



Physiological intracytoplasmic sperm injection does not improve the quality of embryos: A cross-sectional investigation on sibling oocytes

Minh Tam Le^{1,2}, Hiep Tuyet Thi Nguyen¹, Trung Van Nguyen¹, Thai Thanh Thi Nguyen¹, Hong Nhan Thi Dang¹, Thuan Cong Dang³, Quoc Huy Vu Nguyen²

¹Center for Reproductive Endocrinology and Infertility, ²Department of Obstetrics and Gynecology, ³Department of Histology and Embryology, Pathology, Hue University of Medicine and Pharmacy, Hue University, Hue, Vietnam

Objective: This study aimed to compare the efficacy of physiological intracytoplasmic sperm injection (PICSI) and intracytoplasmic sperm injection (ICSI) in terms of the fertilization rate and embryo quality using sibling oocyte cycles.

Methods: This prospective, cross-sectional study collected data from 76 couples who underwent their first cycle at the Hue Center for Reproductive Endocrinology and Infertility, Vietnam, between May 2019 and November 2021. The inclusion criteria were cycles with at least eight oocytes and a sperm concentration of 5×10^6 /mL. Sperm parameters, sperm DNA fragmentation (SDF), fertilization, and the quality of cleavage-stage embryos on day 2 and blastocysts on day 5 were examined.

Results: From 76 ICSI cycles, 1,196 metaphase II (MII) oocytes were retrieved, half of which were randomly allocated to either the PICSI (n=592) or ICSI (n=604) treatment group. The results showed no significant difference between the two groups in terms of fertilization (72.80% vs. 75.33%, $p=0.32$), day 2 cleavage rate (95.13% vs. 96.04%, $p=0.51$), blastulation rate (52.68% vs. 57.89%), and high-quality blastocyst rate (26.10% vs. 31.13%, $p=0.13$). However, in cases where SDF was low, 59 cycles consisting of 913 MII oocytes produced a considerably higher blastulation rate with PICSI than with ICSI (50.49% vs. 35.65%, $p=0.00$). There were no significant differences between the pregnancy outcomes of the PICSI and ICSI embryo groups following embryo transfer.

Conclusion: Using variable sperm quality provided no benefit for PICSI versus ICSI in terms of embryo outcomes. When SDF is low, PICSI appears to be able to produce more blastocysts.

Keywords: DNA fragmentation; Embryonic structures; Fertilization *in vitro*; Hyaluronic acid; Sperm injections, intracytoplasmic

Introduction

The presence of a hyaluronic acid (HA) receptor in the plasma membrane of the sperm head indicates that the sperm has matured

and is capable of binding HA in the extracellular matrix of cumulus cells encircling the oocyte [1]. Sperm with HA receptors can reach the cytoplasm of the oocyte during natural fertilization [2]. Two sperm selection methods have been developed based on the interaction between spermatozoa and HA: (1) recovering spermatozoa attached to HA-coated Petri dishes [3] and (2) selecting spermatozoa that swim slowly in a HA medium [4].

The capacity of mature spermatozoa selected with HA to fertilize is equivalent to that of mature spermatozoa selected with the hemizona binding assay. Moreover, the proportion of sperm with normal morphology was found to be greater in the HA-binding group than in the group of fresh sperm [5]. HA is believed to play a role in the selection of mature spermatozoa [6] can be used to select sperm without causing DNA damage [7-10]. An inverse correlation exists between

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Corresponding author: **Minh Tam Le**

Department of Obstetrics and Gynecology, Hue University of Medicine and Pharmacy, Hue University, 06 Ngo Quyen street, Hue, Vietnam

Tel: +84-0989-228-779 Fax: +84-234-3822173 E-mail: leminhtam@hueuni.edu.vn

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the rate of HA-binding and protamine deficiency, as well as between DNA fragmentation and poor sperm morphology [11]. Furthermore, Parmegiani et al. [8] and Huang et al. [12] demonstrated reduced DNA fragmentation in spermatozoa selected in HA solution as opposed to swim-up and in HA-coated dishes as opposed to density gradient centrifugation, respectively. Razavi et al. [13] found that spermatozoa recovered from HA-coated dishes had the same level of DNA fragmentation as spermatozoa recovered from unselected dishes.

