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Utility of Magnetic Activated Cell Sorting (MACS) in Assisted Reproduction

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ABSTRACT

Background: Magnetic activated cell sorting **(**MACS) has mechanisms to reduce the degrees of Deoxyribonucleic acid (DNA) fragmentation done to the sperm sample in sperm processing pre-injection since no density gradient is involved or heavy centrifugation steps involved. If DNA fragmentation is not eliminated from sperms, poor cleavage rate, bad embryo quality, poor implantation and low pregnancy rates may occur. A MACS is a nano-sized mixture of particles with high magnetic properties coated with glycoproteins for morbid/DNA fragmented spermatozoa depletion before Intra Cytoplasmic Sperm Injection (ICSI). Several investigations have demonstrated that employing the MACS approach improves the outcomes of ICSI in patients who have significant levels of sperm DNA fragmentation.

Aim of the study: To prove that the assessing Utility of Clemente Magnetic Activated Cell Sorting in ICSI cycle (especially recurrent implantation failure) would improve cleavage rate, Embryo quality, implantation and hopefully pregnancy rates.

Patients and Methods: We determined if MACS is suitable for male factor having a high DNA Fragmentation Index (DFI), undertaking ICSI or in vitro fertilization (IVF), recurrent miscarriage attributable to male factor, and early pregnancy loss caused by male factor.

Result: The use of MACS techniques in ICSI resulted in increasing fertilization rate, cleavage rate, blastocyst formation rate, number of Grade A embryos and rates of pregnancy as the use of MACS in sperm processing helps in increasing motility, especially progressive motility, and reducing sperm with abnormal morphological defects.

Conclusion: The study's findings showed that using the MACS approach improved ICSI results in patients who had high levels of sperm DNA fragmentation.

Keywords: *MACS; Sperm DNA fragmentation; ICSI; Male Infertility.*

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Authorship: *All authors have a substantial contribution to the article.*

INTRODUCTION

In an in vitro fertilisation process known as intracytoplasmic sperm injection (ICSI), one single sperm is injected right inside an oocyte. Because there were insufficient numbers of motile, morphologically normal sperm in the male partner's ejaculate throughout the previous decade, ICSI was used more frequently worldwide to alleviate severe male fertility problems.[1] Clinical gestation and living birth rates still remain at around 30–40% despite several advancements in assisted reproduction technology (ART) throughout the past three decades.[2] ART). The sperm chromatin structure assay (SCSA) found criteria for a poor pregnancy result following ART when DNA fragmentation index (DFI) was >30%.[3]

The use of insufficient or poor sperm, like those with poor sperm morphology or DNA damage, may contribute to low implantation rates or pregnancy rates with ICSI embryos. Current adopted techniques do not offer accuracy in semen selection and sorting for specific parameters required for a successful fertilization**.** Hence, we demonstrate in this document the outcome of using Clemente Magnetic Nanoparticles in sorting sperms for a higher fertilization potential free of DNA damage.[4] Our Particles are not like traditional MACS, we target three different receptors response to DNA fragmentation within the sperm (Sperm Tail, Head and Acrosomal region). On the other hand, MACS traditional system only targets P-S (Phosphatidylserine) exposed sites in early apoptosis/necrosis. Thus, our DFI reduction is far

superior to traditional MACS. In addition to the targeting and separation based on 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.[5]

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.[6]

A magnetic activated sperm enrichment (MaSE) is Nano-sized mixture of particles with high magnetic properties coated with glycoproteins for morbid/DNA fragmented Spermatozoa depletion before Intra Cytoplasmic Sperm Injection (ICSI). So, MACS is suitable for male factors with high DFI who are having ICSI or In vitro fertilisation (IVF), as well as for male factors who are responsible for early pregnancy losses and recurrent miscarriages.[7]

Study hypothesis: Fertilization and pregnancy rate as ICSI outcomes are lower in spermatozoa cases with high sperm DNA fragmentation than spermatozoa with low sperm DNA fragmentation.

The study aims at assessing Utility of Clemente Magnetic Activated Cell Sorting in ICSI cycle (especially recurrent implantation failure) to improve cleavage rate, Embryo quality, implantation and hopefully pregnancy rates.

