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Comparative Analysis of Microfluid Sperm Sorting Chip with Density Gradient Approaches for Intrauterine Insemination Cycles

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Abstract

The primary goal of sperm preparation prior to IUI is to eliminate viruses, antibodies, leucocytes, and debris from sperm, as well as inhibitors of sperm capacitation, factors like reactive oxygen radicals, and prostaglandins. Microfluidic sperm sorting has come up as an alternate tool to centrifugation-based conventional procedures for in vitro fertilisation in recent years. The purpose of this prospective study is to examine the impact of microfluidic chip sperm processing and density gradient centrifugation procedures on embryo development in teratozoospermic patients.

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Introduction

Fertility is regarded as a crucial event for individuals during their courtship period. Human fertility is accountable for the biological replenishment and preservation of the human species because every community restores and grows through the natural process of fertility. The conviction that fertility was one of the major purposes of creation throughout human history has evolved into a societal expectation that plays a crucial role in defining and assessing an individual's position in society. Numerous aspects of reproduction have emerged throughout the course of societal growth and evolution. For this reason, in numerous instances, the inability to bear children remains a valid reason for strained relationships between

couples and families, even in the face of social and economic advancements and changes in society.

Married couples experience significant anxiety when they are unable to conceive following a year of consistent, unprotected sexual activity (Da Ros and Graziottin, 2018). The objective of the research is to examine the properties of sperm and conduct a comparative analysis of blastocyst and implantation rates among couples with teratozoospermia who choose to pick their sperm using either the density gradient method or the microfluidic sperm sorting technique.

The failure of a married couple to become parents after a year of trying is known as clinical infertility. Thirty to fifty percent of instances of infertility are thought to be caused by male factors. Testicular dysfunction, endocrinopathies, lifestyle factors (like obesity, alcohol consumption, tobacco use, etc.), gonadotoxic exposures, ageing, and congenital anatomical factors are a few of the causes of infertility or diminished fertility. A thorough medical history, a targeted physical examination, and specific laboratory testing, such as semen analysis, are all part of the screening process for male infertility (Eisenberg et al., 2023).

The incapacity to conceive a healthy pregnancy following at least a year of sexual activity without protection is known as infertility (Leslie et al. 2023). The reason behind it could be unknown, or it could be mediated by one or both genders (20% - 40% of couples), or by one or both genders (35% - 40% of couples). For women, among the most common reasons are endometriosis, tubal blockages, and ovulatory dysfunction; for men, the most prevalent causes are abnormalities in the production of sperm and function, as well as obstructions in sperm ducts.

The spermatozoon contributes three components to the oocyte during fertilization:

1. Its DNA,
2. The oocyte-activating factor (likely phospholipase C or PLC zeta, a soluble sperm factor that triggers egg calcium oscillations and activation),
3. The centriole, which guides the formation of the mitotic spindles leading to cell division of the early embryo (Salle and Minc, 2022).

In order to become fertile, sperm cells must become more motile to be able to reach the oocyte at the fertilization locus. They also need to go through the process of capacitation in the female tract, which is indicated by changes in the fluidity and composition of the plasma membrane, dynamic remodeling of the cytoskeleton, especially actin, and increased phosphorylation of a specific set of proteins by various kinases.

It is now evident that some aberrant spermatozoa can successfully fertilize oocytes during in vitro fertilization (IVF) by sperm microinjection into the oocyte (ICSI) (Campos et al. 2023). These adverse conditions can seriously impair post-fertilization processes, early embryonic development, and implantation, suggesting that spermatozoa have a role that goes beyond sperm penetration. There are single structural deficiencies affecting all ejaculated sperm. These monomorphic variants of teratozoospermia cause severe infertility, are uncommon, and are genetically inherited as an autosomal recessive trait.

