

## Role of magnetic-activated cell sorting in sperm preparation and highly fertilization outcome

Osama M. Badr<sup>1\*</sup>, Shokry A.E.E..El Shershaby<sup>2</sup>, Ebrahim A. Sabra<sup>3</sup> and Emad A.E. El Shazly<sup>3</sup>

1,3,-Animal Biotechnology Department, Genetic Engineering and Biotechnology Research Institute, University of Sadat City.

2-International Islamic Center for Population Studies and Research, Al-Azhar University.

\*Corresponding author E-mail: [osama.badr@gebri.usc.edu.eg](mailto:osama.badr@gebri.usc.edu.eg)

Received: April 14, 2024; Accepted: April 28, 2024; Available Online : May 7, 2024

DOI: 10.21608/AJBS.2024.283352.1072

### ABSTRACT

Infertility is a popular problem with major psychological and physiological effects. The Magnetic Activated Cell Sorting technique (MACS) in an intracytoplasmic sperm injection (ICSI) cycle represents one of the modern assisted reproductive techniques. Therefore, the aim of this study was to investigate the role of MACS-ICSI in sperm preparation and highly fertilization outcome. The current findings indicated that couples coping with male infertility brought on by DNA sperm fragmentation can increase the percentage of high-quality embryos produced and the likelihood of conception by using the MACS-ICSI approach.

**Keywords:** ICSI, MACS, Infertility, Fertilization.

### INTRODUCTION

According to Simionescu *et al.* (2021) the inability to conceive after a minimum of a year of attempting is known as infertility. Infertile women are also those who are fertile but regularly miscarry. The World Health Organization defines infertility as what happens after a 24-month period of trying to conceive (Deshpande and Gupta, 2019).

Bonte *et al.* (2019) reported that the “intracytoplasmic sperm injection” (ICSI) has emerged as the most widely used assisted reproductive treatment for a variety of infertile causes. The total fertilization failure (TFF), despite estimates of normal fertilization rates of 70%, still happens in 3%–5% of ICSI cycles (Bhattacharya *et al.*, 2013). Numerous methods have been developed for isolating sperm and preparing them for ICSI, however no randomized

comparative study has evaluated the effect of MACS separation on ICSI cycle success. There aren't enough clinical trials to compare sperm selection techniques, and their efficacy is still up for debate. A retrospective analysis of the literature showed that MACS produced significantly more fertilized eggs and high-quality embryos (Rezaei *et al.*, 2021).

The present study focuses on comparing semen parameters, including processing, fertilization, cleavage, and ideally pregnancy rates, between traditional semen preparation methods and (MACS) for ICSI of super ovulated women. Unlike conventional MACS, the particles utilized in this research target three distinct receptors associated with DNA breakage within sperm tail, head, and acrosomal region. In contrast MACS system only targets areas exposed to phosphatidylserine (P-S) during the early

stages of apoptosis or necrosis. Besides targeting and separating based on DNA fragmentation index, DFI, markers, this methodology reduces DNA damage during sperm processing pre-injection, as it does not involve density gradient or heavy centrifugation steps (Hasanen *et al.*, 2020). MACS technique helps in sperm selection and it is also employed for embryo sexing, identifying genetic diseases through surface markers on gametes and embryonic cells (Gil *et al.*, 2013). MACS utilized for depleting morbid /or DNA fragmented spermatozoa prior to applying ICSI and in vitro fertilization (Abou El-Ela *et al.*, 2022).

## MATERIALS AND METHODS

### Patient selection:

This study included 100 cycles from 100 couples that were enrolled in Waladwebent Fertility Center's ICSI programmed. Patients with a history of complete or partial failure of fertilization following ICSI, as well as cases of severe oligo-astheno-teratozoospermia, non-obstructive azoospermia, or completely immotile spermatozoa, were chosen for this investigation.

### Technical approach of all male subjects:

#### 1-Complete semen analysis: (WHO, 2020)

##### A-Sample collection

After 2-7 days of abstinence, samples of semen were obtained by masturbation; longer abstinence intervals (10 days) result in reduced motility, while shorter periods provide low volume and density (WHO, 2020). To reduce collection mistakes, the container needs to be sterile, clean, and wide-mouthed, and it should be from a non-toxic patch to spermatozoa. Within an hour of collection, the semen samples should be evaluated and kept at room temperature or body temperature (WHO, 2020).

### B- Physical examination:

#### B.1- Appearance of the ejaculate:

Visual appearance was used to estimate appearance. A typical semen sample appears uniformly gray-opalescent.

#### B.2-Liquefaction:

While the semen was melting, the specimen container was set up in an incubator at 37 °C. At room temperature, a typical semen sample liquefies in 60 minutes, however it frequently does so in 15 minutes. Complete liquefaction doesn't always happen in 60 minutes, and this needs to be noted (Hancock and McLaughlin, 2002 &WHO, 2020).

#### B.3-Semen viscosity:

Using a disposable plastic pipette, the viscosity was measured by gently aspirating.

#### B.4-Semen volume:

It was measured by scooping the entire ejaculated semen into a graduated glass pipette that has been heated to sterility (Rouge, 2002).

#### B.5- Odor:

Certain meals and medications may have an impact on the odor of semen. When pee contaminates semen, it can be unpleasant in certain genital infection situations or ferrous in urine cases (WHO, 2020).

#### B.6-Semen pH:

Using pH paper, the semen's pH was measured within the range of 6.0 to 10.0. After liquefaction at a uniform time- ideally after 30 minutes, but at least an hour after ejaculation- the pH should be determined. The following must be followed:

1. Evenly distribute a drop of semen onto the pH paper.
2. Give the impregnated zone around 30 seconds to stabilize its color.
3. To determine the pH, compare the color with the calibration strip.

## **Role of magnetic-activated cell sorting in sperm preparation and highly fertilization outcome**

### **C- Microscopic examination:**

This includes the analysis of the motility, morphology, and concentration of sperm.

