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

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ABSTRACT

Infertility is a popular problem with major psychological and physiological effects. Regarding to modern assisted reproductive techniques that use Magnetic Activated Cell Sorting (MACS) in an intracytoplasmic sperm injection (ICSI) cycle, the aim of this study was to find a current review of the principal sperm separation techniques along with their consequences and significance. According to the current findings, couples coping with male infertility brought on by DNA sperm fragmentation may find that the MACS–ICSI approach increases the percentage of high-quality embryos produced and increases the likelihood of conception.

***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 current data focused on semen to compare the parameter after processing, fertilization, cleavage, and ideally pregnancy rates associated to traditional semen preparation procedures and MACS for the ICSI of super ovulated women. Unlike typical MACS, the particles used in this study target three distinct receptors that respond to DNA breakage within the sperm (the sperm tail, head, and acrosomal region). However, the conventional MACS system only targets P-S exposed areas during the

early stages of apoptosis or necrosis. In addition to the targeting and separation based on DNA fragmentation index (DFI) markers, this methodology is considered a gentle technique that reduces the degree of DNA damage done to the sperm sample in sperm processing pre-injection since no density gradient is involved or heavy centrifugation steps involved (Hasanen *et al.,* 2020). The technique is widely adopted in various clinics of our customers worldwide. Such technology will not only aim at sperm selection but also will be applied to sexing of embryos, gametes, genetic diseases identification via surface markers on gametes and embryonic cells (Gil *et al.,* 2013). A MAC is Nano-Sized mixture of particles with high magnetic properties coated with glycoproteins for morbid/DNA fragmented Spermatozoa depletion prior to Intra Cytoplasmic Sperm Injection (ICSI) and in vitro fertilization (IVF) (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 two to seven days of abstinence, semen samples 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 batch that has been shown to be non-toxic 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 is measured by scooping the entire ejaculate 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. Mix the semen sample well.

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.

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 µl semen is transferred into a sterile glass slide and covered with a 24 ×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 × 10 x ocular) should be taken after the initial microscopic examination, which should be performed at 100 x (10 x objective × 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 moving 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:

Sodium azide removal (washing procedures). Hold the tube with the magnetic particles 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 + the 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 ten minutes, hold the particle sperm solution up against the magnet. While the particles are still up against the tube and magnet walls, decant the supernatant. (The supernatant contains ready-to-use sperm).

5- Proceed to ICSI with the supernatant.

Preparation of semen by a Centrifugation Method as follow:

1. Mix the 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 µ1 drop of buffered solution with 80 IU/ml of hyaluronidase (Life Global, Europe) for 30 to 45 seconds, the oocyte was taken out and put in a 100 µl drop of global total w/HEPES Buffer (Life Global, Europe). Hyaluronidase is an enzyme that breaks down a part of the connective tissue components that hold cells 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 (Life Global, Europe). One half of the denudated oocyte was then inserted into an ICSI injection dish containing 10 µl 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) cytoplasmic granularity, (ii) cytoplasmic color, (iii) vacuoles in the ooplasm, (iv) large perivitelline space (PVS), (v) PVS granularity, (vi) fragmented polar body (PB), (vii) zona pellucida (ZP) abnormalities and (viii) oocyte shape abnormalities. 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 ºC with 6% CO_2 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 (PVP). PVP, a viscous solution is used to slow down motile sperm, which 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 µl. 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 (Life Global, Europe) in a culture plate covered with sterile, warm, equilibrated global oil (Life Global, Europe). The culture dish was kept at 37 ºC, 6% CO_2, and 90–95% humidity until fertilization.

Follow up was done considering the following:

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: Blastomeres of the same size and without fragmentation.

Grade B: Up to 10% of cytoplasmic fragments and mildly uneven blastomeres.

Grade C: Big granules and up to 50% shards of unevenly sized blastomeres.

Grade D: Big black granules and uneven blastomeres with substantial fragmentation.

To recipients, Day 3 embryos were transferred (MACS-ICSI) in accordance with 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 visible). Fourteen days after embryo transfer, serum-HCG was determined as a chemical pregnancy test (considered positive if 20 IU/L).