Table 1. Terminology associated with Semen Quality

Aspermia	Absence of semen (no ejaculation or backward ejaculation)
Asthenozoospermia	Percentage of motile spermatozoa less than the lower reference limit
Asthenoteratozoospermia	Percentage of spermatozoa that are morphologically normal and increasingly motile below the minimum reference limits
Azoospermia	The ejaculate contains no spermatozoa
Cryptozoospermia	Spermatozoa are lacking in fresh preparations but visible in centrifuged pellets
Haemospermia	Erythrocytes detected in the ejaculate
Necrozoospermia	The ejaculate contains a small percentage of living spermatozoa and a high percentage of immotile spermatozoa
Oligoasthenozoospermia	The total quantity of spermatozoa and the proportion of increasingly motile spermatozoa are below the lower reference limits
Oligoteratozoospermia	Total spermatozoa and the proportion of morphologically normal spermatozoa are below the lower reference limits
Teratozoospermia	The proportion of spermatozoa with normal morphology falls below the lowest reference limit
Oligozoospermia	Overall quantity of spermatozoa below the lower reference criteria

The treatment plan includes ARTs (Assisted Reproductive Technologies) such as intrauterine insemination (IUI),*in vitro* fertilisation (IVF), and intracytoplasmic sperm injection (ICSI), as well as lifestyle modifications, surgical and medical management of underlying conditions, fertility counselling, and fertility medications. The quantity of embryos implanted and whether sperm, eggs, or embryos ought to be fresh or frozen are two procedure-related factors that have been the focus of current efforts to improve mother and baby outcomes connected to IVF/ICSI.

Natural sperm selection in humans is an intensive procedure that results in only the highest quality sperm reaching and fertilising the egg. In comparison to other mammalian species, the human ejaculate contains a diverse pool of sperm that varies in form, size, and motility. Assisted reproductive technologies (ART) have long used either a basic swim-up approach, density gradients to prepare sperm, or microfluidic sperm sorting. Both approaches produce highly motile sperm populations.

Several methods are currently being researched to simulate some of the natural selection processes that are present in the female reproductive system. This paper examines the efficacy of existing and new sperm selection approaches for increasing the assortment of sperm available for assisted reproductive technologies.

The objectives of the proposed research work are:

- To study sperm characters - sperm count, sperm motility, and sperm morphology
- To study the comparison of blastocyst rates and implantation rates in couples having teratozoospermia opting for sperm selection either by the density gradient method or the microfluidic sperm sorting method.

One of the most essential elements for a successful IVF pregnancy is sperm quality. The technique used to process sperm is a major factor in determining their quality. Numerous popular sorting methods have been thoroughly compared in a number of studies, including conventional swim-up, hyaluronic acid, density gradient centrifugation, and magnetic-activated cell sorting. Research has demonstrated that different sperm processing techniques result in various levels of

sperm damage, specifically affecting sperm motility, concentration, morphological characteristics, viability, and DNA integrity.

Materials and Methods

Eighty semen samples from patients undergoing Indira IVF were used in a prospective trial for semen analysis. The performance took place between October 1, 2021, and June 30, 2022. Each participant provided written consent and signed it. Only fresh semen samples with an initial volume of 1.5 ml, an average sperm concentration of ≥20 × 106/ml, and overall sperm motility of ≥30% were used in the study in order to perform the three distinct sperm preparation processes on the same sample. For two to five days, men were advised not to ejaculate. Every participant on the spot delivered a sample of semen by masturbating into a sterile cup. In order to mitigate the impact of potential confounding variables, this study solely incorporated semen samples obtained through laboratory masturbation. An engraved glass measuring cylinder was used to calculate the volume of semen. Semen concentration, motility, morphology, and volume were assessed following liquefaction. Then, three aliquots were taken from each chosen semen sample, and each aliquot was treated using one of the following methods: density gradient, pellet swim-up, semen swim-up, or density gradient followed by a swim-up. Sperm motility, morphology, and count were measured after processing. Furthermore, all samples underwent testing for sperm DNA fragmentation both before and following semen processing.

This prospective study would involve couples who met the inclusion criteria and underwent IVF procedures at Indira IVF Hospital Pvt Ltd, Udaipur, between October 1, 2021, and June 30, 2022. The women who have a minimum of five mature oocytes and are between the ages of 21 and 35 were chosen for this study. The WHO 2010 standards and Kruger's rigorous requirements (sperm morphology less than 4%) are used to define teratozoospermia. The mature oocytes will be fertilised using semen samples from infertile men who have teratozoospermia.

