J. Product. & Dev., 27(3): 399- 415(2022)

COMPARISON BETWEEN PICSI AND ICSI AND ITS EFFECT ON BLASTOCYST FORMATION, A PROSPECTIVE RANDOMIZED TRIAL.

Ayat Samir[1]; Momen A. Kamel[2] and Bahget A. El-Feky[1]
[1] *Department of Animal Biotechnology, Genetic Engineering and Biotechnology Research Institute, University of Sadat City, Sadat City, Egypt.*
[2]*Department of Obstetrics and Gynecology, Faculty of Medicine, Assuit University, Egypt.*
***e.mail**.: bahget_elfeky@yahoo.com; momen.kamel@ aun. edu.eg; ayasamer@yahoo.com*
***Ethics approval No.** H16REA89*

ABSTRACT

Although ICSI may not provide a significant advantage over in vitro fertilization (IVF) in cases of non-male factor infertility, it is recommended to for search another method to increase the baby take-home rate in Assisted Technology. To assess the efficacy of two ready-to-use systems: intracytoplasmic sperm injection (ICSI) and Physiological intracytoplasmic sperm injection (PICSI) in a randomized manner on embryo development and blastocyst formation. This study was designed as a prospective, randomized trial. Couples receiving an ICSI or PICSI treatments with fresh embryo transfer were enrolled in this parallel two-groups, randomized trial. The study included 100 infertile women. Their age was between 25 to 39 years. Freshly ejaculated sperms for the treatment after at least 3 days of sexual abstinence. Couples were randomly randomized (1:1) to receive either PICSI or a standard ICSI procedure. PICSI and ICSI showed no statistically significant difference as regards fertilization rate, implantation rate and embryo transfer rate but showed statistically significant in blastocyst formation, clinical pregnancy and pregnancy continuation.

***Conclusively,** according to these findings, the PICSI technique had a higher chance of achieving pregnancy than the ICSI technique. As a result, the approach is preferable to be used in laboratory routine work than ICSI to avoid the selection of immature sperm, which leads to increased clinical pregnancy in male factor infertility, especially with DNA fragmentation. To back up this claim, prospective and randomized trials should be used.*

Keywords: ICSI, PICSI, blastocyst formation, pregnancy.

INTRODUCTION

Various techniques and procedures have swiftly arisen in the field of assisted reproduction during the last three decades, yet the success rate did not increase as expected and remains consistent **(Worrilow *et al.*, 2006).** The traditional criteria of spermatozoa, such as concentration, motility, and morphology, are generally used to choose the best spermatozoa with high quality and low damage during Assisted Reproductive Technology (ART). Because they do not demonstrate decreased DNA and oxidative damage in cells, these metrics are not absolute markers **(Celik-Ozenci *et al.*, 2004).** Also, Male infertility is commonly due to deficiencies in the semen, characterized by decreased spermatogenesis, sperm DNA damage, loss of sperm motility and abnormal sperm morphology **(Yovich & Stanger., 1984 and Palermo *et al.*,1992).**

ICSI is a well-established laboratory technique used worldwide to treat infertility. ICSI was originally introduced to overcome the most severe forms of male factor infertility. Since its advent, the use of this method of fertilization has increased steadily, even though the proportion of infertile couples diagnosed with male factor infertility has remained stable**(Jain & Gupta., 2007)**.

There is an increasing understanding of the risks of slowing sperm mobility with polyvinylpyrrolidone (PVP), a treatment often employed before ICSI **(Balaban *et al.*, 2003 and Boulet *et al.*, 2015)**. In today's IVF lab, finding the perfect spermatozoa for ICSI is an exciting challenge. When there are a limited number of oocytes available for injection, effective sperm selection becomes crucial **(Parmegiani *et al.*, 2010).** In addition to, the success of intracytoplasmic sperm injection is jeopardized when spermatozoa are injected with DNA fragmentation (DF) and oxidative damage (ICSI) **(Zini e*t al.*, 2008).**