#### **C.1-Sperm concentration:**

Using a pipette, a predetermined volume of 10  $\mu$ l semen is transferred into a sterile glass slide and covered with a 24  $\times$  50 mm cover slip. For best viewing, the sample is stretched out by the weight of the cover slip. A further magnified image at 400 x (40 x objective  $\times$  10 x ocular) should be taken after the initial microscopic examination, which should be performed at 100 x (10 x objective  $\times$  10 x ocular) to assess the mucus strand, sperm aggregation, and dissemination of spermatozoa on slide. In a 400X microscope, one sperm is about equivalent to one million per ml of seminal fluid (Jequier, 2011).

#### **C.2 -Sperm motility:**

The surface of a warm, dry, and microscopically clean slide is covered with a drop of well-mixed, undiluted semen before a cover slip is placed on it. After then, the slide can rest on a bench or a tiny stage until the fluid movement stops. Next, the semen drop is examined at 400 times magnification, ideally using a phase contrast-equipped microscope. At least five distinct microscopic fields are used to count both motile and immotile sperm; 200 spermatozoa should be evaluated at the very least. The mean value is used to compute the % motility (WHO, 2020).

#### **C.3 - Categories of sperm movement:**

- 1- Progressive motility, PR,: Spermatozoa can move actively.
- 2-Non-progressive motility, NP,: Every other motility pattern without any progression.
- 3- Immobility, IM,: No movement (WHO, 2020).

### **C.4- Sperm morphology:**

A comprehensive method is used for the evaluation of sperm morphology in accordance with stringent standards, beginning with the fabrication of clean microscope slides, the accurate preparation of a thin semen smear, and the evaluation of slides (Mortimer and Menkveld, 2001).

#### **C.5- Select a spermatozoon:**

Immediately for injection from the point where the mature sperm chooses the center drop and the previously prepared sperm droplet meets. To be picked, mature spermatozoa should move their tails but not move forward or advance. Immature spermatozoa shouldn't be picked; they should be able to move freely. To be picked, mature spermatozoa should move their tails but not move forward or advance. Immature spermatozoa shouldn't be picked; they should move freely (Nadalini *et al*, 2014 and Romany *et al.*, 2017).

### **2. Semen preparation techniques:**

All males were subjected to Sperm preparation by 2 methods:

#### **MACS technique:**

##### **Procedure:**

Removal of Sodium azide (washing procedures). The tube with the magnetic particles was holded against the magnet (ideally on a rack or with a rubber band) until the particles press up against the tube wall. Usually in four minutes. While the particles are still in the magnetic field and being pressed against the tube wall, decant, or aspirate out the supernatant. Re-suspend the particles with the preferred washing buffer after removing the magnet (Young *et al.*, 2010 and Van Thillo *et al.*, 2011).

Turn the Clemente Associates suspension of liquid and particles upside down until the particles are evenly distributed. Repeat step 1 once more. As in steps two and three, resuspend the particles.

The particles are operational. The process for ICSI (Abou El-Ela *et al.*, 2022).

- 1- To prepare sperm, use density gradient centrifugation or wash with extender HTF, either with or without BSA from Irvine Scientific.
- 2- To each ml of 5 million sperm/ml (1 ml containing 5M and 225 ul particle suspension), add 225 ul of particles.
- 3- Mix the sperm and particles gently for half an hour at room temperature.
- 4- For 10 minutes, hold the sperm solution particles up against the magnet and decant the supernatant which contains ready-to-use sperm.
- 5- Proceed to ICSI with the supernatant.

#### **Preparation of semen by a Centrifugation Method:**

1. Mix the semen sample well.
2. Dilute the whole semen sample 1:1 with enriched media.
3. Pour the diluted suspension into several centrifuge tubes, ensuring that each tube contains no more than three milliliters.
4. Centrifuge at 300–500g for 5–10 minutes.
5. Aspirate carefully, then discard the supernatants.
6. Gently pipette the mixed sperm pellets back into 1 ml of enriched media.
7. Repeatedly centrifuge at 300–500g for 3–5 minutes.
8. Gently extract and dispose of the supernatant.
9. Gently pipette the sperm pellet back into suspension in a few enriched media that is suitable for final disposition (Boomsma *et al.*, 2019).

#### **Technical approach of female subjects:**

Oocyte collection, identification, grading, and denudation

#### **1- Stimulation protocols and oocyte retrieval:**

The cases were treated with an appropriate superovulation program (long or short

protocol), to obtain enough eggs. After receiving an hCG injection, oocytes were extracted 34–36 hours later and sent for ICSI. The patient arrived in the operating room fasting to have the oocytes collected, and general anesthesia was used during the process. To get rid of antiseptic residue, the vagina can be cleaned with antiseptics and then irrigated with regular saline. However, there is no assurance that the antiseptic will be eliminated by irrigation; if it comes into touch with the oocytes, it could have harmful effects. As an alternative, all mucus in the vagina can be removed using gauze swabs soaked in regular saline; this cleaning technique has not been associated with an increase in pelvic infections. To confirm the position of the uterus, the quality of the endometrium, the location and accessibility of the ovaries, and the number of follicles to be aspirated, a vaginal transducer was inserted into the vagina and the pelvis was extensively scanned. Following the computerized needle guidance that the Labotect aspiration pump had set, the ultrasonic probe was introduced into the vagina and directed towards the posterolateral part of the vaginal fornix (Stevenson and Lashen, 2008).

#### **2- Oocyte Collection: -**

The Oocyte Cumulus Cells (OCC) complexes were separated Under a dissecting microscope, (Nikon SMZ 800 Stereo Microscope) cleaned and put into four wells after being rinsed in global total with HEPES Buffer (Life Global, Europe). (Nunc) dishes containing the same medium.

#### **3- Denudation:**

After being in a 100 µl drop of buffered solution with 80 IU/ml of hyaluronidase for 30-45 seconds, the oocyte was taken out and put in a 100 µl drop of global total w/HEPES buffer. Hyaluronidase is an enzyme that breaks down a part of the connective tissue components that hold cells

## **Role of magnetic-activated cell sorting in sperm preparation and highly fertilization outcome**

together. A sterile stripper pipette was used to gently aspirate the oocyte in and out to remove the remaining corona cells manually. Upon completion of denudation, the oocyte was rinsed in global total w/HEPES buffer. One half of the denudated oocyte was then inserted into an ICSI injection dish containing 10  $\mu$ l micro drops of the global total w/HEPES Buffer (Life Global, Europe), while the other half was placed in an additional ICSI injection dish.

