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Original Research Article

Optimizing sperm function: examining the correlation between sperm preparation techniques and membrane integrity plus capacitation dynamics

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ABSTRACT

Background: Sperm preparation is a critical step in Assisted Reproductive Technology (ART) that significantly impacts sperm quality and fertilization potential. Membrane integrity and capacitation are the most significant parameters to assess sperm function, but methods like Swim-Up (SU) and Density Gradient Centrifugation (DGC) could influence these values by inducing mechanical damage.

Methods: Ninety normozoospermic semen samples were obtained from ART patients and randomly divided into three groups (n=30 per group). Samples were processed by SU, DGC, or Microfluidics (MF) techniques. Initial motility and post-processing membrane integrity were evaluated according to routine protocols, including the Hypo-Osmotic Swelling Test. Statistical analysis was done with one-way ANOVA.

Results: Initial motility was uniformly high across all groups (SU: 99%, DGC and MF: 100%). However, post-processing membrane integrity varied significantly ($p < 0.0001$), with MF showing the highest integrity ($86.03 \pm 1.98\%$), followed by SU ($78.44 \pm 2.70\%$) and DGC ($67.52 \pm 3.72\%$). Microscopic analysis corroborated these findings, indicating superior morphological preservation in the MF group.

Conclusion: Microfluidics significantly outperforms traditional sperm preparation methods in preserving sperm membrane integrity at the cost of no motility compromise. Its adoption into ART protocols can potentially enhance sperm selection and deliver better treatment outcomes for fertility treatment.

Keywords: Microfluidics, Sperm preparation, Membrane integrity, Assisted reproductive technology

INTRODUCTION

Membrane integrity and capacitation are important in the process of fertilization. Capacitation is characterized by physiological changes in sperm, including membrane reorganization and protein modification, resulting in hyperpolarization of the plasma membrane potential, needed for acrosome reaction and successful fertilization.¹ Protein tyrosine phosphorylation during capacitation influences sperm viability and sensitivity to acrosomal exocytosis, suggesting an association between molecular signalling and sperm physiology.² Capacitation-induced displacement of lipid and membrane proteins regulates

signalling pathways, which are responsible for sperm fertilizing capacity.³ Integrity of the mitochondria is vital for sperm function during capacitation, with implications for motility, hyperactivation, and fertilization competence.⁴ Additionally, cross-talk between apoptosis signalling and the calpain-calmodulin system participates in capacitation, suggesting complex mechanisms in fertilization.⁵ Centrifugal force has the potential to influence hyperactivation of sperm in the oviduct. The laboratory centrifugation technique utilizes the power of centrifugal force to separate sample constituents based on density.⁶ Studies have shown that the use of centrifugal force in sperm preparation protocols, e.g., density gradient

centrifugation commonly used in Assisted Reproductive Technology (ART), has the potential to influence sperm motility patterns, including hyperactivation. Centrifugation also has the potential to induce changes in sperm membrane integrity, mitochondrial activity, and biochemical content, which have the potential to influence their ability to undergo hyperactivation.⁷ Sperm membrane integrity is vital for fertility since it has a significant impact on sperm function. Moreover, functions of the sperm plasma membrane are inextricably linked with key reproduction processes, e.g., capacitation of sperm, acrosome reaction, and sperm merge with the ovum.⁸

Several research studies have explored the effect of diverse semen preparation techniques on membrane integrity. Assessment of stallion sperm-membrane integrity emphasized the need to test membrane integrity to resolve sperm motility, particularly in doubtful cases.⁹ A study of ovine semen also illustrated that sperm selection techniques employing colloidal silica enhanced sperm quality, with colloidal silica-silane being superior in eliminating spermatozoa with acrosomal pathologies.¹⁰

Another study of Bali bull semen concluded that ejaculate volume had no significant influence on plasma membrane integrity and acrosomal integrity of fresh semen and frozen semen samples.¹¹ These results together stress the importance of choosing proper semen preparation techniques to ensure membrane integrity, and thus the quality and viability of sperm for successful fertilization.

METHODS

Study design and ethical clearance

This study was conducted at Andrology Laboratory, Prasanth Fertility and Research Centre, Chennai, India. Samples were obtained from male partners who is undergoing ART treatments in Prasanth Fertility and Research Centre, Chennai, India.

All participants included in the study were informed about the study and signed informed consent forms were retrieved. Only sperm samples that would normally be discarded after a successful ART procedure were used. Ethical approval to conduct this study was obtained from Chennai Meenakshi Multispeciality Hospital Ethics Committee, Chennai, India. (Ref No: CMMHEC/24/15).

