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Novel Method to Improve In vitro fertilization (IVF) and Intracytoplasmic sperm injection (ICSI) outcomes

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ABSTRACT

Hyaluronan is a constituent of the cumulus oophorous complex, which envelops the oocyte. It is widely acknowledged as a pivotal marker for the quality and maturity of sperm. For male factors that have a high DNA Fragmentation Index (DFI), those having in vitro fertilization (IVF) or Intracytoplasmic sperm injection ICSI, those who have repeated miscarriages because of male factors, and those who lose their pregnancies early because of male factors. The current study's robust result demonstrates the effectiveness of the HA (Physiological intracytoplasmic sperm injection PICSI)) selection system. Based on available and historical data, we can surmise that sperm chosen using the HA technique prior to ICSI offer a higher predictive value for developing viable embryos, thereby aiding in the optimization of treatment outcomes. The HA binding method for sperm selection can be used in routine laboratory settings to boost conception rates and lower the risk of genetic issues by inexpensively replicating a physiologic choice of the male gamete. Clinic abortion rates have been successfully lowered by 50% with the use of this procedure for patients with oligo-asthenoteratozoospermia and for couples who experienced consecutive implantation failures. The primary outcomes of this research are an increase in the quantity of blastocysts extracted each cycle, the quantity of Grade A embryos, the rate of cleavage, the quality of the embryos, the rate of implantation, and ideally the rate of pregnancy. It can be inferred that sperm selected using the HA approach before ICSI had a better chance of becoming viable embryos. The employment of HA (PICSI) media techniques in ICSI led to higher rates of fertilization and pregnancy because they help to minimize sperm with aberrant morphological flaws and promote motility, notably progressive motility. This study's recommendation is to use hyaluronan when selecting spermatozoa to produce high-quality embryos with high pregnancy rates.

1. Introduction

Infertility occurs when a couple tries for a year without using contraception and still is unable to conceive. Due to its tendency to impact two people rather than one, infertility is an uncommon medical disease [1]. The best chances of becoming pregnant naturally are often found in women under 30 who have never given birth, have had infertility for no more than two years, do not have endometriosis, tubal disease, anovulation, or infertile partners [2]. Infertility affects 8% of males who are of reproductive age [3]. Male infertility has been studied using a simple laboratory test called semen analysis. According to Esteves et.al. [4], it looks at parameters like volume, pH concentration, motility, morphology, and the quantity of leukocytes.

High rates of DNA damage are generally linked to infertility and low semen parameters [5]. Dulama et.al [6] reported evidence of a connection between abnormal sperm motility and morphology and DNA damage. These elements may make it more difficult for sperm cells with damaged DNA to fertilize, decrease the viability of the resulting embryo, and raise the risk of abortion. Several studies have found that sperm had aberrant morphologies and DNA damage [7]. Unknown causes of infertility account for about 25% of cases [8]. Even after multiple IVF treatments, a very small percentage of people are unable to conceive. Recurrent implant failure (RIF) has an unknown cause. The quality of the embryo and sperm selection can be enhanced by a variety of techniques, such as: i. apoptosis (glass wool and magnetic cell sorting); ii. surface charge (electrophoresis and zeta potential); iii. membrane maturity (HA binding); and iv. ultramorphology (high magnification). By using the previously described techniques, higher quality sperm can be selected [9]. Younger patients experiencing repeated IVF failures were more likely to have male factor infertility, which is defined by a low intracytoplasmic sperm injection (ICSI) fertilization rate [10]. Compared to other cutting-edge sperm selection techniques, hyaluronic acid (HA)-containing media for ICSI have been shown to offer better specificity and reduced biological risk [10]. It has been proposed that sperm selection with HA could boost the implantation rate in ICSI cycles since spermatozoa's ability to bind HA is related to sperm membrane development and fertilizing

