ORIGINAL RESEARCH ARTICLE

Comparison study of microfluidic sperm selection chips with density gradient centrifugation and upstream methods in sperm selection

DOI: 10.29063/ajrh2026/v30i1.6

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Abstract

This study compared microfluidic sperm selection chips with density gradient centrifugation and swim-up methods using 60 semen samples. All techniques improved sperm motility and reduced DNA fragmentation, abnormal morphology, and leukocyte levels. The microfluidic chip yielded sperm with the best motility, morphology, and DNA integrity, though with a lower recovery rate and concentration than traditional methods. Progressive motility recovery rates were similar across groups. Despite lower sperm yield, microfluidic chips offer a promising option for selecting high-quality sperm in fertility treatments. *(Afr J Reprod Health 2026; 30 [1]: 44-53).*

Keywords: Microfluidic selection chip, DNA fragment rate, total sperm motility, progressive motility, abnormality rate.

Résumé

Cette étude a comparé les puces de sélection spermatique microfluidiques à la centrifugation sur gradient de densité et à la méthode de swim-up en utilisant 60 échantillons de sperme. Toutes les techniques ont amélioré la motilité des spermatozoïdes et réduit la fragmentation de l'ADN, les anomalies morphologiques et le taux de leucocytes. La puce microfluidique a permis d'obtenir des spermatozoïdes présentant la meilleure motilité, morphologie et intégrité de l'ADN, bien que son taux de récupération et sa concentration soient inférieurs à ceux des méthodes traditionnelles. Les taux de récupération de la motilité progressive étaient similaires entre les groupes. Malgré un rendement spermatique plus faible, les puces microfluidiques représentent une option prometteuse pour la sélection de spermatozoïdes de haute qualité dans les traitements de fertilité. *(Afr J Reprod Health 2026; 30 [1]: 44-53).*

Mots-clés: : Puce de sélection microfluidique, taux de fragmentation de l'ADN, motilité totale des spermatozoïdes, motilité progressive, taux d'anomalies

Introduction

Sperm selection constitutes a critical determinant in assisted reproductive technology (ART), particularly in *in vitro* fertilization (IVF) procedures where sperm quality critically determines fertilization success rates and subsequent embryonic development.[0] It is necessary to explore how to more effectively screen high-quality sperm in clinical practice, with the aim of improving the success rate of assisted reproductive technology. While traditional sperm screening methods, such as the swim-up method and density gradient centrifugation (DGC), are widely used in clinical settings, but remain clinical mainstays. Firstly, centrifugation may cause mechanical damage to sperm, affecting their viability and function.[0]Secondly, the swim-up method requires a prolonged processing duration and it is difficult to ensure the efficiency of sperm selection, which may lead to the residual presence of unqualified sperm, thereby affecting the fertilization results.[0]Therefore, there is a need to re-evaluate the current sperm selection techniques in clinical practice and to seek a new type of sperm selection technology to minimize sperm DNA damage and select sperm with better morphology. In recent years, the rise of microfluidic technology has provided new options for sperm selection. Microfluidic technology presents a paradigm shift through laminar flow-mediated cell sorting. By exploiting hydrodynamic properties and sperm

rheotaxis characteristics, microfluidic chips enable precise spatial separation of motile, morphologically normal spermatozoa while simultaneously reducing leukocyte co-isolation.[4]

This study aims to investigate the advantages and disadvantages of microfluidic sperm selection chips in sperm selection, and to conduct a comparative analysis with sperm selected by traditional density gradient centrifugation and swim-up methods. The analysis will include indicators such as total sperm motility, progressive motility, rates of sperm recovery, progressive motility recovery, sperm morphology abnormality, and DNA fragmentation, and leukocyte peroxidase to explore the strengths and weaknesses of microfluidic sperm selection chips in sperm selection.

Methods

Instruments, equipments, and experimental materials.

Instrument and equipment: Ultra-clean workbench, computer-assisted sperm analysis instrument, microscope, Low-speed centrifuge, CO_2 incubator, Flow cytometer, etc.

Disposable supplies: sperm counting chamber,10 CM Petri dish, comfortable straw, test tube, Disposable pipette tips, test tube rack, Straw rack, pipette tips, glass slides, cover slips, 1 ml disposable syringes, glass beakers, etc.

