The Chitransh Academic & Research
An International Journal of Multidisciplinary Research

An Online Peer Reviewed / Refereed Journal
Volume1 | Issue 1 | January 2025
ISSN: 3049-303X (Online)
Website: www.thechitranshacadmic.in

EVALUATION OF MICROFLUIDIC AND DENSITY GRADIENT SPERM SELECTION TECHNIQUES IN ICSI CYCLES DURING IVF

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ARTICLE DETAILS

Research Paper

Received: **05/01/2025**

Accepted: **20/01/2025**

Published: **30/01/2025**

Keywords: *Microfluidic, Pregnancy, Sperm, Mortality, Blastocysts*

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ABSTRACT

Improving embryo quality and pregnancy outcomes while minimising stress on gametes is a goal of ongoing infertility therapy evolution. This retrospective comparison research examined two methods for preparing sperm for intracytoplasmic sperm injection (ICSI) in in vitro fertilisation (IVF) cycles: traditional density gradient centrifugation and the ZyMot microfluidic sperm selection device. The study lasted 12 months. An equal number of 320 infertile couples were assigned to the control and research groups. While the statistical analysis did not reveal a significant difference in the fertilisation rate ($p = 0.85$ between the study and control group), it did find a significant difference in the blastocyst rate ($p = 0.046$ between the study and control group) and clinical pregnancy ($p = 0.052$). In addition to potentially improving the workflow in conventional IVF, decreasing involvement by lab staff, and providing more constant incubation conditions, microfluidic preparation of spermatozoa appears to enhance outcomes and may be used more widely for ICSI.

I. INTRODUCTION

The successful use of intracytoplasmic sperm injection (ICSI) in 1992 led to a steady growth in its utilisation. More than half of all assisted reproductive cycles performed annually now use in vitro fertilisation (IVF), and some programs use it exclusively. Fertilisation success and reduced miscarriage rates may be possible outcomes of ICSI using normal, highly progressive sperm. For people undergoing fertility therapy, optimising these characteristics can greatly impact the success of a cycle. There is evidence that total motile sperm count, outperforming all other WHO 2010 cut-off values, is the best predictor of successful embryo formation, pregnancy outcomes, and the likelihood of miscarriage.

To prepare a semen sample for in vitro fertilisation, there are a number of options for separating the motile sperm. Density gradient centrifugation (DGC), swim-up wash, and straight swim-up are the three most used sperm selection procedures. There are limits to each of these approaches. While the swim-up procedures can extract a population of highly motile sperm, they often yield modest quantities. For the purpose of selectively isolating motile sperm from detritus, the density gradient approach uses centrifugation to create sperm pellets, whereas the swim-up wash method uses washing to remove seminal plasma. However, centrifugation can damage sperm plasma membranes and increase DNA damage because it increases the generation of reactive oxygen species (ROS). A higher rate of paediatric cancer may be linked to more DNA damage in human sperm, according to some studies. Therefore, there is a growing push to find safer ways to extract motile sperm, ideally ones that cause less damage to DNA and less reactive oxygen species (ROS).

Emerging microfluidics technology has diagnostic, forensic, and fertility-related uses. For assisted reproductive technology (ART) operations, microfluidic sperm selection has demonstrated potential in choosing motile sperm with decreased reactive oxygen species (ROS) and DNA fragmentation. An increase in the number of high-quality blastocysts, euploid blastocysts, and surplus high-quality blastocysts to freeze are some of the better laboratory and clinical outcomes reported by studies utilising microfluidic sperm selection for in vitro fertilisation (ICSI).