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potential [11]. Horta et.al [12] described hyaluronic acid (HA) as a highly hydrophilic and anionic polysaccharide consisting of covalently bound D-glucuronic acid and N-acetyl glucosamine repeating disaccharide units. Hyaluronic acid (HA) envelops human oocytes in the natural environment and consequently aids in the process of sperm selection. In actuality, the egg can only be fertilized by mature spermatozoa that have extruded their unique receptors to bind to and digest HA. It has been found that spermatozoa can bind HA in vitro when they have finished nuclear maturation, cytoplasmic extrusion, and plasma membrane modification.This validates HA's function in vivo as a "physiologic selector" [13]. Furthermore, these spermatozoa associated with HA exhibit minimal levels of chromosomal aneuploidies, excellent nuclear morphology, and DNA fragmentation [11]. Therefore, choosing HA-sperm before intracytoplasmic sperm injection (ICSI) contributes to improving the therapeutic result [14].

This study sought to determine whether sperm selection using hyaluronic acid (HA) in cases of azoospermia could enhance the number of blastocysts retrieved per cycle, the quality of the embryos, the rate of fertilization, and, ideally, the rates of implantation and pregnancy in intracytoplasmic sperm injection (ICSI) cycles.

2. Materials And Methods

2.1. Study Design

In this experiment, 200 patients were categorized and sorted according to their male companion in the following ways: Testicular sperm samples from the control group were prepared using a traditional washing technique, while the study group's testicular sperm samples were prepared using a hyaluronan method based on mature sperm separation. All subjects were referred to the Fertility Clinic at the International Islamic Center for Population Studies and Research, Al-Azhar University, Cairo, Egypt, for assisted reproduction Additionally, the study was conducted from November 2020 to April 2021 and was given the go-ahead by the Al-Azhar University Faculty of Medicine in Cairo, Egypt's Quality Education Assurance Unit (REC number: 0000038). All patients underwent an ICSI-TESE cycle after being educated about the process and providing written consent. All male patients with azoospermia underwent testicular biopsy, whereas all female partners underwent the same surgery. A female element is one of the inclusion requirements. Males tend to be between 25 and 40 years old. absence of a history of persistent sickness

2.2. Sperm Sample

Traditional method of extracting testicles from the vaginal tunica and gently incising small protruding portions (50-750 mg of testicular tissue) over 0.5-1 cm in the avascular regions of the top, middle, and lower anterior surface of the albuginea tunica under mild testicular pressure is how testicular sperm extraction is done. A tiny portion of testicular tissue is removed during an open surgical procedure. Through a ½-inch incision, a little amount of testicular tissue is removed and placed in culture media. The sperm-containing seminiferous tubules are subsequently extracted [15].

2.3. Traditional methods for Sperm preparation

Mix the sample well and dilute the entire 1 + 1 (1:2) with the supplemented medium. After diluting the suspension, distribute it across numerous centrifuge tubes. After 5–10 minutes of centrifuging at a speed of 300–500 g, gently remove and discard the supernatants. Return the pellets with gentle pipetting to one milliliter of supplemented media. For three to five minutes, centrifuge the mixture at 300–500 g. Aspirate with caution, then discard the supernatant. Shake the sample well. Next, dilute the entire sample 1 + 1 (1:2) using the supplemented medium. Divide the suspension among several centrifuge tubes after it has been diluted. After 5–10 minutes of centrifuging at 300–500 g, remove and discard the supernatants with caution. Return the pellets with care into one milliliter of enriched media using a pipette. The mixture should be centrifuged at 300–500 g for 3–5 minutes. Aspirate the supernatant carefully and discard it. Droplets of testicular cell suspension, ten microliters of HEPES-buffered growth media, and one droplet of 10% polyvinylpyrrolidone (PVP; Sigma) were added to the injection plate.Covering the plate was filtered mineral oil (Sigma) that had undergone embryo testing [16].