In nature, sperm migration through the female reproductive system is a highly selective mechanism that selects mature sperm with a high level of DNA integrity. Intracytoplasmic sperm injection (ICSI), in contrast, overcomes most barriers in natural sperm selection. Using external morphology to select sperm, it is not possible to identify sperm with DNA fragmentation, which may have impacts on embryological and clinical outcomes [14-16]. Several advanced methods of sperm selection are currently being investigated to imitate the dynamics of natural selection. In this context, sperm selection based on the maturation of the cell membrane (physiological intracytoplasmic sperm injection [PICS]) is often employed [17]. PICS selects sperm based on an oocyte's ability to bind HA, which improves the success rate of ICSI. Analyses have been conducted on mature spermatozoa with no or minimum DNA damage and no aneuploidy. Previous trials compared the effectiveness of PICS to that of conventional ICSI. Parmegiani et al. [8] demonstrated that when HA was employed, the embryo development rate and proportion of high-quality embryos were significantly greater than when ICSI was employed. Mokanszki et al. [18] evaluated the efficacy of PICS using an HA-binding assay threshold (HBA score) and reported significantly higher fertilization rates in the PICS group with HBA > 60%, implantation rates in the PICS group with HBA ≤ 60%, and clinical pregnancy rates in each PICS group than in the ICSI group.

However, several other studies have reported that PICS does not improve the success of *in vitro* fertilization (IVF), even in patients with unexplained infertility [19-21]. It was reported that the effectiveness of PICS in improving treatment outcomes for unexplained infertility was uncertain [22]. Thus, it is unclear whether HA should be employed to select sperm for enhancing ICSI performance or whether physiological sperm selection is effective in certain situations. The goal of this study was to assess the fertilization rate and embryo quality of PICS and ICSI cycles utilizing sibling oocytes.

Methods

1. Study design

This prospective, cross-sectional study collected data from 76 couples who underwent their initial IVF cycle at the Center for Reproductive Endocrinology and Infertility, Hue University of Medicine and

Pharmacy, Vietnam, between May 2019 and November 2021. The inclusion criteria were cycles that had at least eight oocytes to ensure the separation of two sibling groups and a minimum sperm concentration of 5×10^6 /mL (to perform an accurate Halosperm test). Sperm parameters, the sperm DNA fragmentation index (DFI), fertilization, and the quality of cleavage-stage embryos on day 2 and blastocysts on day 5 were evaluated. The embryo cleavage rate was defined as the proportion of embryos with at least two blastomeres to those with two pronuclei 44 to 48 hours following ICSI. A blastocyst was recorded based on the presence of an embryonic cavity. At 116 to 118 hours after ICSI, a high-quality blastocyst possessed the following characteristics: a cavity that filled the blastocyst's volume, a densely packed inner cell mass, and a trophectoderm consisting of many cells that formed a cohesive epithelium. The blastulation rate was defined as the number of blastocysts to cleavage-stage embryos, while the quality blastulation rate referred to the proportion of healthy blastocysts to cleavage-stage embryos. This study compared the percentage of embryo development between PICS and ICSI using different sperm subgroup characteristics, including morphology, motility, and DNA fragmentation. Inability to ejaculate, sperm collected by cryopreservation or surgery, individuals with extremely low sperm counts (less than 5×10^6 /mL), severe varicocele, or azoospermia were among the exclusion criteria. This study also excluded IVF cycles involving gamete donors and women with severe endometriosis (grades 3 and 4).

2. Semen analysis

Sperm samples were collected after 3 to 5 days of. Using the 2010 World Health Organization guidelines, semen samples were tested for concentration, sperm motility, vitality, and morphology following 30 minutes of liquefaction at 37 °C.

3. Sperm DNA fragmentation test

All semen samples were examined with Halosperm HT-HS10 (Halotech DNA S.L.) for fragmented DNA. The sperm sample was combined with an agarose microgel and smeared onto a microscope slide before being refrigerated at 40 °C. After agarose was dried at room temperature, the slide was submerged in a denaturation solution for 7 minutes. Following this, the sample was incubated in a lysis solution for 25 minutes. The slide was cleaned with distilled water, dehydrated in successive 70% and 100% ethanol washes for 2 minutes each, and then air-dried and stained with Giemsa. Using a Carl Zeiss Primo Starlight microscope at $\times 1,000$ magnification, 500 sperm were counted on each slide. The DFI was computed as the proportion of spermatozoa with DNA fragmentation per 500 spermatozoa; those with DNA fragmentation were detected with small halos, without halos, or degraded.

4. Semen preparation

Sperm samples were produced using a two-layer density gradient centrifugation procedure with 300 to 400 \times g for 15 minutes and 45% and 90% Sil-Select Plus (Fertipro). Before ICSI, sperm samples were twice cleaned in 3 mL of Spermrinse medium (Vitrolife). During each step of washing with Spermrinse medium, sperm samples were centrifuged at 300 to 400 \times g for 10 minutes, and the supernatants were discarded. In preparation for the sperm selection procedure, 0.3 mL of residual sperm medium was retained.