II. REVIEW OF LITERATURE

Bhat, Ghulam et al., (2024) One method for selecting high-quality sperm is microfluidics. The inability to conceive is a common problem in many animal species, including humans. The success or failure of assisted reproduction depends on the quality of the sperm, just as defects in the male counterpart cause infertility. Miniature quantities of liquids (in microlitres) are the focus of microfluidics, which employs laminar flow streamlines in small-scale microchannel networks. Microfluidic sperm selection designs that mirror in vivo circumstances have been created in chip forms. Here, motility and sperm behavioural traits are used to select and analyse sperm. In contrast to traditional methods of sperm selection, this one allows for the production of high-quality motile sperm cells with intact or minimally damaged DNA, which in turn increases the success rate of insemination in cattle and improves the success rate of assisted reproduction procedures like intracytoplasmic sperm injection (ICSI) and in vitro fertilisation (IVF). Furthermore, by adjusting the flow velocity in microfluidic chips, one may manage the concentration of sperm that is accessible to the oocyte. The technological hurdles in this field include the following: the requirement for comprehensive knowledge of the reproductive physiology of domestic animals, the scarcity of research on the topic, the commercialisation of chips, and the creation of microfluidic instruments that are completely functional and particular to individual species. Ultimately, the use of a microfluidic system in sperm selection through assisted reproduction holds significant potential for improved results in in vitro fertilisation and intracytoplasmic sperm storage. Improving this technology for the future will need tweaks to chip designs that are both affordable and species-specific, as well as being commercially viable. Before microfluidic sperm selection may be used more widely in in vitro techniques, extensive investigations in several animal species are required.

Ozaltin, Selin et al., (2023) The sperm chip technique, also known as the microfluidic sperm sorting approach, uses a disposable chip to pick sperm without the use of chemicals. A novel, gentler method of sperm processing has been developed to improve the morphology and motility of sperm and to decrease the DNA fragmentation rate in sperm that is already very fragmented to almost undetectable levels.

We set out to determine if intracytoplasmic sperm injection (ICSI) patients' clinical pregnancy rates were affected by sperm chip procedures. Patients who had fresh embryo transfer (ET) on either Day 3 or 5 following ICSI were the subjects of this prospective randomised cohort research. Based on the results, 102 patients were assigned to the study group and given in vitro fertilization (ICSI) using sperm separated by sperm chips, whereas 111 patients were assigned to the control group and given ICSI using sperm separated by swim-up rods. In terms of fertilisation rate, there was no significant difference between the groups in patients who had ET on the 3rd or 5th day. Both the control group and the group that received sperm chips were able to successfully acquire Grade 1 embryos in the patients who underwent embryo transfer on Day 3. The research group showed a statistically significant increase ($p = 0.050$) in the number of Grade 1 embryos transplanted on Day 5. Nevertheless, there were no notable variations in the clinical pregnancy rates across the groups for patients who were transferred on both the third and fifth days. Although there is an advantage to blastocyst quality when sperm selection is done using the sperm chip technology, there is no benefit to clinical pregnancy success when utilizing this technique.

Ozaltin, Selin et al., (2022) The sperm chip technique, also known as the microfluidic sperm sorting approach, uses a disposable chip to pick sperm without the use of chemicals. The goal of developing this novel, mild method of sperm processing was to increase motility and improve morphology, while simultaneously decreasing DNA Fragmentation (DFI) in sperms that had previously shown significant levels of fragmentation to almost undetectable levels. We set out to determine how the sperm chip technology, a microfluidic sperm sorting procedure, affected the clinical pregnancy rates of patients who had intracytoplasmic sperm injections (ICSIs). This prospective randomised cohort research examined the patients who had fresh embryo transfer (ET) on either Day 3 or 5 following ICSI. The results showed that 102 patients were part of the study group and had in vitro fertilisation using the sperm chip technique, whereas 111 patients were part of the control group and had in vitro fertilisation using the swim-up technique. In patients who had embryo transfer on the third or fifth day, there was no discernible difference in the fertilisation rate between the control group and the sperm chip group. Both the control group and the group that received sperm chips were able to successfully obtain Grade 1 embryos from patients who underwent embryo transfer on Day 3.

There was a statistically significant increase in the number of Grade 1 embryos transplanted on Day-5 in the research group (p = 0.050). Patients transplanted on days 3 and 5 did not differ significantly from one another in terms of clinical pregnancy rate.

Baldini, Domenico et al., (2021) The most often used approach for fertilisation in assisted reproductive technology (ART) is intracytoplasmic sperm injection (ICSI), which aims to prepare sperm cells to choose competent spermatozoa with the highest fertilisation potential. Because of this, finding the ideal spermatozoa is a crucial task. Several techniques have recently been devised to simulate the female reproductive tract's natural selection mechanisms. Despite the abundance of research aimed at determining the election strategy, several questions and arguments persist. From the most fundamental procedures to those catering to sperm cells with diminished motility, this review covers it everything when it comes to sperm cell selection for in vitro fertilisation (ICSI). In addition, the most cutting-edge microfluidics-based strategy for sperm selection will be discussed, along with several methods that take use of certain sperm membrane properties. Lastly, we will examine a novel approach to sperm selection that utilises a micro swim-up performed directly on the ICSI plate.