Advanced sperm selection procedures are becoming more popular in ART, especially in ICSI cycles. Advanced sperm selection approaches are presented to increase the likelihood of selecting structurally intact and mature sperm with high DNA integrity for fertilization. Surface charge, sperm apoptosis, sperm birefringence, ability to bind to hyaluronic acid, and sperm morphology under ultra-high magnification are among the strategies used. The goal of these strategies is to improve ART outcomes **(Lepine *et al.*, 2019).** One of the indications of sperm maturation is the creation of HA binding sites in sperm cell membranes **(Huszar *et al.*, 1997).** The HA-binding approach reduces the probability of chromosomal aneuploidy and DNA fragmentation in spermatozoa **(Parmegiani *et al.*, 2012).**

Hyaluronic acid (HA) and its binding proteins have also been employed in research to assess hyaluronan localization in the placenta in pre-eclampsia **(Matejevic *et al.*, 2001).** This strategy is based on research that shows mature and structurally sounds sperm bind to hyaluronic acid, which is plentiful in the cumulus oophorus' extracellular matrix, and hence have a higher fertilization potential (**Novoselsky *et al.*, 2021)**. In contrast to immature spermatozoa, mature hyaluronan-bound spermatozoa have a reduced number of apoptotic markers such as caspase-3 **(Cayli *et al*., 2004),** decreased DNA fragmentation (DF) **(Yagci et al., 2010),** proper expression of heat shock protein A2 (HspA2) **(Huszar *et al.*, 2007),** and a lower frequency of chromosomal aneuploidies **(Aitken *et al.*, 1994).** As a result, hyaluronan-binding assay (HBA) is thought to be a good strategy for sperm selection and ICSI fertilization rate improvement.

A few studies compared PICSI and ICSI to see which method was best and how it affected blastocyst formation. **Ní Dhuifin *et al*** ( ) reported that HAB-sperm selection for in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) improves clinical outcomes or reduces miscarriage rates zzcsz In cases of male factor infertility, another authors showed that PICSI produces higher-quality embryos, higher fertilization rates (85.26% vs. 71.33%), and higher clinical pregnancy rates (66% vs. 40.67%) than ICSI (**Hassan *et al.*, 2022). Hasanen *et al.*, (2020**) found that PICSI and Magnetic Activated Cell Sorting (MACS) are efficient techniques for sperm selection in cases with abnormal sperm DNA fragmentation. However, MACS is preferred when females are younger than 30 years, while PICSI is preferred in older females **(Hasanen *et al.*, 2020).**

Therefore, the aim of this study to investigate the effect of selected sperm from PVP (ICSI) and sperm slow media (PICSI) on embryo development and blastocyst formation and to improve ICSI outcomes.

MATERIALS AND METHODS

The present study is a prospective cohort clinical trial during the period from November 2019 to October 2020. It was conducted at El-Gezera Hospital for IVF/ICSI, Egypt. The study included 100 infertile women in enrolled for IVF /ICSI cycle, age range from 25 to 40 years old, who were invited to participate in their fresh embryo transfer study.

The patients were given a gonadotropin-releasing hormone (GnRH) agonist, "Decapeptyl," 0.1 mg subcutaneously (Ferring, Germany). The dose of gonadotropin was administered according to the ovarian response of age and BMI and was modified based on the follicular response. Oocytes were

collected 34–36 hours after triggering with 10.000 HCG and were assessed for oocyte maturity.

By masturbation semen samples were collected and left to liquefied for 20 minutes at room temperature into a sterile cup (BD falcon sterile sample containers from VGDUSA) and semen samples were centrifuged and layered over 'sperm wash' media (Origio, Denmark). The swim-up method was used to capture the best motile spermatozoa. Semen morphology, concentration, and motility were evaluated using an inverted microscope (Olympus, Japan ix 70) according to World Health Organization (WHO) guidelines from 2010.