5. Controlled ovarian hyperstimulation

Utilizing a gonadotropin-releasing hormone antagonist protocol, women who underwent IVF cycles were treated with controlled ovarian hyperstimulation. The starting dosage of recombinant follicle-stimulating hormone (follitropin alfa) was based on the antral follicle count and anti-Müllerian hormone level. Then, 35 to 36 hours after an intramuscular injection of 10,000 IU of human chorionic gonadotropin (hCG) (Pregnyl; Merck Sharp & Dohme Limited), follicles were aspirated with an ultrasound-guided single-lumen needle (Vitrolife).

6. Physiological intracytoplasmic sperm injection

PICSI dishes (Origio) were prepared by soaking hyaluronan microdots in 10 μ L of culture medium droplets GMOPS PLUS (Vitrolife). To immobilize the sperm, drops of 10% polyvinylpyrrolidone (PVP) (Vitrolife) were applied to the disk. Then, 2 μ L of purified sperm suspension was added to the hyaluronan microdot-containing droplets. To optimize sperm binding, the dishes were covered with 3 to 4 mL of Ovoil (Vitrolife) and incubated at 37 °C for 15 minutes.

7. Intracytoplasmic sperm injection and embryo culture

After using 80 IU of HYASE (Vitrolife) to denude the oocyte cumulus complex, mature oocytes were identified. Three hours after retrieval, ICSI was performed using a mature oocyte and sperm that had been previously prepared. The injected oocyte was cultivated in a single drop of 20 μ L of G-TL (Vitrolife) covered by 3 mL of Ovoil (Vitrolife) under conditions of 6% CO₂ and 5% O₂. Next, 16 to 18 hours after the injection, fertilized oocytes were detected by the presence of two pronuclei. On days 2 and 5, embryos were evaluated according to the Istanbul consensus. A high-quality blastocyst was defined as possessing a densely packed inner cell mass and trophoctoderm composed of many cells forming a cohesive epithelium [23].

8. Embryo vitrification and thawing

Day 5 blastocysts were chosen for vitrification. Utilizing the Cryo-

top device and commercially available medium (Kitazato), vitrification was performed according to the manufacturer's instructions. The blastocysts were stored in liquid nitrogen for storage.

The embryos were thawed using the warming solution (Kitazato) per the manufacturer's instructions. Before transfer, embryos were cultured for 2 hours in 20 μ L of G-TL (Vitrolife) prepared the previous night at 6.0% CO₂ and 5.0% O₂.

9. Embryo transfer

To prepare the endometrium, 4 mg of oral estradiol (Progynova; Bayer) was administered twice daily beginning on day 2 of the subsequent cycle. Progesterone (Crinone Gel 8%; Merck KGaA) was administered vaginally at a dosage of 90 mg twice daily to induce secretory transformation. Embryos were transferred on days when the endometrial thickness was at least 7 mm. Embryos were immersed for 15 to 30 minutes in 1 mL of Embryogluce (Vitrolife) before being put into the Kitazato catheter and then transferred to the uterus under transvaginal ultrasound monitoring.

10. Clinical follow-up

On the 14th day after embryo transfer, serum β subunit of human chorionic gonadotropin (β -hCG) levels were measured, and a value of more than 50 mIU/mL was considered β -hCG-positive. Two weeks later, transvaginal ultrasound was performed. Four weeks after embryo transfer, the appearance of a gestational sac and fetal cardiac activity were considered to indicate clinical pregnancy. Miscarriage or pregnancy loss was confirmed by ultrasound.

11. Statistical analysis

Statistical analysis was conducted using SPSS version 22.0 (IBM Corp.). Numeric data were presented as mean standard deviation, and frequencies were expressed as a percentage when comparing results between the PICSI and ICSI groups. Samples were classified into subgroups based on their morphology, motility, and sperm DNA fragmentation (SDF) as follows: normal morphology \geq 4% and normal morphology < 4%; progressive \geq 30% and progressive < 32%; DFI < 30% (low SDF) and DFI \geq 30% (high SDF). The chi-square or Fisher exact test, as well as the independent-samples *t*-test, were used to analyze categorical variables. A *p*-value < 0.05 was considered statistically significant in all tests.

12. Ethical statement

The present study was approved by the Ethics Committee of Hue University of Medicine and Pharmacy, with approval number H2020/030. All patients agreed to participate in this study and signed an informed consent form.

13. Availability of data and material

The dataset used and/or analyzed during the current study is available from the corresponding author upon reasonable request.

