SPERM PREPARATION TECHNIQUE FOR INTRA CYTOPLASMIC SPERM INJECTION (ICSI): COMPARATIVE STUDY

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Abstract

Background: It has been proposed that the mechanical force of centrifugation produces reactive oxygen species (ROS) that compromise the functional and structural integrity of sperm cells. Many oxygen radicals produced by spermatozoa and leukocytes affect the likelihood of sperm-oocyte fusion during repeated centrifugations. Thus, it is preferable to separate the sperm from the other components using techniques like the swim-up approach or micro swim-up approach.

Methods: 400 couples were classified and grouped as follow: **Group 1**: prepared by standard swim-up approach which split into two Subgroups (include 100 normal male factor and 100 abnormal male factor). **Group 2**: prepared by mini swim up technique using sperm gradient media which divided into two Subgroups (include 100 normal male factor and 100 abnormal male factor).

Results: Pregnancy rate was higher (42.0%) in mini swim up group (normal male factor) compare to (41.0%) in swim up group (normal male factor), (46.0%) in mini swim up group (abnormal male factor) compare to (36.0%) in swim up group (abnormal male factor.

Conclusions: Sperm preparation has been reported to result in the enrichment of sperm with intact chromatin, which is anticipated to enhance the odds of generating a healthy pregnancy, even though both swim-up and density gradient approaches have been proven to be successful in creating a sperm population with low proportion of apoptotic sperm.

Keywords: Male Infertility; Sperm DNA fragmentation; ICSI

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1. INTRODUCTION

15% of all couples attempting conception suffer from infertility, and in around 50% of those instances, the male component is the only or a significant reason. (1). The conventional tests of sperm count, motility percent, and morphology may not detect sperm abnormalities compromising the integrity of the male genome even though semen analysis is frequently utilized to predict fertility. (2). A modest amount of ROS is produced by spermatozoa under physiological settings. In the male reproductive tract, ROS synthesis and antioxidant scavenging activities often maintain balance. Nevertheless, too much ROS may harm healthy spermatozoa by damaging their DNA. (3). A single sperm is inserted into an egg directly during an in vitro fertilization procedure known as intracytoplasmic sperm injection (ICSI). ICSI has been used more often over the last ten years to treat severe male infertility issues caused by a lack of motile and morphologically healthy sperm in the male partner's ejaculate. (4). Clinical gestation and live-birth rates remain at about 30–40% despite numerous advancements in ART methods over the past three decades. Numerous techniques are utilized to prepare spermatozoa for ICSI, but the choice is heavily influenced by the quality of the semen, specifically its motility, concentration, and morphology, in order to obtain a greater quantity of high-quality spermatozoa, even from the poorest men. (5). Many oxygen radicals produced by spermatozoa and leukocytes affect the likelihood of sperm-oocyte fusion during repeated centrifugations. Thus, it is preferable to separate the sperm from the other components using techniques like the swim-up method or mini swim-up method. (6). For clinical

practice, the methods for choosing the most effective spermatozoa are crucial. To maximize the recovery of the sperms, the ideal semen preparation method may be chosen by making minor adjustments to each approach. (7). Simple washing of spermatozoa led to the development of separation methods based on various concepts, such as filtration, migration, or density gradient centrifugation. The traditional swim-up method is the easiest and least expensive. (8). In contrast to the tiny swim-up approach, the swim-up technique actually results in a larger degree of non-sperm components (such as detritus and germs) and the diffusion of other substances (such as prostatic zinc) from the semen into the overlying media. (9). After using the two major approaches, there are some discrepancies in the existence and generation of ROS as well as the sperm DNA damage that is linked to high levels of ROS. (10). The functional and structural integrity of sperm cells may be harmed by ROS produced during the mechanical force of centrifugation, according to certain theories. (11). In this work, we sought to provide a current overview of the primary sperm separation techniques, as well as their consequences and significance for contemporary assisted reproduction. Consequently, the objective of the current research was to assess the optimal sperm selection technique in order to increase the pregnancy rate using the ICSI Method.

Study Design: 400 couples who were referred to assisted reproduction at the Walad We Bend Fertility Center between March 2019 and September 2020 made up the research population. Male semen analysis resulted in the following classification and grouping of the participants under study:

Group 1: prepared by traditional swim up Method which split into two Subgroups (include 100 normal male factor and 100 abnormal male factor).

Group 2: prepared by mini swim up Method using sperm gradient media (which divided into two Subgroups (include 100 normal male factor and 100 abnormal male factor).

Inclusion criteria: no gender factor Males vary in age from 25 to 40, and they have no history of serious chronic illnesses. No history of testicular or scrotal surgery.

