lifestyle influences on sperm health, including potential molecular markers of sperm quality assessment (Jungwirth et al., 2018; Laqqan et al., 2020; Jenkins et al., 2017).

While techniques such as MACS, microfluidic sorting, and advanced proteomic profiling had promising results, due to the absence of standardization of methodologies, substantially high costs,

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and a lack of large studies on the evidence, they are not used commonly in the clinic (Agarwal et al., 2022; Simon et al., 2017; Esteves et al., 2021). In this regard, it is essential that there be full evidence-based guidelines stating recommendations for various sperm selection techniques in different male infertility etiologies. In the future, research should be directed to clinical trials that will establish the efficacy of new emerging techniques in sperm selection, identify indications for their use, and finally assess their cost-effectiveness to facilitate their wider application in ART (Vander Borght & Wyns, 2018; Quinn et al., 2018; Prasad et al., 2016). Thus, the following review will critically assess the current literature reporting on sperm selection techniques for use in ART, both traditional and emerging ones, and identify their strengths and weaknesses, while identifying future directions. We perform a synthesis of evidence about the impact of these methods on the outcome of ART to provide the overview of the field, including constructive recommendations for further research and clinical practice. The ensuing review thus sets the future study course in focusing on gaps, working out better protocols, and ultimately increasing success rates for couples trying ART.

2. Sperm Selection Techniques

These conventional and advanced sperm selection techniques have been changing assisted reproduction in steps by improving the outcomes and thus providing solutions for conventionally untreated male infertility factors through standard diagnostic or therapeutic measures. In general, the aims of effective sperm selection methodologies are to isolate sperm cells with the best motility, morphology, and genetic integrity; this improves fertilization success rates and embryo quality in ART (Agarwal et al., 2022; Agarwal & Majzoub, 2017). These techniques include the conventional Swim-Up and Density Gradient Centrifugation (DGC) to more advanced Magnetic-Activated Cell Sorting (MACS), and microfluidics, and other newer techniques. Each of these has various advantages and limitations, making any particular method to be preferable depending on the underlying factor of infertility and specific needs of ART.

2.1. Conventional Sperm Selection Methods

2.1.1. Swim-Up Technique

Swim-Up is the oldest and one of the easiest and cost-effective sperm selection methods; it finds wide usage due to the simplicity of the approach, and it is very effective in selecting motile sperm (>90%). In this method, a sperm sample is layered underneath a medium; hence, motile sperm swim upwards into the medium leaving the immotile or less viable cells behind (Esteves et al., 2015; Bashiri et al., 2014). Because motility is a criterion for natural selection, Swim-Up enriches the sample with active motile sperm, which correlates with enhanced fertilizing potential (Simon et al., 2017). Therefore, this technique has the advantage of being able to isolate sperm with minimal DNA fragmentation, since those highly motile sperm bear lower levels of DNA damage (Henkel et al., 2014; Moskovtsev et al., 2012). While effective, the Swim-Up method possesses some drawback and limitation in cases of poor motility of sperm, as seen in severe male factor infertility. Because only a fraction of motile sperm swim up into the medium, yields are usually low and the technique is not usually applied in oligospermic or asthenozoospermic individuals (Agarwal et al., 2022). There are researchers suggesting that pellet swim-up can be used to reduce cell stress caused by multiple centrifugation of other methods and improve the recovery rate of mature spermatozoa (Fasano et al., 2022). Nevertheless, in patients with severe male factor infertility, the pelleted spermatozoa have been documented to create increased ROS and thus reduced motility (Charles et al., 2024). Studies have demonstrated that Swim-Up, while adequate for IUI, is limited in yield and hence restricted in its utilization for intensive ART processes such as ICSI or IVF, where higher quality sperm samples may be in demand (Esteves et al., 2019; De Vos et al., 2019).