Results

The clinical and cycle characteristics of the couples are shown in Table 1. From May 2019 to November 2021, 76 couples were selected to undergo either PICS or ICSI. In addition to typical semen analysis parameters, the average sperm DFI was calculated to be 23.48% ± 15.10% (range, 4.4% to 76.4%). The majority of cases were due to female-factor infertility, with polycystic ovary syndrome being the most common cause. The mean number of retrieved oocytes was 20.04 ± 7.27 (range, 8 to 43), of which 15.75 ± 5.31 (range, 7 to 31) were metaphase II (MII) oocytes.

The outcomes of fertilization and embryo development using two

Table 1. General characteristics of study participants in infertile couples with IVF

Characteristic	Results (n = 76)
Male age (yr)	35.71 ± 5.07 (28–51)
Female age (yr)	31.93 ± 4.17 (25–44)
Infertility duration (yr)	4.20 ± 2.19
Primary infertility	46 (60.53)
Secondary infertility	30 (39.47)
Male BMI (kg/m ²)	23.75 ± 2.78 (17.19–33.70)
Female BMI (kg/m ²)	21.32 ± 2.57 (15.19–32.89)
Semen analysis	
Concentration (10 ⁶ /mL)	36.09 ± 14.18 (5–86)
PR (%)	32.32 ± 10.75 (5–58)
Viability (%)	82.34 ± 8.66 (23–92)
Normal morphology (%)	3.89 ± 1.92 (1–14)
Abnormal head (%)	88.67 ± 5.59 (78–97)
Abnormal neck: tail (%)	52.07 ± 11.43 (30–92)
DFI (%)	23.48 ± 15.10 (4.4–76.4)
Indication for IVF	
With male factor	11 (14.47)
With PCOS	39 (51.32)
With tubal factor	18 (23.68)
With endometriosis	6 (7.89)
With low ovarian reserve	4 (5.26)
With ≥ 2 factors	15 (19.74)
Main findings of IVF cycles	
AMH (ng/mL)	4.82 ± 3.02 (1.40–21.23)
FSH day 2 (mIU/mL)	6.33 ± 1.44 (4.06–10.50)
Total no. of retrieved oocytes	20.04 ± 7.27 (8–43)
No. of obtained MII oocytes	15.75 ± 5.31 (7–31)

Values are presented as mean ± standard deviation (range) or number (%). IVF, *in vitro* fertilization; BMI, body mass index; PR, progressive; DFI, DNA fragmentation index; PCOS, polycystic ovary syndrome; AMH, anti-Müllerian hormone; FSH, follicle-stimulating hormone; MII, metaphase II.

techniques are summarized in Table 2. In each cycle, half of the oocytes were randomly assigned to one of two treatment groups: PICS (n = 592) and ICSI (n = 604), based on inclusion and exclusion criteria. The fertilization rate (72.80% vs. 75.33%, *p* = 0.32), cleavage rate on day 2 (95.13% vs. 96.04%, *p* = 0.51), blastulation rate (52.68% vs. 57.89%, *p* = 0.13), and good-quality blastocyst rate (26.10% vs. 31.58%, *p* = 0.08) did not show statistically significant differences between the PICS and ICSI groups.

Table 3 presents the comparison between embryo culture outcomes from using PICS and ICSI in different subgroups of spermatozoa concerning morphology and motility. No significant variation was found between PICS and ICSI in the subgroups with normal and defective sperm morphology and motility.

The association between the degree of SDF and the outcomes of PICS versus ICSI was also evaluated. As shown in Table 4, in the low SDF subgroup (DFI < 30%), 913 MII oocytes subjected to 59 cycles had a significantly higher blastulation rate with PICS as compared to ICSI (50.49% vs. 35.65%, *p* = 0.00). However, in the high SDF group, no significant difference was observed between PICS and ICSI. The relationship of the fertilization rate with these four factors—sperm morphology, motility, DNA fragmentation, and blastocyst quality—are presented in Figure 1, respectively.

Table 5 shows the results of embryo transfer for 31 cycles with embryos following PICS and 63 cycles with embryos following ICSI. Although the percentage of hCG-positive patients in the PICS group was somewhat higher than in the ICSI group (61.19% vs. 57.14%), this difference was not statistically significant (*p* = 0.53). Additionally, the frequencies of clinical pregnancy and stillbirth between the two groups did not demonstrate a statistically significant difference.