PATIENTS AND METHODS

Study Design: The study population consisted of 200 couples. Due to the male factor, all female partners undergo ICSI. They are split into two groups: the first group receives an injection of sperm prepared using the traditional Intracytoplasmic sperm method (traditional ICSI-100 cases), and the second group by sperm prepared by the Magnetic Activated Cell Sorting technique (MaSE ICSI-100 cases). The study was approved by the quality education assurance unit at Al-Azhar University's Faculty of Medicine in Egypt (REC number: 0000036). The results of this study were summarized in the following tables and figures. Data are collected during the time period (1/11/2020 - 28/04/2021). Inclusion criteria include; No female factor, Male factor with high DFI but No history of chronic medical illness and No previous testicular or scrotal operation.

Semen Sample: Masturbation was used to collect semen samples following a three to seven-day interval of sexual abstinence. A physical inspection was performed, which included volume, colour, odour, as well as liquefaction. Using a light microscope, microscopic inspection was carried out to assess the sperm's morphology, motility, concentration, and existence of additional cellular components (Olympus, C 21, Japan). Sperm was classified as immotile, non-progressive, or progressive motile.

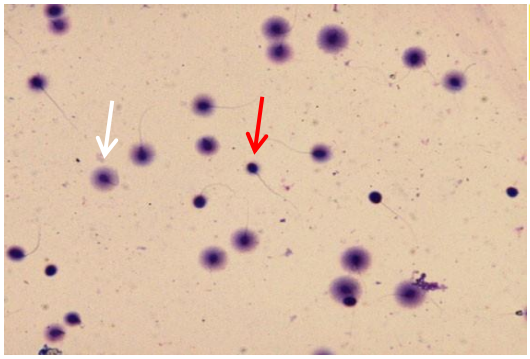

Fig.1: The light micrograph shows sperms with fragmented DNA (red arrow) and sperms without fragmented DNA (white arrow) (Magnification x 10) stained with halosperm G2 kit (Halotech DNA).

1-Sperm preparation by Centrifugation Method for first group which injected by sperm prepared by traditional technique:
Following microscopic examination, sperm samples for ICSI have been processed. We added 1 ml of sperm gradient media (Pure sperm, Nidacon, Sweden) to a fresh sample and centrifuged it for 10 min at 1800 rpm (Heraeus 300, Osterode, Germany), removing the supernatant. Next, we added 2 ml of sperm washing media (Sage, Denmark) to the sperm in the resultant pellet and centrifuged for 10 min at 1800 rpm to obtain the required number of motile and morphologically healthy sperm for assisted reproduction.[8]

2-Sperm preparation by Centrifugation Method for second group which injected by sperm prepared by Magnetic Activated Cell Sorting technique:
Sodium Azide removal (washing steps) -Hold the tube containing the magnetic particles against the magnet until they congregate against the tube wall (ideally with a rubber band or in a rack). Normally, it takes 4 min. -Whereas the particles are still held against the tube wall by the magnetic field and are decanted or aspirated out of the supernatant. -Remove the magnet and resuspension the particles in the suitable washing buffer **[9].** Invert the Clemente Associates particle and liquid suspension gently until the particles are dispersed. -Repeat step one. -Resuspend the particles as directed in steps 2 and 3 of the ICSI technique to make them ready for usage.

1- Sperm can be prepared by washing with extender Human tubal fluid (HTF) with or without Bovine serum albumin (BSA) from Irvine Scientific or Density Gradient Centrifugation.

2- To make one dose, mix 225 µl of particles with 5 million sperm/ml (1 ml of 5M plus the 225 µl of particle suspension).

3- For 30 min at room temp, gently mix the particles and sperm together.

4- For 10 min, put the particle sperm solution against the magnet. While the particles are still up against the tube and magnet's walls, decant the supernatant. The supernatant contains sperm that is ready to be used.

5- With the supernatant, proceed to ICSI.