### **4 -The oocyte grading:**

The oocyte was rapidly evaluated for maturity (Quality) using an inverted Nikon Integra 3 microscope equipped with Hoffman optics, a hot stage, and automatic manipulators RI. The evaluation was based on the grading system. Just before injecting sperm, the oocyte's morphology was evaluated. The following dysmorphisms of oocytes were noted: (i) granularity of cytoplasm, (ii) color of cytoplasm, (iii) ooplasm vacuoles, (iv) large perivitelline space and granularity, (v) fragmented polar body, (vi) abnormalities in zona pellucida and oocyte shape. Before being injected, oocytes must be seen to distinguish between germinal vesicles (GV), immature oocytes (MI), and mature oocytes (MII). Maturity was determined by the release of the first polar body, which was recorded, and these oocytes were used for ICSI (Ekart *et al.*, 2013). The mature oocytes were split in half and injected with sperm prepared using MACS (MACS-ICSI) and traditional preparation (traditional ICSI). The sperm-injected half of the oocytes was then cultured in a culture medium at 37 °C with 6% CO<sub>2</sub> until the ICSI process.

### **Intracytoplasmic sperm injection (ICSI) procedure:**

#### **Preparation of the dish for injection:**

The gametes for ICSI are stored on a unique electrostatically coated dish. In the

middle of the plate is a 10-microdroplet of polyvinylpyrrolidone which is a viscous solution that is used to slow down motile of sperm and facilitates easier sperm capture using an injection pipette before ICSI. The oocytes for ICSI will be inserted in four droplets of a culture media containing 10  $\mu$ l. To minimize surface evaporation during ICSI, 3-5 milliliters of sterile, equilibrated mineral oil are carefully placed into the dish to cover these droplets. After that, the dish is put back into the incubator for almost half an hour, at which point the oocytes are transferred into the culture droplets in preparation for ICSI.

### **Intracytoplasmic sperm injection ICSI:**

The PVP droplet is visible when the ICSI dish is placed on the microscope stage. A tiny amount of PVP is aspirated when the injection pipette is lowered into the droplet. The next step involves mechanically immobilizing sperm by scoring the tail of one of the best-moving sperm with the needle. Next, aspirate the sperm into the injection pipette, starting with the tail. The injection pipette is lifted above the dish, and the dish is moved to reveal the first droplet carrying an oocyte. The injection pipette is dropped into this droplet after the oocyte holding pipette. The oocyte is kept securely with the polar body at the 12 or 6 o'clock position by gently moving the holding pipette to the side of the oocyte and applying a small amount of suction pressure. With a swift motion, the injection pipette penetrates the oocyte from the side that is opposite the holding pipette. The oolemma typically tents slightly, but this gives way as the needle moves closer inward. A tiny amount of the cytoplasm flows backwards into the injection needle when the needle tip enters the cytoplasm, and the micro-injector makes a tiny suction movement. To ensure that the needle has penetrated the oolemma, this

procedure is required. Additionally, some data points to the necessity of this cytoplasmic agitation for oocyte activation, which initiates the processes that will result in fertilization. The sperm immediately follows the aspirated cytoplasm, which is then ejected back into the oocyte. After injecting the oocyte, remove the needle. The oocyte is released when the holding pipette's negative pressure is released. To restore visibility to the core PVP droplet, the needles are extracted from the drop and elevated above the plate. The sequence of sperm picks up and oocyte injection is repeated until all oocytes are injected. Following injection, the oocyte was cleaned and placed in global total media in a culture plate covered with sterile, warm, equilibrated global oil. The culture dish was kept at 37 °C, 6% CO<sub>2</sub>, and 90–95% humidity until fertilization.

**Follow up must be done considering the followings:**

1-Fertilization rate; 2- Cleavage rate; and 3- Embryo grading.

Assessment of fertilization and embryo's quality: After microinjection, fertilization was evaluated 16–18 hours later. We checked the injected oocytes for pronuclei and for any indications of injury. If there were two pronuclei (2PN) and the second polar body had been extruded, the oocytes were considered fertilized. An acceptable number of embryos were transferred to recipient individuals approximately 72 hours following microinjection. According to Halvaei *et al.* (2016), embryo was graded after 72 hrs. following injection into:

**Grade A:** Includes blastomeres of the same size and without fragmentation.

**Grade B:** Blastomeres up to 10% of cytoplasmic fragments and mildly uneven.

**Grade C:** Blastomeres with big granules and up to 50% shards of unevenly size.

**Grade D:** Blastomeres are uneven with big black granules and with substantial fragmentation.

To recipients, Day 3 embryos were transferred (MACS-ICSI) in accordance with the 'American Society of Reproduction' guidelines. Overproduction of high-quality embryos was cryopreserved. After 6-7 weeks of amenorrhea, a transvaginal ultrasound scan of the uterus was performed to ascertain whether a clinical pregnancy had been established (intrauterine gestational sac is visible). Fourteen days after embryo transfer, serum-HCG was determined as a chemical pregnancy test (considered positive if 20 IU/L).

**Statistical analysis:**

Analysis of data was carried out using SAS. For comparing between the studied parameters in each studied group the Paired T-test and Mc Nomar's test were used. The None paired T test and Fischer exact test were used to compare the mean changes in different studied groups regarding the studied parameters. The significant level was set at [P<0.05].

The study was approved by 'Ethics Committee of International Islamic Centre for Population Studies and Research' (IICPSR), Al-Azhar University, Cairo, Egypt (Gomez *et al.*, 2023).

**Confidentiality:**

Every patient who was admitted for the study had his privacy respected. The names of study participants will not appear in any report or publication derived from the project's data collection.