2.1.2. Density Gradient Centrifugation (DGC)

DGC is a widely used sperm selection technique and based on the cell density, it separates healthy and motile sperm from debris, leukocytes, and morphologically abnormal spermatozoa

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(Muratori et al., 2019; Henkel et al., 2021). Centrifugation would render the sample layer over a colloidal silica gradient, thus allowing the high-quality motile sperm to collect at the bottom layer. Indeed, various studies have shown that DGC effectively selects sperm with high motility and intact DNA to improve ART outcomes compared to unprocessed semen (Aitken et al., 2014; Esteves et al., 2020). Of particular note, DGC has been quite successful in IVF and ICSI settings where a high yield of motile and morphologically normal sperm is crucial for successful fertilization and embryo quality. However, there are some disadvantages to DGC, including oxidative stress that could be generated during the centrifugation procedure. The generation of oxidative stress is also well documented to result in DNA fragmentation in spermatozoa, which will influence fertilization and subsequent embryo development (Sharma et al., 2021; Tremellen, 2018). Mainly targeting motility and morphology but not DNA fragmentation, DGC is not effective for the patients mentioned above because DNA fragmentation is >30 % (Moskovtsev et al., 2012). Given these caveats, DGC has remained a mainstay in ART except that it is usually combined with advanced selection methods to further ensure the integrity of the selected sperm.

2.1.3. Comparison of Swim-Up and DGC

Swim-Up and DGC represent two efficient options for motility- and morphology-based sperm selection. However, Swim-Up and DGC significantly differ in their application and limitations. Swim-Up results in minimal generation of oxidative stress and represents the treatment of choice when motility is fair enough; however, the low yield associated with it limits its utility in ICSI and IVF (Henkel et al., 2014; Bashiri et al., 2014). Conversely, DGC allows for favorable advantages for procedures that require a higher volume of motile sperm, such as IVF, although it could introduce oxidative stress, affecting DNA integrity in vulnerable individuals (Esteves et al., 2019). Comparative studies show that the Swim-up method results in greater sperm viability and motility, but DGC has greater total motile sperm counts (Ricci et al, 2009). However, clinical pregnancy rates (CPR) and live birth rates (LBR) are similar between these methods (Charles et al., 2024). Conventional methods hence are usually selected according to the specific needs of the ART and the sperm quality profile of the patient.

2.2. Advanced Sperm Selection Techniques

2.2.1. Magnetic-Activated Cell Sorting (MACS)

Magnetic-Activated Cell Sorting (MACS) is the most recent and sophisticated technique for the sorting out of non-apoptotic sperm, though separation of apoptotic cells is based on the expression of phosphatidylserine—a marker for apoptosis. For MACS, magnetic microbeads are conjugated with Annexin V, which binds Annexin V only to apoptotic sperm (Henkel et al., 2014; Anbari et al., 2021). Similarly, upon exposure to a magnetic field, human vital cells are isolated. So far, this technique has been efficient in increased oxidative stress and DNA fragmentation cases, as it discards harmed or dying sperm while enriching the samples with DNA-intact, viable sperm (Esteves et al., 2019), thus provides a comprehensive evaluation of sperm molecular properties (Zhang et al., 2024). There is conflicting evidence regarding MACS. Although there is a trend that improves ART outcomes by enhancing fertilization rates, achieving reduced duration, cost-effective treatments and better embryo quality in severe male factor infertility cases (Zini & Agarwal, 2018; Said et al., 2021), studies also underline that MACS show minimum statistically significant change in CPR or LBR (Romany et al., 2014; Ziarati et al., 2019). A recent study by Mantravadi and Rao compared MACS and TESA outcomes in patients with >30% sperm DNA fragmentation. No significant differences were detected in blastocyst formation, miscarriage rate and LBR, although the MACS group had a higher implantation rate (Mantravadi and Rao, 2024). Recent studies have investigated the combination of MACS with DGC to further improve the selection efficacy, since DGC isolates motile sperm, while MACS ensures that their DNA is intact. Combining these two may ensure a synergistic effect for high-risk cases (Agarwal et al., 2022; Raheem et al., 2020).