Sibling oocytes were randomly assigned to be fertilized with normal ICSI or HA-ICSI after retrieval. In an ideal method, each fertilization method would receive an equal number of oocytes. Sperm cells were placed in a PVP-containing medium (Origio, Denmark) for ICSI and selected using morphologic and motility parameters using a conventional light microscope. Specified spermatozoa were injected into the cytoplasm of oocytes. We used a commercial medium containing HA for PICSI (SpermSlow; Origio, Denmark). It is expected that spermatozoa expressing HA receptors were observed moving slowly, non-progressively, and with their tails beating in this viscous medium. A pipette was used to add a 1-5 µL droplet of prepared spermatozoa to a 5-10 µL droplet of medium in the plate. Slowed spermatozoa were observed in the plate after 15 minutes of incubation at 37 C under oil (Irvine Scientific, Santa Ana, USA). For cleavage stage embryos, the Society for Assisted Reproductive Technology (SART) was used, while for blastocysts, Gardner's scales were used.

A value of more than 5 IU/ml in serum HCG was defined as a positive pregnancy 14 days following oocyte retrieval. An ultrasonography image of the gestational sac was used to diagnose clinical pregnancy. A viable pregnancy was defined as one with a gestational age of more than 7 weeks with ultrasound evidence of fetal heart activity.

Sample size calculations

A power calculation estimated that to defect an effect size of 3.5% difference in pregnancy rate between the studied groups, with a p-value <0.05 and 80% power, confidence level 0.95, a sample size of 39 patients in each group was needed (78 patients total). However, 100 patients were attempted in this research work to avoid a non-response rate. This was calculated using G power 3.1 (**Hsieh *et al.*, 1998).**

Consent

Informed consent was taken from participants before the implementation of the study.

Statistical analysis

Cross tabulation was used for comparing categorical variables using the Chi-Square test. Quantitative variables were expressed as the Median (IQR) and qualitative variables were expressed as the frequency and percentage. The Mann–Whitney U test was used for comparing continuous data. The correlation between continuous variables was estimated by the Spearman's rank correlation coefficient. The results were accounted statistically significant at a P-value ≤0.05. Statistical analyses were carried out using SPSS, version 25.0.

RESULTS AND DISCUSSION

Intracytoplasmic sperm injection (ICSI) has been regularly employed in assisted reproduction technique (ART) cycles for over 20 years **(Kupka *et al.*, 2014).** ICSI has effectively treated male factor infertility (**Avalos-Durán *et al.*, 2018),** but ICSI bypasses some natural fertilization check-points and some processes deviate significantly from the physiological process **(Parmegiani *et al.*, 2012)**, various concerns about the technique's safety may arise **(Oehninger, 2011).** ICSI outcomes are being improved by isolating mature, structurally intact, and non-apoptotic spermatozoa with excellent DNA integrity, according to current research **(Medicine, 2015).**

In the present study, we compared an alternate product that incorporates hyaluronate, a chemical occurring naturally in the reproductive system, with the selected sperm from PVP (ICSI). The present study included one hundred patients, fifty cases for PICSI and fifty cases for ICSI. The mean female age in PICSI and ICSI groups was 33(28.75-39) and 33(28.75-39), respectively, with no statistically significant difference (P = 0.409).

There is no statistically significant difference in sperm concentration, sperm motility, or abnormal forms of sperms in cases included in PICSI compared with cases included in ICSI **(**Table 1).

Table 1: Male profile

Variable	**PICSI**	**ICSI**	**P-value**
	Median (IQR)		
Sperm concentration (M)	27(9.75-45.75)	25(15-35)	0.876
Sperm motility (%)	25(10-46.25)	22(13.75-40)	0.501
Abnormal forms of sperm (%)	90(85-90)	90(85-90)	0.742

ICSI: Intracytoplasmic sperm injection; PICSI: physiological intracytoplasmic sperm injection; The Mann–Whitney U test was performed for continuous variables.
IQR, interquartile range.