Culture medium: Sperm processing solution such as 80%, 40% SpermGrad (sperm gradient separation solution), and sperm culture medium G-IVF-Plus (washed sperm fertilization solution).

Sperm morphology: Papanicolaou staining reagent, sperm DNA fragmentation staining kit (Rui-Ji staining method), semen leukocyte staining kit (peroxidase staining method)

Research methods

The experimental group used a microfluidic sperm separation chip. (Patent’s number 202121653474.8 Authorization Announcement NumberCN 216141524 U）Screening sperm. Control group 1 used density gradient centrifugation, while control group 2 used the swim-up method for sperm selection.

Steps:

Sixty semen samples were consecutively collected from male partners of infertile couples (age range: 22–49 years; abstinence duration: 2–7 days) undergoing ART evaluation at the hospital's Reproductive Center. Participants underwent standardized semen collection procedures: 1) Pre-collection hygiene protocols (hand and genital disinfection); 2) Masturbatory ejaculation into pre-warmed sterile containers; 3) Immediate incubation at 37 °C for 60 minutes to complete liquefaction. Semen analysis was conducted in three times using computer-assisted sperm analysis methods within 5 minutes after liquefaction. Each qualified sample (volume ≥1.5 mL; concentration $\geq 15\times10^6$/mL) was divided into three 0.8 mL aliquots for parallel processing: microfluidic sperm separation chip method (experimental group), density gradient centrifugation method (control group 1), and swim-up method for sperm selection (control group 2).

Exclusion criteria: 1) Teratozoospermia (percentage of normal morphology sperm below the reference value of 4%), 2) azoospermia (no sperm in the semen), 3) cryptospermia (no sperm observed in the fresh semen slide preparation, but sperm can be seen in the centrifuged sediment). Semen quality assessment is based on the WHO Fifth Edition “WHO laboratory manual for the examination and processing of human semen”.

Sperm selection

The experimental group employed a microfluidic sperm separation chip for sperm selection. The principle is to simulate physiological conditions, optimize the sperm separation and enrichment process, and effectively select sperm using fluid dynamics and the motility characteristics of sperm. It can remove low-quality or abnormally shaped sperm to improve overall sperm quality. Steps: 1)

Place the microfluidic sperm separation chip into a clean, sterile 10 cm petri dish; 2) Aspirate 0.8 ml of liquefied semen sample using a 1 ml syringe and insert the syringe vertically into the sample inlet. Apply slight pressure to ensure a tight connection between the syringe and the inlet; slowly advance the syringe until the sample is visible at the observation port, then stop pushing the syringe; 3) Load 1 ml syringe to aspirate 1 ml of sperm culture medium G-IVF-Plus (washed sperm), add a small amount at the collection port, and slowly add the remaining sperm culture medium G-IVF-Plus (washed sperm) to the upper collection chamber until the solution covers the entire membrane surface; 4) Cover the petri dish and transport it to an incubator at 37°C for incubation; 5) Use a new 1 ml syringe to remove the needle and aspirate the gamete solution or sperm wash culture medium containing selected sperm from the collection port or microporous membrane after 30 minutes of incubation (do not touch the microporous membrane directly); 6) Transfer the collected culture medium to a test tube and place it in the incubator for later use. Take 12 µL of the selected sperm sample for semen analysis and biochemical examination, labeling it with a name tag; 7) Record the relevant data of the selected sperm (volume, concentration, motility, DNA fragmentation rate, abnormality rate, and leukocyte peroxidase, etc.).

Control group 1 used density-gradient centrifugation to select sperm, which is based on differences in density by establishing a gradient of varying concentrations in a centrifuge tube. The advantage of this method is its ability to effectively remove cellular debris, white blood cells, and other impurities from the semen, thereby obtaining high-quality sperm. Steps: 1) Pre-balance the culture medium in an incubator according to the instructions, allowing 80% and 40% SpermGrad (sperm gradient separation solution) to equilibrate at room temperature. 2) Before processing the semen, add 0.7ml of the prepared high-density gradient centrifugation solution to the bottom of a round-bottom tube, then gently layer 0.7ml of the low-density gradient centrifugation solution on top, creating a distinct interface in the tube. Add the liquids gently to avoid mixing. 3) Use a pipette to place 0.8ml of liquefied semen onto the prepared gradient solution, ensuring no mixing occurs at the liquid interface, and centrifuge at 300g for 15 minutes. 4) Centrifuge the sperm pellet at 200g for 5 minutes to wash it once. 5) Discard the supernatant using a Pasteur pipette, then add 0.8ml of sperm culture medium G-IVF-Plus (washed sperm for fertilization) to create a sperm suspension. Take 12 µL of the selected semen sample for semen analysis and biochemical testing, labeling it with the name tag. 6) Record relevant data of the selected sperm (volume, concentration, motility, DNA fragmentation rate, abnormality rate, and leukocyte peroxidase levels, etc.).

