Scientific Journal for Damietta Faculty of Science **11**(2) 2021, 10-21
DOI: 10.21608/SJDFS.2018.194790
ISSN Print 2314-8594
ISSN Online 2314-8616

https://sjdfs.journals.ekb.eg/

Effects of Magnetic-Activated Sperm Cell Sorting Technique in Blastocyst Formation After Intracytoplasmic Sperm Injection

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Received: 20 November 2021 /Accepted: 13 December 2021

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Abstract

Many sperm preparation procedures are used in intracytoplasmic sperm injection (ICSI), including migration, density gradient centrifugation, and filtering. Magnetic-activated cell sorting (MACS) is a type of magnetic separation that has been used in a variety of biomedical and biological studies.
To examine the fertilisation rate, cleavage, and development of Blastocystes in two sperm preparation procedures for ICSI, MACS and density gradient centrifugation (DGC).
The research was carried out in the Royal Center in Mansoura, Dakahlia Governorate, Egypt. Fifty patients receiving ICSI had their sperm samples taken. The sperm were separated into two groups using traditional DGC. One group served as DGC, while the other served as MACS. The number of oocytes harvested, the number of metaphase II oocytes injected, fertilisation rates, cleavage rates, and embryo quality were all compared.
In terms of fertilisation rate and cleavage, there was a statistically significant difference between the MACS and DGC groups. In terms of high quality, there is a statistically significant difference between the two groups in terms of the quantity and rate of good quality embryo A and B. The number of MII oocytes and age had a statistically significant negative connection. Furthermore, in the DGC group, there was a statistically significant negative connection between sperm DNA fragmentation (SDF) and fertilisation rate.
MACS sperm selection of human spermatozoa is a safe, straightforward, and effective procedure for sperm preparation in a clinical environment for ICSI..

Keywords: Intracytoplasmic sperm injection, sperm preparation, magnet activated cell sorting, density gradient centrifugation, blastocysts.

Introduction

Infertility is defined as a sexually active, non-contracepting couple's failure to conceive naturally within a year [1].
Infertility, defined by the World Health Organization as the failure to conceive after one year of unprotected intercourse, is a frequent

problem that affects 10-15% of couples in industrialized and developing nations [2].

Infertility is a serious health issue with an estimated frequency of 48.5 million couples globally.

In 50 percent of involuntarily childless couples, both men and women are affected by infertility, and a male-infertility related factor is discovered alongside defective sperm parameters. Infertility is most common when both spouses have decreased fertility. A fertile partner may compensate for the man's reproductive problem, so infertility is most common when partners have reduced fertility[1].

Infertility is treated using assisted reproductive technology (ART). Gamete Intrafallopian Transfer (GIFT), Intrauterine Insemination (IUI), Zygote Intrafallopian Transfer (ZIFT), IVF, and ICSI are some of the technologies used. In 2012, the total number of successful IVF and ICSI births recorded globally was 5 million [5]

After previous therapies have failed, the National Institute for Health and Care Excellence (NICE) recommends in vitro fertilization (IVF) as the definitive therapy for protracted unresolved infertility. IVF can be used to treat both female and male infertility [4]

Migration, Density Gradient Centrifugation (DGC), and filtering procedures are the most common methods of sperm preparation. While the approach for migration is based on spermatozoa movement, the method for (DGC) and filtration methods is based on a combination of motility and retention at phase boundaries, as well as adherence to filtration matrices [6]

Magnetic Activated Cell Sorting (MACS) is a type of magnetic separation that has been used in a variety of biomedical and biological studies. In addition, cellular treatments for human autoimmune illnesses such rheumatoid arthritis, diabetes, multiple sclerosis, and systemic lupus erythematosus are being researched [7].

MACS is a magnetic-based sperm cell selection system that can distinguish between early apoptotic and non-apoptotic spermatozoa. Phosphatidylserine (PS) has been externalized on the sperm plasma membrane by apoptotic cells. PS has a strong affinity for Annexin V and apoptosis [8].

MACS micro size particles with high magnetic properties coated with annexin v, PNA and LcA.

Annexin V conjugated paramagnetic microbeads bind to sperm cells. Magnetically tagged apoptotic or dead spermatozoa are kept in the column after the material is passed through a magnetic column [9].

Because it cannot remove leukocytes, immature germ cells, seminal plasma, and other impurities from the semen sample, therefore must be supplemented with density gradient centrifugation [(10)].

The goal of this study was to see if there is a link between Blastocysts production and two sperm preparation methods: Magnetic Activated Cell Sorting (MACS) and Density Gradient Centrifugation (DGC) for Intracytoplasmic Sperm Injection (ICSI).

Apoptotic sperm with significant quantities of fragmented DNA can be separated from the remainder using magnetic-activated cell sorting (MACS), enhancing the overall quality of the seminal sample. The goal of this retrospective study was to see how practical MACS approach was in improving reproductive outcomes in patients undergoing intracytoplasmic sperm injection who had high levels of sperm DNA fragmentation (SDF) (ICSI)

In ICSI cycles, the use of Annexin –v MACS and density gradient centrifugation were shown to improve clinical pregnancy rates.

