Assisted sperm fusion insemination improves fertilization rates and increases usable embryos for transfer: a clinical sibling-oocyte study

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Objective: To evaluate the impact of assisted sperm fusion insemination (ASFI), a microinsemination method using sperm bound to the zona pellucida (ZP-sperm) without penetrating the oocyte membrane, compared with conventional intracytoplasmic sperm injection (C-ICSI), on fertilization and embryonic development.
Design: Prospective clinical sibling-oocyte study.
Subjects: A total of 197 oocytes from 24 patients who underwent 35 oocyte retrieval cycles from January 2023 to April 2024 were analyzed. Patients who underwent retrieval of both, at least 2 metaphase II (MII) oocytes and at least 1 immature or degenerated oocyte, were recruited.
Intervention: Metaphase II oocytes were alternately allocated to 2 groups: ASFI and C-ICSI groups. To obtain ZP-sperm for ASFI, immature or degenerated oocytes were incubated with 10,000 motile sperm for 3 hours. After harvesting the ZP-sperm, it was pressed onto the membrane of an MII oocyte for 10 seconds in the ASFI group. Conventional intracytoplasmic sperm injection was performed conventionally in the C-ICSI group.
Main Outcome Measures: The rates of 2 pronuclei (2PN), degeneration, blastocyst formation, and usable embryos, defined as the total number of transferred or cryopreserved embryos divided by the number of MII oocytes, were compared between the 2 groups.
Results: The 2PN rate of the ASFI group was 88.0% (73/83), which was significantly higher than that of the C-ICSI group (70.2% [80/114]). In addition, a significantly lower degeneration rate was observed in the ASFI group (0% [0/83]) than in the C-ICSI group (8.8% [10/114]). The blastocyst formation rate was equivalent in the 2 groups (ASFI group, 63.9% [39/61]; C-ICSI group, 62.0% [44/71]). However, the usable embryo rate was significantly higher in the ASFI group (45.8% [38/83]) than in the C-ICSI group (28.1% [32/114]).
Conclusion: The ASFI group yielded significantly higher 2PN and lower degeneration rates than the C-ICSI group. Consequently, the ASFI group experienced a higher number of embryos usable for implantation, although there was no significant difference in the blastocyst formation rate between the 2 groups. Further studies with a larger number of cases will be needed for more general application of these findings. (F S Rep® 2025;6:17–24. ©2024 by American Society for Reproductive Medicine.)

Key Words: ICSI, fertilization, zona pellucida, acrosome reaction, degeneration

Intracytoplasmic sperm injection (ICSI), which is a microinsemination technique in which a single sperm is injected into an oocyte, was first introduced in 1992 (1). Since then, it has developed as a major technique in assisted reproductive technology for treating couples with male factor infertility. However, fertilization failure because of high degeneration rates after ICSI sometimes occurs because of damage caused by direct penetration of the plasma membrane by the injection pipette or by aspiration of cytoplasm to break the plasma membrane (2, 3). To solve this problem, a method using a piezo drive unit was reported, in which sperm can be injected into the oocyte cytoplasm without cytoplasmic aspiration (4). This piezo-assisted ICSI improved the survival and fertilization rates compared with

Received October 2, 2024; revised November 15, 2024; accepted November 19, 2024.
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F S Rep® Vol. 6, No. 1, March 2025 2666-3341

https://doi.org/10.1016/j.xfre.2024.11.006

conventional ICSI (C-ICSI) (4–7). However, degeneration after ICSI has not been completely eliminated despite using piezo-assisted ICSI because the plasma membrane still needs to be broken for injecting the sperm into the oocyte cytoplasm (8). To entirely eliminate degeneration after ICSI, the sperm should penetrate into the oocyte cytoplasm without breaking the oocyte membrane, i.e., fusion of the sperm and oocyte is required.