1. Select a spermatozoon from the junction between the prepared sperm droplet and the Mature Sperm Select central drop directly for injection. -Mature spermatozoa should exhibit tail movement but no forward motility/progression (to be selected). -Immature spermatozoa should be moving freely (are not to be selected).[10,11]

Technical approach of female subjects

Oocytes were aspirated using transvaginal ultrasonography (US) while under general anaesthesia in order to collect human cumulus cells, roughly 34–36 hrs following the administration of human chronic gonadotropin (HCG) (Labotect aspiration pump, Germany). A single-lumen 17-gauge oocyte pick-up needle (Reproline Medical, Germany) was used to aspirate the follicles at a negative pressure of 115–120 mm Hg. In sterile tubes, 14 ml of follicular fluid was aspirated (Falcon, Boen Healthcare Co., China). The Oocyte-cumulus cell complexes have been separated using a dissecting microscope (Zeiss Stemi 2000-C Stereo Microscope), washed with Gamete Buffer media (Cook, Limerick, Ireland), and afterwards put into four plates with fertilisation medium (Cook, Limerick, Ireland). They were then incubated for about a half-hour at 37 ºC with 6% CO2 (C60, Labotect, Germany). For denudation, the oocyte has been put in a 100 µl drop of buffered media having hyaluronidase enzyme 80 IU/ml (Life Global, Europe) for 30 to 45 s, after which it has been removed and put in a 100 µl drop of gamete buffer media. The corona cells have been then eliminated by gently aspirating the oocyte in and out of a sterile-drawn pipette. Once the denudation process has been finished, the oocyte has been washed in Gamete Buffer (Cook, Limerick, Ireland) and afterwards put in 10 µl microdrops of the fertilisation media (Cook, Limerick, Ireland) in injection plates, coated with 3ml of sterile equilibrated mineral oil. An inverted microscope with Hoffman optics (Olympus 1x71, Japan), a heated stage, and automatic manipulators (Narishige, Japan) were used for the oocyte grading in order to rapidly assess the oocyte for maturity and quality in accordance with the grading system. The stages of maturation were prophase I, metaphase I (MI), metaphase II (MII), and post-mature. The naked oocytes have been incubated in a culture medium containing 6% CO2 at 37^{o}C till the ICSI procedure was performed.[12]

ICSI procedure

Samples have been incubated until it was time for injection after semen analysis and sperm preparations as previously mentioned. A single spermatozoon that was morphologically normal and stabilzed in polyvinylpyrolidone (PVP) (Irvine, USA) has been injected into every oocyte. Individual sperm that had undergone ICSI were analysed and assessed. Using an injection needle and holding pipette, the injection technique has been performed in a sterile dish. Injection of intracytoplasmic sperm has been carried out in accordance with Van Steirteghem's protocol.[12] The injected oocyte was then washed and put in global total media (Life Global, Europe) in a culture dish coated with sterile warm equilibrated global oil (Life Global, Europe) at 37^{o}C in a 6% CO2 environment with a humidity level of 90–95% till fertilization.

The embryo quality and fertilisation have been assessed ±17 hours following microinjection. We checked the injected oocytes for pronuclei and for any signs of damage. Two pronuclei (2PN) and the extrusion of the second polar body were indicators that an oocyte had undergone fertilization **[13].** The cell count and shape of every embryo have been scored for transfer and grading roughly 72 hours following injection based on the proportion of nucleate fragments and equality of blastomeric size. The best day-3 embryos (Grade 1) have then been transferred to the uterus using an embryo transfer (ET) catheter (Labotect, Germany) in 30µl of Global medium (Life Global, Europe) with 10% human serum albumin (HSA). 48-72 hours following oocyte retrieval.[14]
Follow up was done considering the following:

1. Fertilization rate.
2. Cleavage rate.
3. Embryo grading.
4. Pregnancy rate.

Evaluation of fertilisation and embryo quality: 16 to 18 hours following microinjection, fertilisation has been evaluated. The injected oocytes have been checked for pronuclei and for any signs of damage. Two pronuclei (2PN) and the extrusion of the second polar body were indicators that an oocyte had undergone fertilisation. Approximately 72 h after microinjection, adequate number of embryos were transferred to recipient subjects. Grading and transfer of embryos take place roughly 72 hours after injection. Each embryo's cell count and morphology have been scored in accordance with the grading.[15]

Grade A: blastomeres of equal size with no fragmentation.

Grade B: up to 10% cytoplasmic fragments, mildly unequal blastomeres.

Grade C: blastomeres with unequal sizes, up to 50% fragmentation, and big granules.

Grade D: blastomeres are unequal, with significant fragmentation and big black granules.