The control group 2 used the swim-up method to select sperm, removing impurities and non-sperm cells from the semen through centrifugation and sedimentation to improve the quality and vitality of the sperm. The basic principle of is to separate sperm from other components using different centrifugal forces, washing solutions, and the motility of the sperm, where highly motile sperm swim from the sediment into the supernatant after centrifugation. Steps: 1) Add 1.5ml of sperm culture medium G-IVF-Plus (washed sperm for fertilization) to a test tube. 2) Add 0.8ml of semen and mix well. 3) Centrifuge at 300g for 10 minutes, remove the supernatant, and add 2ml of sperm culture medium G-IVF-Plus (washed sperm for fertilization), then centrifuge at 200g for 5 minutes. 4) Discard the supernatant and slowly add 1ml of sperm culture medium G-IVF-Plus (washed sperm for fertilization) along the wall of the tube. 5) Place the test tube in a beaker, tilt it at a 45° angle, and incubate in the incubator for 30 minutes. 6) After the swim-up, use a Pasteur pipette to extract the sperm and place it in a new test tube for storage in the incubator, and take a 12 µL sample of the selected semen for semen analysis and biochemical examination, labeling it with a name tag. 7) Record the relevant data of the selected

sperm (volume, concentration, vitality, DNA fragmentation rate, abnormality rate, and leukocyte peroxidase, etc.).

Detection Method Routine semen analysis involves taking a 12 μL sample and placing it on a sperm counting chamber of a computer-assisted sperm analysis system for analysis, followed by recording sperm concentration, motility, and other indicators. Sperm DNA is assessed using a sperm DNA fragmentation staining kit (Ree-Gi staining method). Five hundred sperm morphology slides are observed under a 400x optical microscope after staining. Leukocytes are evaluated using a semen leukocyte staining kit (peroxidase staining method), and after staining, leukocytes are observed under a 40x microscope. Sperm morphology assessment uses Papanicolaou staining to evaluate the rate of abnormalities, preparing semen smears, air drying, fixing, and staining, followed by observing the morphology of the sperm head, neck/midpiece, and tail under an oil immersion lens. A total of 200 sperm are evaluated.

All semen samples underwent baseline evaluation through computer-assisted sperm analysis and biochemical profiling prior to processing. Following initial characterization, each specimen was aliquoted into three equal 0.8 mL volumes for parallel processing via distinct sperm selection modalities: the experimental group uses a microfluidic sperm separation chip method, control group 1 uses density gradient centrifugation for sperm selection, and control group 2 uses the swim-up method for sperm selection. After the sperm selection in all three groups, routine analysis of the semen is conducted, along with tests for DNA fragmentation, morphological abnormalities, and leukocyte peroxidase detection.

Detection items: Total sperm motility before screening and after screening using three methods. Total motility = progressive motility (PR) + non-progressive motility (NR), concentration, sperm morphology abnormality rate, DNA fragmentation rate, leukocytes, etc. Sperm morphology: Abnormal sperm includes defects in the sperm head, neck and midpiece defects, tail abnormalities, and excessive cytoplasmic residue. During the counting process, any sperm with unclear abnormal morphology is considered abnormal. Immature round sperm and sperm without heads or tails are not counted. Sperm abnormality rate = number of abnormal sperm / 200 × 100%. The sperm DNA fragmentation rate; a lower value indicates better integrity of sperm DNA. The normal value for leukocytes in seminal fluid is $<1.0 \times 10^6$/ml; a value higher than normally indicates the presence of an infection.

Outcome variables: The core indicators compared in this study include-total sperm motility, progressive motility, sperm concentration, abnormal morphology rate, DNA fragmentation rate, leukocyte peroxidase level before and after screening, as well as sperm recovery rate and progressive motile sperm recovery rate.

