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Differences in clinical outcomes between men with mosaic Klinefelter syndrome and those with non-mosaic Klinefelter syndrome

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Abstract

Purpose: This study compared the clinical outcomes of men with Klinfelter syndrome based on karyotype.

Methods: The authors analyzed the outcomes of microdissection testicular sperm extraction (micro-TESE) performed on 57 patients with Klinfelter syndrome (KS) at our clinic.

Results: The average ages of the non-mosaic and mosaic KS groups were 32.2 ± 4.8 and 45.9 ± 13.1 years, respectively. The sperm retrieval rates of the non-mosaic and mosaic KS groups were 46.5% (20/43) and 50.0% (7/14), respectively. The fertilization rates after intracytoplasmic sperm injection did not significantly differ between the non-mosaic and mosaic KS groups. The mosaic KS group had higher cleavage and blastocyst development rates than the non-mosaic KS group (72.2% vs. 96.2% and 30.5% vs. 44.7%, respectively). The group using motile sperm had better outcomes than the group using immotile sperm. The embryo transfer outcomes of the non-mosaic and mosaic KS groups did not significantly differ (clinical pregnancy rate: 28.0% vs. 20.7%, miscarriage rate: 14.3% vs. 33.3%, production rate per transfer: 22.0% vs. 13.8%, and production rate per case: 58.8% vs. 57.1%).

Conclusions: Compared with the non-mosaic KS group, the mosaic KS group had significantly better intracytoplasmic sperm injection outcomes because of the higher utilization rate of motile sperm.

KEYWORDS
intracytoplasmic sperm injection, Klinfelter syndrome, microdissection sperm extraction, mosaic karyotype

1 | INTRODUCTION

Klinefelter syndrome (KS), characterized by an extra X chromosome, is a common cause of azoospermia. Approximately 90% of chromosome aberrations in KS are associated with the most common karyotype (47, XXY). Meanwhile, the remaining 10% present with mosaicism with a normal karyotype (46, XY/47, XXY) or mosaicism with a higher order of aneuploidies (48, XXXY; 49, XXXXY) in rare cases.[1] In addition to azoospermia, KS is phenotypically characterized by a small testis and penis, gynecomastia in late puberty, absence of pubic and body hair, and visceral obesity (feminine distribution of adipose tissues).[2] A cross-sectional karyotypic study of newborns in Denmark reported that the incidence of KS is approximately 1 in 500–1000 birth males. By contrast, the number of patients diagnosed with KS is less than expected, and several patients are believed to be undiagnosed.[3] One possible reason is that the patients have few or minor symptoms other than hypogonadism. Therefore, they are not examined by a health care provider unless infertility is suspected. Approximately 60%–70% of patients are undiagnosed for the rest of their lives.[4,5] In addition, in cases involving 46, XY/47, XXY, which is a mosaic type with a normal karyotype, hypogonadism does not manifest occasionally, and ejaculated sperm is observed in some cases.[6] Smplaski et al. compared testicular volumes, hormone levels, and sperm concentrations between non-mosaic and mosaic KS. The results showed that men with mosaic KS had a significantly better testicular volume and ejaculated sperm concentration than men with non-mosaic KS.[2]

Microdissection testicular sperm extraction (micro-TESE) is now commonly performed for azoospermia associated with KS. Several studies have reported that the sperm retrieval rate of micro-TESE in patients with KS is approximately 30%–70%.[7-9] Two recent systematic reviews showed that in patients with KS, the sperm retrieval rates were 44% and 43% and the live birth rates were 43% and 47%.[10,11] However, the clinical outcomes of micro-TESE in men with KS based on karyotype are still unknown. Therefore, this retrospective study aimed to compare men with non-mosaic KS and those with mosaic KS.