On Day 3, embryos were transferred to recipient subjects according to the guidelines of the American Society of Reproduction. Excess good-quality embryos were cryopreserved. A Serum-HCG has been measured as a chemical pregnancy test 14 days following embryo transfer (regarded positive if 20 IU/L), and a transvaginal ultrasonography scan of the uterus has been performed following 6-7 weeks of amenorrhea to assess if a clinical pregnancy was achieved (a gestational sac inside the uterus is evident).[15]

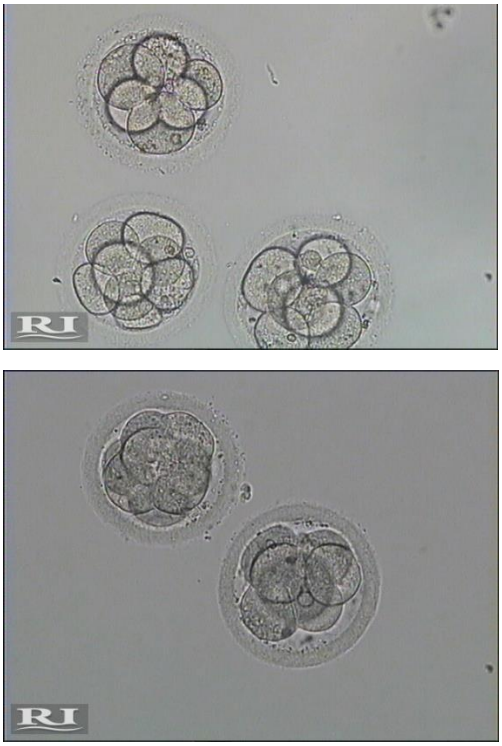

Fig. 2: 8-Cells Embryos

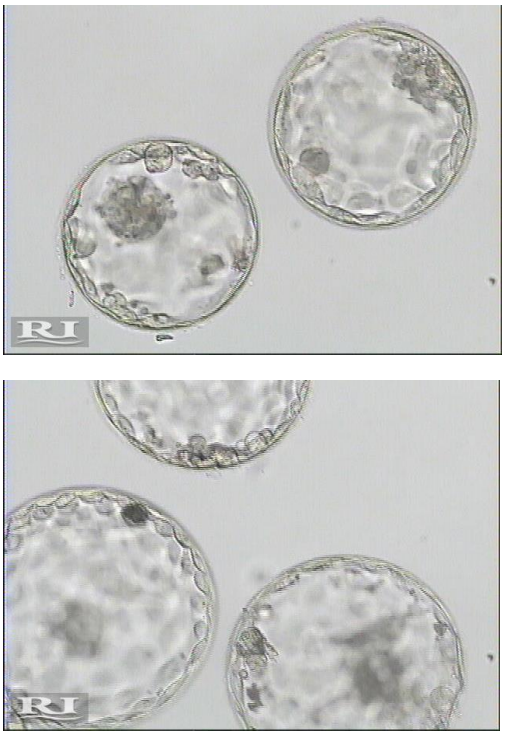

Fig. 3: Blastocyst Embryos

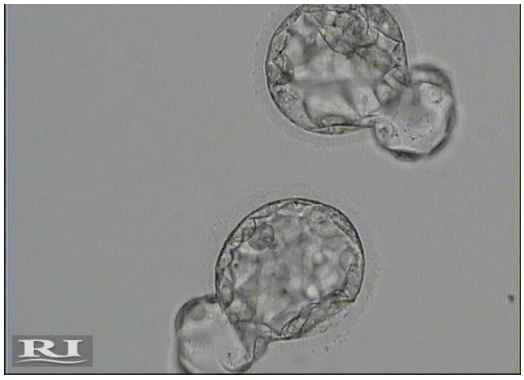

Fig. 4: Hatched Blastocyst Embryos

Statistical analysis

The International Islamic Centre for Population Studies and Research (IICPSR), Al-Azhar University, Cairo, Egypt's ethics committee gave its approval for the study. All subjects provided their written informed consent, and the data was managed and analysed in a confidential, anonymous manner. A statistical analysis software package was used to analyse the data.[16] When comparing the studied parameters within each group under study prior to and following therapy, the paired T-test and McNemar's test have been employed.