2. MATERIAL AND METHODS

All male partner subjected to:

1-For semen assessment:

After 2 to 7 days of abstinence, semen samples are obtained through masturbation. The container was sterile, clean, and wide-mouthed to reduce collecting errors, and it needs to be from a batch that has been shown to be safe for spermatozoa. Within an hour after collection, the semen specimen should be kept at body temperature or at room temperature. Physical examination as regards the appearance of the ejaculate, liquefaction, viscosity, volume, odor, and semen pH were done. And microscopic examination as regards concentration, motility, and abnormal forms. Were done according to WHO, 2020 (12).

2-Sperm preparation methods:

a-Swim up: Semen samples were initially transferred to distinct 15-mL centrifuge tubes in a brief manner. The G-IVF plus solution (Vitrolife Sweden AB, V. Frölunda, Sweden) was then carefully put on top of the semen in a 2 mL layer. The tubes were placed at a 45° angle and incubated for 40–60 minutes at 37 °C. 1.5 mL of the supernatant was added to a fresh tube after incubation, and the tube was centrifuged at 300 ×g for 6 min. The sperm pellet was then re-suspended in 2 mL of G-IVF plus and subjected to further centrifugation at 300 ×g for 6 minutes after the resultant supernatant was discarded. The sperm pellet was then re-suspended in 0.5–1 mL of the culture media for later usage after the resultant supernatant was discarded. (13).

b-Mini swim up using with Sperm gradient: 1.5 mL of 45 and 90% Sperm Grad (Vitrolife Sweden AB, V. Frölunda, Sweden) were combined to create a two-layer gradient in 15 mL centrifuge tubes. Single tubes containing the semen samples were placed on top of the gradient and centrifuged at 300×g for 20 min. After discarding the resultant supernatant, the pellet was re-suspended in 2 mL of G-IVF plus and centrifuged at 300×g for 6 min. The sperm pellet was then resuspended in 0.5–1 mL of the culture medium and the excess supernatant was once again discarded. (13).

3-Examination by Halo sperm G2 stains to detect DNA fragmentation: On a prepared slide, intact, unfixed spermatozoa are submerged in an inert agarose microgel. In those sperm cells with fractured DNA, a first acid therapy denatures the DNA. The lysing solution then eliminates most of the nuclear protein and, in the lack of significant DNA damage, creates nucleoids with significant haloes of spread DNA loops that emerge from a central core. However, the dispersion halo in the nucleoids from spermatozoa with fragmented DNA is either absent or hardly visible. Sperm classification: Score at least 300 sperm in each sample in accordance with the standards (14).

All female partner subjected to:

Oocytes collection: 34–36 hours after the dose of human chronic gonadotropin (HCG), transvaginal ultrasonography (US) was performed while the patient was under general anesthesia (Germany's Labotect aspiration pump). Follicles were aspirated utilizing a single lumen 17-gauge oocyte pick-up needle at a negative pressure of 115–120 mm Hg (Germany's Reproline Medical). A 14 ml sample of follicular fluid was aspirated into sterile tubes (China's Falcon, Boen Healthcare Co.). The Oocyte-cumulus cells complexes were separated using a dissecting microscope (Zeiss Stemi 2000-C Stereo Microscope), washed in Gamete Buffer media

(Ireland's Limerick and Cook), and then put into four dishes containing fertilization medium (Ireland's Limerick and Cook). These steps were repeated for about a half-hour at 37 oC with 6% CO2 (C60, Labotect, Germany). For denudation, the corona cells were eliminated from the oocyte by gently aspirating it in and out of a sterile drawn pipette after the oocyte had been incubated for 30 to 45 seconds in a 100 µl drop of buffered containing hyaluronidase enzyme 80 IU/ml (European Life Global). After the denudation process was finished, the oocyte was cleaned with Gamete Buffer (Ireland's Limerick and Cook) and then put in 10 µl micro drops of the fertilization medium in injection plates, coated with 3ml of sterile evenly balanced mineral oil (European Life Global).For the oocyte grading, Using a heated stage, automated manipulators, and an inverted microscope with Hoffman optics (Olympus 1x71, Japan), the oocyte's quality and maturity were swiftly evaluated (Narishige, japan). The phases of maturation were prophase I (GV), metaphase I (MI), and metaphase II (MII). Before the ICSI treatment, the mature oocytes were cultured in a culture medium at 37 ºC with 6% CO2. (15). ICSI procedure: Samples were incubated until it was time for injection after semen analysis and sperm preparation as previously mentioned. A single immobilized, morphologically normal spermatozoon was inserted into each oocyte in polyvinyl pyrolidone (PVP) (Irvine, USA). According to the Van Steirteghem protocol, the injection operation was completed in a sterile dish utilizing a holding pipette and injection needle. After being injected, the oocyte was cleaned and placed in global total media (Life Global, Europe) on a culture plate covered with sterile warm equilibrated global oil (Life Global, Europe) at 37 oC in a 6% Co2 atmosphere with (90 - 95%) humidity until fertilization. (16).