Data processing

Data processing and statistical analysis were conducted using SPSS25 statistical software. The method of variance analysis was used, with measurement data expressed as $x \pm s$. Inter-group comparisons were performed using multiple comparisons of variance analysis, with $P<0.05$ indicating statistical significance. This study was approved by the Medical Ethics Committee of Jingzhou Hospital Affiliated to Yangtze University, with the approval number: 2025-132-01, and all subjects signed the informed consent form.

Results

Comparative analysis of sperm quality parameters

As shown in Table 1, all three methods improved sperm quality post-selection, with the microfluidic chip group (experimental group) showing the most notable advantages: its total sperm motility (88.42±12.25%, up 77.4% from pre-selection) and progressive motility (63.57±18.71%, up 112.7% from pre-selection) were significantly higher than those of the density gradient centrifugation group (Control Group 1, 64.2±16.65% and

37.35±19.96%) and swim-up group (Control Group 2, 71.95±15.51% and 28.66±21.02%) (all P<0.05); its DNA fragmentation rate (2±0.57%), abnormal morphology rate (36.79±10.47%), and white blood cell peroxidase level (0.15±0.14%) were the lowest among the three groups (all P<0.05 vs. control groups); while its sperm concentration (12.82±13.93%) was comparable to Control Group 1 (22.73±22.2%, P>0.05) and only lower than Control Group 2 (28.31±21.49%, P<0.05), showing no excessive loss due to high selectivity.

Comparison of rates of sperm recovery and forward motility recovery

As shown in Table 2, the three groups differed significantly in total sperm recovery rate but not in progressive motility recovery rate: the experimental group had a significantly lower total recovery rate (15.35±15.2%) than Control Group 1 (27.03±20.59%) and Control Group 2 (37.22±22.27%) (both P<0.05), which is attributed to the microfluidic chip's high-selectivity mechanism that excludes low-quality sperm; however, its progressive motility recovery rate (35.48±32.73%) was not significantly different from the two control groups (39.32±45.97% and 37.05±50.41%, both P>0.05), meaning it still meets the demand for clinically usable sperm in assisted reproduction despite lower total yield.

Discussion

Sperm processing serves as a pivotal component in assisted reproductive technology (ART), employing biochemical and biomechanical strategies to isolate motile, morphologically intact spermatozoa through systematic elimination of seminal plasma constituents (e.g., prostaglandins, zinc ions), cellular debris, and pathogenic contaminants (leukocytes, bacterial flora, reactive oxygen species). Research shows that after selection, the number and density of normally shaped sperm are increased, thereby improving the success rate of assisted reproduction[6,7]. Sperm kinematic competence, particularly progressive motility driven by flagellar beating mechanics, serves as a critical biomarker for male fertility assessment. In this study, the microfluidic chip group exhibited significantly higher total sperm motility (88.42±12.25%) and progressive motility (63.57±18.71%) than both the density gradient centrifugation group (Control Group 1, 64.2±16.65% and 37.35±19.96%) and swim-up group (Control Group 2, 71.95±15.51% and 28.66±21.02%) (all P<0.05).

Utilizing the motility of sperm, microfluidic chips consist of multiple optimized microchannels based on the movement characteristics of sperm and the biochemical environment to achieve the best selection results[8].Research shows that using microfluidic chips achieve selective enrichment of sperm populations with highly motile and morphologically normal sperm, thereby increasing the success rate of *in vitro* fertilization[9].
Using a microfluidic sperm sorter exhibit superior DNA integrity, enhanced acrosomal reactivity, and reduced oxidative stress markers[0].

By replicating oviductal fluid dynamics through laminar flow gradients, these systems enable natural selection of functionally competent spermatozoa, with the quality of the selected sperm significantly superior to that obtained through upstream selection methods[11]. Sperm DNA fragmentation index and teratozoospermia index are important indicators for assessing sperm quality, with a higher DNA fragmentation rate closely associated with male infertility. As shown in the results, the microfluidic chip group had the lowest DNA fragmentation rate (2±0.57%) and morphological abnormality rate (36.79±10.47%) among the three groups (both P<0.05 vs. control groups). Studies have shown that conventional centrifugation has a potentially harmful effect on sperm DNA integrity, causing sperm damage[12-14].