2.4. Innovative technique utilizing mature sperm separation

After removing the ready-to-use media from storage at a temperature between 2 and 8 °C, let it sit at room temperature for 10 minutes. With extreme caution, the pipette creates a junction between the media central drop and the sperm drop by adding 5 µl of previously prepared and cleaned sperm near the two 10 µl drops of ready-to-use media that are positioned at the center and rim of the dish. Incubate for ten to fifteen minutes at 37 °C. After the incubation period, surround the center drop with 5–10 µl of oocyte holding media drops per holding media drop. Two to five millimeters of the sperm preparation medium should be sucked into the injection pipette and ten to twenty millimeters into the holding pipette. After a minute, release the pipettes so that the Mature Sperm Select medium can be aspirated. Take a sample of the mature sperm, about 2-4 mm. Select the material to be added to the injection pipette from the outside rim drop.Select a spermatozoon from the prepared sperm droplet where the mature sperm converges. Select the central drop directly to inject (De Geyter, C., 2021). Classification of Sperm: Mature spermatozoa should wag their tails but not go ahead or advance in order to be selected. The immature spermatozoa should be allowed to move; picking them is not necessary. Human chronic gonadotropin (HCG) was administered to the patient under general anesthesia, and around 34 to 36 hours later, oocytes were aspirated using a transvaginal ultrasound (US) to obtain human cumulus cells (Labotect aspiration pump, Germany). Using a single lumen, 17-gauge oocyte pick-up needle, folicles were aspirated at a negative pressure of 115–120 mm Hg. Follic fluid was aspirated into sterile, fourteen milliliter tubes. Using a Zeiss Stemi 2000-C stereo microscope, the oocyte and cumulus cell complexes were divided. After cleaning them in Gamete Buffer media, they were placed in four dishes with Fertilization Medium (Cook, Limerick, Ireland) and incubated for approximately half an hour at 37 oC with 6% CO2 (C60, Labotect, Germany). The oocyte underwent denudation by being incubated in a 100 µl drop of buffered hyaluronidase enzyme solution containing 80 IU/ml for 30 to 45 seconds. The oocyte was then removed and placed in a 100 µl drop of Gamete Buffer medium. Ultimately, a sterile drawn pipette was used to collect the corona cells, and the oocyte was gently aspirated. After denudation was finished, the oocyte was placed into injection plates with 10 µl microdrops of the

fertilization media and washed with Cook's Gamete Buffer (Limerick, Ireland). Eventually, the injection dishes were filled with 3ml of sterile, equilibrated mineral oil. The oocyte was quickly rated for maturity and quality using an automated manipulator, a heated stage, and an inverted microscope equipped with Hoffman optics (Olympus 1x71, Japan) in compliance with the grading technique (Narishige, japan). Mature phases being recorded were prophase I, metaphase I (MI), metaphase II (MII), and post-maturity. The naked oocytes were cultivated in a medium containing 6% CO2 at 37 ºC before to the ICSI procedure [17].

2.5. Procedure of ICSI:

Samples were incubated until injection time following the previously described sperm preparation and semen analysis. Each oocyte received an injection of one immobilized, morphologically normal spermatozoon in polyvinyl pyrolidone (PVP) (Irvine, USA). We examined and evaluated each sperm that had ICSI. A sterile dish was used for the injection procedure, which involved holding a pipette and using an injection needle. After following Van Steirteghem's protocol, an intracytoplasmic sperm injection was performed.[18] 2020). After injection, the oocyte was washed and put in a culture plate with sterile, warm, equilibrated global oil under global total media (Life Global, Europe). The culture dish was maintained until fertilization at 37 ºC, 6% CO2, and 90–95% humidity. A quality assessment was carried out 17 hours after microinjection to evaluate the embryo and fertilization. We examined the injected oocytes for signs of damage and pronuclei. The oocytes were said to have fertilized if there were two pronuclei (2PN) and the second polar body had been extruded [19]. For the purpose of embryo grading and transfer, each embryo's cell count and morphology were graded around 72 hours after injection based on the percentage of nucleate fragments and blastomeric size equality. Subsequently, the best day-3 (Grade 1) embryos were transferred to the uterus in 30µl of Global medium (Life Global, Europe) with 10% human serum albumin (HSA) using an embryo transfer (ET) catheter from Labotect in Germany. 48–72 hours following the eggs' retrieval [20]. A follow-up was conducted with the following in mind: rate of fertilization. 2. Rate of cleavage. - Pregnancy rate based on embryo grade. Assessment of fertilization and embryonic quality: 16–18 hours after microinjection, the effectiveness of fertilization was assessed. We examined the injected oocytes for signs of damage and pronuclei. The oocytes were deemed fertilized if they had two pronuclei (2PN) and the second polar body had been extruded. About 72 hours after microinjection, an appropriate number of embryos were transferred to recipient persons. embryo transfer and grading About 72 hours after injection, each embryo's cell count and shape were rated using a grading system [21].