Subzonal insemination (SUZI) is another method that has been reported for injecting sperm into the perivitelline space (9), although it is no longer performed because of the low fertilization rate, which could be because it ignored an essential part of the fertilization process (10). One of the essential factors in the fusion process during fertilization is the acrosome reaction of the sperm head, which involves disruption of the acrosomal cap of the sperm after the merger between the plasma membrane and outer acrosomal membrane (11). Because all sperm attached to the oocyte membrane have already undergone the acrosome reaction (12), we hypothesized that sperm bound to the zona pellucida (ZP), which is an outer layer of the oocyte, may have undergone the acrosome reaction. In previous studies, we revealed that 98% of motile sperm bound to the ZP (ZP-sperm) underwent the acrosome reaction and fertilization was achieved by pressing ZP-sperm onto the oocyte membrane (13, 14). We designated this novel microinsemination method as assisted sperm fusion insemination (ASFI). In those previous studies, we performed ASFI on unfertilized oocytes that did not show the second polar body 6 hours after conventional insemination and evaluated the outcomes of fertilization compared with rescue ICSI. Rescue ICSI involves performing ICSI on unfertilized oocytes after conventional insemination (15). Evaluation showed that ASFI achieved an equivalent fertilization rate and a lower degeneration rate than rescue ICSI.

However, a concern with ASFI is the risk of polyspermy, similar to that with rescue ICSI, because ASFI is performed on oocytes that do not show a second polar body 6 hours after conventional insemination. Moreover, it was technically unclear in our previous studies whether the oocyte was fertilized by pressing the sperm onto the oocyte membrane during the ASFI procedure or through conventional insemination. Performing ASFI without prior conventional insemination is necessary to address these concerns. Therefore, this study aimed to investigate whether ASFI without prior conventional insemination can enhance fertilization and embryonic development compared with C-ICSI.

MATERIALS AND METHODS

This prospective, nonrandomized study was conducted using sibling oocytes at a single center (Yanaihara Women's Clinic) between January 2023 and April 2024. A total of 197 oocytes from 24 patients were recruited. This study was approved by the Ethics Committee of Yanaihara Women's Clinic (22-001), and written informed consent was obtained from all patients.

Patient selection

Patients who required microinsemination, on the basis of their clinician's judgment or at the patient's request, were eligible for this study. Patients who met the following criteria were excluded: age of <18 years; age of >50 years; and severe oligozoospermia. Oocyte retrieval results that met the following criteria were included: more than 1 metaphase II (MII) oocyte retrieved and at least 1 immature (germinal vesicle or metaphase I) or degenerated oocyte retrieved to obtain ZP-sperm.

Sperm preparation

Semen samples from male patients were obtained by masturbation after 2–7 days of ejaculatory abstinence and were liquefied at room temperature. Sperm concentration and motility were assessed using the Sperm Motility Analysis System (DITECT, Tokyo, Japan) on the basis of the World Health Organization criteria (16). Semen was processed by density gradient separation using the Isolate sperm separation kit (FUJIFILM Irvine Scientific, Santa Ana, CA) or SepaSperm (Kitazato Corporation, Shizuoka, Japan). However, because the originally used Isolate became unavailable because of supply constraints, an equivalent-quality SepaSperm was used as a substitute. After centrifugation at $300 \times g$ for 20 minutes, the supernatant was removed, and the pellet was washed with 2.5 mL of HTF Medium (FUJIFILM Irvine Scientific) with 10% serum supplement (Dextran Serum Supplement; FUJIFILM Irvine Scientific). The supernatant was removed after centrifugation at $300 \times g$ for 5 minutes, and 0.2 mL of HTF Medium with 10% serum supplement was pipetted over the pellet to facilitate swimming up of the sperm. The sample was incubated for 20 minutes at 37 °C with 6% CO_2, at which point 0.1–0.2 mL of the upper layer of medium was carefully collected.

Ovarian stimulation and oocyte retrieval

The ovarian stimulation method was selected according to the patients' condition on the second or third day of their menstrual cycle. For the mild stimulation method, 150 IU of human menopausal gonadotropin or recombinant follicle-stimulating hormone was administered every 2 days with letrozole starting from day 3 of the menstrual cycle until the diameter of the dominant follicles reached >17 mm. The high-dose stimulation method used the long protocol of gonadotropin-releasing hormone agonists with human menopausal gonadotropin or recombinant follicle-stimulating hormone daily. When the dominant follicles reached >17 mm in diameter, either or both of 10,000 IU of human chorionic gonadotropin and gonadotropin-releasing

hormone agonist were administered as maturation triggers, followed by oocyte retrieval 36 hours later. The retrieved oocytes, including MII, immature, and degenerated oocytes, were incubated in 1.0 mL of the insemination medium (Universal IVF Medium; CooperSurgical, Trumbull, CT) that had been equilibrated in a 2-well dish (2-well well dish for in vitro fertilization; Nakamedical, Tokyo, Japan) for 2–3 hours at 37 °C in 6% CO_2 and 5% O_2.