Discussion

The usefulness of utilizing HA in sperm selection has been demonstrated in a number of previous studies [8,18,24]. Parmegiani et al. [8] found that the total rate of good-quality embryos was significantly

Table 2. Comparison of the outcomes of embryo culture following PICS versus ICSI

	PICS	ICSI	<i>p</i> -value ^{a)}
No. of MII oocytes	592	604	
Fertilization rate	431/592 (72.80)	455/604 (75.33)	0.32
Cleavage rate	410/431 (95.13)	437/455 (96.04)	0.51
Blastulation rate	216/410 (52.68)	253/437 (57.89)	0.13
Good-quality blastocyst rate	107/410 (26.10)	138/437 (31.58)	0.08

Values are presented as number (%).

PICS, physiological intracytoplasmic sperm injection; ICSI, intracytoplasmic sperm injection; MII, metaphase II.

^{a)}Statistical test using chi-square test.

Table 3. Comparison of the result of embryo culture following PICSI and ICSI in terms of sperm morphology and motility subgroups

Outcome	Sperm morphology				Sperm motility			
	Normal morphology ≥ 4% (n = 40)		Normal morphology < 4% (n = 36)		PR ≥ 32% (n = 42)		PR < 32% (n = 34)	
	PICSI (317 MII oocytes)	ICSI (325 MII oocytes)	PICSI (275 MII oocytes)	ICSI (279 MII oocytes)	PICSI (337 MII oocytes)	ICSI (347 MII oocytes)	PICSI (255 MII oocytes)	ICSI (257 MII oocytes)
Fertilization rate	231/317 (72.87)	252/325 (77.54)	200/275 (72.73)	204/279 (73.12)	245/337 (72.70)	273/347 (78.67)	186/255 (72.94)	183/257 (71.21)
Cleavage rate	218/231 (94.37)	241/252 (95.63)	192/200 (96.00)	197/204 (96.57)	233/245 (95.10)	263/273 (96.34)	177/186 (95.16)	175/183 (95.63)
Blastulation rate	138/218 (63.30)	159/241 (65.97)	78/192 (41.67)	94/197 (47.71)	128/233 (54.94)	155/263 (58.94)	88/177 (49.72)	98/175 (56.00)
Good-quality blastocyst rate	71/218 (32.57)	89/241 (36.91)	36/192 (18.75)	49/197 (24.87)	64/233 (27.47)	88/263 (33.46)	45/177 (25.42)	50/175 (28.57)
				<i>p</i> -value ^{a)}		<i>p</i> -value ^{a)}		<i>p</i> -value ^{a)}

Values are presented as number (%).
 PICSI, physiological intracytoplasmic sperm injection; ICSI, kintracytoplasmic sperm injection; PR, progressive; MII, metaphase II.
^{a)}Statistical test using chi-square test.

higher in the PICSI group (95.0% ± 0.8%) compared to the ICSI group (84.0% ± 1.1%, *p* < 0.001). The proportion of top-grade embryos was also significantly greater in the PICSI group than in the ICSI group (35.8% vs. 24.1%, *p* = 0.046). While there was no statistical significance, there were tendencies toward increased rates of fertilization, implantation, and pregnancy in the PICSI group [8]. Furthermore, when deciding whether to use PICSI or ICSI during cycles, it is important to measure the HBA score of sperm in fresh semen. PICSI is considered a new and effective procedure that can significantly improve clinical outcomes in patients with a low HBA score [18].

Contrary to our expectations, PICSI did not provide any additional benefits in terms of fertilization and subsequent embryo cleavage compared to ICSI cycles. The difference in blastocyst development between PICSI and conventional ICSI was not statistically significant. This finding was also documented in a study conducted by Majumdar and Majumdar [22] in 2013. Two previous studies had also used HA-containing media to select spermatozoa and had similarly found that this did not significantly increase the fertilization rate and the proportion of high-quality embryos [19,20]. There are numerous possible explanations for this finding. One possible theory is that the mechanical act of removing adhering sperm from the PICSI plate may damage the sperm, which could be exacerbated by the toxicity of PVP [21]. As another possibility, we discovered that the technique of sperm selection in the PICSI dish followed by sperm injection into the oocytes, took significantly longer than conventional ICSI. When oocytes spend an extended period of time outside, their quality can decrease. As Liu et al. [20] reported, a longer injection time was seen in the medium containing HA (Sperm Slow) group than in the ICSI group. The authors suggest that a possible approach would be to limit the number of oocytes that are injected into each dish, thereby minimizing the amount of time that oocytes are exposed to conditions outside of a controlled incubator [20].