2 | MATERIALS AND METHODS

This retrospective study included 57 patients with azoospermia caused by KS who underwent micro-TESE at our clinic between January 2011 and December 2021. All patients underwent a clinical work-up with physical examination and endocrine profile testing, including scrotal ultrasonography and the assessment of testicular volume and serum testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone levels. The semen collected via masturbation after 2–7 days of abstinence period was analyzed at least twice to diagnose azoospermia. The G-banding technique was performed to diagnose chromosomal abnormalities. This study included patients with non-mosaic KS (47, XXY) or mosaic KS with the normal male chromosome (46, XY/47, XXY). Mosaic ratio was defined as the proportion of 47, XXY:46, XY, calculated by (number of 47, XXY cells)/(number of total cells). Patients with AZF microdeletions were excluded from the analysis, except for AZFc gr/gr deletion, which is considered to be less associated with azoospermia.[12]

Micro-TESE was performed based on a previously described technique.[13] In brief, one sagittal incision in the tunica albuginea was made to allow visualization of the testicular parenchyma without affecting the testicular blood supply. During micro-TESE, the collected testicular tissue was minced on a glass slide using scalpel no. 22 (FEATHER Safety Razor Co., Ltd., Osaka, Japan), covered with a cover glass and evaluated under a microscope (magnification: ×400) for sperm exploration. A portion of the collected tissue was sent for histopathological evaluation. The testicular tissue for cryopreservation was moved in a 5-mL tube with 2 mL of Handling Medium (Origio Japan K.K., Yokoyama, Japan). After blood was removed, the tissue was minced finely using ophthalmic scissors (double-pointed, threaded, 115 mm) in a 60 × 15-mm organ culture dish (CORNING, New York, USA). The tissue was finely minced using a Surflow indwelling needle (Terumo Co., Ltd.) attached to a 1-mL syringe (Terumo Co., Ltd., Tokyo, Japan) until the tissue was fine enough to be aspirated, mixed with the medium, and washed at 200 *g* × 10 min. After removing the supernatant, Universal IVF Medium (Origio Japan K.K., Yokoyama, Japan) was added, and the Arctic Sperm Cryopreservation Medium, a sperm freezing solution (Fujifilm Wako Pure Chemicals Corporation, Osaka, Japan), was added to achieve a ratio of 3:1. The sperm sample was then dispensed into mini crystal straws (IVM Technologies, Rambouillet, France), placed under liquid nitrogen vapor for 15 min, and then frozen and stored in liquid nitrogen.

ICSI was performed using the thawed sperm collected via micro-TESE, and fertilization was confirmed 18–20 h after ICSI. The early embryos were evaluated according to the Veeck classification[14] and blastocysts according to the Gardner classification.[15] Embryos on days 2 or 5–6 of culture were cryopreserved. In cases where only immotile sperm were found, pharmacological stimulation using theophylline solution (GM501 SpermMobil, Gynemed, Sierksdorf, Germany) was used to stimulate and select viable sperm. If no motile sperm was found after stimulation with theophylline, immotile sperm were used for ICSI.

Frozen and thawed embryo transfer (ET) under transvaginal ultrasonography was performed with either natural ovulation cycles or hormone replacement cycles using a previously reported method.[16] Cleavage-stage embryos were transferred into the uterine cavity at day 2 after ovulation, or progesterone supplementation was initiated. Blastocysts were transferred at days 4, 5, or 6 based on the implantation window.

The sperm retrieval rate via micro-TESE, fertilization rate, cleavage rate, blastocyst development rate with ICSI, clinical pregnancy rate, miscarriage rate, and live birth rate after embryo transfer were compared between the non-mosaic and mosaic KS groups.

All statistical data were calculated using the Student's *t*-test or the chi-square test and were analyzed using Excel (Office 365, Microsoft, the USA) and EZR (Saitama Medical Center, Japan), which is a graphical

user interface for R (The R Foundation for Statistical Computing). *p*-Values <0.05 were considered statistically significant.