Subjects and Methods

The research had conducted at the Royal Fertility Center in Mansoura, Egypt's Dakahlia province. The participants in this trial were 50 couples who were treated with ICSI for male infertility. The women in the couples that took part in this trial had to be under 35 years old and have a normal ovarian response to controlled ovarian hyperstimulation. Work began 2019 and lasted until 2021. In this study, 50 couples were divided into two groups: those treated with ICSI for male infertility and those prepped for sperm DNA fragmentation.

First group: Sperm samples from 25 patients were obtained using Density Gradient Centrifugation (DGC), and mature oocytes were fertilized using ICSI utilizing spermatozoa that had been chosen using (DGC).

Second group: Magnetic Activated Cell Sorting was used to prepare sperm samples from 25 individuals (MACS). ICSI was used to fertilize the mature oocyte with spermatozoa chosen by

Magnetic Activated Cell Sorting (MACS).

Inclusion criteria:

The following criteria were used to choose the subjects:

- Females under the age of 35.
- Males under the age of 35.
- Sperm DNA fragmentation is a concern for males.
- Males with a high sperm quality rating (concentration ,motility and morphology).
- Female body mass index (BMI) of 30 kg/m2 Was preferred.
- Follicle stimulating hormone (FSH) concentrations of less than 10 ml/m2.
- Being a candidate for ICSI for any cause, including infertility that cannot have explained, tubal factor and ovulation malfunction, or mild male factor infertility.

Exclusion criteria:

- Ovarian poly cysts in females (PCO).
- Endometriosis is a condition that affects women.
- Varicocele is a condition that affects men.
- Males have abnormal sperm.

Methods:

- A thorough medical history was taken focusing on the type and duration of infertility as well as any risk factors such as varicocele and endocrine abnormalities.
- Comprehensive clinical examination was considered to rule out any major infections that might influence fertility.
- Semen analysis: Computer aided semen analysis (CASA) was performed in accordance with WHO guidelines (12).

DNA fragmentation:

- Strand breaks in sperm and DNA have been assessed using a variety of procedures, including terminal deoxynucleotidyl transferase–mediated fluorescein–dU TP nick end labelling (TUNEL), Sperm chromatin structure assay (SCSA), and acridine orange staining (AOT), among others.

Preparation of semen sample for ICSI by :

• DGC of the prepared semen samples: the lower and higher gradients were carefully stacked, and the seminal ejaculate was put on top. The sample was centrifuged for 20 minutes at 1600 rpm. Clear seminal plasma was preserved on the highest section of the gradient preserved, followed by a clear separation of white blood cells, detritus, and other cells. Because of their density and motility, immature, defective sperm were visible along the gradient. Normal sperm that are highly motile travel aggressively to the bottom of the gradient and are collected in a pellet (13). (Figur1).

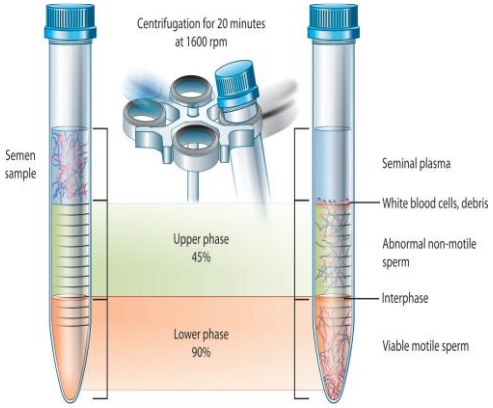

Fig. 1: Density gradient centrifugation (Otsuki,. et al, 2008).

Magnetic activated cell sorting (MACS) of the prepared semen samples:

Magnetic Aactivated Cell Sorting (MACS) is a relatively new sperm preparation technology for ART. It enables the isolation of apoptotic spermatozoa, which can cause ART failure even in individuals with otherwise normal sperm parameters.

It had used to prepare sperm from isolated non-apoptotic spermatozoa using annexin-V kits [(14).]

MACS is a magnetic-based sperm cell selection technology that can distinguish early apoptotic from non-apoptotic spermatozoa. On the sperm plasma membrane, apoplastic cells have externalized phospholipid phosphatidylserine (PS). Apoptotic sperm cells connect to Annexin V conjugated paramagnetic microbeads because PS has a high affinity for Annexin V. Annexin V has a great affinity for phosphatidyl serine, but it can't get beyond the sperm membrane intact. The highly specific antibodies to annexin V are attached to colloidal superparamagnetic beads (50 nm in diameter) and utilised to separate the cells.

MACS detects dead and apoptotic spermatozoa. Annexin V binding to spermatozoa suggests

that the sperm membrane integrity has been disrupted. Magnetically tagged apoptotic or dead spermatozoa are kept in the column after the material is passed through a magnetic column. Because it cannot remove leukocytes, immature germ cells, seminal plasma, and other contaminants, this approach is supplemented with density gradient centrifugation.

