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Review

Effects of sperm processing techniques on IVF pregnancy rates: a mini-review

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***Abstract*:** Many factors associated with assisted reproductive technologies significantly influence the success of pregnancy after *in vitro* fertilization (IVF) either directly or indirectly. These factors include sperm processing techniques, egg retrieval, intrauterine artificial insemination, intracytoplasmic sperm injection, and embryo transfer. Among these technologies, sperm quality is one of the most critical factors for a successful IVF pregnancy. The method used for sperm processing plays a crucial role in determining the quality of sperm. Several widely used sorting techniques, such as conventional swim-up, density gradient centrifugation, magnetic activated cell sorting, and hyaluronic acid, have been extensively compared in various studies. Previous studies have shown that each sperm processing method causes varying degrees of sperm damage, particularly in sperm motility, concentration, morphological features, viability, and DNA integrity. However, sperm processing techniques have been developed slowly, and the impact of these methods on pregnancy rates is still unclear. Further exploration is needed. In this review, we aim to compare the results of different sperm processing techniques concerning sperm quality and IVF pregnancy rates. We will also discuss possible clinical approaches, such as microfluidics and integrated approaches, for testing and improving sperm quality.

Keywords: conventional swim-up, density gradient centrifugation, hyaluronic acid, magnetic activated cell sorting, microfluidics, sperm

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Introduction

Infertility can be clinically attributed to reproductive issues in either men or women. Studies suggest that one-third of infertility cases are caused by male reproductive issues, one-third by female fertility problems, and one-third by a combination of male and female factors with unknown causes.[1] Statistically, more than 48.5 million couples worldwide have been unable to conceive a child for 5 years or longer.[2] Approximately 30–50% of infertility cases are caused by male infertility, specifically low sperm quality.[3–5] Assisted reproductive technologies (ARTs)[6] have made significant contributions to global infertility for over 40 years. Due to advancements in ARTs, an increasing number of couples with infertility issues have been able to achieve successful pregnancies and birth healthy children. However, only one-third of couples who undergo ART cycles have been able to achieve live births, and despite the wide range of research and efforts made, *in vitro* fertilization (IVF) appears to remain relatively immature. Despite this, ART has played a significant role in approximately 10 million births worldwide since its first clinical use.[7]

The birth rate of babies born through IVF is predominantly influenced by sperm quality. Abnormal sperm parameters, such as low sperm concentration, poor motility, abnormal morphology, and sperm DNA damage, are the major reasons for low sperm quality.[5] For example, the level of reactive oxygen species (ROS) is

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significantly higher in 40–88% of sperm from infertile men. This increase induces oxidative stress, which negatively impacts essential sperm functions, such as the maturation process, hyperactivation, and the acrosome reaction.[8,9] The high level of ROS increases midpiece defects in sperm with abnormal shapes or missing tails, which can damage capacitation and the acrosome reaction. In addition, a high level of ROS can induce poor DNA integrity, which is a leading cause of low pregnancy rates in IVF and a high disease rate in offspring conceived through ART.[10,11] It has been reported in a meta-analysis that reduced fertility during natural conception is linked to decreased DNA integrity. This suggests that higher levels of ROS and poor DNA integrity have a negative effect on fertility and the development of future offspring.[12] As a result, the selection and optimization of sperm processing methods are crucial for improving sperm quality. These methods should principally meet several requirements: (i) they should be fast, easy, and cost-effective; (ii) they should isolate and enrich the maximum possible number of motile and live sperm; and (iii) they should not damage sperm motility, morphological features, or DNA integrity. Currently, conventional sperm sorting methods for ART, such as conventional swim-up (CSW), and density gradient centrifugation (DGC), are based solely on motility and morphology, without taking into account DNA integrity or ROS levels. In this article, we will provide an overview of these conventional methods and compare their impact on IVF pregnancy rates. Additionally, we will discuss several emerging techniques, such as microfluidic-based methods, that are rapidly being developed and show promising improvements in sperm selection.