3 | RESULTS

Table 1 shows the baseline characteristics of patients with KS. Among 57 patients with KS who presented with azoospermia, 43 were diagnosed with non-mosaic KS (47, XXY) and the remaining 14 with mosaic KS (46, XY/47, XXY). The non-mosaic KS group was younger than the mosaic KS group (32.2 ± 4.8 vs. 45.9 ± 13.1 years). There were statistically significant differences in the physical examination results and endocrine profiles between the non-mosaic and mosaic KS groups. Men with non-mosaic KS had smaller testicular volume than men with mosaic KS (4.2 ± 1.1 vs. 13.9 ± 7.7 mL, respectively [$p < 0.01$]). Similarly, the FSH (38.0 ± 13.6 vs. 17.5 ± 16.5 mIU/mL), luteinizing hormone (20.5 ± 9.8 vs. 8.3 ± 7.6 mIU/mL), and testosterone (3.3 ± 1.9 vs. 3.6 ± 1.1 pg/mL) levels significantly differed between the non-mosaic and mosaic KS groups. Hence, the non-mosaic KS group had more severe hormonal statuses (high gonadotropin and low testosterone levels) than the mosaic KS group. However, the sperm retrieval rate via micro-TESE did not differ between the non-mosaic and mosaic KS groups (46.5% vs. 50.0%).

Next, the baseline characteristics of the sperm-retrieved group and the without sperm-retrieved group were compared. Table 2 depicts the baseline characteristics between the sperm-retrieved group and the without sperm-retrieved group. The patients with mosaic KS in the sperm-retrieved group had a significantly higher testicular volume than those in the without sperm-retrieved group (13.9 ± 7.7 vs. 7.2 ± 4.1 mL). However, the testicular volume did not significantly differ in patients with non-mosaic KS. The hormonal status was more severe in without sperm-retrieved group than sperm-retrieved group with significantly lower testosterone (2.0 ± 1.4 vs. 3.2 ± 1.9 pg/mL) and higher FSH (40.6 ± 10.7 vs. 38.0 ± 13.6 mIU/mL) levels than the sperm-retrieved group. However, the results did not significantly differ. The mosaic KS group was more likely to present with this tendency than the non-mosaic KS group (FSH levels: 25.2 ± 6.3 vs. 17.5 ± 16.5 mIU/mL and testosterone levels: 2.6 ± 1.2 vs. 3.6 ± 1.1 pg/mL). However, the results did not significantly differ due to the small sample size. Moreover, chromosomal analysis revealed that the mosaic ratio of the sperm-retrieved group (4.3% XXY) was significantly lower than that of the without sperm-retrieved group (38.6% XXY). Hence, the sperm-retrieved group had a higher proportion of 46XY cells than the without sperm-retrieved group.

Table 3 presents the clinical outcomes of ICSI using frozen and thawed sperm obtained via micro-TESE between the non-mosaic and mosaic KS groups. In total, 20 couples in the non-mosaic KS group underwent 38 ICSI cycles, and 7 couples in the mosaic KS group underwent 25 ICSI cycles. Similar to male participants, the female participants in the non-mosaic KS group were significantly younger than those in the mosaic KS group (33.9 ± 4.6 vs. 41.0 ± 5.1 years). In the mosaic KS group, 100% of the participants used motile sperm (25 cycles on 7 patients). Meanwhile, only 51.3% of the participants in the non-mosaic KS group used motile sperm. The results are listed

TABLE 1 Baseline characteristics of the non-mosaic and mosaic Klinefelter syndrome groups.

	Non-mosaic group	Mosaic group	*p*-Value
Number of cases	43	14	–
Age	32.2 ± 4.8	45.9 ± 13.1	<0.01
Testicular volume[a] (mL)	4.2 ± 1.1	14.0 ± 7.5	<0.01
FSH level (mIU/mL)	38.0 ± 13.6	17.5 ± 16.5	<0.01
LH level (mIU/mL)	20.5 ± 9.8	8.3 ± 7.6	<0.01
Testosterone level (pg/mL)	3.3 ± 1.9	3.6 ± 1.1	<0.05
Sperm retrieval cases (%)	20 (46.5)	7 (50.0)	0.82

Note: Values were expressed as average ± standard deviation.

Abbreviations: FSH, follicle-stimulating hormone; LH, luteinizing hormone.

[a]Testicular volumes were calculated using the average volume of the right and left testes.

TABLE 2 Comparison of patients with non-mosaic and mosaic Klinefelter syndrome between the sperm-retrieved group and the without sperm-retrieved group via micro-TESE.