Embryo quality and fertilization were evaluated 17 ±hours after microinjection. We examined the injected oocytes for pronuclei and other indications of damage. The presence of two pronuclei (2PN) and the protrusion of the second polar body were signs of fertilization in an egg. (17). Around 72 hours after injection, each embryo's cell count and shape were graded for transfer and grading based on the percentage of nucleate fragments and the equality of blastomeric size in each embryo. The best day-3 embryos (Grade 1) were then transplanted to the uterus 48–72 hours following oocyte extraction utilizing an embryo transfer catheter (Labotect, Germany) in 30 l of Global medium (Life Global, Europe) containing 10% human serum albumin (HSA). (18). If embryo transfer was postponed to day 5, embryos are assessed for quality and grading before E.T. (19).

The American Association of Reproduction recommended transferring day 3 or day 5 embryos to recipient individuals. Cryopreserved embryos of high grade were overproduced. In order to determine if a clinical gestation had been obtained, a transvaginal ultrasonography scan of the uterus was carried out after 6-7 weeks of amenorrhea (deemed positive if 20 IU/L of serum-HCG was detected) (intrauterine gestational sac visible).

STATISTICAL ANALYSIS:

Software for statistical analysis was used to examine the data. Paired T-test and Mc Namar's test were utilized to compare the investigated parameters in each study group before and after treatment. (20).

ETHICAL APPROVAL:

The study was approved by Faculty of Science, Suez Canal University. Egypt. All individuals gave their written informed permission before the data were maintained and processed in a private, anonymous manner.

3. RESULTS

Table (1): General characters and stimulation protocols distribution of the female patients

Parameters	Mean ± SD (n=400):
Age/ years	28.8 ± 5.5
BMI (Kg/m^2)	28.6 ± 3.5
Infertility duration /years	6.6 ± 2.5
FSH (IU/L)	5.1 ± 1.6
LH (IU/L)	4.1 ± 1.4
PRL (µg/L)	20.8 ± 9.8
E2 (pg/ml)	35.9 ± 16.3
TSH (mIU/L)	3.2 ± 1.12
Antagonist Protocols	74 (37%)
Long Protocols	84 (42%)
Short Protocols	42 (21%)

The median age of female patients was 28.8 ± 5.5. The median BMI was 28.6 ± 3.5 and the median duration of infertility was 6.6 ± 2.5. The mean of FSH (IU/L) hormone was 5.1 ± 1.6, the mean of LH (IU/L) hormone was 4.1 ± 1.4, the mean of PRL (µg/L) hormone was 20.8 ± 9.8, the mean of E2

(pg/ml) hormone was 35.9 ± 16.3 and the mean of TSH (mIU/L) hormone was 3.2 ± 1.12. the distribution of stimulation protocols in the Female Studied patients showed Female patients who underwent the Antagonist regimen made up, on average, 74 (37%). Female patients who underwent the Long protocol on average were 84 (42%) while those who underwent the Short protocol on average were 42 (21%).

Table (2): Sperms parameters after Processing among normal male factor group (n=200):

Parameters	Original semen	Swim up (N=100)	Mini Swim up (N=100)	P value
	Mean ± SD	Mean ± SD	Mean ± SD	
Sperm concentration ($\times10^6$/ml)	38.6 ± 8.3	18.1 ± 7.8	20.8 ± 7.5	**≤ 0.01***
Sperm motility/ml	51.3 ± 2.2	63.2 ± 5.5	64.3 ± 5.4	> 0.1
Sperm Progressive motility/ml	31.3 ± 2.2	43.8 ± 3.7	44.1 ± 2.7	> 0.1
Sperm Head defects	31.3 ± 2.2	30.4 ± 6.8	25.8 ± 6.5	**≤ 0. 001***
Sperm Midpiece defects	21.3 ± 2.2	20.3 ± 7.8	20.8 ± 8.2	> 0.1
Sperm Tail defects	11.3 ± 2.2	10.7 ± 2.3	10.8 ± 2.3	> 0.1
Sperm DNA fragmentation index	12. 6 ± 1.3	12.0 ± 1.8	11.0 ± 1.5	**≤ 0. 001***
ROS index	10. 6 ± 1.2	10.1 ± 0.8	10.0 ± 0.5	> 0. 1

In normal male factor group, There were statistically significant differences in Mini Swim up group compared in Swim up group, reading Sperm concentration, Sperm Head defects, DNA fragmentation index (P < 0. 01*). However, there weren't statistically significant differences in Mini Swim up group compared in Swim up group, reading Sperm motility, abnormal forms, Midpiece defects, Tail defects and ROS index.