Conventional swim-up

The swim-up procedure was first described by Mahadevan and Baker[13] as a technique for the separation of sperm. Since then, it has been widely used worldwide and is considered the standard technique for couples with fertility problems. However, despite its continued use in some IVF laboratories, its application is limited due to the disadvantages outlined in Table 1.

The CSW technique relies on the strong motility of the sperm cells that have been isolated and washed. Notably, this process can yield a high concentration (more than 90%) of motile sperm. Therefore, animal breeding industries take advantage of this technique for endangered animal species.[14] It is also widely used in the laboratory.[15] However, some of the motile sperm may remain trapped in the deep layers of the cell pellet, reducing the overall yield. Moreover, considering that the initial step for cell pellets is necessary, this method generates cell debris and leukocytes, which are known to produce high levels of ROS.[16] Lipid peroxidation caused by ROS can severely impair sperm function.[17]

To overcome the disadvantages of the traditional swim-up method, direct swim-up has been developed. This method is better able to separate motile sperm from liquefied semen containing cell debris and leukocytes. However, this technique is best suited for high-quality sperm and may decrease the yield of motile sperm to some extent. The direct swim-up process involves dividing a semen sample into several aliquots and placing each in a tube with culture medium. The highest number of motile sperm is obtained by combining all aliquots into a single tube.[18] When compared to the CSW technique, direct swim-up has been shown to yield a significantly higher percentage of motile spermatozoa. However, there are reports that the use of purified hyaluronic acid (HA) can increase calcium-influx into spermatozoa, leading to an acrosome reaction.[19] The quality of the sperm significantly impacts IVF pregnancy rates. Further experimental tests and clinical data are necessary to determine the link between direct swim-up and IVF pregnancy rate.

Density gradient centrifugation

Compared to the swim-up method, DGC obtains a higher number of normal sperm with strong motility and capability by distinguishing sperm from cell debris and leukocytes. DGC can be divided into continuous and discontinuous gradients.[20,21] In this process, liquefied semen is placed on top of the density media. Cell debris and leukocytes remain at the top layer, poorly motile sperm or immobile cells move to the middle layer, and highly motile sperms penetrate the media boundary and reach the bottom. The centrifuge time, speed, and density volume of media can vary according to different kinds of semen

Table 1. Comparation of different sperm separation methods.

Method	Advantages	Disadvantages
CSW	- Easy to perform - Purified recovery of highly motile spermatozoa	- Low yield - Increased spermatozoa damaged by ROS - Decreased normally chromatin-condensed spermatozoa
DGC	- Purified recovery of highly motile spermatozoa - High yield - Cell debris and leukocytes are largely decreased - ROS are significantly reduced	- More time-consuming - Potential risk of endotoxins - Difficulty in stable pH and temperature
MACS	- Sperm with high quality - Maintain nuclear DNA integrity - Separation of non-apoptotic sperm	- Uncertain improvement in pregnancy rates - PS externalization may happen after acrosome reaction - The impact of microbeads on ICSI
HA	- Purified mature sperm - Mimic a natural selection - More sperm with normal morphology - Lower level of DNA fragmentation and rates of chromosomal aneuploidy	- Insufficient studies to confirm that it improves pregnancy rate - Requirement of experienced embryological skills

CSW, conventional swim-up; DGC, density gradient centrifugation; HA, hyaluronic acid; ICSI, intracytoplasmic sperm injection; MACS, magnetic activated cell sorting; PS, phosphatidylserine; ROS, reactive oxygen species.

samples. By increasing the centrifuge speed, more highly motile sperm and particles with low density are enriched at the bottom. Therefore, high-quality sperm require a shorter centrifuge time. While a larger density volume is favorable for sperm isolation, the yield of motile sperm cells will decrease. Besides, there are mainly two types of centrifuge rotors. It is also essential to analyze the specific effects of different rotors on sperm quality and pregnancy rates after DGC. A retrospective analysis of 352 artificial insemination cycles has been conducted, and the participants have been divided into two groups according to the different rotors used during gradient centrifugation: a horizontal rotor group and an angled rotor group. Sperm samples have been subjected to DGC, and the effects on sperm quality and pregnancy outcomes have been compared between the two groups. However, it has been indicated that there is no difference in sperm quality and pregnancy outcome by using the two types of centrifuge rotors, as shown in Table 2.[22]