	Non-mosaic group			Mosaic group		
	Sperm-retrieved group (*n* = 20)	Without sperm-retrieved group (*n* = 23)	*p*-Value	Sperm-retrieved group (*n* = 7)	Without sperm-retrieved group (*n* = 7)	*p*-Value
Testicular volume (mL)	4.2 ± 1.1	5.1 ± 2.5	0.14	13.9 ± 7.7	7.2 ± 4.1	<0.05
FSH level (mIU/mL)	38.0 ± 13.6	40.6 ± 10.7	0.49	17.5 ± 16.5	25.2 ± 6.3	0.27
LH level (mIU/mL)	20.5 ± 9.8	19.8 ± 6.4	0.78	8.3 ± 7.6	16.7 ± 14.1	0.19
Testosterone level (pg/mL)	3.2 ± 1.9	2.0 ± 1.4	<0.05	3.6 ± 1.1	2.6 ± 1.2	0.12
Age of male participants	32.2 ± 4.8	35.0 ± 5.6	0.09	45.9 ± 13.1	40.4 ± 7.5	0.35
Mosaic ratio[a] (%)	–	–	–	4.3 (9/210)	38.6 (81/210)	<0.001

Abbreviations: FSH, follicle-stimulating hormone; LH, luteinizing hormone; micro-TESE, microdissection testicular sperm extraction.

[a]Mosaic ratio represents the proportion of 47, XXY:46, XY, calculated as (number of 47, XXY cells)/(number of total cells).

TABLE 3 Clinical outcomes of ICSI using frozen–thawed sperm obtained via micro-TESE between the non-mosaic and mosaic Klinefelter syndrome groups.

	Non-mosaic group		Mosaic group	Odds ratio[a]		*p*-Value[a]	
Number of patients (ICSI cycles)	20 (38 cycles)		7 (25 cycles)	–		–	
Age of female patients	33.9 ± 4.6		41.0 ± 5.1	–		<0.01	
Age of male patients	32.2 ± 4.8		45.9 ± 13.1	–		<0.01	
Percentage of motile sperm used (%)	51.3 (143/279)		100 (70/70)	–		<0.001	
Fertilization rate (%)	79.9 (223/279)		75.7 (53/70)	1.28		0.44	
Cleavage rate on day 2 (%)	72.2 (161/223)		96.2 (51/53)	0.10		<0.001	
Blastocyst development rate (%)	30.5 (39/128)		52.6 (20/38)	0.39		<0.05	
	Use of motile sperm[b]	**Use of immotile sperm[b]**		**Use of motile sperm**	**Use of immotile sperm**	**Use of motile sperm**	**Use of immotile sperm**
Fertilization rate (%)	81.8 (117/143)	77.9 (106/136)	75.7 (53/70)	1.44	1.1	0.41	0.71
Cleavage rate on day 2 (%)	84.6 (99/117)	58.5 (62/106)	96.2 (51/53)	0.22	0.06	0.03	<0.001
Blastocyst development rate (%)	39.0 (30/77)	17.6 (9/51)	52.6 (20/38)	0.57	0.19	0.16	<0.001

Abbreviations: ICSI, intracytoplasmic sperm injection; micro-TESE, microdissection testicular sperm extraction.

[a]Odds ratio and *p*-values were calculated in comparison to the mosaic group. All ICSI procedures were performed using motile sperm in the mosaic group (25 cycles on 7 patients).

[b]The results are listed on an individual oocyte basis because, in some patients, motile and non-motile sperm were used in the same cycle.

on an individual oocyte basis because, in some cases, motile and non-motile sperm were used in the same cycle. The ICSI results revealed that although the fertilization rate did not differ between the non-mosaic (79.9%) and mosaic (75.7%) KS groups, the non-mosaic KS group had a significantly worse cleavage rate at day 2 than the mosaic KS group (72.2% vs. 96.2%). Similarly, the non-mosaic KS group had a significantly worse blastocyst development rate on day 5 or 6 than the mosaic KS group (30.5% vs. 52.6%). Additional analysis was performed by dividing the non-mosaic KS group into two subgroups: a group using motile sperm and a group using immotile sperm. As shown in the lower columns in Table 3, patients using motile sperm had a higher cleavage rate at day 2 than those using immotile sperm (84.6% vs. 58.5%), which was no difference with the mosaic KS group (96.2%). Similarly, patients using motile sperm had a higher blastocyst development rate than those using immotile sperm (39.0% vs. 17.6%).