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Although these studies agree that these techniques combined can lead to the best results as far as ART is concerned, mainly in those cases with high DNA fragmentation or with recurrent failures of the ART treatment, a recent systematic review did not find any statistically significant change in overall ability for filtered sperm to result in pregnancy relative to traditional methods (Ribas-Maynou et al., 2022). MACS sperm sorting has significant differences compared to conventional methods mainly in high SDF levels, and therefore may help to overcome miscarriage rates in autologous ICSI and achieve higher pregnancy rates and LBR (Pacheco et al., 2020).

2.2.2. Microfluidic Sperm Sorting

Microfluidic sperm sorting is an emerging technology that selects sperm through the reconstitution of natural mechanisms of selection by microfluidic channels, letting the most motile sperm pass through while subjecting them to minimum mechanical and oxidative stress (Schuster et al., 2021). Microfluidics have become an emerging tool for processing low-volume samples and this technology is a hot topic about infertility diagnosis and treatment (Bouloorchi Tabalvandani et al., 2024). Unlike conventional techniques, microfluidics has no need for centrifugation; hence, the result can lower the risk of DNA damage drastically (Quinn et al., 2018; Samuel et al., 2020). This method therefore offers an advantage in the settings of ART, as highly motile, morphologically entitled, and DNA-intact sperm samples will be produced, which is an essential condition for successful IVF and ICSI results (Esteves et al., 2020; Simon et al., 2017). Indeed, microfluidic sorting has been shown to be very helpful in cases of male infertility with high levels of DNA fragmentation because it enriches samples with sperm that possess lower fragmentation indices and better morphology compared to other methods (Schuster et al., 2021; Bianchi et al., 2022). Banti et al. have documented that the use of sperm sorting microfluidic chip can improve blastocyst formation, utilization, and euploidy rates following ICSI in comparison to the DGC method. Moreover, no significant association was found between the presence or absence of male factor infertility (Banti et al., 2024). Researchers have reported promising results from microfluidics—better fertilization rates, improved embryo development, and superior live birth outcomes in the ART setting; thus, establishing the former as low-stress and highly effective techniques of sperm selection (Agarwal et al., 2022).

2.2.3. Zeta Potential Selection

Zeta potential selection in sperm selection is a novel method involving the use of electrical charge on the sperm to distinguish mature, high-quality cells from immature or damaged ones. Mature sperm with intact membranes exhibit more negative zeta potential, which could be utilized in order to retreat these cells. Selection of zeta potential for research purposes has been shown to decrease DNA fragmentation and produce a high-quality sperm population, promising in various ART applications (Simon et al., 2017; Keating et al., 2021). Zeta potential selection is less clinically utilized than MACS or microfluidics, but early studies have reported improved outcomes with respect to fertilization rates and embryo quality when zeta-selected sperm are used. These results indicate that zeta potential could be one of the selection factors in specific cases of sperm selection.

2.2.4. Hyaluronic Acid (HA) Binding Assay (PICSI)

The HA Binding Assay selects for DNA integrity and functional maturity of sperm through natural selection of HA by mature sperm. This technique involves using HA-coated plates, to which mature, viable sperm selectively bind, thereby mimicking the natural selection processes of the female reproductive tract (Aitken et al., 2014; De Vos et al., 2019). Indeed, to this day, most studies have shown that the HA-binding sperm are usually those with low DNA fragmentation and hence could serve as ideal ones in ICSI, for which DNA integrity is considered one of the important factors regarding the quality of embryos and the respective pregnancy outcomes (Ramasamy et al., 2015; Said et al., 2021). The HA Binding Assay has been noted to improve outcomes from ART, mainly by increasing embryo quality and pregnancy rates. The effective binding of HA into ICSI procedures for

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the non-invasive isolation of mature sperm supplements the treatment and embryo development in cases of male infertility related to high DNA fragmentation (Simon et al., 2017; Bashiri et al., 2014).