The effect of HA-based selection of normal sperm was also presented by Prinosilova et al. [5]. Their results showed that when mature spermatozoa are chosen with HA, their capacity to fertilize normally was similar to that observed when the hemi-zona assay was used. Moreover, the percentage of sperm meeting strict normal shape criteria was higher in the HA-binding group than in the initial semen sample [5]. Erberelli et al. [25] examined PICSI and ICSI cycles in couples with the moderate to severe male factor fertility. The researchers concluded that teratozoospermia cases could benefit from the PICSI technique and suggested using PICSI in all cases of abnormal morphology spermatozoa [25]. Kim et al. [24] also found support for the advantage of PICSI when a medium containing HA is used in cases of severe teratozoospermia cases (≤ 1% of sperm with normal morphology). The fertilization rate and ratio of good-quality embryos were significantly higher in the PICSI group than in the ICSI group

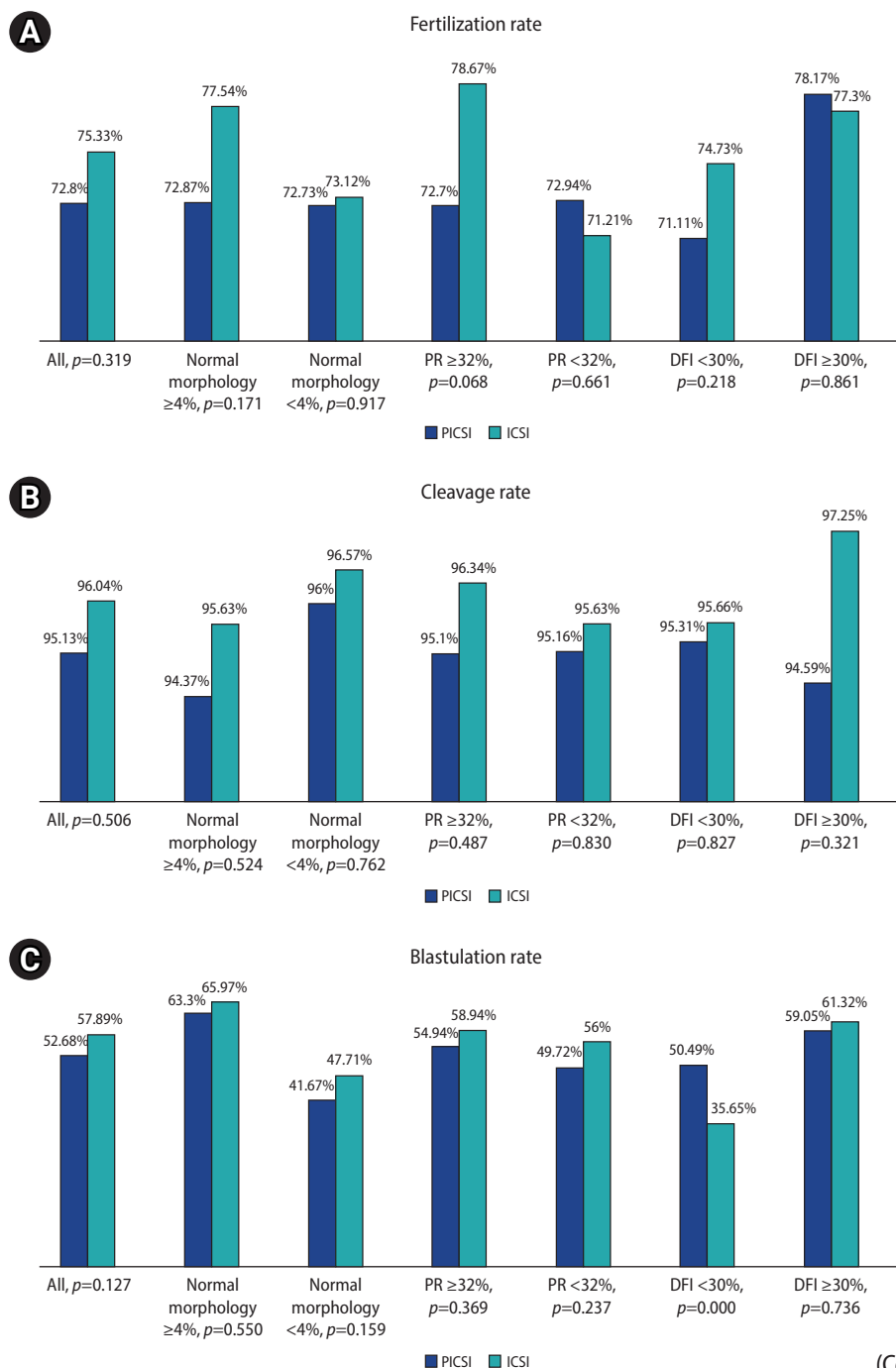
Table 4. The relationship between embryo culture results and the sperm DNA fragmentation following PICSI and ICSI groups