The advantages and disadvantages of DGC are summarized in Table 1. However, it has been observed that sperm isolated by DGC exhibit relatively high levels of DNA fragments, detected by live terminal deoxynucleotidyl transferase-mediated fluorescein-2'-deoxyuridine 5'-triphosphate (dUTP) nick end labeling.[23] The underlying mechanisms behind this phenomenon remain unknown. Two types of spermatozoa, immature and senescent mature spermatozoa, are known to contain damaged DNA. Immature spermatozoa are vulnerable to ROS due to the unpacked chromatin, whereas DNA damage in senescent mature spermatozoa could occur due to mitochondrial lesions in an apoptosis-induced process.[24,25] DGC can enrich motile sperm with high density at the bottom, including DNA-damaged senescent mature spermatozoa with reasonable motility. Therefore, while DGC is effective in concentrating normal sperm, it may also collect DNA-damaged sperm, potentially increasing the risk of ART failure.

Table 2. The effects of different sperm sorting techniques on sperm quality and pregnancy rates of IVF.

Method	Rotation	Sperm quality		Pregnant rate		Reference
DGC	Horizontal rotor	Ratio of forward motile sperm (%)	89.2 ± 8.5	Clinical pregnancy rate (%)	16.5 (30/182)	22
		Sperm concentration (×10⁶/ml)	47.8 ± 14.3	Abortion rate (%)	3.3 (1/30)	
		Number of forward motile sperm (×10⁶)	13.6 ± 6.3	Live birth rate (%)	96.7 (29/30)	
		Ratio of normal sperm (%)	18.5 ± 8.2			
	Angle rotor	Ratio of forward motile sperm (%)	90.1 ± 8.8	Clinical pregnancy rate (%)	12.4 (21/170)	
		Sperm concentration (×10⁶/ml)	43.4 ± 15.1	Abortion rate (%)	9.5 (2/21)	
		Number of forward motile sperm (×10⁶)	12.9 ± 6.1	Live birth rate (%)	90.5 (19/21)	
		Ratio of normal sperm (%)	14.2 ± 5.7			
CSW		Sperm concentration (×10⁶/ml)	75.73 ± 52.09	Clinical pregnancy rate (%)	14.6 (56/328)	26
		Motility	60.67 ± 16.65	Live birth rate (%)	10.9 (42/328)	
		Progressive motility	49.25 ± 17.81	Miscarriage (%)	3.6 (14/328)	
		Inseminated total progressive motile sperm count (×10⁶)	40.18 ± 36.47			
Density gradient CSW		Sperm concentration (×10⁶/ml)	63.67 ± 41.75	Clinical pregnancy rate (%)	12.5 (45/316)	
		Motility	62.42 ± 15.89	Live birth rate (%)	10.8 (39/316)	
		Progressive motility	49.25 ± 17.98	Miscarriage (%)	1.7 (6/316)	
		Inseminated total progressive motile sperm count (×10⁶)	24.82 ± 22.63			
MACS		Sperm concentration (×10⁶/ml)	28.9 ± 26.1 (39)	Clinical pregnancy rate (%)	65.8 (25/38)	27
		Sperm motility (%)	17.8 ± 13.8 (39)	Implantation rate (%)	53.6 (30/56)	
		Normal sperm morphology (%)	2.7 ± 2.8 (39)	Early miscarriage rate (%)	2.6 (1/38)	
Control		Sperm concentration (×10⁶/ml)	35.8 ± 36.3 (41)	Clinical pregnancy rate (%)	59.0 (23/39)	
		Sperm motility (%)	23.2 ± 21.5 (41)	Implantation rate (%)	55.6 (30/54)	
		Normal sperm morphology (%)	2.6 ± 2.6 (41)	Early miscarriage rate (%)	5.1 (2/39)	
HA		Bound sperm (%)	59.69 ± 7.24	Clinical pregnancy rate (%)	34 (32/93)	28
		Released sperm (%)	31.16 ± 4.81	Miscarriage rate (%)	13 (4/32)	
		Progressive sperm (%)	55.00 ± 5.00	Live birth rate (%)	17 (26/152)	
Control		Bound sperm (%)	45.23 ± 12.48	Clinical pregnancy rate (%)	7 (5/69)	
		Released sperm (%)	17.72 ± 2.57	Miscarriage rate (%)	100 (5/5)	
		Progressive sperm (%)	36.67 ± 3.33	Live birth rate (%)	0 (0/115)	
PVP				Clinical pregnancy rate (%)	21.6 (21/96)	
				Abortion rate (%)	14.3 (3/21)	
				Live birth rate (%)	94.7 (18/19)	