Semen Analysis

Male partners will undergo a 2- to 7-day period of sexual abstinence followed by masturbation to obtain semen samples. After incubation, the semen analysis is scheduled to take place 15–30 minutes later. Under a compound microscope, the sperm count and motility of ten microliters of the raw semen sample will be assessed on a Makler counting chamber. To evaluate the morphology of the sperm, a semen smear will be produced and stained with a Diff-Quik stain. The samples that met the requirements for teratozoospermia were used in the investigation. With computer-generated random number sampling, they were randomised to either the study group (density gradient) or the Microfluidic Sperm Sorter chips.

Sperm Preparation Using a Microfluidic Sorting Chip

Preparation of Sperm Suspension: Following ejaculation, the semen samples were permitted to liquefy before being diluted 1:1 using HEPES medium. All that is required for a sperm sorting equipment is 500 uL of dilution. Following this, the diluted samples were stored in an incubator at 37 degrees.

Preparation of Qualis Sperm Sorting Device: The 60 mm dish was fixed with the Qualis dish. Following this, 100 uL of medium was poured into well A and allowed to pass through the channels and into channels B, C, and D by waiting for a little while. A medium was added to chambers B, C, and D. Following that, the entire medium was removed from each well.

Loading of sperm sample and sorting procedure: It was planned to add 20 uL of medium to chambers C and D and 100 uL of medium to chamber B. A diluted sperm sample of 65 uL was added to chamber A. Based on laminar flow, sperms eventually moved from well A to chambers D and C. Immotile sperms flowed with the medium and gathered in well D, while motile sperm swam in the direction of well C. The final sample was collected from well C into a 5 mL tube for further processing.

Sperm Preparation Using the Density Gradient Centrifugation Method

Density Gradient is a colloidal suspension of silica particles enhanced with HTF medium and stabilised with covalently bound hydrophilic silane. In this case, the density gradient medium is the Puresperm-II kit. This kit's components made it possible to separate motile sperm from the semen sample very effectively. Three categories of components make up the Puresperm-II kit:

1. Puresperm-A: 80% sterile colloidal silica particles stabilized with covalently bound hydrophilic silane.
2. Puresperm-B: 40% sterile colloidal silica particles stabilized with covalently bound hydrophilic silane.
3. Puresperm-C: Wash solution is a HEPES-buffered HTF medium.

To prevent the spermatozoa from suffering a cold shock, the operation began with all the kit’s components maintained at 37 degrees. Following an analysis of the prewash concentration and motility, 1 mL of Puresperm-A was added to the conical centrifuge tube's bottom, followed by 1 mL of Puresperm-B. Semen, between 1.5 and 2 millilitres, was put atop Puresperm A and B so as not to disrupt the interface between the two layers. The first centrifugation, which takes 20 minutes at 2000 RPM / 648 G (RCF) based on the specimen's viscosity, was conducted. Using a transfer pipette, the supernatant was disposed of after 20 minutes without disrupting the sperm pellet. The sperm pellet was then mixed with 3 mL of Puresperm-C, and the pellet was reconstituted with a finger tap. The second centrifugation occurred at 317 G (1400 RPM) (RCF). The supernatant was then carefully disposed of. To the pellet, 0.4 mL of HEPES medium was added, and it was maintained at 37 degrees in an incubator for the next step. The Fornix Spermfuge SF 800 was utilised.

3.6.2 ICSI Procedure: After two hours of sperm extraction and preparation using both methods, physically normal sperm were chosen to undergo the intracytoplasmic sperm injection (ICSI) process. After two hours of oocyte retrieval, the ICSI process was carried out with the assistance of an Olympus IX73 inverted microscope and a Narishige ON3 micromanipulator. A micromanipulator is utilised to precisely manipulate gametes. The injector system and the manipulator make up its two components. The micromanipulator is used to manipulate the gametes at a precise level. It consists of 2 parts, the manipulator and the injector system.

- Manipulator: It is made up of two joystick units linked to three axes of motion (X, Y, and Z). The arms can hold injectors

for manipulating sperm and eggs.