**RESULTS**

**1- Comparison between sperm parameters after processing among male studied groups:**

The present investigation looked at the properties of the sperm in male study

## Role of magnetic-activated cell sorting in sperm preparation and highly fertilization outcome

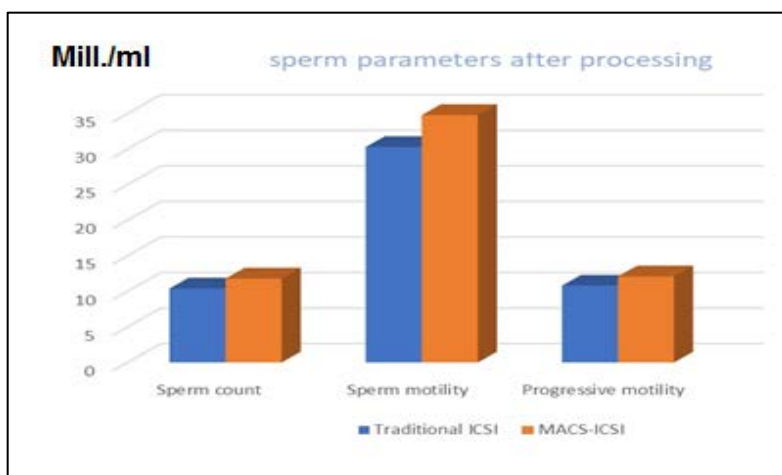
participants. It was found that the sperm count incidence was greater ( $11.8 \pm 3.5$  mill/ml) in the MACS-ICSI group than in the Traditional ICSI group ( $10.4 \pm 3.8$  mill/ml), but the differences were not statistically significant ( $P > 0.05$ ). The MACS-ICSI group had a greater incidence of motility ( $34.8 \pm 9.2$ /ml) compared to the Traditional ICSI group ( $30.3 \pm 9.8$ /ml), and

this difference was very high significant ( $P \leq 0.01^{**}$ ). Data in Table (1) and Figure (1) indicated that the incidence of progressive sperm motility was greater ( $12.1 \pm 2.7$ /ml) in the MACS-ICSI group than in ICSI group ( $10.8 \pm 3.7$ /ml) and exhibited a very statistically significant difference ( $P \leq 0.01^{**}$ ).

**Table 1. Sperm parameters after processing by traditional ICSI and MACS-ICSI.**

Parameters	Traditional ICSI	MACS-ICSI	P value
	Mean $\pm$ SD	Mean $\pm$ SD	
Sperm count (mill/ml) %	$10.4 \pm 3.8$	$11.8 \pm 3.5$	<b><math>P &gt; 0.05</math></b>
Sperm motility/ml %	$30.3 \pm 9.8$	$34.8 \pm 9.2$	<b><math>P \leq 0.01^{**}</math></b>
Progressive motility/ml %	$10.8 \pm 3.7$	$12.1 \pm 2.7$	<b><math>P \leq 0.01^{**}</math></b>

(\*\*\*) highly significant  $P \leq 0.001$ , (\*\*\*) very significant  $P \leq 0.01$ , (\*) significant  $P \leq 0.05$ , (\$) not-significant  $P > 0.05$ .



**Fig. 1. Analysis of sperm parameters after processing by traditional ICSI & MACS-ICSI.**

### 2- Morphological variations of sperms after processing among male studied groups:

The results of the current study (Fig. 2 and Table 2) indicated that there was a morphological difference after processing (using MACS-ICSI and ICSI) where the abnormal forms of sperms in ICSI group were ( $98.7 \pm 1.3\%$ ), while they were ( $97.7 \pm$

$1.1\%$ ) in the MACS-ICSI group. This variation was very statistically ( $P < 0.01^{**}$ ). Also, the incidence of head defects was higher in the Traditional ICSI group ( $90.4 \pm 6.8\%$ ) than in the MACS-ICSI group ( $85.8 \pm 6.5\%$ ). This difference was highly significant ( $P \leq 0.001^{***}$ ). Furthermore, the incidence of midpiece defects was higher in the Traditional ICSI group ( $60.3 \pm 7.8\%$ )

than in the MACS – ICSI group ( $55.8 \pm 8.2\%$ ). This variation was highly significant ( $P < 0.001^{***}$ ). Moreover, ICSI group had a greater incidence of tail defects morphology

( $20.7 \pm 12.4\%$ ) than the MACS-ICSI group ( $15.7 \pm 12.3\%$ ) with ( $P < 0.01^{**}$ ) indicated that this difference was very significant.

**Table 2. Morphological analyses of sperms after processing among male studied groups (n=100).**

Defects	Traditional ICSI	MACS-ICSI	P value
	Mean $\pm$ SD	Mean $\pm$ SD	
Abnormal forms (%)	$98.7 \pm 1.3$	$97.7 \pm 1.1$	$P \leq 0.01^{**}$
Head defects (%)	$90.4 \pm 6.8$	$85.8 \pm 6.5$	$P \leq 0.001^{***}$
Midpiece defects (%)	$60.3 \pm 7.8$	$55.8 \pm 8.2$	$P \leq 0.001^{***}$
Tail defects (%)	$20.7 \pm 12.4$	$15.7 \pm 12.3$	$P \leq 0.01^{**}$

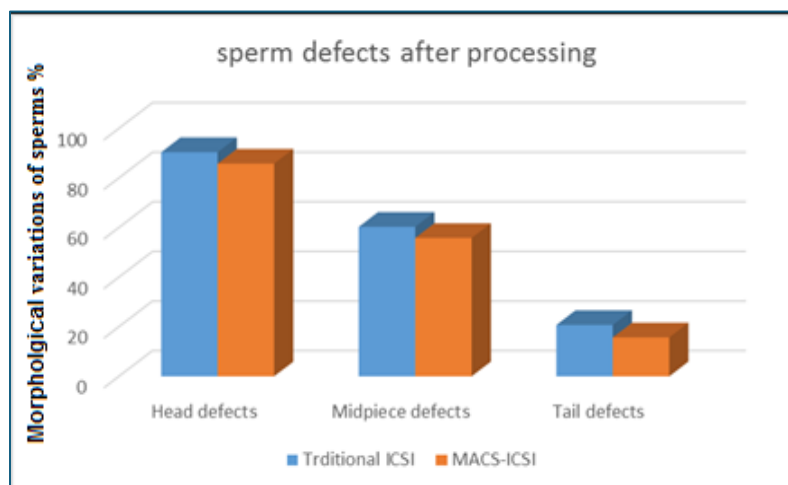
Data are presented as means  $\pm$  SD.