In addition, there was no significant difference in egg number, fertilized eggs, and fertilization rate in cases included in PICSI compared with cases included in ICSI. Similar results from Miller et al. showed that the exploratory endpoints of fertilization showed no differences between groups **(Miller *et al.*, 2019**). However, an inconsistent study with the good result found that injecting oocytes with HA-chosen spermatozoa resulted in a greater fertilization rate **(Nasr-Esfahani *et al.*, 2008).**

In the present study, the number of embryos transferred was similar in both groups, as was the cleavage rate and implantation rate in the two groups. However, there was a significant difference between the PICSI and ICSI groups in blastocyst rate and grading. Although it was extracted that sperm picked using the HA approach before ICSI had a higher predictive value for forming competent embryos, assisting in treatment optimization **(Balaban *et al.*, 2003).**

Dissimilarly, HA-containing medium in ICSI cycles showed that fertilization and cleavage rates were advanced (**Van den Bergh *et al.*, 2009)**. Selection of HA bound sperm shows advantages in terms of fertilization and following embryo cleavage in ICSI cycles, as it unverified our outcomes (**Parmegiani *et al.*, 2010).** Several studies have been conducted (**Worrilow *et al.*, 2007;Worrilow *et al.*, 2010**). Because of the increased intensity of advanced maturity and genetic integrity associated with hyaluronan bonded (HB) sperm, using HB sperm in ICSI may help to improve paternal input to the embryo and hence clinical results **(Javed *et al.*, 2015).**

Table 2: **Female profile and clinical outcomes**

Variable	PICSI	ICSI	P-value
	Median (IQR)		
Eggs number	13.5(7.75-19)	13(9-18.5)	0.522
Fertilized eggs	8.5(5-13.25)	10(6-12)	0.399
Fertilization rate	80(66.67-88.76)	80(71.07-95)	0.399
Blastocyst formation (N.)	4(2-8)	2(0.75-4)	0.001**
Cleavage rate	80(70.24-88.76)	81.67(72.61-97.75)	0.320
Blastocyst rate	60(50-70.35)	33.33(15.62-57.85)	P<0.000**
Embryo transfer	2(2-3)	2(2-3)	0.137
Pregnancy status	25 (50)	20 (40)	0.315
implantation rate	25 (50)	20(40)	0.315
Pregnancy continuation	22 (88)	13(65)	0.113

ICSI: intracytoplasmic sperm injection; PICSI: physiological intracytoplasmic sperm injection; The Mann–Whitney U test was performed for the continuous variable.

IQR, interquartile range.

.**Significant at the 0.01 level (2-tailed).

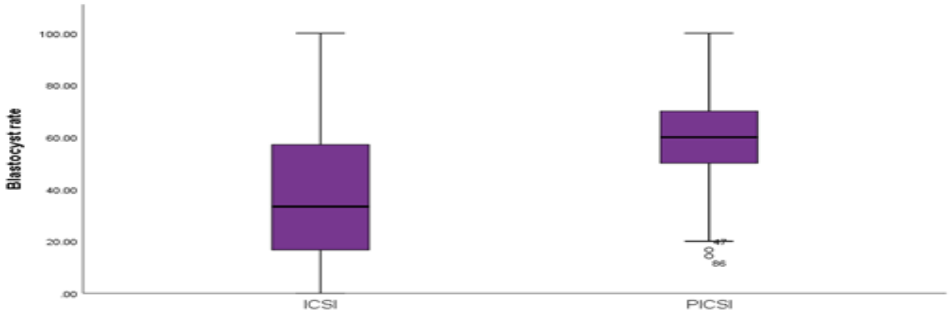

Figure 1: Boxplot of Blastocyst rate show difference between two techniques.

The median and IQR of blastocyst rate in PICSI and ICSI are 60(50-70.35) and 33.33(15.62-57.85), respectively with highly significance difference. The median blastocyst formation in PICSI and ICSI was 4(2-8) and 2(0.75-4), respectively, P<0.001**. There is a high blastocyst rate in PICSI cases 60(50-70.35) as compared with ICSI cases 33.33(15.62-57.85), P<0.000**.

Consequently, by increasing blastocyst formation, the probability of fertilized eggs is higher, as a result of which the pregnancy rate will increase. And this appears in Table 3. There is a high positive correlation between blastocyst formation and fertilized eggs, (r=0.738, P<0.000) (Figure 3), (r=0.775, P<0.000), respectively.