2.2.5. Raman Spectroscopy and Artificial Intelligence (AI)

Raman spectroscopy and AI are considered novel technologies in sperm selection, as they can provide non-invasive evaluation and accurate sorting. By employing Raman spectroscopy, one could study the sperm at a molecular level without destructing their viability based on the detection of specific markers related to DNA integrity and metabolic profiles that enhanced ART selection (Castillo et al., 2021; Rajamanickam et al., 2022). AI is powered by machine learning algorithms, where improvements in selection processes are automated to pick out subtle indications of sperm quality that might be overlooked in a manual selection process (Nixon et al., 2019; Weng et al., 2020). Indeed, Raman spectroscopy and AI remain experimental and require further research to clinically validate them; however, they seem to hold promise for improving the outcome of ART. These technologies have the potential to provide further support to non-destructive, precision-based selection processes in the future of ART (Laqqan et al., 2020).

2.3. Comparative Analysis of Techniques

Each sperm selection method has its particular advantages and limitations, and their applications are highly contextual under ART. The conventional approaches, Swim-Up and DGC, are proficient in the isolation of sperm bearing well-motility and normal morphology since these parameters are involved in fertilization success. Swim-Up is indicated in relatively high motility conditions, giving sperm with minimal DNA fragmentation. However, DGC separated sperm based on density and thus gave higher yields of motile sperm suitable for intensive ART procedures like IVF, and as such, has a broader applicability (Esteves et al., 2015; De Vos et al., 2019). However, these two techniques are documented not to be suitable for individuals with a high degree of DNA fragmentation or oxidative stress, since neither of the prepared sperm involves direct selection concerning DNA integrity, hence limiting the outcomes in high-risk cases (Sharma et al., 2021; Moskovtsev et al., 2012). The most critical issue here is that DGC and Swim-Up are often inadequate on their own to produce desired results and need supplementation with advanced techniques in order to achieve a better outcome, especially in patients whose sperm quality is poor.

Advanced techniques such as MACS, microfluidic sorting, and the HA Binding Assay have increased the efficiency of selection techniques to improve sperm selection with intact DNA, either targeting apoptotic sperm or cells with compromised integrity. For example, MACS has been useful in a number of clinical cases to reduce DNA fragmentation and oxidative stress in sperm samples. This is because MACS selectively removes apoptotic sperm with damaged cell membranes. Indeed, evidence has shown that MACS brings added value to DGC in the improvement of clinical ART outcomes, more so in cases of high oxidative stress (Zini & Agarwal, 2018; Said et al., 2021). According MACS may be preferred in patients with repeated implantation failure or poor embryo development since it consistently provides lower DNA fragmentation levels from sperm, indicative of higher pregnancy and live birth rates (Raheem et al., 2020).

Another advanced technique that recently gained much attention is microfluidic sperm sorting due to its high integrity of sperm DNA selection in simulations of natural processes of sperm selection in microchannels. In contrast to the centrifugation-based techniques, microfluidics minimizes oxidative stress and mechanical strain, thereby reducing DNA fragmentation significantly (Samuel et al., 2020; Quinn et al., 2018). Microfluidic selection usually offers better quality embryos and reduces miscarriage rates in IVF and ICSI through the selection of sperm with lower DNA fragmentation indices and better morphology. By nature, microfluidics are non-invasive and hence used in those ART cases in which minimum stress should fall on the sperm (Bianchi et al., 2022). It is one of the major reasons it is being used for complete male infertility cases and repeated ART failure (Schuster et al., 2021). Both the HA Binding Assay and Zeta Potential Selection represent new approaches to the selection of sperm in relation to maturity and integrity, each based on different physiological properties. The HA Binding Assay isolates mature sperm capable of binding to HA,

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which correlates with DNA integrity and reduced fragmentation. This method has given promising results in intracytoplasmic sperm injection, where selected sperm improve embryo quality and clinical pregnancy rates (Simon et al., 2017). In turn, Zeta Potential Selection separates mature and high-quality sperm from immature or damaged ones by electrical charge characteristics. Though less ubiquitously applied than other advanced methods, it has shown efficacy in reducing DNA fragmentation and yielding sperm populations more likely to support successful ART outcomes (Keating et al., 2021).