Statistical analysis:

The data were analysed using statistical analysis software package **(SAS)**. Paired T-test and Mc Nomar's test were used to compare between the studied parameters in each studied group. None paired T test and Fischer exact test were also 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 participants. It was found that the sperm count incidence was greater (11.8 ± 3.5 mill/ml)) in the MACS–ICSI group than in the Traditional ICSI group (10.4 ± 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 ± 9.2/ml) compared to the Traditional ICSI group (30.3 ± 9.8/ml), and this difference was very high significant (P ≤ 0.01**). Additionally, as shown in Table (1) and Figure (1), the incidence of progressive sperm motility was greater (12.1 ± 2.7/ml) in the MACS-ICSI group than in the Traditional ICSI group (10.8 ± 3.7/ml) and exhibited a very statistically significant difference (P ≤ 0.01**).

Table 1. Comparison between sperm parameters after processing by traditional ICSI and MACS–ICSI.

Parameters	Traditional ICSI	MACS-ICSI	P value
	Mean ± SD	Mean ± SD	
Sperm count (mill/ml) %	10.4 ± 3.8	11.8 ± 3.5	**P > 0.05**
Sperm motility/ml %	30.3 ± 9.8	34.8 ± 9.2	**P ≤ 0. 01****
Progressive motility/ml %	10.8 ± 3.7	12.1 ± 2.7	**P ≤ 0.01****

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

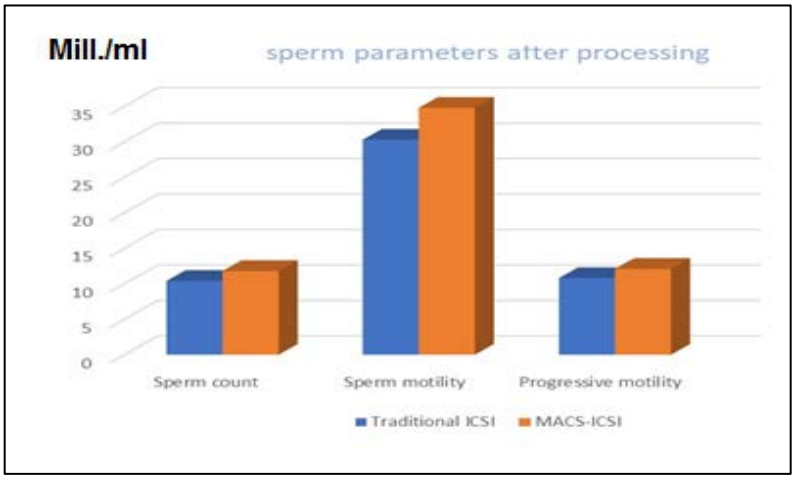

Fig. 1. Analysis of sperm parameters after processing by traditional ICSI & MACS –ICSI.

2- Comparison between 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 Traditional ICSI) where the abnormal forms of sperms in the Traditional ICSI group were (98.7 ± 1.3%), while they were (97.7 ± 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 ± 6.8%) than in the MACS-ICSI group (85.8 ± 6.5%). This difference was highly statistically significant (P ≤ 0.001***). Furthermore, the incidence of midpiece defects was higher in the Traditional ICSI group (60.3 ± 7.8%) than in the MACS – ICSI group (55.8 ± 8.2%). This variation was highly statistically significant (P < 0.001***). Additionally, the Traditional ICSI group had a greater incidence of tail defects morphology (20.7 ± 12.4%) than the MACS – ICSI group (15.7 ± 12.3%) with (P < 0.01**) indicated that this difference was statistically very significant.

Table 2. Comparison between morphological analyses of sperms after processing among male studied groups (n=100).