Coculture of immature and degenerated oocytes with sperm

To obtain motile ZP-sperm for ASFI, immature and degenerated oocytes were cocultured with prepared sperm. Briefly, a droplet with 100 μL of the insemination medium was made in the inner well of the 2-well dish and covered with mineral oil (Oil for Embryo Culture; FUJIFILM Irvine Scientific). The outer well was filled with 3.9 mL of the insemination medium, and the droplet was equilibrated at 37 °C in 6% CO_2 and 5% O_2. Two to 3 hours after oocyte retrieval, 1 or 2 cumulus-oocyte complexes containing immature or degenerated oocytes were transferred to the droplet and incubated with 10,000 motile sperm. After incubation for 2 hours, the cumulus cells surrounding these oocytes were removed by pipetting without hyaluronidase. Subsequently, incubation continued for an additional hour to increase the number of sperm bound to the ZP as ZP-sperm because there is a possibility of sperm detachment while removing the cumulus cells.

C-ICSI and ASFI procedures

The cumulus cells surrounding MII oocytes were removed using hyaluronidase (ICSI Cumulase; CooperSurgical) within 4–5 hours after oocyte retrieval. For preparation of the C-ICSI and ASFI dish, multiple microdroplets were prepared and covered with the mineral oil. A microdroplet of 7% polyvinylpyrrolidone (7% PVP ready-to-use solution; CooperSurgical) was used for preparation of the injection pipette, and 5 microdroplets of the manipulation medium (Multipurpose Handling Medium; FUJIFILM Irvine Scientific) containing 30% serum supplement were used for preparation of the injection pipette, rinsing of oocytes, ZP-sperm retrieval, C-ICSI, and ASFI. The MII oocytes were alternately allocated to the ASFI and C-ICSI groups on the basis of the order in which they were aspirated into the pipette without any morphological examinations, and oocytes were allocated equally to each group. When the number of MII oocytes was an odd number, 1 additional oocyte was assigned to the C-ICSI group to minimize the risk of no fertilization. The ASFI procedure was performed as previously described (13, 14). Briefly, a motile ZP-sperm of the immature or degenerated oocyte was aspirated tail first into an injection pipette. The injection pipette was pushed across the ZP of the MII oocyte into the perivitelline space, after which the tip of the pipette was pressed onto the oocyte membrane. The sperm head was pressed against the oocyte membrane using the bevel of the pipette, avoiding the vicinity of the polar body, for 10 seconds. Subsequently, the injection pipette was gently removed without interrupting sperm motility (Fig. 1). In contrast, ICSI was conducted in accordance with the conventional method (1). Both insemination methods were performed by 2 embryologists.