( $^{***}$ ) highly significant  $P \leq 0.001$ ,

( $^{**}$ ) very significant  $P \leq 0.01$ ,

( $^{*}$ ) significant  $P \leq 0.05$ ,

( $^{\$}$ ) not-significant  $P > 0.05$ .



**Fig. 2. Analysis of sperm defects after processing by traditional ICSI & MACS –ICSI.**

### 3- ICSI outcomes among studied groups:

Comparing the results of MACS-ICSI and Traditional ICSI among the studied groups after processing (Table 3 and Fig. 3) indicated that although the differences were non-significant ( $P > 0.05$ ), the incidence of number of collected oocytes was higher ( $9.3 \pm 3.1$ ) in ICSI group than in the MACS-ICSI group ( $8.3 \pm 2.1$ ). In ICSI group, the

incidence of mature oocytes was greater ( $8.2 \pm 1.1$ ) than in the MACS-ICSI group ( $8.1 \pm 1.1$ ). These variations were non-significant, though ( $P > 0.05$ ). Fertilized oocyte incidence was ( $5.5 \pm 2.1$ ) in ICSI group and ( $6.2 \pm 2.5$ ) in the MACS-ICSI group, these variations were also not significant ( $P > 0.05$ ).

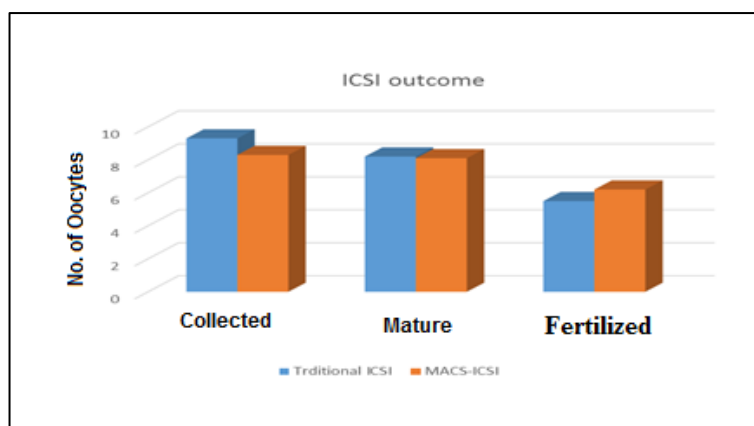


## Role of magnetic-activated cell sorting in sperm preparation and highly fertilization outcome

**Table 3. Traditional ICSI and MACS –ICSI outcomes among studied groups.**

Parameters	Traditional ICSI (N=730)	MACS-ICSI (N=720)	P value
	Mean ± SD	Mean ± SD	
Collected oocytes %	9.3 ± 3.1	8.3 ± 2.1	(P > 0.05)
Mature oocytes %	8.2 ± 1.1	8.1 ± 1.1	(P > 0.05)
Fertilized oocytes%	5.5 ± 2.1	6.2 ± 2.5	(P > 0.05)

Number of cases=100. Data are presented as means ± SD. (\*\*\*) highly significant  $P \leq 0.001$ , (\*\*) very significant  $P \leq 0.01$ , (\*) significant  $P \leq 0.05$ , (\$) not-significant  $P > 0.05$ .



**Fig. 3. Traditional ICSI and MACS –ICSI outcomes among studied Groups.**

#### 4- Cleavage rates among studied Groups:

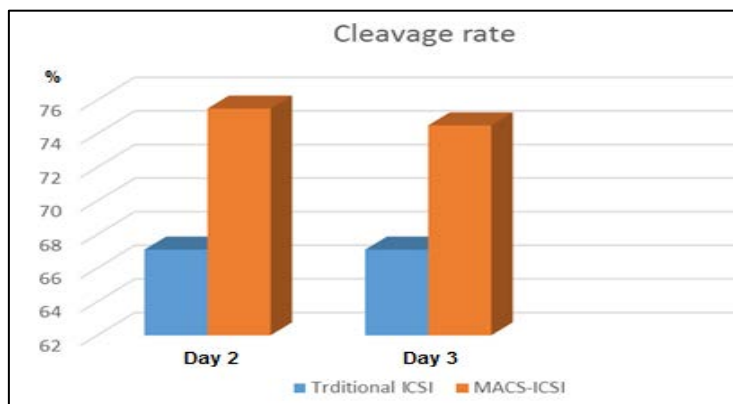
The percentage of the cleavage rate on days 2 and 3 was lower in ICSI group

(67.1±8.1%), while they were higher in the MACS –ICSI group (74.5±8.0%). This difference was very significant ( $P \leq 0.01^{**}$ ).

**Table 4. Cleavage rates among studied Groups.**

Parameters	Traditional ICSI (N=730)	MACS-ICSI (N=720)	P value
	Mean ± SD	Mean ± SD	
Cleavage on (D2) %	67.1± 8.1	74.5 ± 8.0	<b>P ≤ 0.01**</b>
Cleavage on (D3) %	67.1± 8.1	74.5 ± 8.0	<b>P ≤ 0.01**</b>

Data are presented as means ± SD. (\*\*\*) highly significant  $P \leq 0.001$ , (\*\*) very significant  $P \leq 0.01$ , (\*) significant  $P \leq 0.05$ , (\$) not-significant  $P > 0.05$ .



**Fig. 4. Cleavage rates among groups.**

#### 5- Embryo grading in the studied groups:

It was lower in ICSI group ( $320.6 \pm 13.4$ ) than in the MACS-ICSI group ( $330.6 \pm 15.4$ ). This difference was highly significant ( $P \leq 0.001^{***}$ ). In ICSI group, the incidence of **Grade B embryos** was higher ( $130.1 \pm 10.2$ ), while in the MACS-ICSI group, it was ( $120.1 \pm 12.2$ ). Moreover, ( $P < 0.001$ ) indicates that these changes were highly significant. The incidence of **Grade**

**C embryos** was greater in ICSI group ( $21.1 \pm 1.1$ ) than in the MACS-ICSI group ( $20.1 \pm 1.1$ ). Furthermore, ( $P \leq 0.001^{***}$ ) indicated that these changes were highly significant. The results revealed that after processing the number of **Grade A embryos** was higher in MACS-ICSI group than ICSI group as shown in Table (5) and Figure (5).