New technologies, including Raman spectroscopy and AI-assisted selection, will bring in and shape a new generation of precision-based sperm selection. Raman spectroscopy can be used to carry out noninvasive analyses of the biochemical composition of human sperm, thereby allowing the clinician to evaluate DNA integrity and metabolic markers important for fertilization without compromising cell viability (Castillo et al., 2021). Backed by advanced imaging, AI demonstrates promise in fully automating the identification of high-quality sperm through subtle morphological and functional markers that enable faster, more accurate selection for ART (Rajamanickam et al., 2022). Though clinical application of these methods is still in its relative infancy, their principle represents advancement toward individualized treatment strategies—ranging a further step into a sophisticated, data-driven approach to sperm selection. On Table 1, the advantages and disadvantages of the previous discussed sperm sorting methods used in assisted reproductive technologies (ART) are summarized.

Table 1. The advantages and disadvantages of the sperm sorting methods used in ART are summarized.

Sperm sorting method	Advantages	Disadvantages	References
Swim-up	-Cost-effective -Non-invasive -Easy to perform -Selects motile sperm with good DNA integrity	-Depends on operator -Sperm with DNA fragmentation remains - Ineffective for cases of low sperm concentration and/ or motility	Vieira et al. (2021), Esteves et al. (2020), Rojas et al. (2020), Bonde et al. (2021)
Density Gradient Centrifugation	-Well-established -Cost-effective -Removes debris, dead sperm, leukocytes -Efficient selection of motile spermatozoa	-Centrifugation needed: stress and count loss -May induce DNA fragmentation	Madaan et al. (2022), Esteves et al. (2020), Mendez et al. (2021), Ghulmiyyah et al. (2019)
MACS (Magnetic-Activated Cell Sorting)	-Sperm with intact membranes -Low DNA fragmentation -Can be automated - Minimum contamination (dead or apoptotic sperm)	-Specialized equipment and reagents -Increased cost -Sperm loss during sorting - Not universally used/ available	El Hajj et al. (2020), Zhang et al. (2021), Bohn et al. (2020), Mehta et al. (2021)
Microfluidics	-Mimics the natural selection process -Non-invasive (minimal stress) -Viable sperm with good motility and morphology	-Increased cost (requires specialized equipment) -Not widely available -Not suitable for all types of male infertility (severe oligo- or/ and astheno-zoospermia)	Berruti et al. (2020), Sadeghi et al. (2021), Paudel et al. (2021), Martínez-González et al. (2020)
Zeta Potential Selection	-Non-invasive	-Early stages of clinical use	Sadeghi et al. (2020), Hadi et al. (2023), Wei et

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	-Reduces sperm loss -High precision in selecting viable sperm	-Expensive equipment -Not for low sperm count or poor motility	al. (2021), Moghaddam et al. (2020)
Raman Spectroscopy	-Non-invasive -Detailed molecular analysis of sperm -Fast, real-time assessment	- Expensive equipment -Experimental technique -Requires expert	Ramos et al. (2022), Tsutsui et al. (2021), Acuna et al. (2021), Zhao et al. (2020)
AI (Artificial Intelligence)	-Analyze sperm motility, morphology, DNA integrity -Rapid, accurate -Increased consistency -Reduced human error (automation)	-High cost -Large datasets to train AI models --Not widely available	Zeng et al. (2022), Hammadeh et al. (2021), Wu et al. (2020), Li et al. (2021)
PICSI (Physiological ICSI)	-Selection based on natural fertilization (hyaluronan binding) -Good for poor sperm morphology or motility	-Laborious -Requires ICSI -Expensive -Limited use to specific infertility cases	Bhattacharya et al. (2021), Saeed et al. (2022), Mosa et al. (2020), Coticchio et al. (2021)