2.6. Statistical analysis:

The primary features of patients are described using tabular representation. Descriptive statistics of the variables taken into account, such as mean and standard deviation (S.D.), may also be included in single or cross-frequency tables. To ascertain if the observed variations between two means or two proportions (percentages) were noteworthy, significance testing was employed [22].

3. Results

In table (1), we found that the incidence of abnormal forms was lower in the Hyaluronan ICSI-TESE group (77.5 ± 1.1) than in the Traditional ICSI-TESE group (88.6 ± 1.3) when testicular sperm morphological analysis was compared between the study groups. These differences were highly statistically significant ($P \leq 0.001$). The incidence of head abnormalities was 75.8 ± 5.5 in the Hyaluronan ICSI-TESE group and 80.4 ± 5.8 in the Traditional ICSI-TESE group. The statistical significance of these differences was high ($P < 0.001$). Midpice defects were more common in the Hyaluronan ICSI TESE group (56.8 ± 7.2) than in the Traditional ICSI TESE group (62.3 ± 7.8), and the differences were statistically significant ($P < 0.001$). Tail faulty morphology was present in 14.3 ± 5.3 cases in the Hyaluronan ICSI-TESE group and 22.6 ± 5.4 cases in the Traditional ICSI-TESE group. These variations also met the statistical significance threshold at ($P < 0.01$).

Table 1: Morphological analysis of testicular Sperm Among male patients.

Parameters	Traditional ICSI –TESE (N=100)	Hyaluronan ICSI-TESE (N=100)	*P*-value
	Mean ± SD	Mean ± SD	
Unusual forms	88.6 ± 1.3	77.5 ± 1.1	≤ 0.001*
Head abnormalities	80.4 ± 5.8	75.8 ± 5.5	≤ 0.001*
Midpice defects	62.3 ± 7.8	56.8 ± 7.2	≤ 0.001*
Tail defective	22.6 ± 5.4	14.3 ± 5.3	≤ 0.01*

Data in Table 2 delineated the overall characteristics of the female partners. Specifically, there was a statistically significant difference ($P \leq 0.01$) in the mean duration of infertility between the Hyaluronan ICSI-TESE group (7.8 ± 2.3) and the Traditional ICSI-TESE group (6.8 ± 1.4). The incidence of harvested oocytes did not differ statistically (P > 0.1) between the Hyaluronan ICSI-TESE group (918.0 ± 3.1) and the Traditional ICSI-TESE group (913.0 ± 3.1). The incidence of mature oocytes in the Hyaluronan ICSI-TESE group was 816 ± 1.1, whereas the Traditional ICSI-TESE group had an incidence of 810.0 ± 1.0. These variations, however, did not reach statistical significance ($P > 0.1$). The incidence of fertilized oocytes in the Hyaluronan ICSI-TESE group was higher at 620.0 ± 1.5 compared to 515.0 ± 2.1 in the Traditional ICSI-TESE group; these differences were statistically significant ($P \leq 0.01$).