- Injector System: In micro-manipulators, two different types of injector systems were used: one to contain oocytes and the other to hold sperm.
- The Narishige IM-9C oocyte-holding injector works on the principle of air control, whereby air is moved through a closed tube system to regulate the oocyte. Holding pipettes (Cook K-HPIP-1035) are the type of pipettes used for oocytes. To function, they are bent at a 35-degree angle.
- The oil in the closed tube controls the sperm-holding injector, which is based on an oil-controlling mechanism. Microinjection pipettes are used to contain sperm (Cook K-MPIP-1035).
- The oocyte's centre was punctured with a single sperm. Oocytes were assessed for blastocyst development after day 5.

Embryo Development: Following 20 hours of ICSI, a fertilisation check will be performed for embryo development. On day five of ICSI, the embryos' continued development and the creation of blastocysts were assessed. On day six, the embryos were further evaluated. Blastocysts were categorised as good, fair, poor, or excellent according to Gardner and Schoolcraft's grading scheme. With the approval of the patient, additional high-quality embryos (AA, AB, and BA) will be frozen. Kitazato vitrification was employed in the process of freezing embryos.

Endometrial preparation and Embryo Transfer: Estradiol valerate, 2 mg three times a day, is used orally in FET cycles to prepare the endometrium for the subsequent hormone-free cycle following ovum pickup. Starting on day two of the natural or provoked cycle, estradiol valerate will be administered. After taking estradiol for 14 days, the female patients were called for an endometrial evaluation. Serum levels of progesterone (P4) and estradiol (E2) will be measured for those with endometrial thickness ≥7 mm and vascularity in zone 3/4 and grade 3/4; progesterone supplementation was initiated if P4 < 1.5 ng/ml. On the fourteenth day of HRT, women whose endometrial thickness was less than 6 mm were recommended to increase their oestrogen dosage; nevertheless, they were not allowed to continue in the study. Those whose endometrial thickness is between 6 and 7 mm will stay on the same dosage and be reevaluated in two to three days. If ET does not reach 7 mm even after 21 days of E2 delivery, and if P4 is greater than 1.5 ng/ml prior to the initiation of progesterone, the cycle was terminated. Before embryo transfer, 100 mg of progesterone will be injected intramuscularly once a day for six days. Using the soft catheter and USG guidance, one or two blastocysts were transferred, based on the patient's preference. For the procedure, a Cook Embryo Transfer catheter was used. To eliminate any potential bias resulting from variations in the clinicians' skill sets, all transfers were carried out by a single physician. For luteal support, progesterone vaginal gel (8%) was administered twice a day. A beta-hCG test was performed on the serum after 14 days following embryo transfer; results of more than 50 IU were deemed to be positive. We assessed the development of the embryonic pole and gestational sac at the 6-week ultrasound. Throughout the pregnancy and delivery, the women received routine follow-up, and every incident that raises concerns was documented.

Inclusion

Patients who have had their written informed consent granted by the European Commission (EC) and who have voluntarily agreed to participate in the study after being fully informed of the advantages, potential hazards, and any

associated pain.