Table (3): Comparison of ICSI results among groups of healthy men (n=200):

Parameters	**Swim up (N=100)**	**Mini Swim up (N=100)**	**P value**
	Mean ± SD	**Mean ± SD**	
Collected oocytes	9.3 ± 3.1	8.2 ± 2.1	> 0.1
Mature oocytes	8.2 ± 1.1	8.1 ± 1.1	> 0.1
Cleavage rate on (D2)	77.1± 8.1	77.5 ± 8.0	> 0.1
Cleavage rate on (D3)	77.1± 8.1	77.5 ± 8.0	> 0.1
Blastocyst formation (D5)	64.5 ± 8.0	67.1± 8.1	**≤ 0.01***
Grade A embryos	6.0 ± 1.4	6.6 ± 1.4	**≤ 0.01***
Grade B embryos	2.5 ± 1.2	2.1 ± 1.2	> 0.1
Grade C embryos	1.1 ± 1.1	0.1 ± 1.1	**≤ 0.001***

There were statistically significant differences in Mini Swim up group compared in Swim up group, reading, Blastocyst formation rate (D5) and Grade A embryos (P □ 0. 001*). However, there weren't statistically substantial variation in Mini Swim up group compared in Swim up group, reading, collected oocytes, Mature oocytes Fertilized oocytes.

Table (4): properties of processed sperms in the aberrant male factor group (n=200):

Parameters	**Original semen**	**Swim up (N=100)**	**Mini Swim up (N=100)**	**P value**
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	
Sperm concentration ($\times10^6$/ml)	28.6 ± 8.3	18.1 ± 7.8	20.8 ± 7.5	≤ 0.001*
Sperm motility/ml	41.3 ± 2.2	43.2 ± 5.5	54.3 ± 5.4	≤ 0.001*
Sperm Progressive motility/ml	21.3 ± 2.2	23.8 ± 3.7	34.1 ± 2.7	≤ 0.001*
Sperm Head defects	51.3 ± 2.2	40.4 ± 6.8	35.8 ± 6.5	≤ 0.001*
Sperm Midpiece defects	41.3 ± 2.1	30.3 ± 7.8	27.8 ± 8.2	≤ 0.001*
Sperm Tail defects	21.3 ± 2.2	20.7 ± 2.3	15.8 ± 2.3	≤ 0.001*
Sperm DNA fragmentation index	42. 6 ± 11.3	38.0 ± 1.8	35.0 ± 11.5	≤ 0. 001*
ROS index	30. 6 ± 1.2	28.1 ± 0.8	20.0 ± 0.5	≤ 0. 001*

There were statistically significant differences in Mini Swim up group compared in Swim up group, reading Sperm count, Sperm motility, Progressive motility, Head defects, Midpiece defects, Tail defects, DNA, ROS (P < 0. 001*).

Table (5): Comparison between ICSI findings among abnormal male factor group (n=200)

Parameters	Swim up (N=100)	Mini Swim up (N=100)	P value
	Mean ± SD	Mean ± SD	
Collected oocytes	15.3 ± 3.1	14.9 ± 2.1	> 0.1
Mature oocytes	13.2 ± 1.1	13.1 ± 1.1	> 0.1
Cleavage rate on (D2)	77.1± 8.1	87.5 ± 8.0	≤ 0.001*
Cleavage rate on (D3)	77.1± 8.1	87.5 ± 8.0	≤ 0.001*
Blastocyst formation (D5)	67.1± 8.1	84.5 ± 8.0	≤ 0.001*
Grade A embryos	6.6 ± 1.4	7.0 ± 1.4	≤ 0.01*
Grade B embryos	2.1 ± 1.2	2.0 ± 1.2	> 0.1
Grade C embryos	1.1 ± 1.1	1.1 ± 1.1	> 0.1