Their pollutants were discovered in the sperm sample (Figur2). After mixing, the mixture was kept at room temperature for 15 minutes to allow annexin v-conjugated magnetic microbead complexes to attach to apoptotic spermatozoa that externalize phosphatidylserine. MACS ART MS columns were inserted in a MiniMACS separator (Fig2), washed once with 1ml MACS ART binding buffer (discarded after flow-through), and the spermatozoa solution was then deposited in the column for magnetic separation. The apoptotic spermatozoa were kept in the column after being coupled to annexin V-conjugated magnetic microbeads. After passing through the column, the spermatozoa that were not magnetically tagged with annexin V-conjugated magnetic microbeads were collected These spermatozoa represent the non-apoptotic population since they did not label After collecting the initial portion of spermatozoa, the column was washed with 0.5 ml MACS ART Binding Buffer to remove any residual unlabeled spermatozoa. This was the result of combining the flow-through fraction with the fraction from the previous stage. This enhanced viable spermatozoa combination was then rinsed with 4 mL of sperm preparation media and centrifuged at 1400rpm for 10 minutes. When the sperm sample has a high concentration of extraneous cells pus cells, round cells, etc., Density Gradient Centrifugation (DGC) was used). (However, if the concentration of extraneous cells is modest, the sperm samples were washed without DGC. Thereafter, the pellet was resuspended in the washing medium and swimmed up (25 minutes at 45-degree tube tilting. Before adding the spermatozoa to the MACS particles, the surface layer was aspirated and the concentration of spermatozoa collected was assessed). (50 ul of particles per 300 ul of 5 million sperm (were added) for a single dosage, or 100 ul of particles (full dose) every 500 ul of 5 million sperm for a complete dose. MACS particle concentration (was adjusted) if a different sperm count is utilised (The particles and sperm were gently mixed) at room temperature for 25 minutes. (Whenever, the particle contact was maintained, hand mixing was applied very gently every 5 minutes (The particle sperm solution was placed against the magnet). (While the MACS were still against the wall or at the bottom of the tube and magnet, the supernatant was decant or aspirate into a new tube). (Consequently, the supernatant contained viable sperm). (THERE IS NO NEED FOR THIS STATEMENT WITHIN THE METHODS SECYTION). For ICSI, proceed as usual. (The same steps were followed for ICSI).

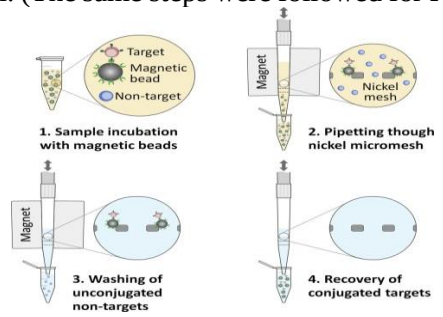

Fig. 2: Magnetically activated cell sorting (MACS) is achieved by attaching antibody-coated

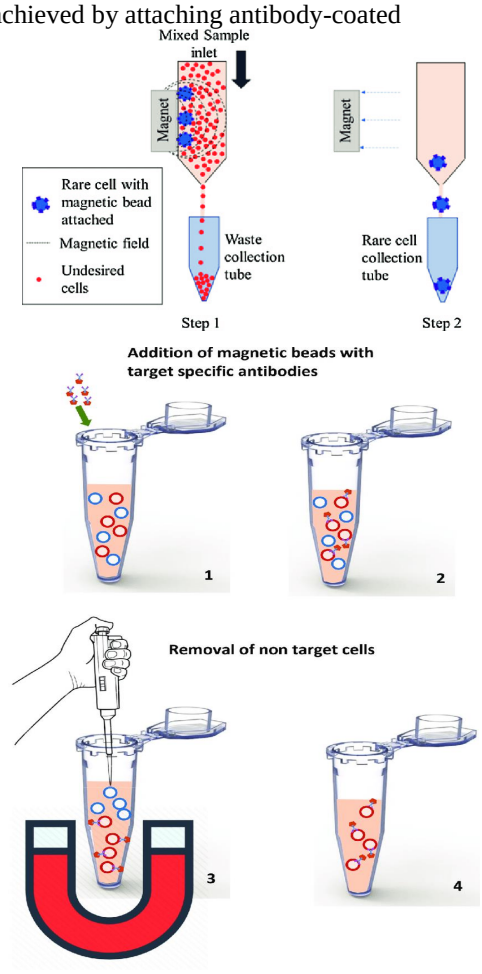

Fig. 3: Magnetic Activatid Cell Sorting(MACS) Miltenyi et al. 1990

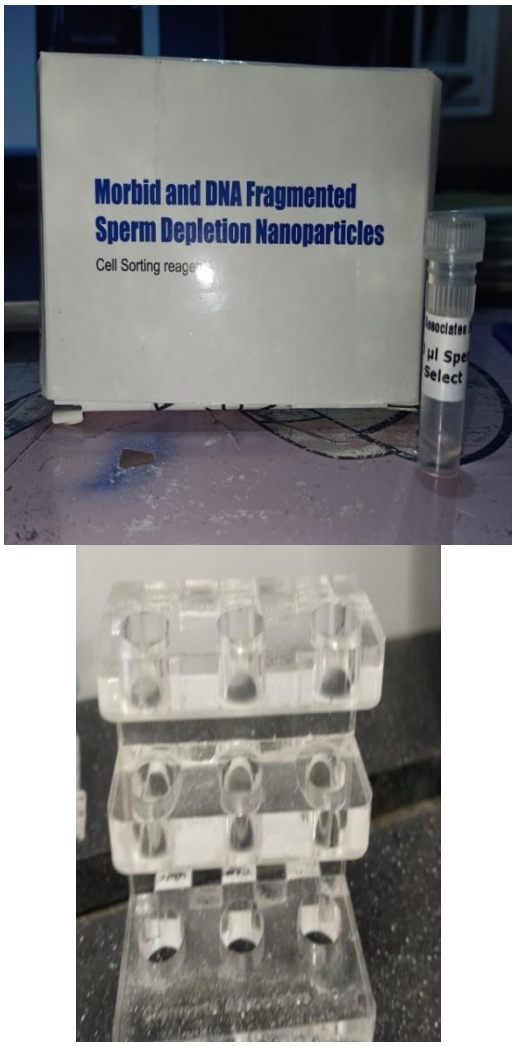

Fig. 4: Photo taken during Magnetic activated cells sorting kits and rack

Intracytoplasmic sperm injection (ICSI) after Magnetic activated cell sorting and Density Gradient centrifugation sperm prepared :