Outcome	DFI < 30% (n = 59)			DFI ≥ 30% (n = 17)		
	PICSI (450 MII oocytes)	ICSI (463 MII oocytes)	p-value ^{a)}	PICSI (142 MII oocytes)	ICSI (141 MII oocytes)	p-value ^{a)}
Fertilization rate	320/450 (71.11)	346/463 (74.73)	0.22	111/142 (78.17)	109/141 (77.30)	0.86
Cleavage rate	305/320 (95.31)	331/346 (95.66)	0.83	105/111 (94.59)	106/109 (97.25)	0.32
Blastulation rate	154/305 (50.49)	118/331 (35.65)	0.00	62/105 (59.05)	(65/106) (61.32)	0.74
Good-quality blastocyst rate	72/305 (23.61)	94/331 (28.40)	0.17	35/105 (33.33)	44/106 (41.51)	0.22

Values are presented as number (%).

PICSI, physiological intracytoplasmic sperm injection; ICSI, intracytoplasmic sperm injection; DFI, DNA fragmentation index; MII, metaphase II.

^{a)}Statistical test using chi-square test.



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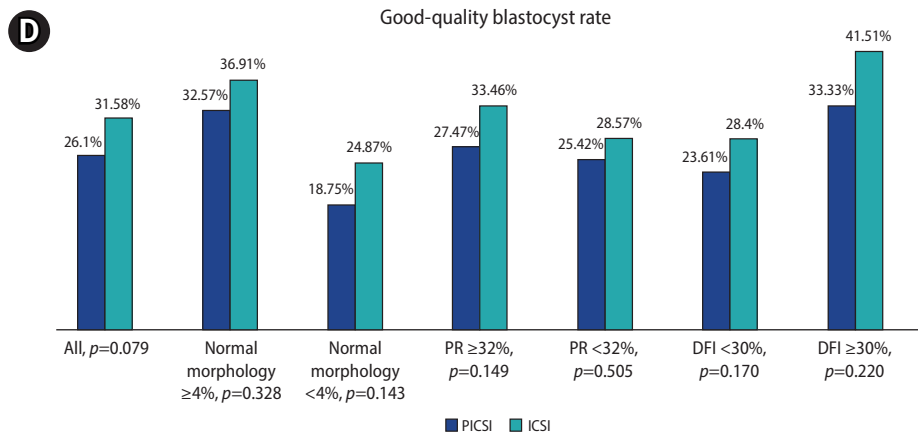


Figure 1. Embryo outcomes of physiological intracytoplasmic sperm injection (PICSI) versus intracytoplasmic sperm injection (ICSI) in all cases and in each subgroup in terms of sperm morphology, motility, and DNA fragmentation. (A) Fertilization rate, (B) cleavage rate, (C) blastulation rate, and (D) good-quality blastocyst rate. PR, progressive; DFI, DNA fragmentation index. Statistical significance is defined at $p < 0.05$.

Table 5. Comparison of transfer embryo results following PICSI and ICSI groups

Outcome	PICSI	ICSI	p-value
No. of transfer embryos cycles	31	63	
No. of embryos transferred per cycle	1.97 ± 0.31	1.90 ± 0.49	0.52 ^{a)}
Endometrium thickness (mm)	9.78 ± 1.76	9.56 ± 1.47	0.83 ^{a)}
β-hCG positive	19 (61.19)	36 (57.14)	0.53 ^{b)}
Clinical pregnancy	16 (51.61)	34 (53.97)	0.42 ^{b)}
Miscarriage	4 (12.90)	11 (17.46)	0.91 ^{b)}

Values are presented as mean ± standard deviation or number (%). PICSI, physiological intracytoplasmic sperm injection; ICSI, intracytoplasmic sperm injection; β-hCG, β subunit of human chorionic gonadotropin. ^{a)}Statistical test using independent-samples *t*-test; ^{b)}Statistical test using chi-square test.

(82.7% vs. 71.7%, $p < 0.001$; and 52.8% vs. 34.0%, $p < 0.001$, respectively). Furthermore, the PICSI group had a lower ratio of poor-quality embryos on day 3. In cases with severe teratozoospermia, PICSI appears to be superior to ICSI in terms of fertilization rate and embryo quality [24]. We also distinguished between normal and abnormal spermatozoa morphology groups, but found no benefit of PICSI in the subgroups with teratozoospermia (normal morphology of sperm < 4%) or normal morphology (normal morphology of sperm ≥ 4%), and motility. This finding may be explained by the embryologist's ability to select high-quality sperm during traditional ICSI by observing and picking them before insemination.