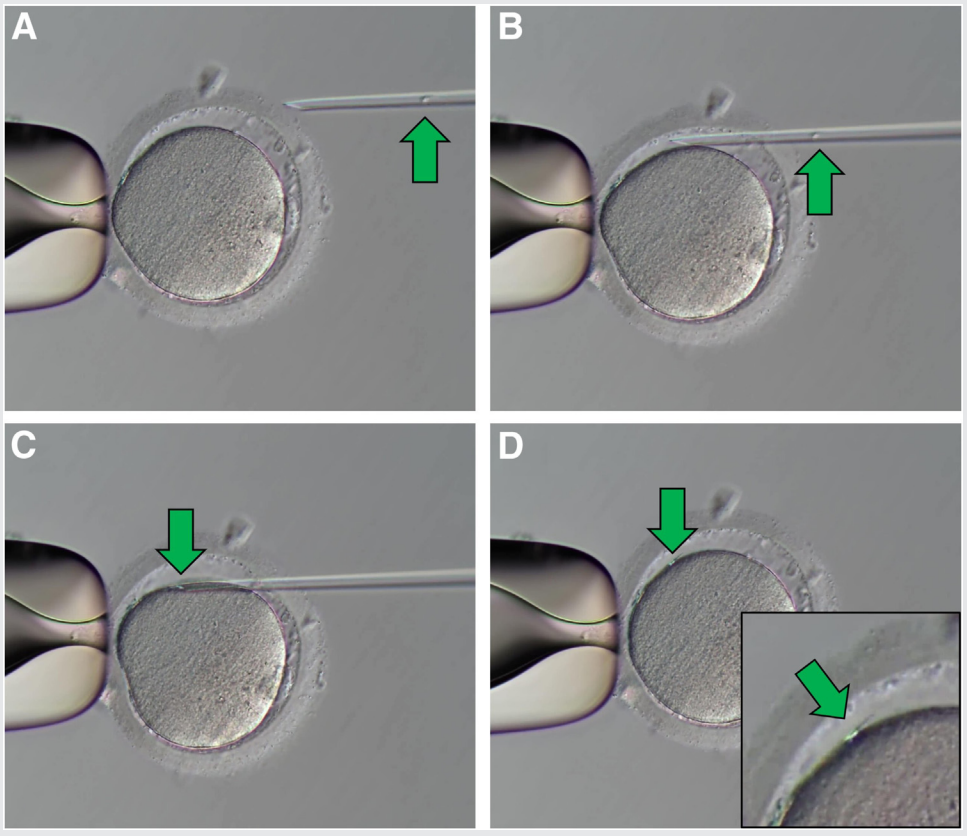
Embryo culture assessment

After both the microinsemination methods, the oocytes were cultured with the culture medium (Continuous Single Culture-NX; FUJIFILM Irvine Scientific) in the time-lapse incubator (EmbryoScope+ time-lapse system; Vitrolife, Göteborg, Sweden) and observed 17–20 hours after the ASFI and C-ICSI procedures. Oocyte survival and the number of pronuclei (PNs) were assessed using images derived from the time-lapse incubator. The scoring criteria for cleavage-stage embryos suggested by Veeck (17) were used; embryos that scored as >6 cells of grade 2 72 hours after insemination were defined as morphologically good cleavage embryos. A subset of these embryos was vitrified 72 hours after insemination according to the patients' decision; the remaining embryos were cultured until the blastocyst stage. The Gardner classification was used for evaluation of blastocysts (18), and full, hatching, or hatched blastocysts of either grade A or B were defined as morphologically good blastocysts. Embryos with >6 cells of grade 3 at 72 hours after insemination or those reaching the full, hatching, or hatched blastocyst stages without grade C for both the inner cell mass and trophectoderm were subjected to transfer or cryopreservation. The usable embryo rate was calculated as follows: the total number of either transferred or cryopreserved embryos divided by the number of MII oocytes subjected to ASFI or C-ICSI. Moreover, the time from completion of the microinsemination to each developmental stage—defined as tPNa, time to pronuclear appearance; tPNf, time to pronuclear fading; t2–t8, time to 2–8 discrete cells; tSB, time to initiation of blastulation; and tB, time to full blastocyst—was evaluated from the records of the time-lapse incubator for embryos that eventually reached the full blastocyst stage.

Statistical analysis

Although this was a sibling-oocyte study, the data could not be treated as paired because more C-ICSI procedures were performed when an odd number of oocytes were retrieved. Therefore, the mean values were compared using the Mann-Whitney *U* test. Proportions were compared using the Fisher exact test because of the small sample sizes for some data. For each proportion, 95% confidence intervals were calculated using the Clopper-Pearson method to evaluate the variability of the results. Analyses were performed as 2-tailed tests. A *P* value of $<.05$ was considered statistically significant. All analyses were conducted using R software, version 3.5.1 (19).

FIGURE 1



Assisted sperm fusion insemination procedure (representative images). (**A, B**) Inserting the injection pipette into the perivitelline space by penetrating the zona pellucida. (**C**) Pressing the head of the motile sperm onto the oocyte membrane for 10 seconds. (**D**) Adhesion of the sperm head to the oocyte membrane while the tail is moving. The bottom right corner of Figure **D** is an enlarged image of sperm adhesion. Green arrows: motile sperm collected from the zona pellucida.

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RESULTS

Patients' characteristics

The characteristics of the recruited patients are shown in Table 1. The mean and standard deviation of patient age was 40.1 ± 3.2 years, and that of male age was 43.3 ± 5.5 years.

TABLE 1

Patient characteristics.