Table 1: Comparison of sperm quality before and after screening with three methods (x ± s, %)

	Total sperm motility%	PR Forward motion %	Sperm concentration %	White blood cell peroxidase %	Sperm DNA fragmentation rate %	Sperm morphology Bart's staining deformity rate %	Normal morphology of PAS staining %
Before filtering	49.84±20.92	29.88±16.4	78.64±65.18	0.85±0.14	9.02±0.88	68.96±4.13	31.04±4.13
Microfluidic chip method	88.42±12.25	63.57±18.71	12.82±13.93	0.15±0.14	2±0.57	36.79±10.47	63.25±10.5
Density-gradient centrifugation	64.2±16.65	37.35±19.96	22.73±22.2	0.35±0.14	5.82±0.61	49.53±6.1	50.47±6.1
Swim up	71.95±15.51	28.66±21.02	28.31±21.49	0.35±0.14	4.58±0.4	46.86±4.78	53.54±4.88
P1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
P2	0.00	0.00	0.14	0.04	0.00	0.01	0.01
P3	0.00	0.00	0.02	0.04	0.00	0.03	0.04
F	56.12	43.51	31.82	23.78	105.22	19.38	19.35
P	0.00	0.00	0.00	0.00	0.00	0.00	0.00

P1: $P_{\text{Before Filtering-Microfluidic chip method}}$

P2: $P_{\text{Microfluidic chip method-Density-gradient centrifugation}}$

P3: $P_{\text{Microfluidic chip method-Swim up}}$

Table 2: Comparison of sperm recovery rates after screening with three methods (x ± s,

	Sperm recovery rate	**Forward motion sperm recovery rate**
Microfluidic chip method	15.35±15.2	35.48±32.73
Density-gradient centrifugation	27.03±20.59	39.32±45.97
Swim up	37.22±22.27	37.05±50.41
P1	0.00	0.63
P2	0.00	0.84
F	18.74	0.12
P	0.00	0.89

P1: $P_{\text{Microfluidic chip method-Density-gradient centrifugation}}$
P2: $P_{\text{Microfluidic chip method-Swim up}}$

Although traditional sperm selection methods such as density gradient centrifugation and swim-up method select for motile and morphologically normal sperm. However, the prolonged sorting time and excessive centrifugation can easily lead to sperm oxidative damage and DNA fragmentation, resulting in decreased sperm quality and affecting the success rate of ART. In contrast, the microfluidic device based on fluid dynamics for sperm separation shows the highest percentage of motility and normal morphology, a key factor for successful fertilization[15].

Microfluidic chip technology can effectively reduce the DNA fragmentation rate of sperm through precise fluid control and optimized separation processes. Research shows that a channel-based biomimetic microfluidic sperm sorter, compared to density gradient centrifugation, separates fewer sperm but has a higher proportion of forward motility and better DNA integrity[0]. The design of microfluidic chips can provide a gentler selection environment, reducing potential damage to sperm DNA, thereby enhancing sperm fertilization ability and embryo development potentia[0].

The detection of leukocyte peroxidase as a supplementary indicator in semen analysis, is of certain significance for assessing semen quality and male fertility. The results further indicated that the microfluidic chip group had the lowest leukocyte peroxidase level (0.15±0.14%) compared to the two control groups (both 0.35±0.14%, P<0.05). Elevated leukocyte concentrations correlate with compromised semen quality, manifesting as reduced sperm count, motility, and increased morphological abnormalities. Activated granulocytes generate reactive oxygen species (ROS), inducing oxidative stress that elevates sperm DNA fragmentation index and impairs fertilization potential[Error! Reference source not found.]. Conventional sperm processing techniques exacerbate this damage during the centrifugation process, which can lead to a decrease in fertilization rates and pregnancy rates in *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI)[0]. In contrast, microfluidic sperm separation chip to select sperm eliminates the need for centrifugation, reducing the production of reactive oxygen species (ROS) during the centrifugation process and decreasing DNA fragmentation. Experimental results indicate that the sperm selected by the microfluidic sperm separation chip have lower leukocyte levels, which can reduce ROS generation.