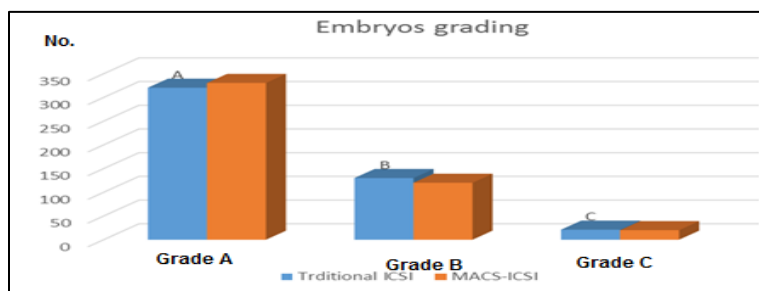
**Table 5. Embryo grading in the studied groups.**

Embryo grading	Traditional ICSI (N=730)	MACS-ICSI (N=720)	P value
	Mean $\pm$ SD	Mean $\pm$ SD	
Grade A embryos %	$320.6 \pm 13.4$	$330.6 \pm 15.4$	$P \leq 0.001^{***}$
Grade B embryos %	$130.1 \pm 10.2$	$120.1 \pm 12.2$	$P \leq 0.001^{***}$
Grade C embryos %	$21.1 \pm 1.1$	$20.1 \pm 1.1$	$P \leq 0.001^{***}$

Data are presented as means  $\pm$  SD.

( $^{***}$ ) highly significant  $P \leq 0.001$ , ( $^{**}$ )

very significant  $P \leq 0.01$ , ( $^{*}$ ) significant  $P \leq 0.05$ , ( $^{\$}$ ) not-significant  $P > 0.05$



**Fig. 5. Embryo grading among groups.**

## Role of magnetic-activated cell sorting in sperm preparation and highly fertilization outcome

### DISCUSSION

The magnetically activated cell sorting, MACS, is a great method for isolating target cells from mixed cell populations. The sorter makes use of magnetic microparticles as well as nanoparticles that coupled with antibodies which are particular to the desired cell membrane protein. After changing their course, the magnetic particle-bound cells lie in a high magnetic energy gradient. MACS were developed to distinguish between apoptotic and nonapoptotic spermatozoa. Because of its significant affinity for phosphatidylserine phospholipids, which are found in the cell membrane and externalize as one of the first signs of apoptosis when membrane integrity is compromised. In the MACS approach, spermatozoa treated with a buffer containing annexin V-conjugated microbeads are separated from non-apoptotic sperm cells using a magnetic field and an affinity column (Cakar *et al.*, 2016).

The aim of the present work was to provide an update on the primary strategies for sperm separation, together with an analysis of their significance and consequences for contemporary assisted reproductive technologies. While several techniques have been developed for sperm isolation and ICSI cycle preparation, no randomized comparative study has evaluated the effect of MACS separation on ICSI cycle success. The present data concentrated on semen to compare the parameter after processing, fertilization, cleavage, and hopefully pregnancy rates associated with traditional semen preparation procedures and MACS for the ICSI of super ovulated women.

There aren't enough clinical trials to compare sperm selection techniques, and their efficacy. The current data compared two distinct sperm selection techniques, Traditional ICSI and MACS-ICSI, and

MACS was found to have significantly better rates of fertilization and high-quality of embryos. Hasanen *et al.* (2020) found that using MACS with density gradient centrifugation, DGC, gave motile spermatozoa which are viable and non-apoptotic more efficiently than the use of traditional sperm preparation methods. Measurements of the activated caspase-3 levels, mitochondrial membrane potential integrity, and externalization of phosphatidylserine residues showed that the combination of DGC and MACS reduced the percentage of apoptotic spermatozoa after preparation more effectively than other techniques (Said *et al.*, 2005). Based on the current MACS-ICSI outcomes, all parameters showed significant differences across all groups, according to the acquired data and there is progressive in the number of sperm motility and motility. This in agreement with the results of Pacheco *et al.* (2020) who used the magnetic-activated cell sorting (MACS) for sperm-selection in cases of high levels of sperm DNA fragmentation.

Hamze *et al.* (2019) had reproduced 3D model mimicking the shape of oocyte by using magnetic Sepharose beads coated with the recombinant zona pellucida (ZP) glycoproteins, and they selected sperms that were reacted to acrosomes and their characteristics were noted using magnetic beads, also their ability to infect hamster oocytes without a zona was evaluated. They found that ZP-beads can provide a tool to investigate the role of specific proteins on egg-sperm interactions becoming a relevant tool as a diagnostic predictor of mammalian sperm function when transferred to the industry. In current study, it was found that there were significant statistical differences in the morphological analyses of sperm after processing (Traditional ICSI and MACS-ICSI) between the two groups under

investigation. These results indicated that the Traditional ICSI group had more head defects than the MACS-ICSI group. Furthermore, compared to the MACS-ICSI group, the Traditional ICSI group had a greater prevalence of midpiece defects. Additionally, there was a significant difference in the incidence of tail defects morphology between ICSI group and the MACS-ICSI group. These findings were consistent with those of Hasanen *et al.* (2020) who found that MACS was an effective technique for sperm selection in cases where there is an abnormal sperm DNA fragmentation.

Lee *et al.* (2013) found that apoptotic sperm can be extracted from sperm using MACS; Phosphatidylserine is externalized from the inner membrane leaflet to the outer membrane leaflet if the membrane structure is damaged, DNA integrity is reduced, and cells are labelled abortive. Annexin V particularly binds to this substance. Different methods were used by MACS to select sperm (Hasanen *et al.*, 2020). The comparison between ICSI and MACS-selected sperm exhibit lower significant difference, decreased apoptosis, and higher early fertilization potential (Jeyendran *et al.*, 2019; Chi *et al.*, 2016; Horta *et al.*, 2016). Grunewald and Paasch, (2013) provided an explanation for an early sperm chromatin de-condensation. Thus, this validates the current study's findings that MACS-selected sperm for females produce better pre-implantation embryogenesis than conventional ICSI-selected sperm.