Table 3: Correlation between variables group in two techniques

Techniques			**Age**	**Fertilized eggs**	**Blastocyst formation (number)**
ICSI	Age-	R	1	-.382	-.564
		P-value		.006**	.000**
	Fertilized eggs	R	-.382	1	.438
		P-value	.006**	.438	.001**
	Blastocyst formation (number)	R	-.564	.001**	1
		P-value	.000**	.000	
PICSI	Age	R	1	-.279	-.373
		P-value		.049*	.008**
	Fertilized eggs	R	-.279	1.000	.892**
		P-value	.049*	.	.000
	Blastocyst formation (number)	R	-.373	.738	1.000
		P-value	.008**	.000**	.

ICSI: Intracytoplasmic sperm injection; PICSI: physiological intracytoplasmic sperm injection.
**. Correlation is significant at the 0.01 level (2-tailed).

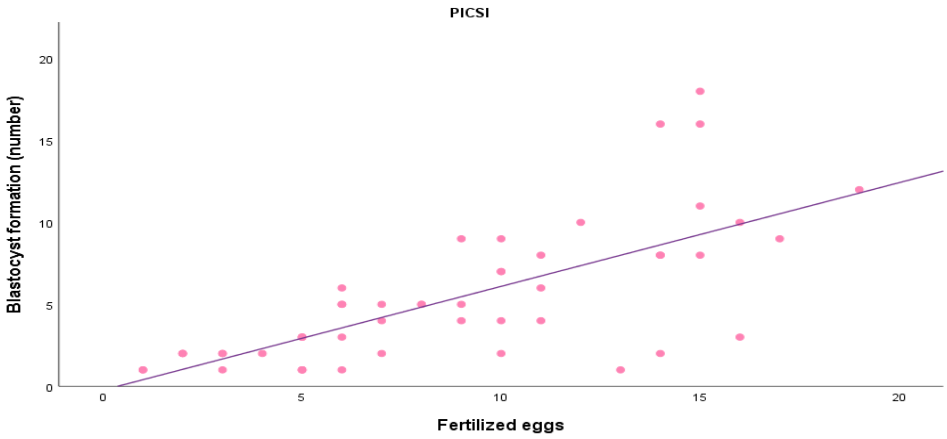

Figure 3: Strong positive correlation between fertilized eggs and blastocyst formation (r=0.738), with increase in fertilized eggs, there is highly increase in blastocyst formation in PICSI group.

There is low negative correlation between age and blastocyst formation, age and fertilized eggs (Figure 4). The results display a median difference in blastocyst rate in PICSI cases 63.15(55.27-69.78) compared with ICSI cases 53.57(33.33-88), P<0.000. PICSI 7(3.5-9.5) has a higher median of blastocyst formation than ICSI cases 4(2-9), P<0.0001 (Table 4).

Table 4: Comparison between factors affects clinical pregnancy in PICSI and ICSI

Variable	Clinical pregnancy Median (IQR)		P-value
	PICSI (25)	ICSI (20)	
Sperm morphology	88(85-90)	88(85-90)	0.732
Sperm concentration (M)	30(15-50)	30(21.25-45)	0.435
Sperm motility (%)	35(17.5-50)	27.5(20-43.75)	0.630
Age	30(25-33.5)	28.5(26.25-32)	0.936
Egg number	19(11.5-22)	18(12.25-20)	0.680
Fertilized eggs	10(6-14.5)	10(6.25-15.75)	0.792
Fertilization rate	81.82(73.21-87.71)	86.11(68.75-93.06)	0.417
Blastocyst rate	63.15(55.27-69.78)	53.57(33.33-88)	0.233
Cleavage rate	86.94(68.75-99.25)	81.82(73.21-87.71)	0.277
Blastocyst formation	7(3.5-9.5)	4(2-9)	0.165
Embryo transfer	2(2-3)	2(2-3)	0.936

ICSI: intracytoplasmic sperm injection; PICSI: physiological intracytoplasmic sperm injection; IQR, interquartile range .

