

Intrauterine Insemination

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ABSTRACT

Microfluidic systems offer precise control over fluids at the microscale level and can assist in fertility research and clinical practice. These systems facilitate efficient sperm sorting and selection via physical and/or chemical forces, enhancing sperm quality and motility for improved success rates in assisted reproductive technologies (ARTs) such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI). Microfluidic platforms also enable noninvasive assessment of oocyte quality and viability, optimizing embryo culture conditions by providing a controlled microenvironment that effectively mimics the physiological conditions of the female reproductive tract. The precise control over environmental variables enables embryo development and implantation, resulting in better ART outcomes than traditional sperm sorting techniques (swim-up and density gradient methods). Microfluidic systems may pose a solution to common clinical issues. Traditional sperm sorting techniques, such as the swim-up and density gradient methods, are susceptible to deoxyribonucleic acid (DNA) fragmentation damage, whereas laminar flow commonly used in microfluidic systems is not. Cryopreserved cells can be controllably thawed with microfluidics to reduce the risk of cellular deformation and maintain viability. Additionally, portable microfluidic sperm sorting devices may allow for more accessible and affordable fertility analyses. The implementation of microfluidic devices may serve as an effective alternative to traditional sperm sorting techniques and aid in ART by allowing for more successful clinical outcomes.

■ INTRODUCTION

The current laboratory processes for the preparation of sperm for assisted reproductive technology (ART) procedures are the swim-up and density gradient methods. The direct swim-up method involves placing sperm on top of a medium that mimics the environment of the female reproductive tract, containing nutrients and buffers that support sperm viability and motility in

a test tube. This allows the sperm with the best motility to swim up onto a separate layer that is collected for use in fertility treatments.^{1,2} This method allows for the selection of the most motile and viable sperm. The density gradient method involves varying densities of a medium solution in a test tube. Sperm is layered on top of the gradient and centrifuged so that sperm with higher motility and normal morphology migrate through the layers of the gradient solution based on their density.¹ This allows for separation between high-quality sperm and cellular debris, contaminants, and low-quality sperm. While these methods are currently the most prevalent for ART sperm preparation procedures, they inevitably tend to succumb to deoxyribonucleic acid (DNA) damage, and while effective, currently have questionable success rates regarding their optimal objective (e.g., the quality of sperm in the sample to begin with and the individual patient's condition and lifestyle since morphology can correlate with lifestyle).¹⁻³

Microfluidic devices are biochip devices that are currently being studied for use in ART. Polydimethylsiloxane (PDMS), a popular and inexpensive biochip material, offers a strong yet cost-effective material for crafting biochips and is preferable for microfluidic devices.^{2,4} Microfluidic devices can include digital microfluidics, utilizing external forces like electricity, magnets, and physical and thermal energy to manipulate droplets for separation and sorting processes.^{4,5} These devices are used in *in vivo* applications, creating environments that mimic natural conditions for sperm sorting based on fitness.⁵⁻⁷ They offer several potential advantages that may help to outdo the traditional sperm sorting methods. They work by allowing for precise control over fluid flow and sperm movement, analyzing much smaller volumes leading to the analysis of cell-by-cell sorting, while providing quick results.⁵ Microfluidic devices may also aid in reducing a common clinical issue caused by defrosting cryopreserved cells and embryos.^{8,9} Cells and embryos can be cryopreserved with no risk of deformation using microfluidic devices to carefully allow for thawing without the risk of cells expanding or deforming.⁸⁻¹⁰

This chapter will discuss the use of microfluidic devices in conjunction with ART and will both answer and raise questions about the implementation of the technique in ART procedures. Once microfluidic devices are proven to have the potential to serve as a more affordable and efficient option to traditional ART techniques, the technology may be ready for widescale use in ART.

■ CLINICAL DISCUSSION

The direct swim-up and density gradient methods are currently selected for ART preparations. It was revealed that the swim-up method, while cost-effective and efficient, may result in sperm DNA damage due to reactive

oxygen species (ROS) and prolonged exposure to immature sperm and cell debris.¹¹ Another consideration is that the swim-up technique is suitable for samples with high sperm count and good motility, yet not all motile sperm may reach the culture medium within the incubation period. The density gradient method, involving two to three washes, can also induce ROS-mediated sperm DNA fragmentation (especially with high centrifugation force), but it is preferable for cases with low sperm count despite being more expensive as it yields higher sperm counts.¹¹ It is important to consider new technologies and techniques that may overcome these barriers.

Microfluidic sorting in ART encompasses sperm, oocyte, and embryo selection.⁸⁻¹⁰ Sperm sorting can utilize velocity manipulation or gravity-induced changes in hydrostatic pressure for concentration determination.⁸ Microfluidic systems emulate the physiological conditions of fallopian tubes, including thermotaxis, chemotaxis, and rheotaxis, while mimicking mucus penetration using polycarbonate membrane filters.¹¹ Oocyte sorting relies on sedimentation rates in sucrose buffer, with proposed devices using dielectrophoresis to separate healthy from unhealthy eggs.⁸⁻¹¹ Microfluidic systems enhance gamete and embryo cryopreservation by minimizing oxidative stress-induced damage and improving survival rates.^{9,11} Integration of microfluidic systems in fertility research and clinical practice offers precise control over gamete manipulation, optimized embryo culture, and enhanced cryopreservation techniques.