Table 2: Overall traits of the 200 female partners:

Parameters	Traditional ICSI –TESE (N=100)	Hyaluronan ICSI-TESE (N=100)	P value
	Mean ± SD	Mean ± SD	
Infertility/years	6.8 ± 1.4	7.8 ± 2.3	≤ 0.01*
Collected number	913.0 ± 3.1	918.0 ± 3.1	> 0.1
Mature oocytes	810.0 ± 1.0	816 .0 ± 1.1	> 0.1
Fertilized oocytes	515.0 ± 2.1	620.0 ± 1.5	≤ 0.01*

The comparison of fertilization and cleavage rate between the study groups was shown in Table 3. Compared to the Traditional ICSI-TESE group (629.9%), the Hyaluronan ICSITESE group exhibited a higher incidence of fertilization rate (713/75.1%) on the first day. A statistical significance was observed ($P < 0.01$) for these differences. A statistically significant difference ($P < 0.01$) was seen in the incidence of cleavage rate on days two and three between the Hyaluronan ICSI-TESE group and the Traditional ICSI-TESE group (599, 97.7% and 590, 98.5%, respectively). Day five indicated a greater incidence of blastocyst formation rate (890-83.1%) in the Hyaluronan ICSI-TESE group compared to the Traditional ICSI-TESE group 310 (65.3%). $P < 0.001$ indicated that these differences were statistically significant. The grading of the embryos throughout the study groups is compared in Table 4. There were 439 (74.4%) more Grade A embryos in the Hyaluronan ICSI-TESE group than in the Traditional ICSI-TESE group (280, 59.6%). $P < 0.001$ indicates that these differences were extremely statistically significant. The incidence of Grade B embryos was lower in the Hyaluronan ICSI-TESE group, at 101 (32.8%). These differences were very statistically significant ($P < 0.001$) compared to 120 (42.9%) in the group receiving Traditional ICSI TESE. The incidence of Grade C embryos was 50 (46.7%) in the Hyaluronan ICSI-TESE group compared to 70 (58.3%) in the Traditional ICSI-TESE group. $P < 0.001$ indicates that these differences were extremely statistically significant.

Table 3: Comparison of the rates of blastocyst and fertilization cleavage among the patients in the studied groups.

Parameters	Traditional ICSI -TESE (N=510)	Hyaluronan ICSI-TESE (N=613)	P-value
	Mean (%)	Mean (%)	
Fertilized rate (D1)	510/810 (62.9%)	613 (75.1%)	≤ 0.01*
Cleavage rate (D2)	480/510 (94.1%)	599 (97.7%)	≤ 0.01*
Cleavage rate (D3)	470/480 (97.9%)	590 (98.5%)	≤ 0.01*
blastocyst rate (D5)	310/475 (65.3%)	490 (83.1%)	≤ 0.001*

Table 4: compares the embryo grading of the patients in the study groups.

Embryo grading	Traditional ICSI-TESE (N=470)	Hyaluronan ICSI-TESE (N=590)	P-value
	Mean (%)	Mean (%)	
Grade A	280 (59.6%)	439 (74.4%)	≤ 0.001*
Grade B	120 (42.9%)	101 (32.8%)	≤ 0.001*
Grade C	70 (58.3%)	50 (46.7%)	≤ 0.001*

In contrast to the Traditional ICSI-TESE group, which had a pregnancy incidence of 43.0%, the Hyaluronan ICSI-TESE group had a pregnancy incidence of 58.0% among the patients in the examined group (Table 5). The differences were extremely statistically significant ($p < 0.01$). Additionally, the results showed the pregnancy rate by ET quality among the groups under study. Pregnancy rates were higher (53.6%) among women who transferred grade A embryos in the Hyaluronan ICSI-TESE group than they were (53.5%) among those who transferred grade A embryos in the Traditional ICSI-TESE group, with a statistically insignificant difference ($p > 0.1$). Women who transferred grade B embryos in the Hyaluronan ICSI-TESE group and those who transferred grade B embryos in the Traditional ICSI-TESE group had different pregnancy rates, but the difference was not statistically significant ($p > 0.1$). The former group had a higher pregnancy rate (18.7%). However, there was a statistically insignificant difference ($p > 0.1$) in the pregnancy rate between women who transferred embryos grade A, b in the Hyaluronan ICSI-TESE group and those who transferred embryos grade A, b in the Traditional ICSI-TESE group. The pregnancy rate among the former was lower, at 27.7%.