Furthermore, the present study also observed that the fertilized oocytes number was lower in ICSI group in compared to MACS –ICSI group. In addition, the

cleavage rate also was lower in ICSI group compared to in MACS–ICSI group. Aboul El-Ela *et al.* (2022) found that the use of an effective and targeted immunolabeling method is crucial for the successful implementation of MACS. Higher oocyte penetration capacity indicated that the use of MACS to separate a non-apoptotic fraction improves sperm fertilization potential in addition to improving the quality of recovered sperm. According to Juliá *et al.* (2021), after MACS separation, the proportion of sperm with normal morphology increased. Patients in the study and control groups had comparable numbers of oocytes retrieved and injected, and neither group experienced any fertilization failures. Additionally, the current study group's embryos showed significantly higher cleavage rates than the control group's embryos, and after ICSI of MACS-selected non-apoptotic spermatozoa. A slightly (but insignificantly) higher clinical pregnancy and average implantation rate was also observed in the study group.

The current data showed a trend for improvement in terms of clinical pregnancy, cleavage rates, and pregnancy implantation rates. These results are consistent with those of Gil *et al.* (2013) and Stimpfel *et al.* (2018) who reported an improvement in pregnancy rates using MACS.

#### **Conclusion:**

The current data showed that the MACS-ICSI approach can help couples struggling with male infertility caused by DNA fragmentation by increasing the Percentage of high-quality embryos produced and increasing the likelihood of conception.

## Role of magnetic-activated cell sorting in sperm preparation and highly fertilization outcome

### REFERENCES

- Abou El-Ela, A.; Kandeel, A.; Hassan, E.; Nasr, M. and Esam, Y. (2022). Utility of magnetic activated cell sorting (MACS) in assisted reproduction. *Al-Azhar Int. Med. J.*, 3(11): 22-29.
- Bhattacharya, S.; Maheshwari, A. and Mollison, J. (2013). Factors associated with failed treatment: An analysis of 121,744 women embarking on their first IVF Cycles. *PLoS One*, 8(12): 1-13.
- Bonte, D.; Ferrer-Buitrago, M.; Dhaenens, L.; Popovic, M.; Thys, V. and De Croo, I. (2019). Assisted oocyte activation significantly increases fertilization and pregnancy outcome in patients with low and total failed fertilization after intracytoplasmic sperm injection: a 17-year retrospective study. *Fertil. Steril.*, 112(2):266-274.
- Boomsma, C.M.; Cohlen, B.J. and Farquha, C. (2019). Semen preparation techniques for intrauterine insemination. *Cochrane Database Syst Rev.*, 219(10):1-48.
- Cakar, Z.; Cetinkaya, B.; Aras, D.; Koca, B.; Ozkavukcu, S.; Kaplanoglu, I. and Cinar, O. (2016). Does combining magnetic-activated cell sorting with density gradient or swim-up improve sperm selection? *J. Assist. Reprod. Genet.*, 33(8):1059-1065.
- Chi, H.J.; Kwak, S.J.; Kim, S.G.; Kim, Y.Y.; Park, J.Y.; Yoo, C.S.; Park, I.H.; Sun, H.G.; Kim, J. W. and Lee, K.H. (2016). Efficient isolation of sperm with high DNA integrity and stable chromatin packaging by a combination of density-gradient centrifugation and magnetic-activated cell sorting. *Clin. Exp. Reprod. Med.*, 43(4):199-206.
- Deshpande, P. S. and Gupta, A. S. (2019): Causes and prevalence of factors causing infertility in a public health facility. *J Hum Reprod. Sci.*, 12(4): 287-293.
- Ekart, J.; McNatty, K.; Hutton, J. and Pitman, J. (2013). Ranking and selection of MII oocytes in human ICSI cycles using gene expression levels from associated cumulus cells. *Human Reproduction*, 28(11):2930-2942.
- Gil, M.; Sar-Shalom, V.; Melendez Sivira, Y.; Carreras, R. and Checa, M.A. (2013). Sperm selection using magnetic activated cell sorting (MACS) in assisted reproduction: A systematic review and meta-analysis. *J. Assist. Reprod. Genet.*, 30(4):479-485.
- Gomez, C.; Goponenko, A.V. and Soulakova, J.N. (2023). Constructing UpSet plot for survey data with weights using SAS and R software. *Communications in Statistics-Simulation and Computation*, 52(6): 2320-2326.
- Grunewald, S. and Paasch, U. (2013). Sperm selection for ICSI using annexin V. *Methods Mol. Biol.*; 927:257-262.
- Halvaei, I.; Khalili, M.A.; Esfandiari, N.; Safari, S.; Talebi, A. R.; Miglietta, S. and Nottola, S. (2016). Ultrastructure of cytoplasmic fragments in human cleavage stage embryos. *J. Assist. Reprod. Genet.*, 33(12): 1677-1684.
- Hamze, J. H.; Gouveia, A.C.; Algarra, B.; Gómez-torres, M.J.; Olivares, M.C.; Romar, R. and Movilla, M.J. (2019). Mammalian spermatozoa and cumulus cells bind to a 3D model generated by recombinant zona pellucida protein-coated beads. *Scientific reports*, 9(1): 1-12.
- Hancock, P. and McLaughlin. E. (2002). British Andrology Society guidelines for the assessment of post vasectomy