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

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

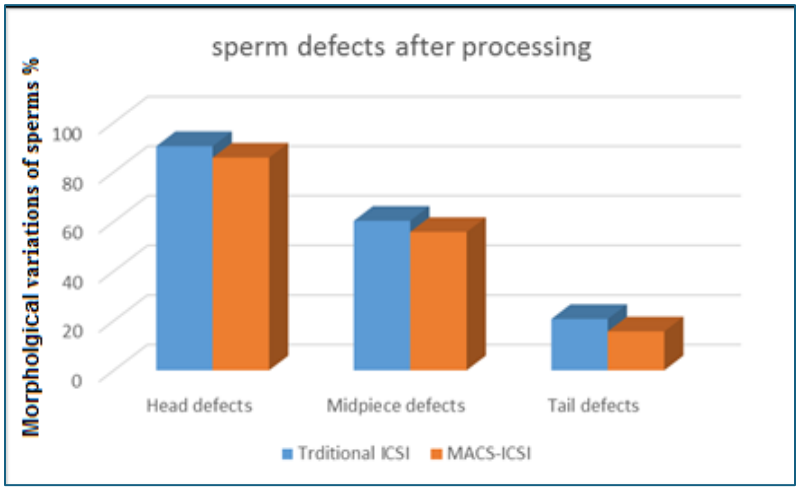

Fig. 2. Analysis of sperm defects after processing by traditional ICSI & MACS –ICSI.

3- Comparison between 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 statistically non-significant (P > 0.05), the incidence of number of collected oocytes was higher (9.3 ± 3.1) in the Traditional ICSI group than it was (8.3 ± 2.1) in the MACS-ICSI group. In the Traditional ICSI group, the incidence of mature oocytes was

greater (8.2 ± 1.1) than in the MACS – ICSI group (8.1 ± 1.1). These variations were statistically non-significant, though (P > 0.05). Fertilized oocyte incidence was (5.5 ± 2.1) in the Traditional ICSI group and (6.2 ± 2.5) in the MACS-ICSI group, these variations were also not significant (P > 0.05).

Table 3. Comparison between 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 ≤ 0.001, (**) very significant P ≤ 0.01, (*) significant P ≤ 0.05, ($) not-significant P > 0.05.

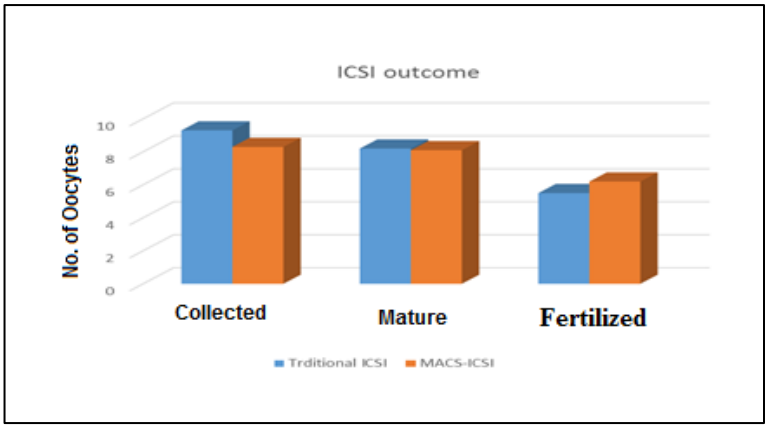

Fig. 3. Comparison between Traditional ICSI and MACS –ICSI outcomes among studied Groups.

4- Comparison between Cleavage rates among studied Groups:

When examining the results of the study groups after processing (Table 4 and Fig. 4) the percentage of the cleavage rate on days 2 and 3 was lower in the Traditional 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 ≤ 0.01)**.

Table 4. Comparison between 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	**P ≤ 0.01****
Cleavage on (D3) %	67.1± 8.1	74.5 ± 8.0	**P ≤ 0.01****

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

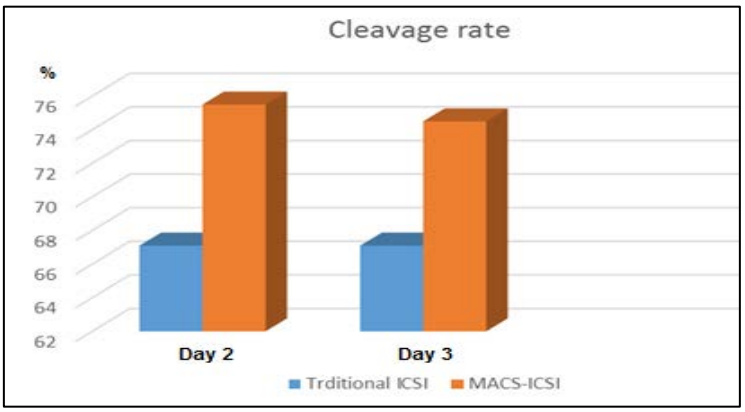

Fig. 4. Illustrate the differences between Cleavage rates among groups.