RESULTS

Parameters	Traditional ICSI (N=100)	MACS-ICSI (N=100)	P value
	Mean ± SD	**Mean ± SD**	
Male age (years)	**38.0 ± 4.1**	**38.0 ± 4.0**	**> 0.01***
Sperm DNA fragmentation (%)	**44.7 ± 9.0**	**45.1 ± 9.1**	**> 0.01***
Sperm count ($\times 10^6$/ml)	**28.6 ± 8.1**	**20.8 ± 7.4**	**≤ 0.01***
Sperm motility/ml	**51.3 ± 2.1**	**64.2 ± 5.4**	**≤ 0.01***
Progressive motility/ml	**21.6 ± 10.5**	**23.6 ± 10.2**	**≤ 0.01***
Abnormal forms	**98.7 ± 1.3**	**97.7 ± 1.1**	**≤ 0.01***
Head defects	**90.4 ± 6.8**	**85.8 ± 6.5**	**≤ 0.001***
Midpiece defects	**60.3 ± 7.8**	**55.8 ± 8.2**	**≤ 0.001***
Tail defects	**20.7 ± 12.4**	**15.7 ± 12.3**	**≤ 0.01***

Table 1: General characters of the male studied patients (n=200)

Parameters	Traditional ICSI (N=100)	MACS-ICSI (N=100)	P value
	Mean ± SD	**Mean ± SD**	
Age/ years	**29.٠ ± 5.6**	**30.٠ ± 4.6**	**P ≤ 0.05***
BMI (Kg/m^2)	**28.8 ± 5.5**	**28.1 ± 5.5**	**P > 0.1**
Infertility duration /years	**6.1 ± 1.6**	**7.1 ± 2.6**	**P ≤ 0.05***
FSH (IU/L)	**5.1 ± 1.6**	**5.1 ± 1.4**	**> 0.1**
LH (IU/L)	**4.1 ± 1.5**	**4.1 ± 1.3**	**> 0.1**
PRL (µg/L)	**19.9 ± 9.9**	**20.9 ± 9.9**	**> 0.1**
E2 (pg/ml)	**33.9 ± 16.3**	**34.9 ± 15.3**	**> 0.1**
TSH (mIU/L)	**3.0 ± 1.22**	**3.1 ± 1.20**	**> 0.1**
Long agonist	**45.1 ± 2.3**	**45.0 ± 2.2**	**> 0.1**
Short agonist	**32.1 ± 2.6**	**31.9 ± 2.7**	**> 0.1**
Antagonist	**19.1 ± 1.2**	**18.9 ± 1.1**	**> 0.1**

Table 2: General characters of the female studied patients (n=200)

Parameters	Traditional ICSI (N=100)	MACS-ICSI (N=100)	P value
	Mean ± SD	**Mean ± SD**	
Total Collected number /case	**954.0 ± 3.1**	**903.0 ± 2.1**	**P ≤ 0.01***
Mature oocytes/case	**810.0 ± 1.0**	**767 ± 1.1**	**P ≤ 0.01***
Fertilized oocytes/case	**510.0 ± 2.1**	**613.0 ± 1.5**	**P ≤ 0.01***
Fertilized rate (D1)	**510 (62.9%)**	**613 (79.9%)**	**P ≤ 0.01***
Cleavage rate (D2)	**500 (61.7%)**	**599 (78.1%)**	**≤ 0.01***
Cleavage rate (D3)	**490 (60.5%)**	**599 (78.1%)**	**≤ 0.01***
blastocyst rate (D5)	**310 (38.3%)**	**490 (63.9%)**	**≤ 0.001***
Grade A embryos	**130 (41.9%)**	**308 (62.8%)**	**≤ 0.001***
Grade B embryos	**120 (38.7%)**	**107 (21.8%)**	**≤ 0.001***
Grade C embryos	**60 (19.4%)**	**75 (15.4%)**	**≤ 0.001***
Pregnancy rate %	**32.0**	**49.0**	**≤ 0.001***

Table 3: Comparison between oocytes factors as ICSI Outcomes among studied group patients (n=200)