The pregnancy rate for each ET in the study group was shown in Table 6. The incidence of pregnancy rate among patients who transferred a single embryo was greater in the Hyaluronan ICSI-TESE group (6.9%) compared to the Traditional ICSI-TESE group (4.7%), with a statistically significant difference ($P < 0.01$). The incidence of pregnancy rate among patients who transferred two embryos was greater in the Hyaluronan ICSI-TESE group (34.5%) compared to the Traditional ICSI-TESE group (32.6%), with a statistically significant difference ($p < 0.01$). The rate of pregnancy among patients who transferred three embryos varied between the Hyaluronan ICSI-TESE group (58.6%) and the Traditional ICSI-TESE group (62.7%), but the difference was statistically insignificant ($p > 0.1$).

Table 5: Pregnancy rate by quality of ET throughout the groups under study.

Embryo quality	Traditional ICSI-TESE (N=43)	Hyaluronan ICSI-TESE (N=58)	P-value
	+Ve Pregnancy	+Ve Pregnancy	
Grade A	23 (53.5%)	31 (53.6%)	> 0.1
Grade B	8 (18. 6%)	11 (18. 7%)	> 0.1
Grade A & B	12 (27.9%)	16 (27.7%)	> 0.1

Table 6: The pregnancy rate among the studied groups (n = 101) in relation to the number of ET.

Embryo transfer (ET) number	Traditional ICSI-TESE (N=43)	Hyaluronan ICSI-TESE (N=58)	P-value
	+ve Pregnancy	+ve Pregnancy	
One embryo	2 (4.7%)	4 (6.9%)	≤ 0.01*
2 embryos	14 (32.6%)	20 (34.5%)	≤ 0.01*
≥ 3 embryos	27 (62.7%)	34 (58.6%)	> 0.1

4. Discussion

Though originally designed to displace the widely used PVP as an agent to lower sperm motility before ICSI, HA's potential as a selective agent was promptly explored [23]. Thus, after selecting boar spermatozoa using HA solution, Patrizio et al. [24] demonstrated a significant increase in embryos

with normal chromosomal counts and a decrease in chromosome abnormalities as compared to regular ICSI. Ji et al. (21) reported that spermatozoa selected by the HA-binding approach have better external and internal structures and show a lower risk of aneuploidy compared to sperm selected using the Hyaluronan-binding system, which shows fragmented DNA. Sperm chromatin compaction increases DNA resilience to environmental stresses and mutations by safeguarding and preserving DNA integrity. Similar results were found in other studies that used HA solution or HA-coated dishes [25;26]. However Horta et al., [12] discovered that those patients undergoing ICSI whose semen had a HA-binding capacity of approximately 65%, as determined by the Hyaluronan binding assay, had a significantly higher rate of live births and pregnancy [27]. This outcome implies that a preliminary semen research is required to evaluate the method's applicability and shows that the process might only be helpful in specific circumstances. Ji et. al., et al. [21] found that use HA-coated plates significantly improved ICSI. Their results also indicated that this strategy might be more advantageous for teratozoospermic individuals. The current study's results were in line with those of Kim et al. [28] who investigated the effects of physiological intracytoplasmic sperm injection (PICSI) chosen by hyaluronan on the quality of cleavage-stage embryos and the frequencies of fertilization in infertile persons. It was discovered that PICSI produced more high-quality embryos than traditional ICSI. Our results, however, were not in line with those of Majumdar and Majumdar [25] who looked at the possibility of injecting HA-bound sperm to treat infertile ICSI patients with normal semen parameters. There was no difference between the ICSI and PICSI groups in terms of fertilization rates, the number of high-quality embryos generated, or clinical pregnancy rates. Although the difference was not statistically significant, the ICSI group did experience a higher pregnancy loss rate than the PICSI group. Furthermore, utilizing the hyaluronic acid binding sperm selection strategy did not result in an increase in the rates of fertilization and pregnancy, as reported by BeckFruchter et al. [29]. Van Den Bergh et al. [30], state that HA is oocyte digestible and does not negatively impact the growth of embryos in culture media [31]. The benefits of the HA binding mechanism for sperm selection seem to be limited to cycles containing a male component. The current investigation's results were in line with those of Parmegiani et .al [11] raising questions about whether hyaluronic acid (HA) plays a role as a "physiologic selector" for spermatozoa prior to intracytoplasmic sperm injection (ICSI). Investigating whether HA sperm selection benefited couples whose ICSI cycles had previously failed was the goal of Scaruffi et al.'s research [32]. The cleavage rate was significantly higher with the second injection of sperm bound to HA (p=0.026) compared to traditional ICSI. He discovered that the selection of HA-bound spermatozoa significantly improved clinical result as compared to additional routine ICSI. The current study's results were consistent with those of Gardner and Balaban [31] when comparing two techniques for sperm selection in ICSI cycles: chemical selection utilizing hyaluronan-treated petri plates (PICSI) and traditional morphological sperm selection (ICSI-PVP). The technique should be included into routine laboratory operations at a low cost to avoid the selection of immature sperm with high rates of peroxidation and DNA fragmentation. Furthermore, the results of a study by Erberelli et.al. [33] evaluating the benefits of a hyaluronan (HA)-based sperm selection process for physiological intracytoplasmic sperm injection (PICSI).