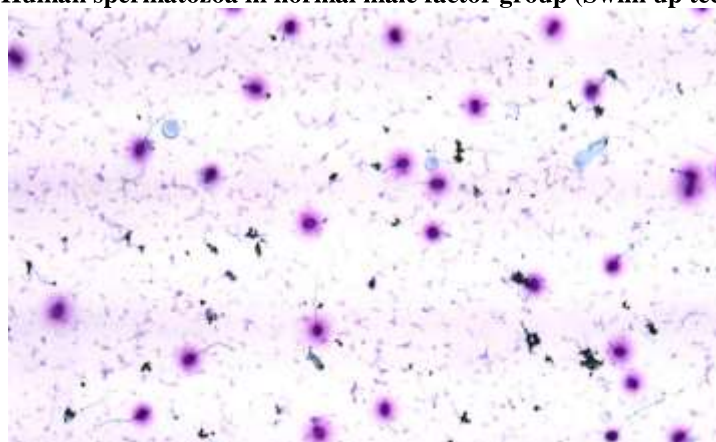
There were statistically significant differences in Mini Swim up group compared in Swim up group, reading Cleavage rate on (D2),Cleavage rate on (D3),Blastocyst formation (D5), Grade A embryos (P < 0. 01*). However, there weren't statistically significant differences in Mini Swim up group compared in Swim up group, reading Collected Oocytes, Mature oocytes, Grade B embryos and Grade C embryos.

Table (6): Comparison between Pregnancy rates among all Studied Groups:

male factor	Swim up	Mini Swim up with sperm gradient	P value
	%	%	
Normal	41.0%	42.0%	**> 0.1**
Abnormal	36.0%	46.0%	**≤ 0.01***

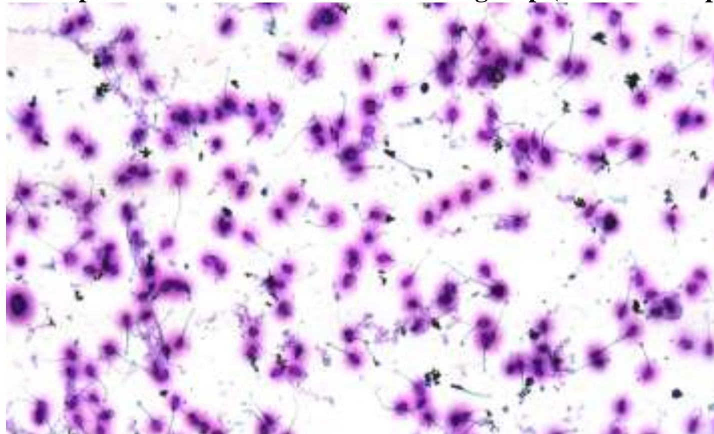
The incidence of Pregnancy rate was higher (42.0%) in mini swim up group (normal male factor) compare to (41.0%) in swim up group (normal male factor). the incidence of Pregnancy rate was higher (46.0%) in mini swim up group (abnormal male factor) compare to (36.0%) in swim up group (abnormal male factor.

I-Human spermatozoa in normal male factor group (Swim up tech).



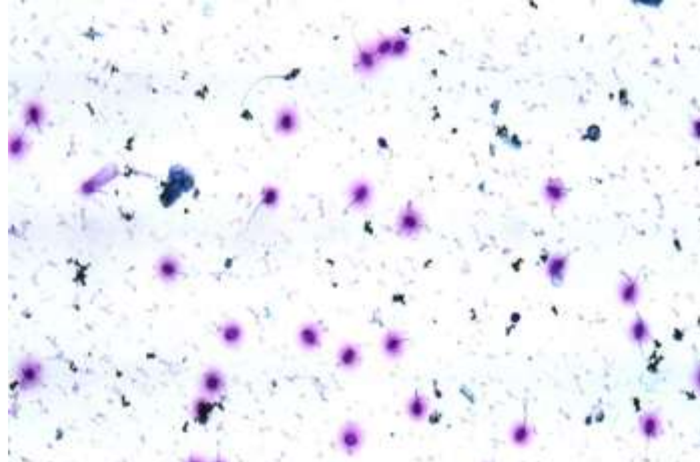
The light microscope showed: Sperms with fragmented DNA: Sperm with small halo (green arrow); Sperm without halo (red arrow). Sperms without fragmented DNA: Sperm with big halo (blue arrow); Sperm with medium-sized halo (yellow arrow). (resolution x 50) stained with halo sperm G2 kit (Halotech DNA).

II- Human spermatozoa in normal male factor group (Mini Swim up tech).