DISCUSSION

In our study, the general characteristics of the male studied patients showed that the mean age of male patients in the MACS-ICSI group was 38.0 ± 4.0, while in the Traditional ICSI group it was 38.0 ± 4.1, with statistically significant differences (P > 0.01). The mean of Sperm DNA fragmentation was 45.1 ± 9.1 in MACS-ICSI group and in Traditional ICSI group was 44.7 ± 9.0 with statistical significant difference (P > 0.01). The Comparison between sperms parameters among male studied patients showed, the incidence of sperm count was 20.8 ± 7.4 in MACS-ICSI group compared with 28.6 ± 8.1 in Traditional ICSI group highly statistical significant difference (P ≤ 0.01). the incidence of sperm motility was higher 64.2 ± 5.4 in MACS-ICSI group compared with 51.3 ± 2.1 in Traditional ICSI group with highly statistical significant difference (P ≤ 0.01). also the incidence of sperm progressive motility was higher 23.63 ± 10.21 in MACS-ICSI group compared with 21.60 ± 10.58 in Traditional ICSI group with highly statistical significant difference (P ≤ 0.01). The Comparison between morphological analysis of Sperms after processing among studied groups, showed the incidence of head defects was lower (85.8 ± 6.5) in MACS-ICSI compared to (90.4 ± 6.8) in Traditional ICSI and these differences have been highly statistically significant (P ≤ 0.001). The incidence of Midpiece defects was lower (55.8 ± 8.2) in MACS-ICSI compared to (60.3 ± 7.8) in Traditional ICSI, and such differences have also been highly statistically significant (P ≤ 0.001). The incidence of tail defects morphology was lower (15.7 ± 12.3) in MACS-ICSI compared to (20.7 ± 12.4) in Traditional ICSI and these differences also were statistically significant (P ≤ 0.01).

The current study's findings were in agreement with those of Dirican et al., who assessed the effect of male fertility therapy when ICSI into human oocytes has been conducted using non-apoptotic MACS-selected spermatozoa. According to strict standards, the study reported that the percent of sperm having normal morphology increased significantly when non-apoptotic spermatozoa were magnetically enriched.[17] Another study by Nadalini et al. that evaluated if the sperm fertilising possibility could be enhanced by choosing a non-apoptotic fraction employing magnetic activated cell sorting (MACS) as well as comparing the findings with the traditional swim-up technique reported that post-MACs significant advancements in progressive sperm motility contrasted to pre-MACs control specimens. Other investigations have found that post-MACs there is a significant enhancement in progressive sperm motility as compared to pre-MACs control samples (DGC only).[10,18&19]

On the other hand, Horta et al. evaluated assessing the impact of MACS on rates of clinical pregnancy, miscarriage, embryo growth, implantation, and fertilisation in couples having intracytoplasmic sperm injection (ICSI), which reported that the effects of MACS on patients with male factor infertility did not differ when compared to individuals who were normozoospermic, demonstrating similar trends in overall clinical findings.[20] Romany et al in another study that evaluated the impact on live-birth delivery rates following (ICSI) of eliminating presumed apoptotic sperm cells from specimens from unselected male using magnetic activated cell sorting (MACS) in

couples having ovum donation (OD). It found that there were a total of 0.07 million motile spermatozoa accessible for microinjection in the MACS group compared to 0.14 million in the control group.[21]

On the other hand, a study by Degheidy et al. evaluated the potential benefit of MACs technology in preventing DNA fragmentation in patients with infertile varicocele before ART. It demonstrated that there was no negative impact on sperm motility overall among post-MACs test samples and pre-MACs control samples. It also revealed that there was no effect on sperm motility progressivity between post-MACs test samples and pre-MACs control samples.[22] In another investigation, it was discovered that there was a post-MACs decrease in sperm motility.[7] Another study by Stimpfel et al. evaluated the advantage of using non-apoptotic spermatozoa chosen by MACS for ICSI procedures for couples where the male factor of infertility was teratozoospermia and the women exhibited good prognosis. The study demonstrated that the percentage of morphologically normal spermatozoa in the MACS-selected non-apoptotic group and the traditionally prepared group didn't differ significantly. Although the difference between the traditionally prepared and MACS-ICSI groups was not statistically significant, there was a tendency for more spermatozoa with aberrant heads in the sample of spermatozoa that had been prepared traditionally.[23]