3. Discussion

Traditional methods currently employed in sperm selection for ART indeed have advanced the field, but at the same time, they are burdened with a host of challenges. For instance, the conventional Swim-Up and DGC remain crucial, though generally limited in targeting key parameters such as DNA integrity, one of the most important factors in fertilization success. Advanced techniques like MACS, microfluidics, and the HA Binding Assay have overcome some of those deficiencies, but inconsistencies in results remain a challenge. Most of the variability arises through procedural differences, skill, and specific characteristics applying to each individual case of infertility (Dorado et al., 2017). For example, the effectiveness of MACS or microfluidic sorting depends on both the expertise of the operator in applying the technique and his ability to obtain viable DNA intact sperm. Standardization of these new techniques, along with best practices, may help reduce such variability, especially when such protocols are introduced for specific infertility etiologies such as high DNA fragmentation and oxidative stress (Esteves et al., 2019).While much has been promised from current sperm selection techniques, all these techniques remain manual and may increase the chances for human error, besides being highly operator-dependent. This dependency suggests a need for approaches that can reduce variability and optimize outcomes through automation, thus potentially enhancing ART success rates. Given such challenges, research is increasingly oriented toward the integration of emerging technologies into ART. For instance, AI and algorithms in machine learning are at the dawn of changing sperm selection by automating analysis of sperm morphology and motility even down to biochemical markers associated with DNA integrity (Zhou et al., 2020). AI-based systems can evaluate very minute parameters that may be overlooked during manual selection; hence, increasing accuracy in selecting high-quality sperm. Concomitantly, non-invasive spectroscopic methods, such as Raman spectroscopy, provide promising perspectives concerning assessing sperm biochemical properties without cell destruction and selecting sperm with ideal metabolic and genetic profiles. It has been revealed that these technologies could make the selection more effective and reduce operator-related variability considerably (Castillo et al., 2021).

It is hereby pointed out that future research in the said field is to be focused on the following lines: There is a real need for clinical studies assessing long-term outcomes in offspring conceived using advanced sperm selection techniques. While most of the available information is concentrated on ART's short-term outcomes—namely fertilization and pregnancy results—the developmental, health, and epigenetic effects of certain sperm selection techniques must be realized to establish the long-term safety and efficiency (Anifandis et al., 2016). This attention to the study of a population over time may show, by means of DNA integrity, if methods like MACS or microfluidic sorting have a positive effect in decreasing genetic problems or developmental disorders in offspring conceived through ART. Further research on the use of sperm selection, including its impact on the genetic and epigenetic components of embryos, will be useful in designing personalized fertility treatments and can take precision in ART to the next level, which may include customized strategies for couples facing specific infertility situations.

With personalized approaches, sperm genetic and molecular profiling can be employed in tailoring selection methods to specific infertility profiles. This extension of success, while minimizing risks related to ART, is possible. Integration of these new technologies with existing practices of ART will, therefore, play an important role in the future in overcoming many conventional pitfalls in sperm selection. Larger studies are also needed in order to provide standardization for a variety of clinical scenarios so that these new tools can be used consistently and thus benefit more patients. In addition, the assessment of the cost-effectiveness of such technologies in real clinical settings will also be important to widely facilitate dissemination. Guidelines and criteria for success benchmarks regarding advanced sperm selection have to be developed based on an evidencing framework that asserts efficacy and safety for both practitioners and patients.

4. Conclusion

Sperm selection techniques form an integral part of assisted reproductive technologies, each with an advantage between the conventional and the advanced ones to be appropriately matched to the different infertility factors. Conventional techniques of Swim-Up and DGC laid the basis for selection based on motility and morphology, whereas MACS, microfluidics, and HA binding developed logos focusing further on DNA integrity and biochemical profiles with enhancing results in ART outcomes for complex cases. In particular, emerging technologies of AI and spectroscopic analysis will further revolutionize sperm selection by bringing automation and precision that may wipe out some of the present drawbacks in ART practices—that is, variability and skill dependency. For such technologies to be fully integrated into the standard protocols of the ART, more extensive research on the long-term safety, efficacy, and cost-effectiveness will be considered essential. Major development and refinement of various sperm selection techniques, by use of technological innovations amply validated by confirmatory empirical evidence, will result in huge improvements in ART outcomes, increasing the chances of success for childless couples and creating healthier future generations. By focusing on an all-around, individualized approach to sperm selection, incorporating the newest available ART practices, the field can continue to evolve with the particular needs of each patient and a new era of reproductive medicine—both precise and patient-centered—can be fostered.

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