Parameters	Value
Age of females, y	40.1 ± 3.2
Age of males, y	43.3 ± 5.5
No. of previous oocyte retrievals, times	7.9 ± 5.5
Semen volume, mL	2.4 ± 1.5
Sperm density, million/mL	62.1 ± 48.8
Sperm motility, %	44.6 ± 19.0

Note: Data are presented as the means ± standard deviations.

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Fertilization and embryonic development

All ZP-sperm that were pressed onto the oocyte membrane adhered to and were incorporated into the oocyte after ASFI. The results of fertilization and embryonic development in both groups are shown in Table 2. The 2PN rate of the ASFI group was 88.0% (73/83), which was significantly higher than that of the C-ICSI group (70.2% [80/114], $P=.003$). The degeneration rate was significantly lower in the ASFI group (0% [0/83]) than in the C-ICSI group (8.8% [10/114], $P=.006$). In contrast, there were no statistically significant differences in the rates of morphologically good cleavage embryos (65.8% [48/73] in the ASFI group and 57.5% [46/80] in the C-ICSI group, $P=.322$), blastocyst formation (63.9% [39/61] in the ASFI group and 62.0% [44/71] in the C-ICSI group, $P=.858$), and morphologically good blastocyst formation (29.5% [18/61] in the ASFI group and 22.5% [16/71] in the C-ICSI group, $P=.426$) between the 2 groups. The usable embryo rate was however, significantly higher in the ASFI group (45.8% [38/83]) than in the C-ICSI group (28.1% [32/114], $P=.016$).

TABLE 2

Number and rate of oocytes, embryos, and blastocysts after fertilization and subsequent embryonic development.

Parameters	ASFI	95% CI	C-ICSI	95% CI	*P*
Total oocytes	83		114		
Fertilized oocytes with 2 pronuclei (%)	73 (88.0)	79.0–94.1	80 (70.2)	60.9–78.4	.003
Fertilized oocytes with 1 pronucleus (%)	2 (2.4)	0.3–8.4	4 (3.5)	1.0–8.7	1.00
Fertilized oocytes with 3 pronuclei (%)	1 (1.2)	0.0–6.5	7 (6.1)	2.5–12.2	.142
Degenerated oocytes (%)	0 (0)	0.0–4.3	10 (8.8)	4.3–15.5	.006
Morphologically good cleavage embryos (%)	48 (65.8)	53.7–76.5	46 (57.5)	46.0–68.5	.322
Oocytes cultured to the blastocyst stage	61		71		
Blastocysts (%)	39 (63.9)	50.6–75.8	44 (62.0%)	49.7–73.2	.858
Morphologically good blastocysts (%)	18 (29.5)	18.5–42.6	16 (22.5)	13.5–34.0	.426
Usable embryos (%)	38 (45.8)	34.8–57.1	32 (28.1)	20.0–37.3	.016

Note: Data are presented as the *n* (%). The morphologically good cleavage embryo rate was calculated as follows: total number of morphologically good cleavage embryos divided by the number of fertilized oocytes with 2 pronuclei. The usable embryo rate was calculated as follows: the total number of either transferred or cryopreserved embryos divided by the number of metaphase II oocytes subjected to ASFI or C-ICSI. ASFI = assisted sperm fusion insemination; CI = confidence interval; C-ICSI = conventional intracytoplasmic sperm injection.

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Embryo morphokinetic parameters

Table 3 shows the morphokinetic parameters of embryos in the ASFI and C-ICSI groups. Among 153 2PN oocytes, 61 developed into full blastocysts, 29 in the ASFI group and 32 in the C-ICSI group. There were no statistically significant differences in terms of tPNa (6.0 ± 1.1 vs. 5.5 ± 1.0 hours, $P=.107$), tPNf (22.0 ± 3.3 vs. 22.2 ± 2.7 hours, $P=.828$), t8 (58.0 ± 11.3 vs. 57.4 ± 9.4 hours, $P=.845$), tSB (95.6 ± 9.1 vs. 95.6 ± 7.7 hours, $P=.739$), and tB (106.5 ± 10.2 vs. 106.7 ± 8.9 hours, $P=.724$) between the 2 groups.

DISCUSSION

In this study, ASFI performed on sibling oocytes without conventional insemination demonstrated a significantly higher 2PN rate and a significantly lower degeneration rate than C-ICSI. Moreover, there were equal embryonic development parameters between ASFI and C-ICSI, and ASFI was associated with an increase in the number of usable embryos for clinical use.