5- Comparison between embryo grading in the studied groups: was lower in the Traditional ICSI group (320.6 ± 13.4) than in the MACS-ICSI group (330.6 ± 15.4). This difference was highly statistically significant **(P ≤ 0.001)**. In the Traditional ICSI group, the incidence of **Grade B embryos** was higher (130.1 ± 10.2), while in the MACS –ICSI group, it was (120.1 ± 12.2). Moreover, (P < 0.001) indicates that these changes were extremely statistically significant. The incidence of **Grade C embryos** was greater in the Traditional ICSI group (21.1 ± 1.1) than in the MACS-ICSI group (20.1 ± 1.1). Furthermore, **(P ≤ 0.001)** indicated that these changes were highly statistically significant. This is displayed in Table (5) and Figure (5).

The results revealed that after processing the number of **Grade A embryos**

Table 5. Comparison between embryo grading in the studied groups.

Embryo grading	Traditional ICSI (N=730)	MACS-ICSI (N=720)	P value
	Mean ± SD	Mean ± SD	
Grade A embryos %	320. 6 ± 13.4	330. 6 ± 15.4	**P ≤ 0.001*** **
Grade B embryos %	130.1 ± 10.2	120.1 ± 12.2	**P ≤ 0.001*** **
Grade C embryos %	21.1 ± 1.1	20.1 ± 1.1	**P ≤ 0.001*** **

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

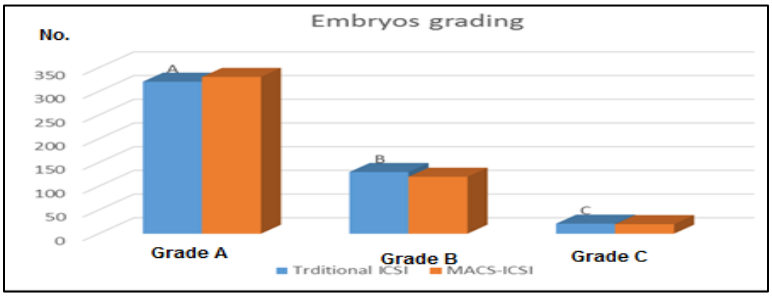

Fig. 5. Illustrate the differences between embryo grading among groups.

DISCUSSION

A great method for isolating target cells from mixed cell populations is magnetically activated cell sorting, or MACS. The sorter makes use of magnetic micro- and nanoparticles that have been coupled with antibodies that are particular to the desired cell membrane protein. After changing their course, the magnetic particle-bound cells lie in a high magnetic energy gradient. The technology known as magnetic-activated cell sorting (MACS) was 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, annexin V labelling can be used as an apoptotic marker. 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 goal of the current study 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, MACS had significantly better rates of fertilization and high-quality embryos. Hasanen *et al.* (2020), found that using MACS with density gradient centrifugation (DGC) yields spermatozoa that are motile, viable, and non-apoptotic more efficiently than the use of traditional sperm preparation methods. Measurements of 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 statistically significant difference in the incidence of tail defects morphology between the Traditional ICSI group and the MACS-ICSI group. These findings are consistent with the findings of Hasanen *et al.* (2020), who concluded that MACS is 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 are used by MACS to select sperm (Hasanen *et al.,* 2020). The comparison between the traditional ICSI and MACS-selected sperm exhibit lower SDF, 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 Traditional ICSI group in compared to MACS –ICSI group. In addition, the cleavage rate also was lower in Traditional 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 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.

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

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المستخلص

العقم مشكلة شائعة ولها أثار نفسية وفسيولوجية كبيرة. فرز الخلايا المغناطيسية النشطة يعتبر من التقنيات الانجابية المساعدة التي تستخدم في دورات الحقن المجهري .هدفت الدراسة الحالية الي دراسة جدوي استخدام فرز الخلايا المغناطيسية النشطة في الحقن المجهري 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 الازواج 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 أعلي.