**. Significant at the 0.01 level (2-tailed).

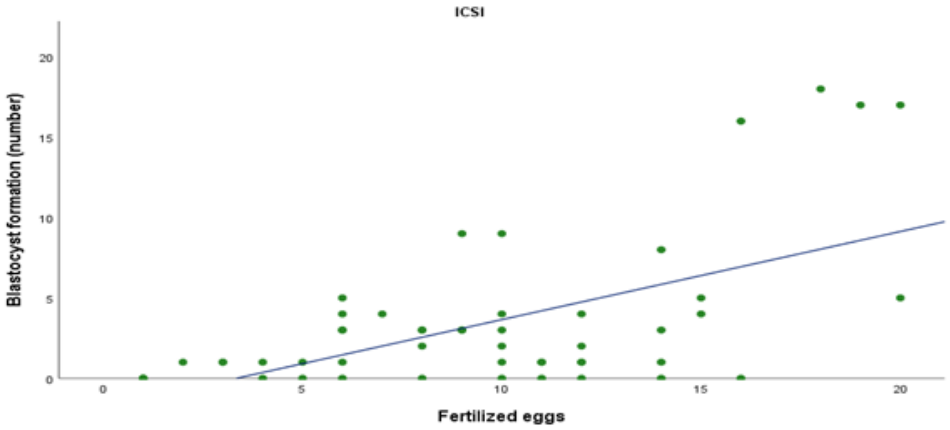

Figure 4: Moderate positive correlation between fertilized eggs and blastocyst formation (r=0.438), with increase in fertilized eggs, there is highly increase in blastocyst formation in ICSI group.

The present data exhibits a higher pregnancy percentage in PICSI cases (25 (50)) compared with ICSI cases (20 (40)) with significance, P=0.315. Our results show higher pregnancy continuation in PICSI cases (22 (88)) compared with ICSI cases 13(65) and a higher miscarriage rate in ICSI cases (7(35)) compared with PICSI cases (3 (12)), P=0.113 (Table 2). This is in line with the findings of **(Azevedo *et al.*, 2013; Lee *et al.*, 2013),** who reported statistically significant differences when comparing PICSI vs. ICSI in terms of miscarriage results and, in the case of **Lee *et al.* (2013),** implantation and clinical pregnancy **(Azevedo *et al.*, 2013; Lee *et al.*, 2013).** These results were in line with the results of a previous study that found a trend toward lower abortion rates in cases when the HA system was employed for sperm selection **(Majumdar & Majumdar, 2013).** In the Worrilow et al. investigation, a statistically significant difference supported PICSI **(Worrilow *et al.*, 2013).** But the results were approved by other researchers. There was no evidence of treatment effects on miscarriage rates being different depending on hyaluronan–sperm binding scores, mother age, or previous miscarriage. That means PICSI does not significantly improve term live birth rates when compared to ICSI **(Miller *et al.*, 2019).**

The median and IQR of pregnancy rates in PICSI and ICSI are 25 (50%) and 20 (40%), respectively with highly significant differences.