In total, 17 of 20 couples who underwent ICSI in the non-mosaic KS group and all 7 couples in the mosaic KS group proceeded with ET. Table 4 shows the clinical outcomes of ET. The clinical pregnancy rates of the non-mosaic and mosaic KS groups were 28.0% (14/50) and 20.7% (6/29), respectively. The miscarriage rates of the non-mosaic and mosaic KS groups were 14.3% (2/14) and 33.3% (2/6), respectively. However, there was no significant difference in the clinical pregnancy and miscarriage rates between the two groups. The live birth rate per transplant in the non-mosaic and mosaic KS groups were 22.0% (11/50) and 13.8% (4/29), respectively. The live birth rates per ET case did not significantly differ between the non-mosaic and mosaic KS groups (58.8% [10/17] and 57.1% [4/7], respectively). The crude cumulative live birth rates per patient who underwent micro-TESE did not significantly differ between the non-mosaic and mosaic KS groups (23.2% [10/43] and 28.6% [4/14]).

4 | DISCUSSION

KS is the most common chromosomal sexual anomaly in men. The typical phenotypic characteristics are low testosterone and high gonadotropin levels and a small testis, which often results in azoospermia.[17] Up to 20% of men with KS present with the mosaic type karyotype and most commonly with the normal karyotype (47, XXY/46, XY).[6] The true prevalence of mosaic forms may be underestimated for two reasons. First, chromosomal mosaicism can be present only in the testes, with the normal karyotype of peripheral leukocytes.[6] Second, men with mosaic KS may be less severely affected than those with non-mosaic KS. Samplaski et al.[3] reported that men with mosaic KS (47, XXY/46, XY) have lower gonadotropin levels, larger testicular volumes, and a higher rate of sperm via ejaculation. Therefore, some of these men may not be tested for KS and, thus, not identified. There are similar reports in other chromosomal abnormalities. For example, in Turner syndrome, patients with the 46, XX/45X mosaic karyotype are reported to have a higher likelihood of spontaneous menarche than those with the 45, X non-mosaic karyotype, which is confirmed as the main predictive

TABLE 4 Clinical outcomes of ET between the non-mosaic and mosaic Klinefelter syndrome groups.

	Non-mosaic group	Mosaic group	Odds ratio	*p*-Value
Number of ET cases	17 (50 cycles)	7 (29 cases)	–	–
Age of female patients	33.4 ± 4.5	37.3 ± 4.2	–	<0.01
Clinical pregnancy per ET (%)	28.0 (14/50)	20.7 (6/29)	1.49	0.47
Spontaneous abortion (%)	14.3 (2/14)	33.3 (2/6)	0.33	0.33
Live birth per ET cycle (%)	22.0 (11/50)	13.8 (4/29)	1.76	0.37
Cumulative live birth per ET case (%)	58.8 (10/17)	57.1 (4/7)	1.07	0.94
Cumulative live birth per micro-TESE case (%)	23.2 (10/43)	28.6 (4/14)	0.69	0.69

Abbreviation: ET, embryo transfer.

factor for spontaneous pregnancy.[18] Similarly, in Down syndrome (21 trisomy), a cohort with mosaic Down syndrome showed higher IQ scores than that with non-mosaic individuals.[19] Moreover, 7% of adults with mosaic Down syndrome had a child, compared with 1% of non-mosaic trisomic probands.[20] These findings suggest that the symptoms associated with chromosome aberrations are alleviated in cases of mosaicism with normal chromosomes. In this study, we found similar phenotypic characteristics. The mosaic KS group had a larger testis and lower FSH and higher testosterone levels than the non-mosaic KS group. However, the sperm retrieval rate from micro-TESE was comparable between the two groups. This is presumably due to the differences in age between the two groups. In the current study, the average age of the mosaic KS group (45.9 years) was approximately 14 years older than that of the non-mosaic KS group (32.2 years). Hence, a specific number of men with mosaic KS probably had ejaculated sperm in the semen during their youth but became azoospermic with age. Therefore, the young men with mosaic KS had not undergone chromosome testing. In general, advanced male age is a negative predictive factor of sperm retrieval in men with KS undergoing micro-TESE.[21-23] Okada et al.[21] also reported a non-linear relationship between sperm retrieval rate and age in KS and that sperm retrieval decreased after the age of 35. However, in this study, there was no difference in the average age between the sperm-retrieved (32.2 years; range 27–41) and without sperm-retrieved groups (35.0 years; range 26–45) in patients with non-mosaic KS. Moreover, there was no significant difference in the sperm retrieval rate of patients aged ≥35 years (41.2% [7/17]) and those <35 years old (50.0% [13/26]). Similarly, recent studies support the lack of an association between age and sperm retrieval rate on micro-TESE.[1,10]