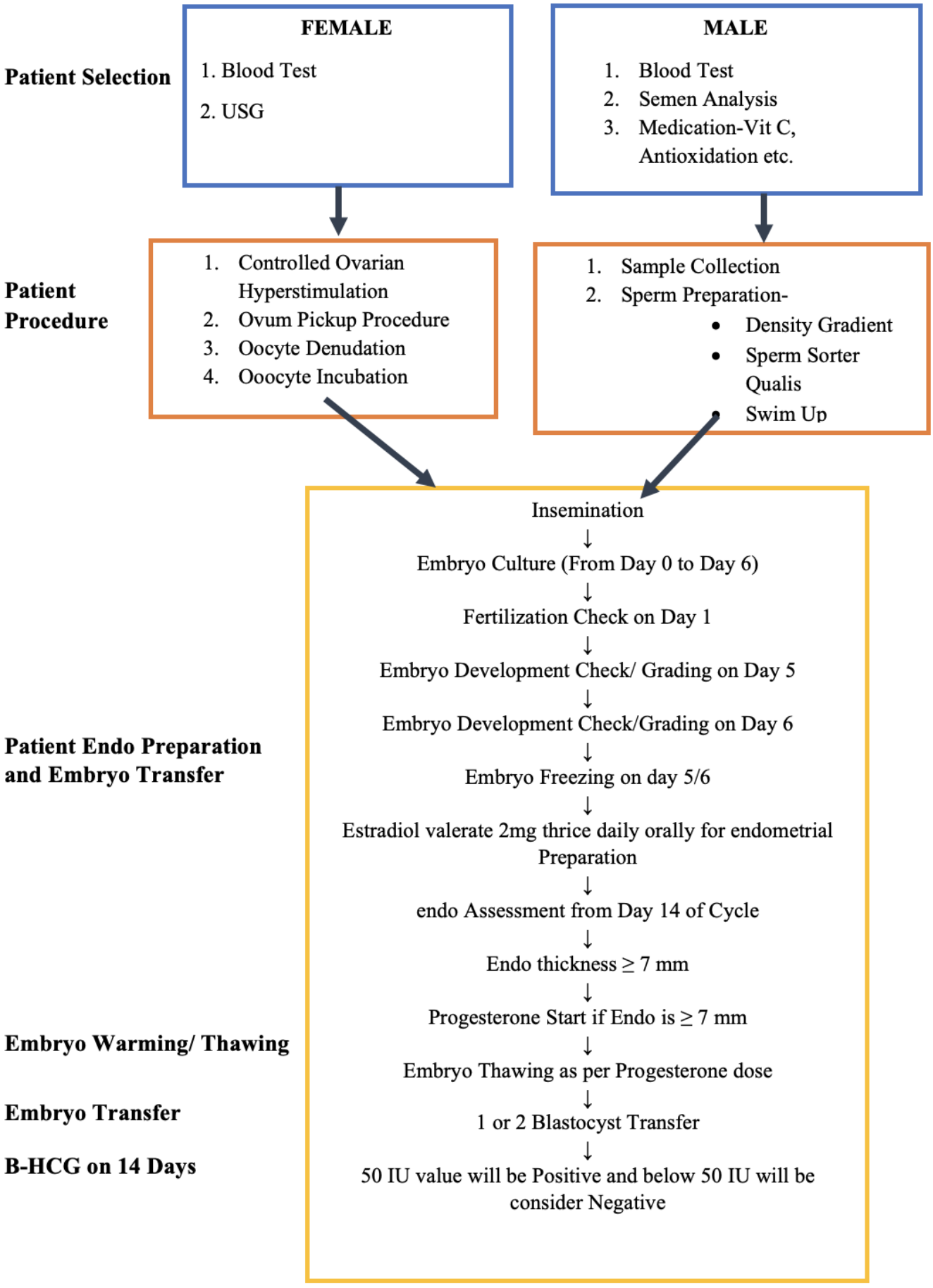
- Female BMI between 18.5 - 30.0 kg/m^2 (both inclusive).
- Normal ovarian reserve (defined as AFC ≥ 8; AMH level ≥ 1.2 ng/ml before the controlled ovarian stimulation (COS) initiation).
- Female age between 21 years and 35 years.
- Negative serological tests for HIV, HBV, HCV, and RPR.
- Patients undergoing their IVF/ICSI cycle with their own oocytes.
- Teratozoospermia (<4% of normal sperm morphology).
- Endometrial thickness more than 7 mm on the day of the start of progesterone during endometrial preparation.

Exclusion

- Patients with uncontrolled diabetes, hypertension, or heart disease.
- Male partner with severe male factor (spermatozoa < 5 million/ml).
- Adenomyosis or any pathological finding affecting the endometrial cavity, illness or unstable medical condition that may put at risk the patient's safety and her compliance in the study.
- If donor sperm is opted by the couple.
- If surgically retrieved sperm will be used for the ICSI procedure.

Statistical analysis

The Shapiro-Wilk test was used to verify the normality of continuous data. ANOVA was used to compare continuous data that were regularly distributed, while the Kruskal-Wallis test was used to analyze continuous data that were non-normally distributed. If a $p < 0.05$ was found, a multi-comparison Bonferroni post hoc test was then performed. To evaluate the correlation between different sperm parameters, Spearman's correlation coefficient was employed. For regularly distributed data, the results were presented as mean ± standard deviation (SD), and as median (interquartile range) for non-normally distributed data, and as a percentage when appropriate.