Participants

Participants included in the study were male partners who have the BMI ranging from 20 kg/m² to 35 kg/m² who are undergoing ART treatments from January 2025 to March 2025. The inclusion criteria were at least one year of infertility, age between 21-35, normozoospermia, motility >90%, sperm count between 50-150 million/ml. The exclusion criteria were as age >35, oligospermia, azoospermia, motility <90%, sperm count less than 50

million/ml or greater than 150 million/ml. The total number of participants who fit criteria is 90.

Semen collection and analysis

90 Samples were collected from the participants through masturbation and were subsequently kept in the incubator for liquefaction at 37°C for 30 minutes. Sperm samples were collected using a clean, wide-mouth plastic container.

To prevent significant temperature changes that could affect the spermatozoa, the specimen container was maintained at an ambient temperature between 20°C and 37°C before collection. According to the WHO 2010 standards, sperm morphology, motility and viability were assessed using optical microscopy in the andrology laboratory.

Experimental design

The semen samples from 90 patients were divided into 3 categories. 1) swim up (SU) n=30, 2) density gradient centrifugation (DGC) n=30, 3) Microfluidics (MF) n=30. Simple randomization using a closed-envelope method was used to randomize the samples across the 3 groups. These 3 methods were routinely used in the andrology laboratory where the study was performed.

Sperm preparation methods

All three sperm preparations are carried out by the standard protocols and the membrane integrity was analyzed using Hypo-Osmotic Swelling Test.

RESULTS

A total of 90 normozoospermic semen samples were analyzed in this study, with participants randomly assigned into three equal groups (n=30 per group) for sperm processing using SU, DGC, and MF techniques.

All samples demonstrated high initial motility, with values of 99% for SU and 100% for both DGC and Microfluidics groups. Post-processing, the sperm were assessed for membrane integrity, a key indicator of their functional viability for fertilization.

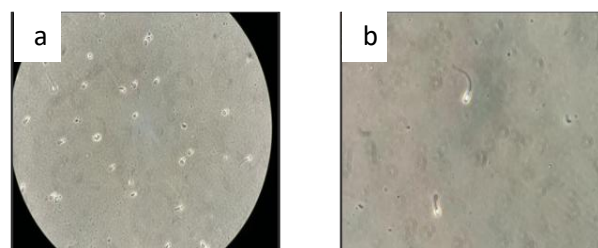


Figure 1: (a and b) Microscopic images of sperm processed by swim up method.

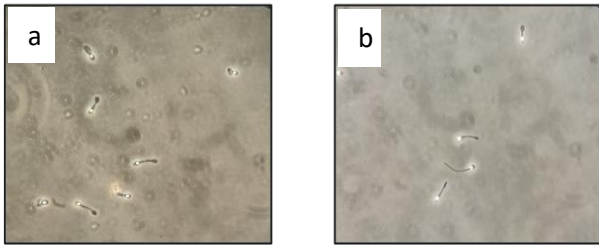


Figure 2: (a and b) Microscopic images of sperm processed by density gradient method.

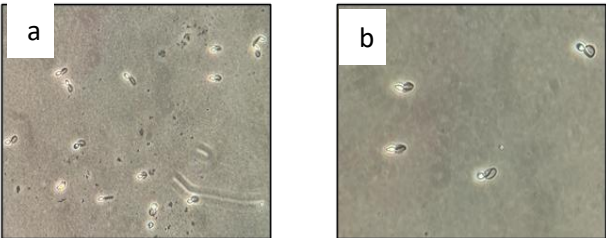


Figure 3: (a and b) Microscopic images of sperm processed by microfluidics.

The post-processing mean membrane integrity percentage was $78.44 \pm 2.70\%$ for the SU group, $67.52 \pm 3.72\%$ for the DGC group, and $86.03 \pm 1.98\%$ for the MF group (Table 1). These values reflect a significant variation in performance among the methods, with the microfluidics method having the highest post-processing integrity and the DGC method the lowest. The 95% confidence intervals also supported these trends, with MF having a narrow range (85.32% to

86.74%), indicating higher consistency and reliability. The DGC method had a wider interval (66.18% to 68.85%), indicating higher variability of outcome. The SU group's interval (77.47% to 79.40%) positioned it as an intermediate effective method (Table 2).

To statistically assess whether these differences were significant, a one-way analysis of variance (ANOVA) was performed. The results revealed a highly significant difference among the three groups, with an F-statistic of 310.49 and a p value less than 0.0001 (Table 3). This strongly indicates that the differences in membrane integrity are not due to random variation but are instead attributable to the sperm preparation method used.

Microscopic analysis was a qualitative confirmation of the quantitative results. Sperm treated with the SU procedure contained a heterogeneous collection of morphological characteristics; some cells contained enlarged tail structures with augmented motility, while others appeared to be damaged (Figure 1).