Intracytoplasmic sperm injection, including the piezo-assisted method, still does not eliminate oocyte degeneration because of the need for breaking the oocyte membrane in the insemination procedure (5–7, 20). Therefore, because degeneration tends to occur in oocytes with high fragility and cytoplasmic viscosity, a less invasive approach is necessary (21, 22). On the other hand, in ASFI, because the sperm is incorporated into the cytoplasm through fusion between the oocyte and sperm, theoretically, the oocyte membrane is not penetrated by the injection pipette, and consequently, it is not broken for fertilization, eliminating the risk of oocyte degeneration. Indeed, none of the oocytes appeared to be degenerated in the present study. Hence, from a fragility perspective, ASFI may be beneficial in patients with fragile oocyte membranes. Although the

TABLE 3

Comparison of morphokinetic parameters between assisted sperm fusion insemination and conventional intracytoplasmic sperm injection.

Parameters	ASFI (n = 29)	C-ICSI (n = 32)	*P*
tPNa	6.0 ± 1.1	5.5 ± 1.0	.107
tPNf	22.0 ± 3.3	22.2 ± 2.7	.828
t2	24.7 ± 3.4	24.8 ± 2.9	.834
t3	33.5 ± 4.8	34.1 ± 4.7	.431
t4	36.0 ± 4.1	36.2 ± 4.5	.659
t5	46.3 ± 8.5	46.0 ± 7.6	.862
t8	58.0 ± 11.3	57.4 ± 9.4	.845
tSB	95.6 ± 9.1	95.6 ± 7.7	.739
tB	106.5 ± 10.2	106.7 ± 8.9	.724

Note: Values are expressed as the means ± standard deviations, in hours. The mean differences were not significantly different for tPNa, early cleavage stages (tPNf to t4), late cleavage stages (t5–t8), and the blastocyst stage parameters ($P>.05$). tB = time to full blastocyst; tPNa = time to pronuclear appearance; tPNf = time to pronuclear fading; tSB = time to initiation of blastulation; t2–t8 = time to 2–8 discrete cells.

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blastocyst formation rate was similar between the groups in this study, the increased usable embryo rate with ASFI is likely because of a reduction in the number of degenerated oocytes. Further studies with varying proportions of subjects, such as those with different infertility causes or age groups, are required to clarify whether this advantage is truly attributable to the microinsemination method.

On the other hand, SUZI has previously been reported as a method for inserting sperm into the perivitelline space without breaking the oocyte membrane (9), although it is no longer performed because it has a low fertilization rate (10). This could be because SUZI likely overlooks the mechanism of fusion with the oocyte and the fertilization process because it involves randomly selecting multiple motile sperm without any process to ensure the selection of sperm that will surely fuse with the oocyte. Proper selection is essential for fusion of the sperm with the oocyte because the acrosome reaction occurs in only 10% of the sperm after the swim-up process (23). In contrast, ASFI solves both problems because it can be performed without breaking the oocyte membrane, and only 1 selected sperm is used.

In addition to avoiding degeneration after ICSI, ASFI has another advantage, namely, that it avoids bringing extra substance into the oocyte cytoplasm. In ICSI, in addition to sperm, various substances such as culture media, PVP, and acrosome enzymes are carried into the oocyte cytoplasm during the sperm injection process. Although PVP is usually used to reduce sperm motility and facilitate handling of sperm in ICSI procedures, prolonged exposure of sperm to PVP can adversely affect sperm viability and morphology, leading to increase in both deoxyribonucleic acid (DNA) fragmentation and abnormal chromatin structure (24). Additionally, using higher concentrations of PVP during the ICSI procedure can impair embryonic development (25). Although the exact effects on fertilization, embryonic development, and subsequent infant outcomes when these substances are directly brought into the oocyte cytoplasm remain unclear (26), efforts should be made to minimize inserting these substances into oocytes as much as possible. In contrast, from this point of view, because fertilization occurs through natural fusion between the sperm and oocyte in ASFI, these substances are not brought into the oocyte cytoplasm.