CSW, conventional swim-up; DGC, density gradient centrifugation; HA, hyaluronic acid; IVF, *in vitro* fertilization; MACS, magnetic activated cell sorting; PVP, polyvinylpyrrolidone.

Magnetic activated cell sorting

MACS was first used by Pesce and De Felici in 1995 to obtain mouse primordial germ cells.[29] Mature sperms express various markers that represent their physiological state, including proliferation, differentiation, and apoptosis.[30,31] Apoptosis of mature sperm induces sperm cell suicide, resulting in morphological and biochemical alterations.[32,33] Sperm apoptosis occurs when phosphatidylserine (PS) transfers from the inner leaflet to the outer leaflet of the spermatozoa membrane.[34] Annexin V is commonly used as an apoptotic marker due to its strong binding affinity to PS. To separate Annexin-positive (apoptotic fraction) and Annexin-negative (nonapoptotic fraction) spermatozoa, MACS involves the conjugation of Annexin V with magnetic microspheres, followed by electrophoretic separation. By subjecting the mixed samples to magnetic circumstance in an iron matrix, nonapoptotic spermatozoa can be enriched.[35] Several reports indicate that this sorting method significantly reduces apoptotic spermatozoa and improves sperm quality.[36] It has been reported that MACS statistically improves pregnancy rates compared to swim-up and density centrifugation in some meta-analyses.[37] However, data from randomized clinical trials suggest that MACS does not improve pregnancy rates after ART.[27] In exploring the underlying mechanisms, several studies have revealed that there are defects in sperm viability and motility in apoptotic sperm.[38–41] However, sperm morphology is not statistically associated with apoptosis, and there is no significant correlation between the percentage of normal sperm and PS externalization.[42–44] Additionally, it remains unclear whether abnormal apoptotic processes start before or after ejaculation, inducing apoptotic markers expression in spermatozoa.[45–57] Therefore, it is essential to further assess the sperm morphology index in nonapoptotic and apoptotic sperm. Simultaneously, the effects of magnetic forces on sperm quality are understudied and require further investigation.

Hyaluronic acid

One of the most widely utilized methods, the process of selecting sperm by binding to HA aims to choose mature sperm. HA is a glycosaminoglycan that is involved in fertilization, although its function in sperm is still not fully understood. It has been reported that HA is linked with several signaling pathways and induces sperm capacitation and acrosome reaction.[48] During fertilization, the combination of HA and sperm influences sperm motility and facilitates sperm penetration, promoting sperm nuclear maturation, plasma membrane remodeling, and acrosome extrusion.[49,50] Only mature sperm express the receptors for HA, which allow them to bind to the oocyte for fertilization. Therefore, HA selection is considered to be the closest thing to natural selection. Through HA selection, sperm with normal morphology, DNA integrity, and a lower rate of chromosomal aneuploidy can be identified.[31] Sperm–HA binding is a selective process that does not occur in all mobile sperms *in vitro*.[50] The sperm–HA binding rate is utilized as a tool to detect the presence of HA receptors in sperm. The data obtained from the ROC curve analysis indicates that a binding rate of 65% is the optimal threshold for *in vitro* screening of hyaluronan-bound (HB) sperm for intracytoplasmic sperm injection (ICSI). Patients with a HA binding rate of less than or equal to 65% are less likely to randomly select HB sperm during the ICSI process.[51] It can be inferred that higher HA binding rates in semen samples increase the likelihood of selecting HB sperm. Therefore, patients with a binding rate greater than 65% have been included in the current research design to confirm the accuracy of the initial trial. Additionally, it is recommended that the procedure is performed by an experienced andrologist.