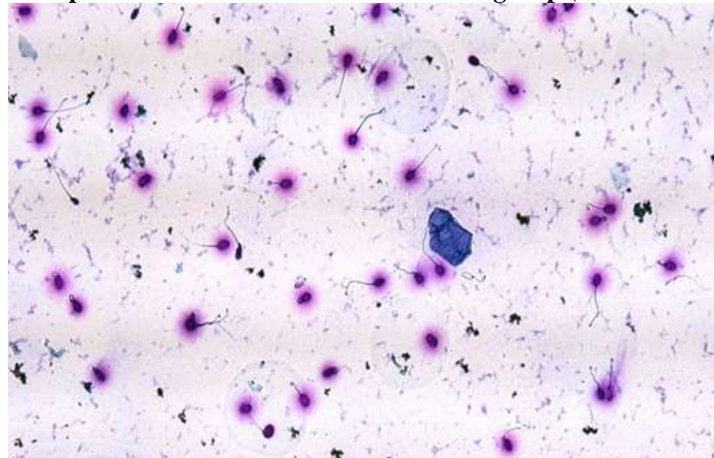
The light micrograph revealed: Sperms with fragmented DNA: Sperm with small halo (green arrow); Sperm without halo (red arrow); Sperm without halo and degraded (orange arrow). Sperms without fragmented DNA: Sperm with big halo (blue arrow); Sperm with medium-sized halo (yellow arrow). (resolution x 50) stained with halo sperm G2 kit (Halotech DNA).

III-Human spermatozoa in abnormal male factor group (Swim up tech).



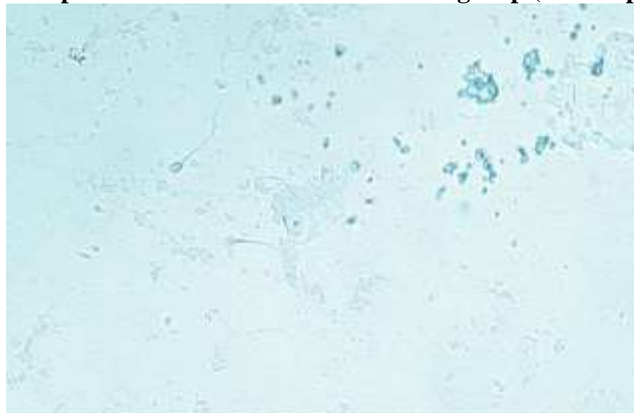
The light micrograph showed: Sperms with fragmented DNA: Sperm with small halo (green arrow); Sperm without halo (red arrow). Sperms without fragmented DNA: Sperm with big halo (blue arrow). Sperm with medium-sized halo (yellow arrow). (resolution x 50) stained with halo sperm G2 kit (Halotech DNA).

IV- Human spermatozoa in abnormal male factor group (Mini Swim up tech).



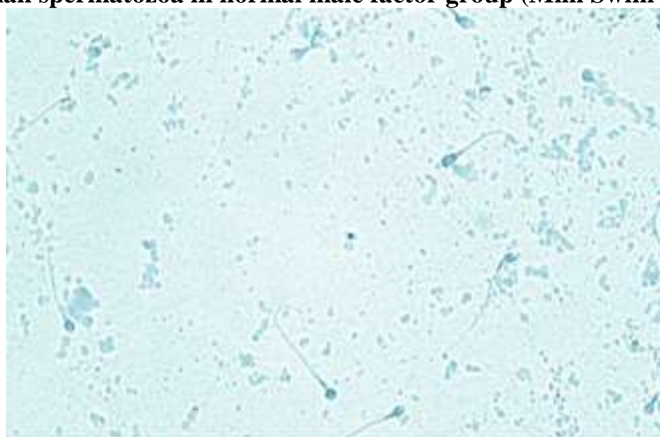
The light micrograph reveals: Sperms with fragmented DNA: Sperm with small halo (green arrow); Sperm without halo (red arrow). Sperms without fragmented DNA: Sperm with big halo (blue arrow); Sperm with medium-sized halo (yellow arrow). (resolution x 50) stained with halo sperm G2 kit (Halotech DNA).

I-Human spermatozoa in normal male factor group (Swim up tech).



The light micrograph showed: Spermatozoa that were affected by oxidative reaction (red arrow); and other where the effect is null (blue arrow). As the number of molecules deposited on the sperm surface increases the color intensity of the reaction increases. (resolution x 50) stained with oxisperm kit (Halotech DNA).

II-Human spermatozoa in normal male factor group (Mini Swim up tech).

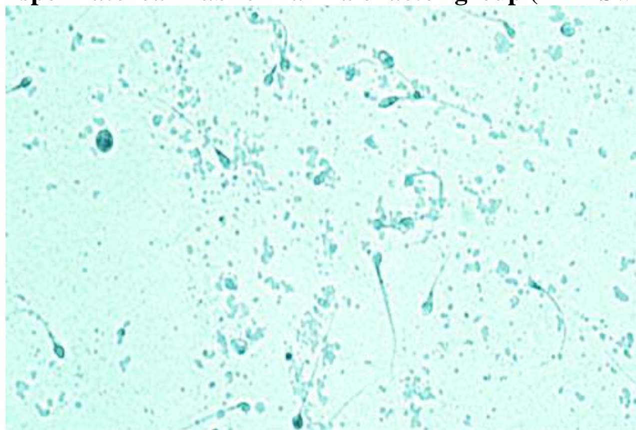


The light micrograph showed: spermatozoa that were affected by oxidative reaction (red arrow). As the number of molecules deposited on the sperm surface increases the colour intensity of the reaction increases. (resolution x 50) stained with oxisperm kit (Halotech DNA).