Thus, the fertilization process in ASFI is similar to that of conventional insemination rather than ICSI because sperm is incorporated into the oocyte through the fusion of their membranes. Additionally, the process of pressing the sperm against the oocyte membrane imitates the behavior of sperm after penetrating the ZP, ensuring that the sperm is incorporated into the oocyte without freely moving in the perivitelline space, as occurs in conventional insemination (27). A previous study reported that a statistically significant delay during early cleavage stages (tPNf to t4) was observed among embryos fertilized by conventional insemination compared with those fertilized by ICSI (28). However, it has also been mentioned that the differences in early cleavage-stage parameters disappeared after normalization to the time point of PNf. We assumed that these results were caused by difficulty in recognizing when the sperm is incorporated into the oocyte during conventional insemination. In contrast, in ASFI, the timing of sperm penetration into the oocyte can be recognized using a time-lapse system (13). In the present study, we observed no statistically significant differences in the time to each developmental stage between the ASFI and C-ICSI groups using a time-lapse system. These results suggest that differentiation during the process of sperm penetration into the oocyte does not impact the timing of each embryo stage.

Although ASFI has some advantages, it should be recognized that it also has some limitations. The first limitation is the need for the ZP for obtaining the sperm needed for ASFI. In cases where immature or degenerated oocytes cannot be obtained at oocyte retrieval, ASFI cannot be performed. Because almost all ZP-sperm have already undergone the acrosome reaction (13), these sperm should play a key role in the fertilization process in ASFI. Although various methods of inducing the acrosome reaction of sperm have been attempted, none of them are particularly effective, with a success rate of approximately 60%–70% at best (29, 30). Therefore, the current approach depends on the ZP to obtain acrosome-reacted sperm. An effective method should be established to either completely induce the acrosome reaction or identify sperm capable of fusing with the oocyte membrane without the use of the ZP. In addition, it was reported that ZP-sperm with higher proportions of morphologically normal sperm, with normal double-stranded DNA and lower DNA fragmentation (31, 32), and embryos derived using these ZP-sperm by ICSI showed higher developmental ability and implantation rates than those by ICSI with conventional sperm selection (33–35). On the basis of these facts, establishment of a method to obtain acrosome-reacted sperm with normal morphology and DNA similar to ZP-sperm is necessary. Second, the small sample size of this study and the alternate allocation of oocytes on the basis of aspiration order may limit randomization and potentially introduce bias into the results. Therefore, ASFI should be validated as a new microinsemination method in future studies involving a large number of patients and improved study design to minimize bias to evaluate its potential for higher embryonic development and a significantly lower degeneration rate than C-ICSI. Additionally, in the future, the methodology of ASFI, including the process of sperm selection and the duration of pressing the sperm onto the oocyte membrane, may change because ASFI is still a developing technique.

Furthermore, a large-scale patient study on pregnancy progression and birth outcomes will finally be required to confirm our findings.

CONCLUSION

In our previous study, which compared ASFI with rescue ICSI, it was not certain whether fertilization occurred during in vitro fertilization or ASFI (13). In the present study,

fertilization was certainly caused by sperm that was attached to the oocyte membrane because sperm that was attached to a degenerated or immature oocyte was selected. This study also revealed that ASFI can have a fertilization rate equivalent to or greater than ICSI. Thus, ASFI can enhance the survival rate of oocytes and increase the number of embryos available for implantation, not only compared with rescue ICSI but also compared with C-ICSI. In conclusion, ASFI may potentially be a novel alternative microinsemination method instead of ICSI for infertility patients.

Acknowledgments

The authors thank Dr. Yasuhisa Araki, Ph.D., and Dr. Yasuyuki Araki, Ph.D., for their invaluable guidance and mentorship in our previous research, which laid the foundation for this study. Their support has been instrumental in the success of our current research. The authors also appreciate the proofreading assistance provided by Dr. Koji Nakagawa, Ph.D., M.D. The authors thank FORTE Science Communications (https://www.forte-science.co.jp/) for English language editing.

CRediT Authorship Contribution Statement

Shota Hatakeyama: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization, Project administration. Kaori Koizumi: Investigation, Writing – review & editing. Goro Kuramoto: Writing – review & editing. Yoriko Horiuchi: Writing – review & editing. Shirei Ohgi: Writing – review & editing. Atsushi Yanaihara: Writing – review & editing, Supervision.

Declaration of Interests

S.H. has nothing to disclose. K.K. has nothing to disclose. G.K. has nothing to disclose. Y.H. has nothing to disclose. S.O. has nothing to disclose. A.Y. has nothing to disclose.

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