Microfluidic chips simulate physiological conditions to selectively enrich motile, morphologically intact spermatozoa with reduced leukocyte contamination and DNA fragmentation. Regarding sperm concentration and recovery rates, the microfluidic chip group's sperm concentration (12.82±13.93%) was comparable to Control Group 1 (22.73±22.2%, P>0.05) but lower than Control

Group 2 (28.31±21.49%, P<0.05); its total sperm recovery rate (15.35±15.2%) was significantly lower than the two control groups (P<0.05), yet its progressive motility recovery rate (35.48±32.73%) showed no significant difference from Control Group 1 (39.32±45.97%) and Control Group 2 (37.05±50.41%) (P>0.05). Compared to conventional methods, microfluidic chips demonstrates procedural simplicity and enhanced efficiency while yielding superior sperm quality parameters: total motility, progressive motility, and DNA integrity (*P*<0.05), resulting in higher embryo formation rates and pregnancy rates in intracytoplasmic sperm injection (ICSI)[0]. Although microfluidic chips-processed specimens exhibit reduced sperm concentrations, the preserved progressive motility ratio and DNA stability compensate for reduced yield, achieving comparable fertilization potential[Error! Reference source not found.]. Experimental results indicate that microfluidic sperm separation chips exhibit higher motility and forward movement, as well as better morphology, lower DNA fragmentation rates, and fewer leukocytes. The sperm recovery rate is low due to low concentration; however, there is no significant difference in the recovery rate of forward-moving sperm among the three methods. The technology's hydrodynamic sorting mechanism effectively segregates progressively motile sperm from static populations through reproductive tract-mimicking fluid dynamics. Notably, in severe oligoasthenozoospermia cases (pre-processing concentration <10×10^6/mL, total motility <10%), microfluidic sperm separation chip method demonstrated limited clinical utility due to critically low recovery rates (<15%), necessitating further validation through multicenter trials with expanded cohorts.

This study has notable strengths. First, its rigorous sample design—60 qualified semen samples were split into three 0.8 mL aliquots for parallel processing, minimizing individual differences. Second, comprehensive indicators, covering not only basic parameters (motility, concentration) but also DNA fragmentation rate and leukocyte peroxidase level. Third, standardized operations: the experimental microfluidic chip has a clear patent (202121653474.8) and detailed procedures, avoiding bias. The study also has limitations from its design and results. It is a single-center study, with samples only from Jingzhou Hospital Affiliated to Yangtze University, limiting result extrapolation. It lacks clinical outcome tracking—only in vitro sperm quality was assessed, with no data on fertilization or pregnancy rates. Additionally, it is less applicable to severe oligoasthenozoospermia samples, with a recovery rate <15%. The results guide practice and policy: Clinically, methods can be selected by patient condition. Policy-wise, health departments should include microfluidic technology in ART catalogs and set standards; support multi-center studies to optimize the chip for severe oligoasthenozoospermia samples, enhancing its clinical utility.Compared with traditional methods, microfluidic chip technology offers higher selectivity and efficiency, allowing for the acquisition of high-quality sperm samples in a shorter time. Sperm selected using the microfluidic sperm separation chip method exhibits higher vitality, better morphology, lower DNA fragmentation rates, and fewer white blood cells, making it suitable for applications in assisted reproductive technologies such as intrauterine insemination, *in vitro* fertilization (IVF), and intracytoplasmic sperm injection (ICSI). In summary, the microfluidic sperm selection chip is an emerging sperm screening technology that provides new options for sperm selection and has promising applications in sperm screening within assisted reproductive technologies.

Conclusion

The microfluidic sperm separation chip is significantly superior to density gradient centrifugation and the swim-up method in screening high-quality sperm (high motility, low DNA fragmentation rate, low morphological

abnormality rate). Although the total sperm recovery rate is relatively low, the progressive motility sperm recovery rate is comparable to that of traditional methods, which can meet the demand for "effective sperm" in assisted reproduction. This technology provides a better sperm screening solution for the field of assisted reproduction, and is particularly suitable for clinical scenarios with high requirements for sperm quality (such as recurrent embryo termination). In the future, multi-center studies are needed to verify its impact on pregnancy outcomes, and the chip design should be optimized to expand its scope of application (such as severe oligozoospermia and asthenozoospermia), promoting it to become one of the routine screening technologies in ART.

Funding

No funding was received for this study.

Acknowledgement

We would like to express our sincere gratitude to all individuals and institutions who contributed to this study.

Competing interest

The authors declare no competing interests.

Contribution of authors

XY X and CH H conceptualised this study. XY X, B H, CH H and LP OY worked on the literature review. XY X and LP OY worked on the data analysis and interpretation of results. All authors worked on the discussion of the findings. All the authors read and approved the final manuscript.

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