In our study, the Comparison between ICSI outcomes among studied groups, showed the incidence of number of Collected oocytes was lower (903.0 ± 2.1) in MACS-ICSI group compared to (954.0 ± 3.1) in Traditional ICSI group but these differences have been statistically significant ($P \leq 0.01$). The incidence of number of mature oocytes was decreased in the MACS-ICSI group (767 ± 1.1) compared to (810.0 ± 1.0) in the traditional ICSI group. However, these differences have been statistically significant ($P \leq 0.01$). However, the incidence of fertilized oocytes was higher in the MACS-ICSI group (613.0 ± 1.5) compared to (510.0 ± 2.1) in the traditional ICSI group. However, such differences have been statistically highly significant ($P \leq 0.01$).

The comparison between fertilization and cleavage rate among studied groups showed that the incidence of fertilization rate on day (1) was higher at 613 (79.9%) in the MACS-ICSI group compared to 510 (62.9%) in the Traditional ICSI group, and such differences have been statistically significant ($P \leq 0.01$). The incidence of cleavage rate on day (2,3) was higher in the MACS-ICST group at 599 (78.1%) and 599 (78.1%), respectively, compared to 500 (61.7%) and 490 (60.5%), respectively, in the traditional ICSI group, and such differences have been statistically significant ($P \leq 0.01$). The incidence of blastocyst formation rate on day (5) was higher at 490 (63.9%) in the MACS-ICSI group compared to 310 (38.3%) in the traditional ICSI group, and such differences have been statistically highly significant ($P \leq 0.001$). Comparison between embryo grading among studied groups showed the incidence of the number of Grade A embryos was higher at 308 (62.8%) in the MACS–ICSI group, compared to 130 (41.9%) in the traditional ICSI group, and these differences have been highly statistically significant ($P \leq 0.001$). The incidence of the number of Grade B embryos was decreased from 107 (21.8%) in the MACS–ICSI group compared to 120 (38.7%) in the traditional ICSI group, and these differences were highly statistically significant ($P \leq 0.001$). The incidence of the number of Grade C embryos was lower at 75 (15.4%) in the MACS–ICSI group compared to 60 (19.4%) in the traditional ICSI group, and such differences have been highly statistically significant ($P \leq 0.001$). A comparison of pregnancy rates among studied group patients showed the incidence of pregnancy rate was 49.0% in the MACS-ICSI group. Compared to 32.0% in the traditional ICSI group with highly statistically significant differences ($P \leq 0.001$).

The current study's findings are in agreement with those of Dirican et al., who assessed the effect of male fertility treatments when ICSI into human oocytes has been conducted with non-apoptotic MACS-selected spermatozoa. The study group's cleavage rates improved statistically significantly when compared to the control group. The study group had a significantly increased chance of chemical pregnancies occurring ($P<0.05$, OR = 1.87). Even though the rates of implantation were statistically equivalent across the two groups, the study group had a slightly higher rate.[17] Another study by Pacheco et al. evaluated the effectiveness of the MACS approach to improve reproductive results in individuals who had undergone intracytoplasmic sperm-injection (ICSI) cycles and had high levels of sperm DNA fragmentation (SDF). In this study, it was found that the MACS group had a higher clinical pregnancy rate per cycle than the control groups, a lower miscarriage rate than the control groups, and a higher live birth rate than the control groups.[24]

Another study by García-Ferreyra et al. evaluated the outcomes of ICSI cycles performed on patients who had normal sperm DNA fragmentation and those performed using non-apoptotic MACS-selected spermatozoa. This study found that the development rates of blastocysts, the number of blastocysts of good quality, and pregnancy rates were all greater in the study groups than in the control groups. In addition, miscarriages were fewer in the study groups than in control groups.[25] Another study by Horta et al. evaluated the impact of MACS on fertilization, implantation, rates of clinical pregnancy, miscarriage, and embryo growth in couples receiving ICSI. This study found that the rate of implantation on EDT5/6 was significantly greater when MACS was used compared to swim-up sperm selection in the study group (MACS) and control group, respectively. In comparison to control groups, the study's pregnancy rates are higher. In addition, miscarriages were 0% and 9.75%, respectively, for the study and control groups.[19] Another study by **Gil** M. et al. evaluated if using MACS as a sperm selection method enhances ART rates of success in couples receiving assisted reproduction treatment. According to the results of this study, patients who had sperm selection employing MACS had a significantly greater pregnancy rate than those who received therapy without MACS (RR=1.50, 95 % CI 1.14–1.98).[26] Another study by Sheikhi A. et al.