III. MATERIALS AND METHODS

Study Design

The research period for this retrospective comparison was from January 1, 2024, to December 31, 2024, a total of 12 months. This research compared the ZyMot microfluidic sperm selection device to density gradient centrifugation as a means of preparing sperm for in vitro fertilisation (IVF) and compared the results to those of the control group.

Sample

The 320 couples that participated in the study were split evenly between the two groups: 160 for the control group and 160 for the study. Participants must be males or females who are prepared to sign a consent form and have a diagnosis of infertility needing in vitro fertilisation (IVF) and who are between the ages of 21 and 40.

Ovarian Stimulation and Oocyte Retrieval

A GnRH antagonist regimen was used to regulate ovarian stimulation in all patients. Once the follicles were large enough, human chorionic gonadotropin (hCG) was administered to induce ovulation. Oocyte extraction, guided by transvaginal ultrasonography, was carried out 34 to 36 hours later. The next step, after retrieval, was to gather sperm samples for ICSI and sperm preparation.

Sperm Preparation Techniques

The semen samples in the control group were processed using a two-layer density gradient centrifugation procedure, with a range of 40% to 80%. The bottom layer's sperm pellet was utilised for in vitro fertilisation (ICSF) following centrifugation and washing.

The experimental group used a disposable ZyMot ICSI Sperm Separation Device, which filters out non-motile sperm via microchannels instead of centrifugation. In cases where not enough sperm were collected, standard gradient processing was used.

Statistical Analysis

The data was examined with the help of IBM SPSS Statistics. Numbers and percentages were used to represent categorical variables, whereas mean ± standard deviation was used for continuous variables. Fertilization, blastocyst utilization, oocyte outcomes, pregnancy rates, and non-parametric comparisons were handled using the Mann-Whitney test, whereas paired t-tests were employed for these outcomes. A significance level of $p < 0.05$ was used.

IV. RESULTS AND DISCUSSION

Below Table 1 show that both the control and study groups were quite similar at the beginning of the trial in terms of demographics and semen characteristics. The control group had an average female age of 35.59 ± 4.52 years, while the study group had an average female age of 34.10 ± 4.91 years.The concentration of sperm before processing was about the same in both groups, with the control group having 39.65 ± 35.52 million/mL and the study group having 40.27 ± 23.80 million/mL.

However, following processing, the concentration of sperm in the control group was greater (24.46 ± 27.09 million/mL) than in the study group (16.28 ± 11.48 million/mL).

Table 1. Baseline Demographic and Semen Characteristics

Parameter	**Control Group (Mean ± SD)**	**Study Group (Mean ± SD)**
Female Age (years)	35.59 ± 4.52	34.10 ± 4.91
Initial Sperm Concentration (millions/mL)	39.65 ± 35.52	40.27 ± 23.80
Post-processing Concentration (millions/mL)	24.46 ± 27.09	16.28 ± 11.48
Oocytes Collected	9.66 ± 6.626	10.95 ± 6.84
Motility A + B (%)	46.84 ± 23.74	57.02 ± 17.40