Patient Selection
FEMALE
1. Blood Test
2. USG
MALE
1. Blood Test
2. Semen Analysis
3. Medication-Vit C, Antioxidation etc.
Patient Procedure
1. Controlled Ovarian Hyperstimulation
2. Ovum Pickup Procedure
3. Oocyte Denudation
4. Ooocyte Incubation
1. Sample Collection
2. Sperm Preparation-
• Density Gradient
• Sperm Sorter Qualis
• Swim Up
Patient Endo Preparation and Embryo Transfer
Insemination
Embryo Culture (From Day 0 to Day 6)
Fertilization Check on Day 1
Embryo Development Check/ Grading on Day 5
Embryo Development Check/Grading on Day 6
Embryo Freezing on day 5/6
Estradiol valerate 2mg thrice daily orally for endometrial Preparation
endo Assessment from Day 14 of Cycle
Endo thickness ≥ 7 mm
Progesterone Start if Endo is ≥ 7 mm
Embryo Warming/ Thawing
Embryo Thawing as per Progesterone dose
Embryo Transfer
1 or 2 Blastocyst Transfer
B-HCG on 14 Days
50 IU value will be Positive and below 50 IU will be consider Negative

Observations

A randomised controlled study was carried out to examine the impact of three different sperm processing procedures on the same cohort of infertile men utilising split sperm samples. The blastocyst formation was the primary result. Secondary outcomes included sperm concentration, rate of progressive motility, and overall sperm motility.

Images of early stages of blastocysts are shown in Figures 1 and 2:

Figure 1. Early Blastocyst Stage 1

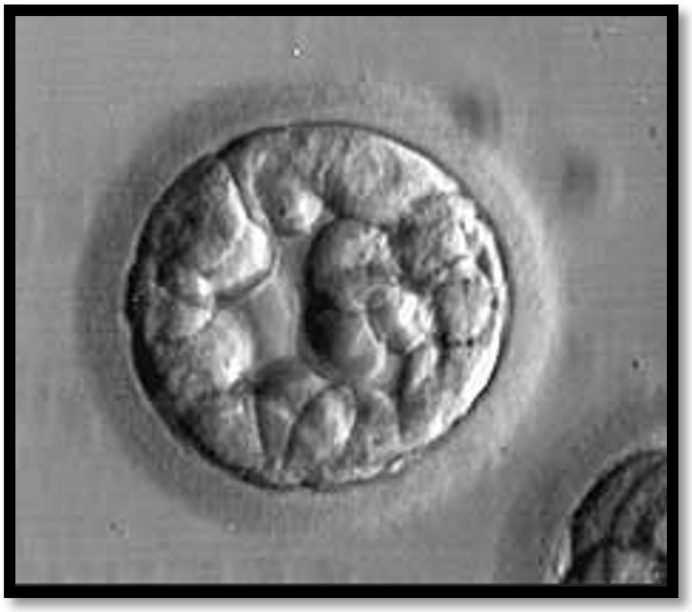


Figure 2. Early Blastocyst Stage II

Table 2. Comparative Analysis of Clinical Features of the Female Patients

Methods	BMI (kg/m2)	% Change - BMI	ET(mm)	% Change - ET	AMH (ng/ml)	% Change- AMH	Embryos Formed	% Change – No. of Embryos Formed	Good Blast Rate	% Change- GBR	β HCG (mIU/ml)	% Change - β HCG	
Swim Up	24.3± 1.33	NA	9.3±0.09	NA	4.71± 0.03	NA	7.97±1.6	NA	43.85± 0.33	NA	2147.71± 0.33	NA	
Density Gradient	23±0.33	-5.35	9±0.06	-3.23	4.45± 0.06	-5.52	8.71±1.3	9.28	40.93± 1.3	-6.65	1608.73± 1.36	-25.10	
Micro Fluidic	24.91± 0.66	2.51	8.93±0.99	-3.96	4.05±0.6	-14.01	9.7±1.36	21.71	50.46± 2.6	15.07	2236.2± 1.63	4.12	