- semen samples. *J. Clin. Pathol.*, 55(11):812-816.
- Hasanen, E.; Elqusi, K.; ElTanbouly, S.; Hussin, A.; AlKhadr, H.; Zaki, H.; Henkel, R. and Agarwal, A. (2020). PICSI vs. MACS for abnormal sperm DNA fragmentation ICSI cases: a prospective randomized trial. *J. Assist. Reprod. Genet.*, 37(10):2605-2613.
- Horta, F.; Crosby, J.; Mackenna, A. and Huidobro, C. (2016). Male factor infertility outcomes using magnetic activated cell sorting in intra cytoplasmic sperm injection cycles. *Andrology*, 5(1):1-6.
- Jequier, A.M. (2011). *Male infertility: a clinical guide*. Cambridge University Press, ISBN: 9780521831475.
- Jeyendran, R.S.; Caroppo, E.; Rouen, A.; Anderson, A. and Puscheck, E. (2019). Selecting the most competent sperm for assisted reproductive technologies. *Fertil Steril.*, 111(5):851-863.
- Juliá, G.M.; Hervás, I.; Navarro-Gómez Lechón, A.; Quintana, F.; Amorós, D.; Pacheco, A.; González-Ravina, C.; Rivera-Egea, R. and Garrido, N. (2021). Sperm selection by magnetic-activated cell sorting before microinjection of autologous oocytes increases cumulative live birth rates with limited clinical impact: A retrospective study in unselected males. *J Biol.*, 10(5):1-11.
- Lee, S. H.; Meng, X. W.; Flatten, K. S.; Loegering, D. A. and Kaufmann, S. H. (2013). Phosphatidylserine exposure during apoptosis reflects bidirectional trafficking between plasma membrane and cytoplasm. *Cell Death Differ*; 20:64-76.
- Mortimer, D. and Menkveld, R. (2001). Sperm morphology assessment historical perspectives and current opinions. *J. Androl.*, 22(2):192-205.
- Nadalini, M.; Tarozzi, N.; Di Santo, M. and Borini, A.J. (2014). Annexin V magnetic-activated cell sorting versus swim-up for the selection of human sperm in ART: is the new approach better than the traditional one? *J. Assist. Reprod. Genet.*, 31(8): 1045-1051.
- Pacheco, A.; Blanco, A.; Bronet, F.; Cruz, M.; García-Fernández, J. and García-Velasco, J.A. (2020). Magnetic-activated cell sorting (MACS): A useful sperm-selection technique in cases of high levels of sperm DNA fragmentation. *J. Clin. Med.*, 9(12):3976 -3985.
- Rezaei, M.; Nikkhoo, B.; Moradveisi, B. and Allahveisi, A. (2021). Effect of sperm selection methods on ICSI outcomes in patients with oligoteratozoospermia. *Am. J. Clin. Exp. Urol.*, 9(2):170-176.
- Romany, L.; Garrido, N.; Cobo, A.; Aparicio-Ruiz, B.; Serra, V. and Meseguer, M. (2017). Obstetric and perinatal outcome of babies born from sperm selected by MACS from a randomized controlled trial. *J. Assist. Reprod. Genet.*, 34(2):201-207.
- Rouge, M. (2002). *Appearance of semen and sperm concentration in Rouge, M. (ed.) collection and evaluation of semen*. Black well science ltd. London: 42-68.
- Said, T.M.; Grunewald, S.; Paasch, U.; Rasch, M. and Agarwal, A. (2005). Effects of magnetic-activated cell sorting on sperm motility and cryosurvival rates. *Fertil. Steril.*, 83(5):1442-1446.
- Simionescu, G.; Doroftei, B.; Maftei, R.; Obreja, B.; Anton, E.; Grab, D.; Ilea, C. and Anton, C. (2021). The complex relationship between infertility and psychological distress (Review). *Exp. Ther. Med.*, 21(4):1-6.

## Role of magnetic-activated cell sorting in sperm preparation and highly fertilization outcome

- Stevenson., T.L. and Lashen, H. (2008). Empty follicle syndrome: the reality of a controversial syndrome, a systematic review. *Fertil Steril.*, 90(3): 691-698.
- Stimpfel, M.; Verdenik, I.; Zorn, B. and Virant-Klun, I. (2018). Magnetic-activated cell sorting of non-apoptotic spermatozoa improves the quality of embryos according to female age: a prospective sibling oocyte study. *J. Assist. Reprod. Genet.*, 35:1665–1674.
- Van Thillo, G.; Guidobono, M.; Young, E.; Ruiz Jorro, M.R.; Vila, M. and Rawe, V. (2011). Biological safety and live births after selection of non-apoptotic spermatozoa during ICSI. *Fertil Steril.*, 96(3):160-161.
- World Health Organization (WHO) (2020). Semen analysis standard procedures. (5<sup>th</sup> ed). WHO laboratory manual for the examination and processing of human semen. Switzerland: WHO Press: 1-271.
- Young, E.; De Caro, R.; Marconi, G; Lombardi, E.; Young, E.; Tiveron, M. and Valcarcel, A. (2010). Reproductive outcome using Annexin V columns for non-apoptotic sperm selection. Conference: 26th Annual Meeting of ESHRE “European Society on Human Reproduction and Embryology”. *Human Reprod.*, 25(1):6-9.

دور استخدام خلايا الفصل المغناطيسي النشطة في تحضير الحيوانات المنوية للحصول علي نسب إخصاب عالية

أسامة محمد بدر<sup>١\*</sup>، شكري عبد العظيم العوضي الشرشابي<sup>٢</sup>، إبراهيم عبد المحسن صبرة<sup>٣</sup>، عماد عبد الرحمن الشاذلي<sup>٣</sup>  
 ١، ٣- قسم البيوتكنولوجيا الحيوانية، معهد بحوث الهندسة الوراثية والتكنولوجيا الحيوية، جامعة مدينة السادات.  
 ٢- المركز الإسلامي العالمي للدراسات والبحوث السكانية جامعة الأزهر.  
 الإلكتروني للباحث الرئيسي: \*osama.badr@gebri.usc.edu.eg البريد

### المستخلص

العقم مشكلة شائعة ولها آثار نفسية وفسولوجية كبيرة. فرز الخلايا المغناطيسية النشطة يعتبر من التقنيات الانجابية المساعدة التي تستخدم في دورات الحقن المجهرية. هدفت الدراسة الحالية الي دراسة جدوي استخدام فرز الخلايا المغناطيسية النشطة في الحقن المجهرية لزيادة معدل الاخصاب وتعزيز معدل الإنقسام وجودة الأجنة وبالتالي زيادة معدلات الحمل للحالات التي تعاني من تكسير في الحامض النووي الخاص بالحيوانات المنوية. خلصت الدراسة الحالية الي ان تقنية فرز الخلايا المغناطيسية النشطة في الأزواج الذين يعانون من تأخر الانجاب الناتج عن خلل او تكسير في الحامض النووي الخاص بالحيوانات المنوية يمكن ان تفيد في الحصول عن نسب اعلي في معدلات الاخصاب وانقسامات الاجنه ونسبة اعلي من الاجنة ذات النوعية الجيده وبالتالي الحصول علي فرص حمل اعلي.