III-Human spermatozoa in abnormal male factor group (Swim up tech).

The light micrograph showed: spermatozoa that were affected by oxidative reaction (red arrow); and other where the effect is null (blue arrow). As the number of molecules deposited on the sperm surface increases the color intensity of the reaction increases. (resolution x 50) stained with oxisperm kit (Halotech DNA).

IV- Human spermatozoa in abnormal male factor group (Mini Swim up tech).



The light micrograph showed: spermatozoa that were affected by oxidative reaction (red arrow); and other where the effect is null (blue arrow). As the number of molecules deposited on the sperm surface increases the color intensity of the reaction increases. (resolution x 50) stained with oxisperm kit (Halotech DNA).

4. DISCUSSION

Hepatorenal condition is one of the likely reasons The management of male factor infertility was revolutionized by ICSI. Considering that the spermatozoon is injected directly into the egg cytoplasm, ICSI removes all natural selection obstacles. Immature spermatozoa may be distinguished from mature ones by visual examination, but since the damage to the sperm DNA itself does not cause any morphological alterations, it is very difficult to distinguish between them. Semen processing is thus crucial before ICSI treatments. (21).

In our study the median age of female patients was 28.8 ± 5.5. The median BMI was 28.6 ± 3.5 and the median duration of infertility was 6.6 ± 2.5. The mean of FSH (IU/L) hormone was 5.1 ± 1.6, the mean of LH (IU/L) hormone was 4.1 ± 1.4, the mean of PRL (µg/L) hormone was 20.8 ± 9.8, the mean of E2 (pg/ml) hormone was 35.9 ± 16.3 and the mean of TSH (mIU/L) hormone was 3.2 ± 1.12. The distribution of stimulation protocols in the Female Studied patients showed 74 (37%), on average, of the patients who received the Antagonist regimen were female. The average number of female patients undergoing the Long procedure was 84 (42%), while the average number of female patients undergoing the Short procedure was 42 (21%).

Our study can be supported by Rao et al. (22) who aimed to study overall live birth rates following IVF/ICSI cycles using swim-up sperm vs. sperm produced by density gradient centrifugation. The study reported that age in female patients in swim up group was 31.8 (4.4%), BMI kg/m2 mean (SD) was 22.4 (3.4%), Agonist protocols were 47.1 (339), Antagonist protocols were 36.3 (261) and other protocols were 16.5 (119).

In normal male factor group, There were statistically significant differences in Mini Swim up group compared in Swim up group, reading Sperm concentration, Sperm Head defects, DNA fragmentation index (P □ 0. 01*). However, there weren't statistically significant differences in Mini Swim up group compared in Swim up group, reading Sperm motility, abnormal forms, Midpiece defects, Tail defects and ROS index. There were statistically significant differences in Mini Swim up group compared in Swim up group, reading, Blastocyst formation rate (D5) and Grade A embryos (P □ 0. 001*). However, there weren't statistically substantial variation differences in Mini Swim up group compared in Swim up group, reading, collected oocytes, Mature oocytes Fertilized oocytes.

For assisted reproductive techniques, populations of highly mobile spermatozoa may be chosen using the swim-up method. Several labs have utilized it as their primary sperm washing method. The elimination of seminal plasma and the selection of highly mobile spermatozoa using the min swim-up approach prior to freezing may thus prevent the viability loss, mobility, and acrosome integrity at cryopreservation, it seems fair to hypothesize. (23). Our study was consistent with Jackson et al. (24) who aimed to study impacts of sperm DNA fragmentation on semen preservation and separation methods. The study reported that Sperm motility (%) was 53 ± 6.5.

In line with our study Baldini et al. (25) who aimed to study a quick and secure method for sperm preparation in ICSI procedures. The study reported that Retrieved MII oocytes (number) in mini swim up group was 5.44 (±2.67), Cleavage rate was 92.10% (±13.69) and Blastocyst rate was 41.2% (±20.69). In line with our study Shirota et al. (26) who aimed to study efficacy of a microfluidic sperm sorter in separating sperm to reduce sperm DNA damage. The study reported that Sperm motility % was 60.3±19.4 (0–88.4) in swim up procedure group while DFI % was 10. ±8.5 (0.6–37.3). However, Sperm concentration 106 /mL was 49.4±46.4 (0–186) in the same group. Study of Bormann et al. (27) reported that According to studies, sperm created using a density gradient and swim-up technique had greater rates of motility and less DNA breakage than sperm created using other techniques.