Each couple had one hall of the mature oocyte fertilised with ICSI using spermatozoa prepared by the (traditional sperm preparation) approach, while the other hall was fertilized with ICSI using spermatozoa chosen by the MACS method (MACS- ICSI).

Fertilized oocytes were cultivated in fertilisation media till the day following ICSI and evaluated for the presence of pronuclei 16-18 hours later. Normally fertilised zygotes (2PN) were grown in the SAGE 1-Step continuous culture media until day 3 or day 5 when they were put into the uterus. On day 3, embryos were classified as good quality if they had 6-10 equally sized blastomeres with less than 15% fragmentation, fair quality if they had unequally sized blastomeres with less than 15% fragmentation or equally and unequally sized blastomeres with 15-50 percent fragmentation, and poor quality if fragmentation accounted for more than 50% of the embryo volume. The embryos were assessed on day 5. Briefly, blastocysts were classified as good quality if they developed, expanded, or began to hatch with good inner cell mass (ICM) and trophoectoderm (TE) morphologies; fair quality if they developed, expanded, or began to hatch with good morphologies of only the ICM or TE; and poor quality if they displayed abnormal morphologies of both structures. Early blastocysts (grade 1) were also included in the last batch. At most, two embryos were transferred per patient. If the embryo transfer was done on day 3, the leftover embryos were cultivated until they reached blastocyst development on day 5/6.

Laboratory Part:

1- Oocyte retrieval was done.
2- The eggs were peeled.
3- The number of oocytes harvested and the number of oocytes in metaphase II were chosen).
4- Metaphase II was divided into two groups:
A group was injected with semen that had been produced using Density Gradient Centrifugation (DGC).
B- A group was injected with semen that had been produced using Magnetic Activated Cell Sorting (MACS).
5- The injected eggs were placed) in the culture plate.
6- The implanted eggs were inserted into the incubator.
7- The number of injected oocytes in the metaphase II oocytes were counted total number of normal metaphase II oocytes injected).
8- 8-On the third day of the injection process, the incubator (was opend) on the injected egg to determine the injection's outcome.
9- 9-Determine cleavage rates (total number of cleaved embryos on day 3/ total number of fertilised oocytes)
10- 10-Embryo quality (on days 3 and 5, the average number of blastomeres per embryo).
11- 11- On day 3, the percentage of fragmentation per embryo (was recorded).
12- Intracytoplasmic sperm injection: The number of collected oocytes, the number of metaphase II oocytes injected, fertilisation rates (total number of normal normally fertilized oocytes/total number of metaphase II oocytes injected), cleavage rates (total number of

cleaved embryos on day 3/total number of normal fertilized oocytes), and embryo quality were all compared (mean number of blastomeres per embryo on day 3&5 and mean percentage of fragmentation per embryo on day 3).

Statistical analysis :

All of the findings were calculated as means plus standard deviation. The statistical significance was determined using the (ANOVA) test in SPSS.

Results

Male and female patients varied in age from 18 to 35 years old, with a mean age of 28.58 years (table 1).
The age of the sperm ranged from 2 to 90 years, with the average age being 42.34 (106/ml). Sperm motility was measured in percentages ranging from 2 to 90%, with a mean of 53.9 percent. The normal forms varied from 0% to 10%, with an average of 2.66 percent (table 2).
MII oocytes in female patients varied from 4 to 82, with a mean of 20.44. The number of MI oocytes ranged from 1 to 14, with a median of 17. The number of GV oocytes ranged from 1 to 19, with a median of 3. The number of BGV oocytes varied from 2 to 15, with a median of 4. The number of atretic oocytes varied from one to twenty, with a median of six (figure 3).
There was a statistically non-significant difference between the analysed groups (non-significantly greater in the MACS group) (table 3).
In There is a statistically significant difference between the tested groups in the quantity of fertilized eggs and fertilization rate (both considerably greater in the MACS group) (table 4)
In terms of cleavage and cleavage rate, there was a statistically significant difference between the examined groups (both were considerably greater in the MACS group) (table 5).
In terms of the number of good quality embryo A (substantially greater in the MACS group), rate of good quality embryo A (notably higher in the MACS group), and rate of good quality embryo B, there were statistically significant variations between the analysed groups (significantly higher in G group). However, there was a statistically non-significant difference in the number of excellent quality embryo B (non-significantly greater in the G group) across the analysed groups (table 6).
There (were) statistically significant differences in the number of blastocysts (notably greater in the MACS group) and blastocyst rate (significantly higher in the MACS group) between the analysed groups (table 7).
Eighty percent of the patients in the study had their embryos transferred on the fifth day (figure 2). The pregnancy test came back positive in 68 percent of the people who took part in the study (figure 3). Age and the quantity of MII oocytes had a statistically significant negative relationship (figure 4).
In the group receiving Density Gradient Centrifugation (DGC), there was a statistically significant negative connection between SDF and fertilisation rate (table 8).
Furthermore, both groups have a statistically significant link between SDF and cleavage or cleavage rate.