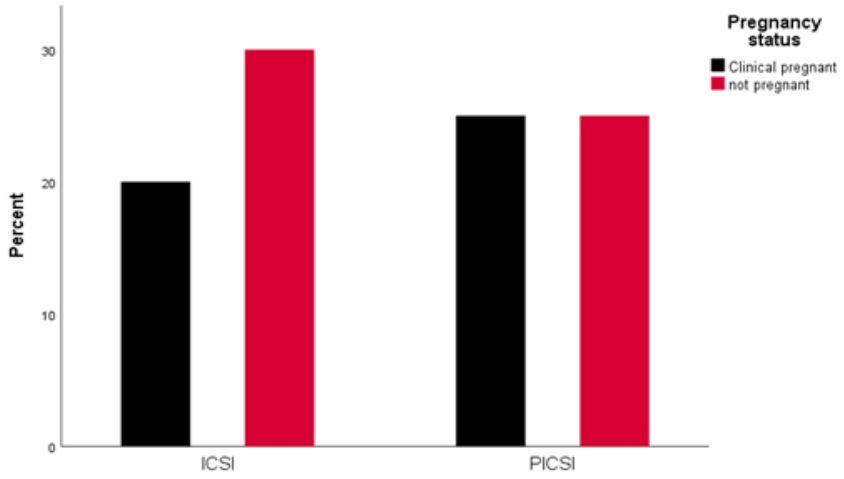

Figure 2: Graph showing pregnancy rate in two techniques

In several research studies, the predictive value of hyaluronic acid binding assays on the outcomes of reproductive treatments has been investigated in several ways. The findings on the rate of fertilization and the quality of the embryos have been inconsistent with our results but consistent with the pregnancy rate **(Nijs *et al.*, 2009; Kovacs *et al.*, 2011; Breznik *et al.*, 2013)**. ICSI with hyaluronan selected sperm physiological ICSI [PICSI] enhanced embryo quality and live birth rates while lowering miscarriage rates, according to several minor clinical studies, including three randomized trials **(Parmegiani, *et al.*, 2012; Mokánszki *et al.*, 2014).**

Miscarriage may be linked to the use of PVP to inject sperm during human ICSI. ICSI with sperm immobilization without PVP had a high rate of clinical pregnancy **(Kato & Nagao, 2012).** In a study by McDowell et al., in the PICSI group, the proportion of couples with clinical pregnancies who miscarried was lower than in the normal ICSI group **(McDowell *et al.*, 2014).** In a relatively similar study, the abortion rate was higher in the ICSI group than in the PICSI group but not significant. The PICSI group had significantly higher clinical pregnancy rates **(Erberelli *et al.*, 2017)**. In terms of clinical pregnancy, implantation, embryo quality, fertilization, and miscarriage, there is no statistically significant difference between PICSI and ICSI as shown by other researchers **(Avalos-Durán *et al.*, 2018).**

DNA damage and aneuploidy in sperm are lower than have been selected for hyaluronan **(Miller *et al.*, 2019).** The same authors added that the use of hyaluronan-based sperm selection for ICSI (physiological ICSI [PICSI])

has been shown to lower the number of miscarriages, but there was no difference in the live birth rate between PICSI and ICSI**.**

The present results matched with a previous report that demonstrated that in patients with aberrant sperm DNA fragmentation, clinical outcomes such as implantation, clinical pregnancy, and continued pregnancy rates improved significantly **(Hozyen *et al*., 2021).**

Hyaluronic acid (HA) is important in the sperm selection system in nature because only mature spermatozoa that have extruded their particular receptors to bind to HA can reach and fertilize the oocyte. In vitro, the role of HA as a "physiological selector" is well understood. It has been shown that spermatozoa that can bind to HA in vitro have completed plasma membrane remodeling, cytoplasmic extrusion, and nuclear maturation (Huszar et al., 2007), and it can be digested by the oocytes due to its natural origin (Balaban et al., 2003). In agreement with the present study, a study by Castillo-Baso (2011) appeared that patients with aberrant sperm parameters were included in the study, and it was predicted that sperm selection utilizing HA-binding could be beneficial **(Castillo-Baso *et al*., 2011).**

The HA-binding approach selects spermatozoa with better internal and exterior structure (**Prinosilova *et al*., 2009).** Because of its ability to prevent genetic issues, HA should be viewed as a viable method for "physiologic" sperm selection before ICSI **(Parmegiani *et al*., 2010),** and another advantage was proved by Javed et al. PICSI dishes are typically used to select sperm when sperm binding is less than 65 percent **(Javed *et al.*, 2015).**

The present study demonstrated that the PICSI group compared with the ICSI group has a higher blastocyst rate of 60 (50-70.35) and 33.33(15.62-57.85), clinical pregnancy 25(50) and 20 (40), a lower miscarriage rate 3 (12) and 7(35), a higher rate of pregnancy continuation 22 (88) and 13(65). Finally, the HA binding technology for sperm selection can be applied in the laboratory and can replicate a "physiologic" choice of the male gamete at a low cost, minimizing the risk of genetic problems. Furthermore, when PICSI is used instead of normal ICSI, more blastocysts are available for vitrification, and patients using PICSI have significantly higher ongoing pregnancy rates. When PICSI is used instead of ICSI, the reproductive outcomes are better.