The results of the present investigation aligned with those of Ji et. al., et al. [21], who examined the possibility of enhancing clinical pregnancy rates (CPR), implantation, rates (IR), and pregnancy loss rates (PLR) by selecting sperm for ICSI based on their ability to attach to hyaluronan. To evaluate the impact of enhanced sperm selection techniques on ART outcomes. He came to the conclusion that using sperm that binds hyaluronic acid improves the chances of a live delivery or pregnancy when using assisted reproductive technologies. The results of the present investigation were in agreement with those of Liu et al. [21] who evaluated the effects of sperm selection and intracytoplasmic sperm injection (ICSI) on subsequent fertilization and embryo development by contrasting the use of polyvinylpyrrolidone (PVP-ICSI) injection with hyaluronic acid-based sperm slow (HA-ICSI). The results of the current investigation were in line with those of Ji et. al. [21] who discovered that, in comparison to testicular sperm or sperm processed from people with faulty SDF, PICSI considerably enhanced the embryological and clinical outcomes. The results of the current investigation were in line with those of Ji et. al. [21], who discovered that, in comparison to testicular sperm or sperm processed from people with faulty SDF, PICSI considerably enhanced the embryological and clinical outcomes. The results of the current study were at odds with those of Miller et. al. [34] comparison of PICSI and traditional ICSI in terms of increasing the live birth rate among couples undergoing reproductive therapy.

5. Conclusion

The current study's robust results demonstrate the effectiveness of the HA (PICSI) selection system. Based on available and historical data, we can surmise that sperm selected using the HA technique prior to ICSI have a higher predictive value for developing viable embryos, which can aid in optimizing treatment outcomes. By simulating a "physiologic" choice of the male gamete at a cheap cost, the HA binding technology for sperm selection can be employed in ordinary laboratory settings to increase pregnancy rates and decrease the likelihood of genetic problems. Clinic abortion rates have been successfully lowered by 50% with the use of this approach for patients with oligo-asthenoteratozoospermia and for couples who experienced multiple unsuccessful implantation attempts.

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Author Contributions

All authors contributed to this work. A. Abou El-Ela prepared the samples and completed the experimental measurements. Both A. kandeel,M.Nasr and E. Hassan shared writing and followed the performance of the experiments. M.Nasr complete the experiments work. A. Abou El-Ela with E. Hassan completed the paper writing, analyzing the data, and validation. A. Abou El-Ela followed the revision and submission of the manuscript for publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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