Table (1): Demographic data of the age (of) male and female studied.

Parameter	Mean ± SD	Range
Age (year)	28.58 ± 5.167	18 - 35

Table (2): Result of sperm analysis.

Parameter	Mean ± SD	Range
Sperm count (10^6/ml)	28.58 ± 5.167	18 – 35
Sperm motility (%)	53.9 ± 14.26	2 – 90

Table (3) : Comparison between modalities regarding oocytes

Parameter	Mean ± SD	Median	Range
MII oocytes	20.44 ± 15.51	17	4 – 82
MI oocytes	4.289 ± 3.487	3	1 – 14
GV	5.343 ± 4.345	4	1 – 19
BGV	5.6 ± 5.367	3	2 – 15
Atretic	7.11 ± 5.201	6	1 – 20

MII oocytes ranged from 4 to 82 with mean 20.44. MI oocytes ranged from 1 to 14 with median 17. GV oocytes ranged from 1 to 19 with median 3. BGV oocytes ranged from 2 to 15 with median 4. Atretic oocytes ranged from 1 to 20 with median 6.

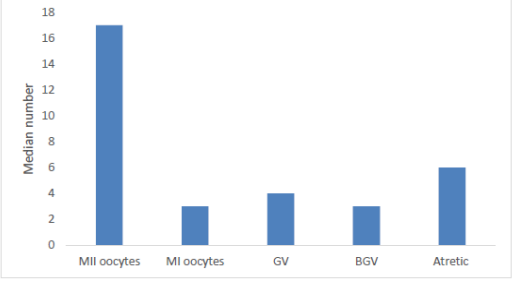

Fig. 5: Chart showing median number of oocyte among the studied participants

Table (4): Comparison between treatment modalities regarding injected

Parameter	Modality		Test	
	Magnetic-Activated Sperm Cell sorting (MACS)	Density Gradient centrifugation (DGC)	t	p
	Mean ± SD	Mean ± SD		
Injected	11.5 ± 7.109	11.36 ± 7.704	0.094	0.925

t independent sample t test

There is statistically non-significant difference between the studied groups regarding injected material (non-significantly higher in MACS group.

Table (5): Comparison between treatment modalities regarding fertilization

Parameter	Modality		Test	
	Magnetic-Activated Sperm Cell sorting (MACS)	Density Gradient centrifugation (DGC)	t/Z	p
	N=50	N=50		
Fertilization: Mean ± SD Median (range)	8.6 ± 5.38 9 (1 – 30)	6.68 ± 6.32 7.5 (2 – 25)	-2.713	0.007*
Fertilization rate (%) Mean ± SD Range	76.98 ± 13.04 28 - 100	55.93 ± 17.92 16.6 – 100	2.085	0.04*

Data recorded as mean± S.D; *P value<0.05 means significant; **P value<0.01 means highly significan Comparison between modalities regarding cleavage

t independent sample t test Z Mann Whitney test *p<0.05 is statistically significant

There is statistically significant difference between the studied groups regarding number of fertilized ova and fertilization rate (both was significantly higher in MACS group)

Table (6):

Parameter	Modality		Test	
	Magnetic-Activated Sperm Cell sorting (MACS)	Density Gradient centrifugation (DGC)	t	p
	N=50	N=50		
Cleavage: Mean ± SD Median (range)	8.6 ± 5.38 9 (1 – 30)	6.68 ± 6.32 7.5 (2 – 25)	-2.63	0.009*
Cleavage rate (%) Mean ± SD Range	80.16 ± 11.8 28 – 100	60.34 ± 16.43 40 – 100	6.926	<0.001**

t independent sample t test *p<0.05 is statistically significant **p≤0.001 is statistically highly significant

There is statistically significant difference between the studied groups regarding cleavage and cleavage rate (both was significantly higher in MACS group)

There is statistically significant difference between the studied groups regarding number of good quality embryo A (significantly higher in MACS group)

There is statistically non-significant difference between the studied groups regarding number of good quality embryo B (non-significantly higher in G group)

Table (7): Comparison between modalities regarding good quality

Good quality embros	Modality		Test	
	Magnetic-Activated Sperm Cell sorting (MACS)	Density Gradient centrifugation (DGC)	t/Z	p
	N	N		
A: Mean ± SD Median (range)	N=49 6.02 ± 3.94 5 (1 – 19)	N=44 3.84 ± 3.06 3 (1 – 15)	-3.224	0.001**
A (%) Mean ± SD Range	 68.71± 16.71 25 – 100	 53.24 ± 15.77 24 – 100	6.926	<0.001**
B: Mean ± SD Median (range)	N=25 2.52 ± 2.06 2 (1 – 9)	N=22 3.27 ± 2.71 3 (1 – 9)	-1.082	0.279
B (%) Mean ± SD Range	 29.49 ± 22.59 20 (7.6 – 100)	 52.14 ± 26.02 50 (10 – 100)	6.926	<0.001**

t independent sample t test Z Mann Whitney test *p<0.05 is statistically significant **p≤0.001 is statistically highly significant