evaluated if removing apoptotic spermatozoa may improve the chances of getting pregnant using (ICSI), which demonstrated that the study group's fertilisation rate differed statistically significantly from that of the control group. On day 3, the study group's number of eight blastomeric non-fragmented (8-grade1) embryos per oocyte (embryo quality) was similarly significantly higher than the controls' number. The study group had a higher pregnancy rate than the control group after intra-cytoplasmic sperm injection. Finally, the study's group live-birth rate was (40.5%) whereas the control group was (27%).[27] Another study by Ziarati et al. evaluated the effectiveness of the MACS method in a prospective randomized study. Ziarati et al. demonstrated that the MACS-DGC group's fertilisation rates were higher than those of the control group. When compared to the control group, the MACS-DGC had a significantly greater proportion of high quality embryos (score A). In addition, the MACS-DGC group's pregnancy and implantation rates were significantly greater than those of the control group (54.54 and 36.3%, respectively, vs. 24.25 and 15.7%, respectively) [28]. The study's findings are in line with other earlier studies suggesting that MACS may enhance the clinical results of ICSI for couples suffering from male factor infertility.[17,26.29,30,31&32]

On the other hand, Juliá G.M. et al. measured the effects of MACS on reproductive results using traditional parameters as well as cumulative live birth rates (CLBR). Results demonstrated that the MACS group had live birth rates (LBR) of 29.3% (27.6%, 31.0%) for each ET and 38.8% (36.7%, 40.9%) for each cycle. Both comparisons were not statistically significant. The MACS groups had a miscarriage rate per ET of 8.2% (7.1%, 9.3%), while the reference group exhibited a rate of 7.5% (7.2%, 7.7%). No statistically significant difference existed between the groups.[33] [

Another study by Romany et al. evaluated the determination of the impact on live-birth delivery rates following ICSI in couples who had undergone ovum donation (OD) of eliminating presumed apoptotic sperm cells from specimens from unselected males using MACS. This study concluded that each study group had similar implantation rates but that the pregnancy rate in the MACS group (79) was less than that of the control group (81). In Per intervention, the pregnancy positive test rate/ET (%) was 64.2 in MACS group while in control group was 71.1. The MACS group had a lower live birth rate/ET than the control group. Compared to the control group, the MACS group had a greater miscarriage rate/ET.[21]

Another study by Stimpfel et al. evaluated the advantages of using non-apoptotic spermatozoa chosen by magnetic-activated cell sorting (MACS) for ICSI procedures for couples when the female factor of infertility was teratozoospermia and the women exhibited good prognosis. This resulted in a similar percentage of fertilised oocytes and cleaved embryos in both groups (standard ICSI and MACS-ICSI). Moreover, there were no significant differences in the quality of the embryos between days 3 and 5. There were no differences in the numbers (ICSI versus MACS-ICSI; 56.5 versus 41.2%, respectively) in the patient group below 31 years old (eight patients) or day 5/6 blastocyst quality (good quality 69.2 versus 42.9%, fair quality 0 versus 42.9%, poor quality 30.8 versus 14.3%) or in numbers (100 versus 95.2%) or day 3 embryo quality (good quality 54.2 versus 45.0%, fair quality 33.3 versus 40.0%, poor quality 12.5 versus15.0%). However, the results showed that in the patient group aged 31 or older, the percentage of good quality day 5/6 blastocysts was greater following MACS-ICSI, despite the quantities of embryos that achieved the blastocyst stage being similar.[23]

CONCLUSION

The study's findings showed that using the MACS approach improved ICSI results in patients who had high levels of sperm DNA fragmentation, as the use of MACS in sperm processing helps in increasing motility, especially progressive motility, and reducing sperm with abnormal morphological defects (head, midpiece, and tail defects), so the use of MACS in ICSI resulted in increasing fertilization rate, cleavage rate, blastocyst formation rate, number of Grade A embryos and pregnancy rates.

Conflict of interest : none

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