On another hand According to earlier research, sperm DNA fragmentation may rise when semen is incubated at 37 °C or at room temperature after being separated by density gradient centrifugation. While producing sperm, spermatozoa that contain intact chromatin may enrich, increasing the chance of having a healthy kid if a significant fraction of the sperm in the semen sample does. The connection between sperm preparation and DNA fragmentation is significant. Thus, more methodological research is advised to determine if higher quantities of DNA seen in an ejaculate may really be passed on to future generations in order to enhance the diagnostic capabilities of sperm DNA damage. (28). In contrast, other research reported that the density gradient and density gradient and swim-up method pellet fractions of sperm had a much greater incidence of sperm with DNA damage than the unprocessed semen. Normozoospermia samples showed the same impact, although it was statistically negligible. (29).

In abnormal male factor group Our results reveal that there were statistically substantial variation in Mini Swim up group compared in Swim up group, reading Sperm count ,Sperm motility ,Progressive motility, Head defects, Midpiece defects, Tail defects, DNA, ROS (P 0. 001*). there were statistically substantial variation in Mini Swim up group compared in Swim up group, reading Cleavage rate on (D2),Cleavage rate on (D3),Blastocyst formation (D5), Grade A embryos (P 0. 01*). However, there weren't statistically substantial variation in Mini Swim up group compared in Swim up group, reading Collected Oocytes, Mature oocytes, Grade B embryos and Grade C embryos.

Both sperm preparation procedures enable creating a sperm population with a low proportion of apoptotic sperm, according to the first research to assess the impact of gradient-density centrifugation and swim-up methods on sperm death utilizing flow cytometry. (30). Our study was consistent with Gosálvez et al. (31) who aimed to study Whether pregnancy after ICSI of donors with viable oocytes can be predicted by DNA fragmentation of neat or swim-up spermatozoa. The study reported that couples with severe female factor infertility age was 24–35 years old; mean ±SD: 29.0±3.6 years. Study of Ahmad et al (32) concluded that it has also been suggested to use percoll density gradient centrifuge for samples of teratozoospermia and cases of idiopathic infertility. Our study can be supported by Oguz et al. (33) who aimed to study the impact of sperm preparation methods such as swim-up and gradient on patients who are infertile. The research found that in the male factor group, post-swim-up improving sperm movement (%) was 59.5± 3.4 and post-swim-up sperm DNA fragmentation (%) was 30.3 ±3.2. (33). Another study showed that Density gradient centrifugation of semen increases the proportion of cells with proper shape and motility. This research also discovered that the discontinuous Pure Sperm reduced the percentage of aneuploid and diploid spermatozoa. (34).

On the other hand, Zini et al., (35). revealed that while the recovery of spermatozoa with improved motility by density-gradient centrifuge and the swim-up approach were similar, the DNA integrity of spermatozoa recovered using the swim-up approach was greater. Another research that contradicted ours indicated that sperm apoptotic DNA fragmentation levels were not improved by semen treatment by density gradient centrifuge, prompting the authors to advise patients with basal DNA fragmentation to utilize other semen processing methods. (36).

In our study the incidence of Pregnancy rate was higher (42.0%) in mini swim up group (normal male factor) compare to (41.0%) in swim up group (normal male factor). the incidence of Pregnancy rate was higher (46.0%) in mini swim up group (abnormal male factor) compare to (36.0%) in swim up group (abnormal male factor. Our study can be supported by Anbari et al. (9) who aimed to study In the ICSI program, microfluidic sperm selection produces sperm of greater quality than the traditional approach. The study reported that Clinical pregnancy was 23.07 % (9/39) in swim up group. Also, our study is consistent with Palini et al. (37) study reported that fertilization rate in micro swim up group was 81.8 (540/660) while pregnancy rate was 41.9 (31/74). While it has been shown that both swim-up and density gradient approaches are useful for collecting sperm populations with a low proportion of apoptotic sperm (38).

On the other hand, another study in disagreement with our study revealed that Swim-up treatment reduces the DFI and HDS of spermatozoa whereas discontinuous gradient removes morphologically defective sperm, although both techniques are efficient for embryo development.

5. CONCLUSION

It has been discovered that sperm preparation produces enriched sperm with intact chromatin, which is expected to increase the likelihood of having a successful pregnancy.

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There are no conflicting interests, according to the authors. The authors alone are accountable for the paper's authorship and content.

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