There is statistically significant difference between the studied groups regarding rate of good quality embryo A (significantly higher in MACS group)

There is statistically significant difference between the studied groups regarding rate of good quality embryo B (significantly higher in G group)

Table (8): Comparison between modalities regarding blastocysts

Parameter	Modality		Test	
	Magnetic-Activated Sperm Cell sorting (MACS)	Density Gradient centrifugation (DGC)	Z	p
	N=50	N=50		
Blastocyst: Mean ± SD Median (range)	4.86 ± 3.21 4.15 (1 – 13)	2.14 ± 2.16 2 (0 – 10)	-4.679	<0.001**
Blastocyst rate (%) Mean ± SD Range	68.04 ± 17.55 67.45 (25 – 100)	38.42 ± 25.17 50 (0 – 100)	-6.149	<0.001**

Data recorded as mean± S.D; *P value<0.05 means significant; **P value<0.01 means highly significan

Table (9) Comparison between treatment modalities regarding day of transfer:

Day of transfer	Test	
	N=50	%
Day 3	10	20
Day 5	40	80

Eighty percent of the studied participants had embryo transfer on fifth day

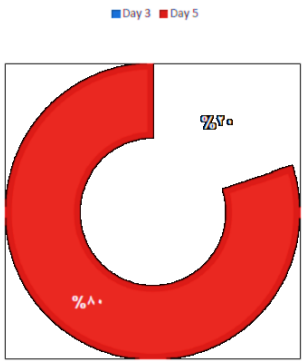

Figure (2): Pie chart showing distribution of the studied participants according to day of ET.

Table (10): Outcome of the Pregnancy test:

Pregnancy test	Test	
	N=50	%
Negative	16	32
Positive	34	68

Pregnancy test was positive in 68% of the studied participants

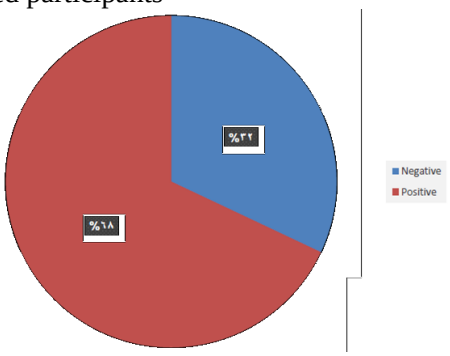

Figure (3): Pie chart showing distribution of the studied participants according to day of ET.

Table (11) Correlation between age and oocytes characteristics:

Parameter	Age (year)	
	r	p
MII	-0.294	0.038*
MI	-0.103	0.539
GV	-0.204	0.279
BGV	-0.444	0.454
Atretic	0.128	0.612

r Spearman rank correlation coefficient (*P) <0.05 is statistically significant

There (was) statistically significant negative correlation between age and number of MII oocytes

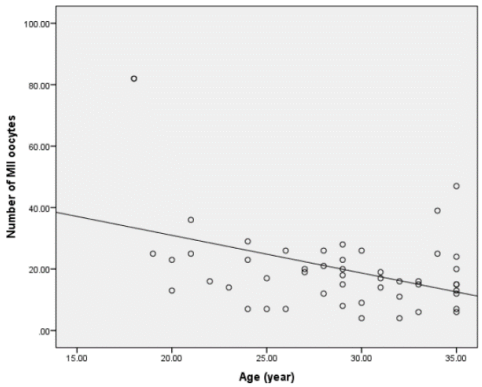

Figure (4): Scatter dot graph showing significant negative correlation between age and number of MII oocytes.

Table (12): Correlation between SDF and fertilization among the studied groups

Parameter	G		MACS	
	r	p	r	p
Fertilization	-0.149	0.302	-0.023	0.876
Fertilization rate	-0.315	0.026*	-0.241	0.091

r Spearman rank correlation coefficient *p<0.05 is statistically significant

Table (13): Correlation between SDF and cleavage among the studied groups

Parameter	G		MACS	
	r	p	r	p
Cleavage	-0.151	0.294	0.029	0.840
Cleavage rate	-0.222	0.122	-0.248	0.082

r Spearman rank correlation coefficient *p<0.05 is statistically significant

Discussion

ICSI is a particular type of in vitro fertilization (IVF) used to treat severe male factor infertility (15).

The DGC method is commonly used to recover a population of sperm with normal morphology and high motility. It resurrects a sperm population with less DNA fragmentation [16].

As the severity of male factor infertility has worsened over the years, scientists and physicians are being encouraged to develop sperm separating processes. Advanced sperm preparation technologies based on cellular and molecular attributes are becoming increasingly popular in this area [17].

MACS is a unique sperm selection process that uses PS externalization via Annexin V-conjugated super paramagnetic microbeads (50 nm) to remove apoptotic spermatozoa from the original population [18].

Separating viable motile sperm from seminal debris and possibly bacterial contamination is crucial for sperm processing. The MACS approach, when used methodically, is incapable of achieving these objectives. Several researchers have used DGC before or after the MACS method [19]. In this line, Tavalaee et al. [20] showed that either MACS before DGC (MACS/DGC) or DGC before MACS (DGC/ MACS) successfully retrieved intact sperm in terms of the aforementioned characteristics above in both methods.