Samples prepared by the DGC procedure, however, contained predominantly a lack of normal tail features, reflecting lowered membrane integrity (Figure 2). Samples treated with the MF procedure, however, always contained morphological characteristics that reflected intact membrane structure, such as well-defined and intact tail regions (Figure 3). These microscopic findings were consistent with the numerical data, reflecting the superior capacity of Microfluidics to preserve sperm integrity during the processing procedure.

Table 1: Comparison of sperm preparation methods based on motility and membrane integrity.

Sperm preparation method	Initial motility %	Post-processing membrane integrity, %	Sample size (N)
Swim-up (SU)	99±0.8	79±1.5	30
Density gradient (DGC)	100±0.5	68±2.2	30
Microfluidics (MF)	100±0.3	86±1.0	30

Table 2: Membrane integrity after different sperm preparation methods.

Group	Mean (%)	Standard deviation (SD)	95% CI (lower-upper)	N
Swim-up	78.44	2.7	77.47 – 79.40	30
Density gradient	67.52	3.72	66.18 – 68.85	30
Microfluidics	86.03	1.98	85.32 – 86.74	30

Table 3: One-way ANOVA.

Source	Sum of squares	Degrees of freedom (df)	F value	P value
Group	5194.98	2	310.49	<0.0001
Residual	727.83	87		

DISCUSSION

The present study sought to evaluate and contrast the impact of three widely used sperm preparation techniques, SU, DGC, and MF on sperm membrane integrity, a critical

parameter linked with fertilization potential in ART. Our findings show that MF is far superior to SU and DGC in terms of sperm membrane integrity after preparation. This difference is statistically significant, with ANOVA showing significance in between-group differences

($p < 0.0001$). Membrane integrity is crucial in a number of basic sperm functions such as capacitation, hyperactivation, acrosome reaction, and successful fusion with the oocyte. Sperm plasma membrane disruption has the potential to impair these functions and, by extension, decrease fertilization success. In this research, while all three methods yielded high initial motility, MF alone maintained the highest percentage of sperm with intact membranes after processing ($86.03 \pm 1.98\%$). DGC, while 100% motility had the lowest membrane integrity ($67.52 \pm 3.72\%$), indicating that its mechanical forces, including centrifugation, have the potential to degrade membrane quality. The SU method performed moderately, with $78.44 \pm 2.70\%$ membrane integrity.

The findings of this research concur with previous research that has already shown concern regarding traditional centrifugation methods. Other research has shown that multiple centrifugations is able to cause oxidative stress, mechanical damage, and fragmentation of spermatozoa DNA.^{6,7} Alternatively, MF technology does away with the need to use high-speed centrifugation and allows precise selection based on the parameters of motility and morphology, hence no stress on the sperm and maintenance of its physiological function.⁷ The microscopic examination in this research also confirmed the quantitative findings. Sperm treated with the MF method largely had intact, swollen tail structures, a sign of strong membrane and mitochondrial integrity, while those under the DGC group commonly presented a breakdown of morphology. This visual proof confirms the advantage of MF not just preserving the membrane but also structural integrity that is vital for successful fertilization.

Clinically, these results justify the use of MF as a first-line method for sperm preparation in ART setups, especially in cases of unexplained infertility or recurrent failure of ART. By enhancing the quality of sperm to be used in procedures like intracytoplasmic sperm injection (ICSI), Microfluidics may help improve embryo development, increase implantation rates, and enhance pregnancy rates.

Nonetheless, this study has some limitations. The focus was limited to assessing membrane integrity without the integration of other sperm functional tests like DNA fragmentation, levels of ROS, or capacitation indicators. In addition, although membrane integrity is a robust surrogate of fertilizing ability, clinical outcome indicators like fertilization rate, embryo quality, and success in pregnancy were not monitored for these samples.

Future studies must include these endpoints to further establish the clinical benefit of MF-based sperm selection. The research presents strong proof to the fact that the method of sperm preparation has an important role in membrane integrity, and MF is a superior method compared to traditional methods. Its inclusion in the standard ART protocols can optimize the selection of functionally competent spermatozoa, thereby optimizing the efficiency and success of infertility treatment.

CONCLUSION

This investigation strongly proves that sperm preparation technique is decisive of the functional quality of sperm, especially membrane integrity, a parameter that has a strong influence on the fertilizing potential. Though the SU and DGC are a part of daily routine in ART labs, they demonstrate considerable limitations, particularly in maintaining post-processing membrane integrity. In contrast, the MF technique not only maintains high initial motility but also exhibits better maintenance of membrane integrity, as assayed both by quantitative estimation and microscopic assessment.

The results highlight the clinical benefit of embracing MF technology for sperm selection in ART treatment. By reducing mechanical damage and improving selection of physiologically intact sperm, MF is a more sophisticated and efficient methodology that can result in higher fertilization rates and improved ART outcomes. Future research incorporating other functional assays and clinical success measures will further establish its place in the optimization of male fertility treatment.

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