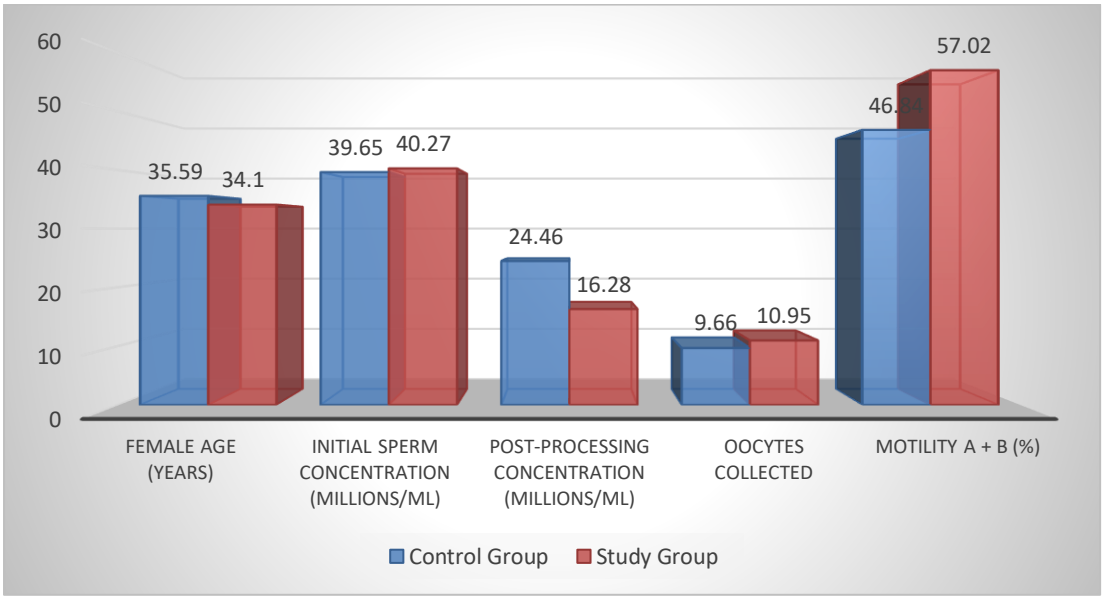

Figure 1. Baseline Demographic and Semen Characteristics

The study group showed greater progressive sperm motility (A + B) with 57.02 ± 17.40% compared to 46.84 ± 23.74% in the control group, even though the post-processing concentration was lower. The study group also had a significantly larger average number of oocytes harvested (10.95 ± 6.84) compared to the control group (9.66 ± 6.63).

Table 2: Comparison of Reproductive Outcomes between Microfluidic and Density Gradient Groups

Parameter	**Study Group (Mean ± SD)**	**Control Group (Mean ± SD)**	**t-value**	**p-value**
Oocytes Collected	10.95 ± 6.84	9.66 ± 6.63	1.526	0.128
Total Blastocysts	2.66 ± 1.71	1.63 ± 0.15	0.302	0.046*
Post-processing Concentration	16.28 ± 11.48	24.87 ± 27.49	3.126	0.002*
Fertilization Rate (%)	80.13 ± 17.01	79.79 ± 17.38	0.153	0.857
Cumulative Pregnancy Rate (%)	51.04 ± 45.80	40.20 ± 42.87	1.891	0.052*
Motility A + B (%)	99.75 ± 2.74	35.00 ± 14.14	30.098	0.001*

*Statistically significant at $p < 0.05$

The reproductive and laboratory outcomes of the two groups, the microfluidic research group and the density gradient control group, are compared in Table 2. The control group collected an average of 9.66 ± 6.63 oocytes, whereas the study group collected 10.95 ± 6.84. Nevertheless, this difference did not reach statistical significance ($p = 0.128$). In the experimental group, the total number of blastocysts generated was 2.66 ± 1.71, which was substantially larger than in the control group, which had 1.63 ± 0.15 ($p = 0.046$). The study group had a sperm concentration of 16.28 ± 11.48 after processing, while the control group had a substantially higher concentration of 24.877 ± 27.49 ($p = 0.002$).

There was very little difference in the fertility rate between the two groups (80.13 percent in the research group and 79.5 percent in the control group; p = 0.857). It is worth noting that the study group had a considerably higher cumulative pregnancy rate (51.04% ± 45.80) than the control group (40.20% ± 42.87) (p = 0.052). In addition, the study group had significantly greater sperm motility (A + B %) following processing (99.75 ± 2.74%) compared to the control group (35.00 ± 14.14%) (p = 0.001).

V. CONCLUSION

This study concludes that microfluidic sperm selection has clear therapeutic benefits over density gradient centrifugation for sperm preparation in in vitro fertilisation (ICSI) cycles, but both methods are successful. There was no improvement in reproductive success for the control group, despite the fact that their sperm concentration was greater after processing. A greater cumulative pregnancy rate, enhanced sperm motility, and higher blastocyst development were seen in the microfluidic group, which maintained identical fertilisation rates. According to these results, boosting IVF outcomes is greatly aided by selecting sperm for quality rather than quantity. As a less harsh and centrifugation-free method, the microfluidic approach has the ability to lessen mechanical stress on sperm and improve embryo development. So, if we want to improve the clinical effectiveness of ICSI therapies, microfluidic sperm selection seems to be a good and viable option.

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