The (sperms) in this investigation were produced using traditional DGC and MACS methods.

The participants in this research varied in age from 18 to 35 years old, with a mean age of 28.58 years. The age of the sperm varied from 2 to 90 years, with a mean of 42.34 (106/ml). Motility in the sperm varied from 2% to 90%, with a mean of 53.9 percent. The normal forms varied from 0% to 10%, with an average of 2.66 percent. The SDF ranged from 6% to 80%, with a median of 24.85%.

Ziarati et al. [21] tested the efficacy of the MACS

technique on sixty-two sperm samples that were divided into two groups and treated using MACS plus density gradient centrifugation (MACS–DGC) as the study group and DGC alone as the control group. They evaluated female age, male age, and sperm parameters (concentration, motility, and morphology) between study (MACS–DGC) and control groups and found no significant variations in these parameters.

MII oocytes varied in size from 4 to 82, with a mean of 20.44. The number of MI oocytes ranged from 1 to 14, with a median of 17. The GV oocytes ranged from 1 to 19, with a median of 3. The BGV oocytes varied from two to fifteen, with a median of four. The number of atretic oocytes varied from one to twenty, with a median of six.

Ziarati et al. [21] discovered that the number of oocytes recovered was not significantly different between MACS–DGC and control groups (12.37 1.02 vs. 11.84 0.83).

There was no statistically significant difference between the examined groups when it came to injected substance. Furthermore, Ziarati et al. [(21)] discovered that the number of oocytes injected was not substantially different between the MACS–DGC and control groups (10.55 0.88 vs. 9.57 0.7).

In our study, there was a statistically significant difference in fertilization rate between the MACS and DGC groups (significantly higher in MACS group). However, Ziarati et al. [21] observed no significant difference in fertilization rates between the MACS–DGC (76.13 4.38) and control (74.78 3.41) groups.

In our investigation, there was a statistically significant difference in cleavage between the MACS and DGC groups (significantly higher in MACS group). In oligoasthenozospermic individuals, Dirican et al. [22] examined fertilization, cleavage, implantation, and pregnancy rates using density gradients and MACS as sperm preparation techniques for ICSI cycles. Using MACS as a sperm selection approach, they discovered greater cleavage and pregnancy rates.

According to the current findings, there was a statistically significant difference between the analyzed groups in terms of the quantity and rate of excellent quality embryo A (notably more significant in the MACS group) and good quality embryo B (substantially lower in the MACS group) (significantly higher in DGC group). The findings are consistent with Simon et al. [23] who found that anomalies in paternal DNA had negatively influenced embryo quality.

Romany et al. [24] examined the quality of embryos, fertilization, implantation, pregnancy, and live-birth rates in patients who had ICSI as part of an ovum donation (OD) program. However, significant differences in all parameters tested using MACS as a sperm selection strategy were not identified.

Because MACS can result in the removal of sperm with fragmented DNA and apoptotic sperm, increased embryo quality in the research group could be attributed to improved sperm quality, which would boost implantation and pregnancy rates. The findings of this study matched those of Troya and Zorrilla [(25),] who found that MACS can enhance the clinical outcome of ICSI in couples with male factor infertility.

In contrast, Romany et al. [25] found no significant differences between MACS and control groups in terms of embryo quality, implantation, or live-birth rates. It's worth noting, though, that before the MACS technique, they utilized swim up instead of DGC.

There was a statistically significant difference between the groups investigated when it came to blastocysts (significantly higher in MACS group).

Eighty percent of the patients in the study had their embryos transferred on the fifth day. The pregnancy test came back positive in 68 percent of the people who took part in the study. Horta et al. [26] looked at how MACS affected fertilization, embryo growth, implantation, clinical pregnancy, and miscarriage rates in couples who had intracytoplasmic sperm injection (ICSI). They discovered that ideal embryo quality on days 3 and 5 was similar in the MACS (case) and swim-up (control) groups, with no significant differences in embryo transfers on either day 3 or day 5.

Age and the number of MII oocytes had a statistically significant negative connection in our study. Also, in the DGC group, there is a statistically significant negative association between SDF and fertilization rate, but in the MACS group, there were statistically non-significant negative correlations between SDF and either fertilization or fertilization rate.

A proportion of sperm may have experienced capacitation during swim up, resulting in the externalization of phosphatidyl serine residue

as a natural capacitation process, and .These sperm are deselected during MACS processes. As a result, in addition to eliminating apoptotic sperm, they may have accidentally destroyed excellent quality sperm with the capacity to undergo capacitation through this procedure [27].

Gil et al. [28] also stated that MACS was an effective treatment that might enhance pregnancy rates but not implantation rates in ART MACS does. according to Troya and Zorrilla [25] does not enhance fertilization rates. These findings align with those of Bounartzi et al. [29] who discovered that DNA fragmentation does not prevent sperm from fertilizing eggs.

Indeed, lowering the sperm DNA fragmentation burden in the ICSI insemination sample is one way to improve ICSI results. Antioxidant treatment for at least three months prior before to ICSI has been recommended to attain this aim [30].

Spermatozoa cells with damaged membranes that demonstrate PS externalization as an expression of apoptosis can be removed via MACS sperm selection. Given this, this approach, rather than only sorting sperm by motility and density, can enhance traditional routine procedures by using molecular selection. Furthermore, as compared to standard methods, MACS can improve sperm quality for ART by removing spermatozoa with apoptotic characteristics [24].