HA has been shown to be capable of selecting spermatozoa with greater DNA integrity and normal morphology [8,9,11,26]. Sperm DNA damage has been linked to the inability to conceive, spontaneous abortion, and assisted reproductive failure [27,28]. Although SDF did not have a negative effect on fertilization in ICSI cycles, it was

associated with the formation of cleavage-stage embryos and blastocysts [29-31]. DNA fragmentation was substantially lower in spermatozoa bound to HA than in spermatozoa after being washed and collected in PVP or spermatozoa from a fresh semen sample [8]. Kirkman-Brown et al. [21] found a correlation between the hyaluronan-based (HAB) score and sperm motility, concentration, fertilization rate, and DNA fragmentation. Although sperm DNA compaction is a weak predictor of clinical pregnancy rates, neither the HAB score nor SDF was indicative of any clinical outcomes [21]. We discovered that when using the PICSI procedure on sperm samples with low DNA fragmentation (DFI < 30%), a higher percentage of blastocysts developed. Therefore, PICSI should be considered in cases of low-level SDF to maximize the number of blastocysts. However, the good-quality blastocyst rates were not significantly different between the two procedures. Furthermore, no statistically significant difference was found between sperm samples with a high degree of DNA fragmentation (DFI ≥ 30%) according to whether PICSI or ICSI was used.

The use of sibling oocytes to evaluate embryos in PICSI and ICSI promotes stability in oocyte quality throughout each cycle. However, because the number of embryo transfer cycles was not large, we assessed the outcomes following embryo transfer using the total number of embryo transfer cycles for each procedure. There was no discernible difference in the hCG-positivity rate, clinical pregnancy rate, or miscarriage rate across the groups. Although PICSI has superiority in fertilization and high-quality embryo rates, a recent study with a small sample size analyzed sibling oocyte cycles and showed no improvement in embryo transfer cycles [32]. A study on male factor infertility demonstrated no difference in biochemical or clinical pregnancy between PICSI and ICSI, although the sample size was also

fairly small [25]. The rates of clinical pregnancy, live birth, and preterm birth did not differ significantly between the PICSI and ICSI groups in a major blinded randomized controlled trial study by Kirkman-Brown et al. [21], with the exception of the stillbirth rate, which was higher in the PICSI group. When Scaruffi et al. [33] evaluated the efficiency of PICSI in cases that had failed in the prior ICSI cycle, they found that HA-ICSI had considerably higher pregnancy and implantation rates than ICSI cycles ($p=0.001$ and $p=0.0001$, respectively). In comparison to ICSI, PICSI recorded statistically significant lower rates of miscarriage (4.3% for PICSI vs. 7.0% for ICSI; odds ratio, 0.61; 95% confidence interval, 0.43 to 0.84; $p=0.003$) [21]. Thus, the effectiveness of PICSI in improving clinical outcomes remains unclear. To evaluate the role of PICSI, more research with larger sample sizes on other types of patients is needed.

In conclusion, a group of sibling oocytes was randomly divided and subjected to both PICSI and ICSI procedures to ensure consistent quality during each cycle. Our findings indicate that although PICSI does not demonstrate any advantages in terms of overall grading and evaluation of specific sperm characteristics during fertilization and embryo development, it appears to have a greater ability to generate blastocysts with minimal SDF. Further studies are required to assess the effectiveness of PICSI by analyzing clinical pregnancy rates, miscarriage rates, and live birth rates in comparison to conventional ICSI.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

ORCID

Minh Tam Le	https://orcid.org/0000-0001-6225-3108
Hiep Tuyet Thi Nguyen	https://orcid.org/0000-0002-8019-9608
Trung Van Nguyen	https://orcid.org/0000-0003-3363-5064
Thai Thanh Thi Nguyen	https://orcid.org/0000-0001-9995-9948
Hong Nhan Thi Dang	https://orcid.org/0000-0003-3872-5456
Thuan Cong Dang	https://orcid.org/0000-0002-2657-6791
Quoc Huy Vu Nguyen	https://orcid.org/0000-0002-4744-7059

Author contributions

Conceptualization: MTL, HTTN, TVN, TTTN, HNTD. Data curation: MTL, TVN, TTTN. Formal analysis: HTTN, TVN, HNTD. Writing-original draft: MTL, HTTN. Writing-review & editing: MTL, HTTN, TVN, TTTN, HNTD, TCD, QHVN.

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