BMI: Body Mass Index; ET: Endometrium Thickness; AMH: Anti Mullerian Hormone; TEC: Total Embryo Count; GBR: Good Blastocyst Rate

Table 3. Comparative Analysis of Clinical Features of the Female Patients

Methods	TSM	% Change- TSM	TSC	% Change- SC	SM	% Change- SM	G1EC	% Change- G1EC	TEC	% Change- TEC
Swim Up	45.6± 0.36	NA	45.5±0.6	NA	2.3±2.6	NA	4.85±0.1	NA	7.97±0.6	
Density Gradient	52.42± 0.13	14.96	46.14±0.3	1.41	2.42±1.3	5.22	4.31±1.6	-11.13	8.71± 0.66	9.28
Microfluidic	53.77±	17.92	45.45±0.03	-0.11	2.45±0.33	6.52	5.1±0.09	5.15	9.7± 0.12	21.71

TSM: Total Sperm Motility; TSC: Total Sperm Count; SM: Sperm Morphology; G1EC: Grade 1 Embryo Count; TEC: Total Embryo Count

Results and Discussion

Intrauterine insemination (IUI) is a frequent treatment option for infertile couples prior to pursuing more complex assisted reproductive techniques. Many factors influence IUI success, including the woman's age, length of infertility, ovarian reserve, and sperm parameters (Varadarajan et al. 2022). One of the most important prognostic indicators is total motile sperm count (Villani et al. 2022). Capacitation occurs when spermatozoa experience a series of metabolic and structural changes inside the female genital canal during the IUI procedure; sperm capacitation is artificially performed utilising specialised procedures (Miyazaki et al. 2023). The goal of sperm processing is to concentrate motile sperm while removing seminal plasma, debris, prostaglandins (PGs), immotile sperm, leukocytes, immature germ cells, and other elements that may be damaging to sperm viability. As a result, the sperm used for IUI must be taken out of the seminal fluid, capacitated, and selected for introduction into the uterine cavity based on morphology and motility (Leung et al. 2022). To prepare the semen sample for IUI, the swim-up technique and density gradient centrifugation are routinely utilised. Motile sperm swim across a prewashed pellet up towards a layer of new medium for selection in the swim-up method (Duracka et al. 2023). Centrifugal forces are utilised in density gradient centrifugation to separate sperm based on their density gradient (Ali et al. 2022). Although these approaches were intended to select a group of high-quality sperm,

there is considerable concern regarding the potential harm these procedures could have on sperm. The tremendous pressure and force applied to sperm during recurrent centrifugation, for example, may impair sperm quality and ultimately destroy their natural features. Sperm in solid pellets contains more reactive oxygen species and has more DNA fragmentation (Vasilescu et al. 2023).

Furthermore, centrifugation-based sperm preparation methods are labor-intensive and time-consuming, and the results can differ between technicians (Feyzioglu and Avul, 2023). To address the limitations of these approaches, the microfluid sperm sorting chips methodology was developed (Jahangiri et al. 2023). In this technology, sperm fluid is pumped into a 0.5 mm wide canal, and viable sperm are swept out of the channel into the chip, allowing healthy sperm to be separated from damaged or dead sperm. This technique enables the correct choice of motile sperm in less time while maintaining overall sperm quality. These sperm also had less DNA fragmentation and fewer reactive oxygen species (Feyzioglu and Avul, 2023).