Although MACS sperm selection has few technical restrictions, we believe that MACS following density gradients is a safe and acceptable approach for selecting quality spermatozoa due to comparable results to regular ART procedures. However, due to practical considerations, severe oligoasthenozoospermic individuals were not included, and so this approach cannot be easily used to this group of patients who can make a difference in a male factor infertility group.

Conclusion

MACS sperm selection of human spermatozoa is a safe, straightforward, and effective procedure for sperm preparation in a clinical environment for ICSI. The current study showed that MACS can help infertile couples with severe male factor select apoptotic sperm and enhance ICSI clinical results.

Studies with a specific group of patients should be conducted in order to determine the benefits of innovative sperm selection procedures, particularly in women of varying ages and oocyte quality. As a result, more research is needed to find advances in prolonged embryo culture and the male infertility issue

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الملخص العربي

عنوان البحث: تأثير فرز الحيوانات المنوية المنشطة مغناطيسيا على تكوين البلاستوسيست بعد الحقن المجهري

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كان الهدف من هذه الدراسة هو مقارنه معدل الإخصاب والانقسام وجودة الأجنة وتكوين البلاستوسيست بعد الحقن في سيتوبلازم البويضات للنساء الخاضعات للحقن المجهري(ICSI) لإعداد الحيوانات المنوية بتقنيتين وهما تقنية فرز الحيوانات المنوية المنشطة مغناطيسيا(MACS) وتقنية الطرد المركزي المتدرج بالكثافة(DGC). تم إجراء الدراسة في مركز رويال للخصوبة بمدينة المنصورة محافظة الدقهلية. وتم تجهيز الحيوانات المنوية من ٥٠ مريض(رجل) للحقن في ٥٠مريضة (سيدة) تراوحت اعمارهم بين ١٨و٣٥ سنة تم عمل مبدئيا تحليل تكسير الحمض النووي(SDF) للرجال ثم تقسيم الى مجموعتين الأولى بتقنية فرز الحيوانات المنشطة مغناطيسيا(MACS) والثانية بتقنية الطرد المركزي بالكثافة(DGC). تمت مقارنة نتائج الحقن في سيتوبلازم البويضات للنساء (الحقن المجهري) (ICSI) فيما يتعلق بعدد البويضات التي تم جمعها وعدد البويضات المحقونة في الطور الثانيMII ، ومعدلات الإخصاب ، ومعدلات انقسام الأجنة وجودة الأجنة وجودة الجنين (متوسط عدد الأجنة في اليوم الثالث والخامس) والنسبة المئوية لكل جنين في اليوم الثالث وقد تم نقل الأجنة المشاركات فى الدراسة فى اليوم الخامس وعمل اختبار للحمل. أظهرت النتائج أن متوسط عدد الحيوانات المنوية كان ١٠٦ / مل. وكان متوسط حركة الحيوانات المنوية ٥٣,٩٪. كما تراوحت الأشكال العادية من ٠ إلى ١٠٪ بمتوسط ٢,٦٦٪. تراوحت نسبة تكسير الحمض النووي(SDF) من ٦ إلى ٨٠٪ بمتوسط ٢٤,٨٥٪. وكان متوسط عدد البويضات فى الطور الثانى ٢٠,٤٤ . وقد كان هناك فرق ذو دلالة إحصائية بين مجموعة الفرز بطريقة MACS وبطريقةDGC فى معدل الإخصاب والانقسام وعدد ومعدل وجودة الأجنة وقد وجد فرق ذو دلالة إحصائية فى عدد البلاستوسيست المتكونة بين المجموعتين وكانت الأعلى عدديا فى مجموعة الفرز بطريقة .MACS ولكن هناك ارتباطات سلبية ليست ذات دلالة إحصائية بين تكسير الحمض النووي(SDF) ومعدل الإخصاب فى مجموعة وتقنية الطرد المركزي المتدرج بالكثافة في معدل الإخصاب والانقسام وعدد ومعدل وجودة الأجنة. وقد وجد فرق ذو دلالة إحصائية فى عدد البلاستوسيست المتكونة بين المجموعتين وكانت الأعلى عدديا فى مجموعة الفرز تقنية فرز الخلايا الحيوانات المنشطة مغناطيسيا وقد كان اختبار الحمل إيجابياً لدى ٦٨٪ من المشاركات فى الدراسة. ووجدت علاقة سلبية ذات دلالة إحصائية بين العمر وعدد البويضات في الطور الثاني. وكذلك فى تكسير الحمض النووي ومعدل الإخصاب في مجموعة الطرد المركزي المتدرج بالكثافة. ولكن هناك ارتباطات سلبية ليست ذات دلالة إحصائية بين تكسير الحمض النووي ومعدل الإخصاب فى مجموعة ولكن هناك ارتباطات سلبية ليست ذات دلالة إحصائية بين تكسير الحمض النووي ومعدل الإخصاب في مجموعة الفرز تقنية فرز الخلايا الحيوانات المنشطة مغناطيسيا. . الخلاصة: فرز الحيوانات المنوية بطريقة فرز الخلايا الحيوانات المنشطة مغناطيسيا أدت الى نتائج أفضل من تقنية الطرد المركزي المتدرج بالكثافة.