Theoretically, HA selection can improve fertilization and pregnancy rates. A meta-analysis has confirmed that HA selection improves embryo quality and implantation rate, which is consistent with other analyses of prospective studies that show improvement in embryo quality.[52–54] However, the clinical data on whether HA selection improves embryo quality and pregnancy rate are still controversial. A previous study showed that HA selection and a control method resulted in fertilization rates of 92% and 86%, and percentages of high-quality embryos of 36% and 24%, respectively.[55] However, two other studies have found no differences in the clinical pregnancy rate between HA selection and a control method, except for a reduction in the miscarriage rate with HA selection.[56,57] To investigate whether HA sperm selection improves ICSI outcomes for couples, both conventional polyvinylpyrrolidone-ICSI (PVP-ICSI) and HA-ICSI are

compared. In ICSI, PVP has been used to immobilize and manipulate spermatozoa. However, it has been reported that prolonged exposure of sperm to PVP for 15, 30, and 60 min significantly affects their viability and morphology, resulting in an increase in DNA fragmentation and abnormal chromatin structure.[58] In addition, the percentage of acrosome-reacted spermatozoa was found to be increased as well. Some authors have shown superior results in terms of fertilization, embryo quality, and implantation rates when HA-bound spermatozoa are injected compared to conventional PVP-ICSI.[57,59] However, other studies have reported inconsistent outcomes.[56] Thus, there are currently insufficient data to support the use of HA selection for high-quality sperm in ART, despite its effectiveness in semen analysis. Some researchers have suggested that purified HA may increase calcium influx into spermatozoa, resulting in an acrosome reaction.[60] However, the roles of HA in the rheological parameters of IVF medium require further study. More researches is needed to provide reasonable explanations for the effects of HA selection on the clinical pregnancy rate in ART.

Microfluidic sperm sorting: A promising future approach to sperm separation

Compared to the current clinical methods, such as swim-up and DGC, microfluidic techniques have been shown to reduce DNA fragmentation and ROS resulting from centrifugation.[61,62] Because of the stable fluid environment, microfluidic technology can avoid mechanical damage to sperm to the greatest possible extent. This technique was first established by Smith and Takayama, who emphasized the potential for improving sperm quality and laboratory efficiency with the adjustment of microfluidic sorting technology.[63,64] Researchers focusing on the study and control of fluids in microfluidics for biomedical applications have investigated picoliters, microliters, and inside channels.[65] Specifically, microfluidic sperm sorting is performed in a flow-free chip with a dual-chambered microfluid. A semen sample is added to the inlet channel after the outlet channel with a microporous membrane is filled with a volume of sperm wash medium. The chip is then incubated at 37°C for half an hour before collecting the sperm from the outlet for further study. It has been reported that microfluidic-sorted sperm can be clinically used due to their high quality and undetectable DNA fragmentation.[16]

The microfluidic sphere can also be utilized in biomimicry-based sperm sorting methods, which closely mimic the *in vivo* environment by modifying the geometry of microconfined areas. At present, the methods for sperm motility evaluation and screening by microfluidic techniques are mainly divided into three types, including microchannel screening, dielectrophoresis force screening, and laminar effect screening.