The sperm specimens of 80 patients with teratozoospermia were separated into three groups, with 10 patients in Swim-Up (control) and the remaining half processed using either a microfluidic chip or a density gradient group. Comparison of the clinical aspects of female and male patients is shown in Table 2 and 3, respectively. Female clinical characteristics were BMI, AMH, and ET. After the preparation techniques, an analysis of the total embryos generated, the good blast rate, and the β-HCG value was made. Among the sperm parameters are count (mL), total motility (%), and morphology (%). The observation shows that the % change in the density gradient approach reduces all parameters of female clinical characteristics except the total embryo count (9.28 %). Whereas the Microfluidic sorting method showed a significant increase in the total embryo count (21.71%), good blast rate (15.07%), and β-HCG (4.12%) value when compared to the density gradient method. The statistics clearly reveal that the endometrial thickness in women patients in the microfluidic group is the smallest (8.9mm) when compared to the Swim-Up (control) (9.3mm) and Density Gradient (9mm) methods. Despite having the least ET, the microfluidic sperm sorting procedures produced the most embryos and the best rate of excellent blastocysts (Table 2). The density gradient approach has the highest sperm count (46.14 mL) compared to the control (45.5 mL) and microfluidic sorting (45.45 mL) in male clinical characteristics. In the density gradient approach, only the Grade 1 embryo count (-11.13%) is reduced. The density gradient methodology had a lower percent change in total sperm motility (14.95%), sperm morphology (5.22%), and total embryo count (9.28%) than the microfluidic method. Among the other groups, the percent change in total sperm motility (17.91%), sperm morphology (6.52%), Grade 1 embryo count (5.15%), and total embryo count (21.71%) is the highest (Table 3). Despite having achieved the highest sperm count, the grade 1 embryo formed through the density gradient method is the least when compared to the control and microfluidic group. The current study concludes that microfluidic sperm sorting produces more and higher grade 1 embryos than the density gradient approach. Microfluidic sperm sorting is a more efficient and better technology in terms of total sperm motility and sperm morphology.

References

- Ali AH, Ajina T, Ali MB, Mehdi M (2022) Efficacy of density gradient centrifugation technique (DGC) in enhancing sperm

cell DNA quality for assisted reproductive technique. Middle East Fertility Society Journal. 27(1): 1-9.

- Campos G, Sciorio R, Esteves SC (2023) Total fertilization failure after ICSI: insights into pathophysiology, diagnosis, and management through artificial oocyte activation. Human Reproduction Update. dmad007.
- Da Ros CT, Graziottin TM (2018) Environmental issues resulting in hypogonadism in Brazilian men. In Bioenvironmental Issues Affecting Men's Reproductive and Sexual Health. 33-40.
- Ďuracka M, Benko F, Chnapek M, Tvrda E (2023) Strategies for Bacterial Eradication from Human and Animal Semen Samples: Current Options and Future Alternatives. Sensors. 23(15): 6978.
- Eisenberg ML, Esteves SC, Lamb DJ, Hotaling JM, Giwercman A, Hwang K, Cheng YS (2023) Male infertility. Nature Reviews Disease Primers. 9(1): 49.
- Feyzioglu BS, Avul Z (2023) Effects of sperm separation methods before intrauterine insemination on pregnancy outcomes and live birth rates: Differences between the swim-up and microfluidic chip techniques. Medicine. 102(46): e36042.
- Jahangiri AR, Ziarati N, Dadkhah E, Bucak MN, Rahimizadeh P, Shahverdi A, Topraggaleh TR (2023) Microfluidics: The future of sperm selection in assisted reproduction. Andrology.1-17.
- Leslie S, Soon-Sutton T, Khan MA (2023) Male infertility. StatPearls.
- Leung ET, Lee CL, Tian X, Lam KK, Li RH, Ng EH, Chiu PC (2022) Simulating nature in sperm selection for assisted reproduction. Nature Reviews Urology. 19(1): 16-36.
- Miyazaki MA, Guilharducci RL, Intasqui P, Bertolla RP (2023) Mapping the human sperm proteome–novel insights into reproductive research. Expert Review of Proteomics. 20(1-3): 19-45.
- Salle J, Minc N (2022) Cell division geometries as central organizers of early embryo development. In Seminars in Cell & Developmental Biology. 130: 3-11.
- Varadarajan N, Khera K, Saxena R (2022) An Overview of Risk Factors, Investigation and Management of Infertility in Women. NeuroQuantology. 20(16): 2481.
- Vasilescu SA, Ding L, Parast FY, Nosrati R, Warkiani ME (2023) Sperm quality metrics were improved by a biomimetic microfluidic selection platform compared to swim-up methods. Microsystems & Nanoengineering. 9(1): 37.
- Villani MT, Morini D, Spaggiari G, Falbo AI, Melli B, La Sala GB, Santi D (2022) Are sperm parameters able to predict the success of assisted reproductive technology? A retrospective analysis of over 22,000 assisted reproductive technology cycles. Andrology. 10(2): 310-321.