CONCLUSION

In comparison to the ICSI group, the PICSI group had a higher rate of blastocyst formation and clinical pregnancy, a lower rate of miscarriage, and a higher rate of pregnancy continuation. A cost effectiveness analysis may be needed for PICSI universal use. Finally, PICSI may be implanted in all

IVF/ICSI a due to achieve a higher blastocyst formation rate and clinical pregnancy rate will reasonable added costs.

Conflicts of interest

The authors declare no conflicts of interest.

Funding information

This work received no specific grant from any funding agency.

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مقارنة بين الحقن المجهري الفسيولوجي والحقن المجهري التقليدي وتأثيره على تكوين الكيسة الأريمية ، تجربة عشوائية مستقبلية

آيات سمير كامل [١] ، مؤمن احمد محمد كامل [٢] بهجت عبد الغفار الفقي [١]
[١]قسم البايوتكنولوجيا الحيوانية ، معهد بحوث الهندسة الوراثية والتكنولوجيا الحيوية ، جامعة مدينة السادات ، مدينة السادات ، مصر.
[٢]قسم أمراض النساء والتوليد ، كلية الطب ، جامعة أسيوط ، مصر

الملخص:
إختيار الحيوانات المنوية الناضجة الذي تحتوي علي أقل نسبه في تكسير المادة الوراثية أهم العوامل التي يعتمد عليها تكوين أفضل أجنه تؤدي إلي حدوث الحمل، حيث أن المرضي الذين يعانون من تكسير المادة الوراثية يؤدي هذا الي تأخر في حدوث الحمل الطبيعي، فشل عمليات الحقن المجهري، وأيضا تكرار الإجهاض.
تمت هذه الدراسة على ١٠٠ مريض، منهم ٥٠ مريض خاضعين للحقن المجهري الفسيولوجي ويتم ذلك بأستخدام وسط غذائي يحتوي علي حمض الهيالورنيك يمكن من خلالة معرفة الحيوان المنوي الأقل في تكسير المادة الوراثية ذلك لتحسين جودة الأجنة وحدوث الحمل، و٥٠ مريض خاضعين للحقن المجهري التقليدي، تقل أعمار السيدات عن ٣٩ سنة، وكان للذكور ذات نسبة أكبر من ٣٥ % في تكسير المادة الوراثية هو العامل الأساسي في الدراسة.

- وقد وجد أنه لا يوجد فروق احصائية بين المجموعتين في كلآ من عدد البويضات والبويضات المخصبة ومعدل الإخصاب مقارنة بمرضى الحقن المجهري التقليدي ولا يظهر فرق كبير بينهم في معدل الإنقسام.
- كما اوضحت ان زيادة نسبة المرضي الحوامل الخاضعين للحقن المجهري الفسيولوجي كان لديهم دلالة احصائية مع كلآ من تكوين الكيسة الأريمية، زياده نسبه الإنغراس، ولديهم نسبة عالية في اكتمال الحمل بنسبه (٢٢(٨٨%))، مقارنة بحالات الحقن المجهري التقليدي (١٣ (٦٥%)) ومعدل إجهاض مرتفع في حالات الحقن المجهري التقليدي (٧ (٣٥%)) مقارنة بحالات الحقن الفسيولوجي (٣(١٢%)).

التوصيه: توصي هذه الدراسة إلي إستخدام الطرق الغير تداخليه لإختيار أفضل الحيوانات المنوية الأقل نسبة في تكسير المادة الوراثية بإستخدام تقنية الحقن المجهري الفسيولوجي في حالات الإجهاض المتكرر وفشل محاولات الحقن المجهري.