Microchannel screening enables sperm to swim freely in the fluid by forming a stable liquid without the need for excessive manual manipulation. This method is direct, minimizing or avoiding mechanical DNA damage to sperm. For example, McCormack *et al.*[66] developed a direct channel for assessing the fertilization capacity of semen samples using fluorescent labeling technology. Screening experiments conducted according to the World Health Organization standard demonstrate that the fluorescence signal of the system exhibited Pearson correlation coefficients of 0.79 and 0.80 with motile sperm concentration and forward motile sperm concentration, respectively. The sensitivity and specificity were 94% and 97%, and 96% and 90%, respectively. Despite its accuracy, the high cost of the device and the complexity of the fluorescence labeling procedure restrict its widespread use.[66] Based on the same principle, Chen *et al.*[67] have also designed a portable analyzer for sperm quality, which integrates Coulter technology to count sperm and evaluate the two indices of sperm motility and concentration. This system does not require fluorescent labeling or sample pretreatment and is a low cost and convenient operation. However, it should be noted that the size of the counting hole is small, measuring only 6 µm. Moreover, microchannel screening only selects sperm based on swimming ability, which may not entirely reflect the natural selection process of sperm under physiological conditions. Therefore, Zhang *et al.*[68] developed a method to simulate the interaction between sperm and cervical mucus under physiological conditions on a chip, allowing for natural selection of sperm and online detection of sperm quality. The method is quick and does not require centrifugation, providing selected sperm that have superior indicators, such as motility, velocity, forward ratio, and normal form percentage, compared to those obtained through presorting and upstream

methods. This also establishes a foundation for the fertilization process under the simulated physiological conditions of the chip.[68]

Dielectrophoresis (DEP) has also been well developed. The principle of this technique is based on inducing polarization in cells through an uneven, alternating electric field. Various cells have unique dipole moments resulting from varying dielectric properties, conductivity, shape, or size. Consequently, different dielectric forces separate these cells within the electric field. The Fuhr group has reportedly utilized various high-frequency electric fields to capture, localize, and screen single human sperm with different activities.[69] Negative DEP occurs when the conductivity of the salt solution exceeds the average conductivity of sperms, thus driving the sperm from the electrode toward the lowest electric field.[69] By using four planar electrodes, a field funnel that impedes the movement of a single spermatozoon can be formed. However, a more effective method would be to use a three-dimensional cage created by an octopole electrode system. The system also has the capability to release surviving captured sperm into designated areas for future experimental operations. However, in stripwise and interdigitated electrodes, rapidly swimming sperm cells can be positioned very effectively in front of a break-electrode through a combination of electric field trapping and field-induced laminar fluid streaming.

When the channel scale is in the micron range and the fluid Reynolds number is low, two parallel laminar flows in close proximity can only mix *via* diffusion. Once the laminar flow is stabilized, motile sperm swim through the laminar flow interface from the stock solution to the collection channel, driven by surface tension and viscosity. Inactive sperm and cell fragments remain in the stock solution and flow until they are discharged, enabling efficient screening of sperm motility. Cho *et al.*[70] integrated horizontal gravity-driven micropumps, which made it possible to conduct human sperm screening using the laminar flow effect. This innovation overcomes a major issue associated with traditional gravity micropumps, where the velocity of the fluid decreases with time. The device is not only small, simple, and disposable, but also an integrated system with sample inlets, outlets, a sorting channel, and a novel passively driven pumping system that ensures a constant flow of liquid. Moreover, it does not need any external power source or controls.[70] To optimize the effectiveness of the chip for screening high-motility sperm, it is necessary to enhance the laminar flow effect. The Tseng[71] group labeled human sperm with the fluorescent dye SYBR-14/PI(Molecular Probes, Invitrogen) and injected samples using an injection pump. By screening the motile sperm using the laminar flow effect, they observed different motile sperm under the microscope and identified and quantified dead/alive sperm using flow cytometry. The flow rate of the system was both controllable and stable, resulting in improved screening efficiency (with a sperm survival rate of 94.8%).[71] In addition, Wu *et al.*[72] utilized a sperm optimization chip made of polydimethylsiloxane (PDMS) with a surface modified with polyethylene glycol methacrylate. This chip retains its hydrophilicity for an extended period of 56 days and exhibits minimal nonspecific adsorption, which effectively avoids channel blockage. Furthermore, the chip surface has the ability to easily infiltrate channels, making it convenient for sample collection. The screened samples showed sperm motility of 74%.[72] However, PDMS is not suitable for clinical applications due to safety concerns, and quartz is not recommended due to its high cost. Thus, Matsuura *et al.*[73] have utilized cycloolefin polymers that are widely recognized in the clinical field to produce sperm screening chips.