■ CASE STUDIES

Oligoasthenoteratozoospermia and Laminar Flow

Patel et al.¹² conducted a study where a sperm sorter was used to analyze sperm quality among 10 patients between the ages of 30 and 40 years with clinically defined idiopathic oligoasthenoteratozoospermia (OTA) and a history of at least one failed in vitro fertilization (IVF) cycle.¹² Sperm morphology was checked before and after using the microfluidic chip. The microfluidic sorter was set up in a dish with four chambers, labeled A-D, and functioned on the principle of laminar flow. Laminar flow was maintained by a viscous force, allowing for the inertial forces to be outcompeted by the viscous force and create two separate flowing streams in parallel. This flow allows for the diffusion of two streams without any physical barriers or mixing. Chamber A was injected with a 1:1 suspension of 65 μL sperm and flushing media mixture, chamber B was filled with 100 μL of flushing media, and chambers C and D contained 20 μL of flushing media. Initial sperm morphology was determined to be $26.0 \pm 1.6\%$ (mean \pm SE) and the initial mean morphology was 2.2 ± 0.2 (mean \pm SE).¹² After 30 minutes of microfluidic sorting, an initial 200 sperm were analyzed, and mean sorted mobility

was $88.5 \pm 1.9\%$ (mean \pm SE) while morphology was $3.4 \pm 0.16\%$ (mean \pm SE). The sperm were collected from the sorting device, and intracytoplasmic sperm injection (ICSI) was performed.¹²

Of the 10 patients, a 72.3% fertilization rate was achieved with a 44.61% blastocyte formation rate.¹² Frozen embryo transfer was performed for 8 out of 10 patients, where a 60% pregnancy rate result was obtained.¹² The authors remarked that their study supports the idea that a microfluidic sperm sorter can be used in clinical applications, leading to a significant difference in sperm quality, but that there is a need for extensive research regarding the DNA fragmentation index of sperm sorted in microfluidic devices versus the density gradient centrifugation method. While there were significant advantages in sperm quality by using the microfluidic sorter, it should be noted that the size of this study was small, and the control was limited.

Laminar Flow Versus Direct Swim-up

Anbari et al.¹³ investigated the efficacy of microfluidic sperm sorting in isolating high-quality sperm for ICSI and compared its outcomes with the direct swim-up method in terms of sperm quality parameters, DNA integrity, embryo development, and clinical outcomes.¹³ The study included 95 patients randomly assigned to two groups: Microfluidic sorting (45 patients) and direct swim-up (50 patients).¹³ In the microfluidic technique, droplets A and B containing Ham's F10 medium with HSA were loaded onto a dish, with droplet A swapped with liquefied semen. Using laminar flow, droplet B had a larger volume, causing fluid flow toward droplet B where sperm were collected. The direct swim-up method involved incubating semen in Ham's F10 medium with HSA, followed by centrifugation and resuspension. Sperm quality and DNA fragmentation were assessed using motile sperm organelle morphology examination (MSOME) and Halosperm. Mature oocytes collected after controlled ovarian hyperstimulation were subjected to ICSI.

The clinical results of the study revealed several significant findings. In the microfluidics group, there were notably higher levels of progressive motility and a greater fraction of poor sperm morphology compared to the direct swim-up group, with *p*-values indicating statistical significance ($p < 0.01$ and $p < 0.001$, respectively).¹³ The microfluidics group exhibited significantly lower rates of DNA fragmentation and immotile spermatozoa compared to the direct swim-up group, as determined by the sperm chromatin dispersion (SCD) test and MSOME. Moreover, the microfluidics group achieved higher rates of high-quality embryo formation ($p < 0.001$), implantation ($p = 0.04$), and pregnancy ($p = 0.05$) compared to the swim-up group.¹³ These findings suggest that the microfluidics technique effectively isolates high-quality spermatozoa, leading to improved laboratory parameters and clinical

■ CONCLUSION

Microfluidic devices have great potential to be used in the aid of ART in the near future. The use of microscale samples for cellular sorting and processing is beneficial due to the reduction of stress on smaller samples than larger ones, but more research is needed to quantify efficiency as opposed to traditional techniques in terms of ongoing pregnancy rates and overcoming complexities in fluid dynamics related to laminar flow in microfluidic devices executing laminar flow. For techniques that use dielectrophoresis to separate cells, it is worth noting that the effects of DNA fragmentation including changes in cellular morphology and function are not well known, and more research is necessary to clinically use dielectrophoretic microfluidics. It would be worthwhile to supplement the density gradient method with microfluidics and to determine if both processes would be more efficient, but the practicality of using both techniques is questionable. Considering other perspectives in conjunction with microfluidic devices may be more practical, as microfluidic devices utilizing fertility-based endocrinological and immunological concepts have been proposed for sperm sorting based on creating a microenvironment similar to the female reproductive tract.¹¹

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