In summary, although the effect of microfluidics on the pregnancy rate in IVF is unknown and different types of microfluidics have limitations, it is still a promising method, especially in combination with other optics. In the future, microfluidics is expected to be applied in other aspects beyond sperm sorting and may be used in livestock reproduction.[74] Future advancements in microfluidic sperm sorting will not only automate the process but also make it accurately comprehensive. By integrating a molecular analytical platform for single cells, the potential for microfluidics in IVF can be maximized.

Discussion

Male infertility has become a growing global issue, and clinical ART is necessary to address the challenge of increasing infertility rates. Semen quality, including semen volume and sperm

count, motility, morphology, and DNA integrity, plays an important role in determining embryo and pregnancy rates. With the advancement of ART, scientists and clinicians are focused on sperm separation techniques that aim to select healthy sperm with intact DNA.

To improve the quality of motile sperm for ART, different sperm separation methods have been developed and applied in combination with pharmacological substances to protect or stimulate sperm functions. In this review, we summarized four widely used methods and one well-established method and their advantages and disadvantages. These methods can make a significant difference in pregnancy rates and may also contribute to the development of more advanced techniques to improve sperm quality. Specific semen samples require different sperm separation methods. For example, semen samples with high ROS levels or genital tract inflammation may not benefit from the swim-up technique but may require gentle methods, such as DGC. The sperm separation medium can also be supplemented with protective substances to improve the quality of the selected sperm. It is also important to consider the dynamic changes in semen and sperm parameters requiring careful pharmacological examination of infertile men *in vivo*.

Regarding the effects of different sperm separation methods on the pregnancy rate of IVF, it has been suggested that there are no significant differences between the CSW and DGC methods (as shown in Table 2).[44,48,49] However, many studies have reported that the other two sorting methods, MACS and HA, achieve significantly better sperm quality than CSW and DGC, despite some disadvantages. There are limited data available on microfluidic sperm separation, and there is still a lack of unified statistical analyses of clinical data on the pregnancy rate when using these different sperm sorting methods. The most concerning question about sperm sorting techniques is whether they maintain DNA integrity. It has been found that sperm DNA damage directly reduces the pregnancy rate and impairs embryonic DNA, even affecting the offspring. Therefore, sperm chromatin structure assay is necessary for detecting the rate of DNA fragmentation in IVF and intrauterine insemination (IUI) cycles. In addition, whole-genome sequencing and analysis should be performed in a timely manner to identify missing or mutant nucleotides. Some studies have shown that sperm sorted by a microfluidic system display a low level of oxidation–reduction potential (ORP).[75] ORP has been discovered as a marker of oxidative stress and redox imbalance in cells and is used to evaluate semen quality.[76–78] However, this conclusion is limited in experimental designs and still needs clinical testing. In conclusion, the selection of an appropriate sperm separation method is critical for the success of ART in infertile men. Further studies comparing and questioning the advantages and disadvantages of these methods will contribute to the improvement of sperm quality and the promotion of successful ART.

To date, it has been believed that achieving successful fertilization through ART requires more than just simple sperm preparation. Couples who have idiopathic infertility often face fertility issues, and even for couples without a diagnosis of infertility, there are several factors that are essential for a successful pregnancy through IVF. With regard to male infertility, the challenge of identifying high-quality sperm still remains.

Declarations

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Not applicable.

Consent for publication
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Cong Zhao: Conceptualization; Writing – review & editing.

Lanming Sun: Conceptualization; Writing – original draft.

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