Notably, we found that the mosaic KS group had a significantly better cleavage rate and blastocyst development rate after ICSI than the non-mosaic KS group, despite the older age of the partner. Hence, the mosaic KS group had a better quality of sperm obtained via micro-TESE than the non-mosaic KS group. The subgroup analysis revealed that the difference was based on the use of motile or immotile sperm. That is, the use of immotile sperm resulted in decreased fertilization and pregnancy rates because a certain proportion of immotile sperm is considered nonviable.[24,25] The reason why more motile sperms are found in the mosaic KS group is not clear. However, it could be attributed to the phenotypic difference between mosaic and non-mosaic KS. In general, most men with non-mosaic KS have a small testis, and the number of seminiferous tubules is few. Furthermore, only a limited lesion of spermatogenesis exists in the seminiferous tubules if any. Subsequently, only a few spermatozoa can be obtained via micro-TESE. In contrast, men with mosaic KS usually have a larger testis than those with non-mosaic KS,[2] resulting in a larger amount of seminiferous tubule, indicating a higher chance of obtaining motile sperm.

Further, the sperm-retrieved group had a lower mosaic XXY ratio than the without sperm-retrieved group (4.3% vs. 38.6%). The sperm retrieval rate in the group with extremely low (<7%) mosaic ratio reached 63.4% (7/11), and the oldest patient was aged 68 (his wife was 40 years old). Conversely, in the group with a high (>50%) mosaic ratio, no patients obtained spermatozoa. This indicates that the sperm-retrieved group had a higher proportion of XY cells in the testis than the without sperm-retrieved group. All male patients with KS who produce spermatozoa present with the mosaic karyotype (XY/XXY) in the testis and occasionally have XY spermatogonial cells, as only XY germ cells can complete meiosis and XXY cells are meiotically incompetent.[26] However, cytogenetic analysis of ejaculated spermatozoa from non-mosaic KS cases revealed the presence of both haploid and diploid spermatozoa. Therefore, some XXY germ cells can complete the meiotic process and produce mature haploid and diploid spermatozoa.[27] In chromosomal normal men, 46, XY germ cells originate in a similar proportion of 23, X and 23, Y spermatozoa. If 47, XXY spermatogonia can produce spermatozoa, the XX pairing will originate from 24, XY and 23, X spermatozoa, and the XY pairing will originate from 24, XX and 23, Y spermatozoa in the same proportion. However, Foresta et al.[27] reported a 23,X−/23,Y-bearing spermatozoa ratio of 2:1. Based on these findings, most spermatozoa did not originate from 46, XY spermatogonia. Interestingly, we found that in the cohort of current study, the mosaic KS group did indeed have a higher percentage of girl newborn (6 girls vs. 4 boys). In contrast, the percentage was the same in non-mosaic group (2 girls vs. 2 boys). However, only some studies have obtained these results; thus, further research should be performed to confirm such findings.

In the current study, the live birth rates per ET of the non-mosaic and mosaic KS groups were 22.0% and 13.8%, respectively. These results are comparable to the whole IVF cohort outcomes

based on female age (22.4% and 18.9%, respectively).[28] Moreover, the cumulative live birth rates of patients who underwent ET were 58.8% in the non-mosaic KS group and 57.1% in the mosaic KS group. This rate was higher than the previously reported one in the cohort with non-mosaic KS (35.9%).[29] Therefore, the possibility of fathering their own child is not extremely low. Future innovations that will improve the success rate of IVF for men with KS are expected.

The current study had several limitations. That is, the number of patients, particularly those with mosaic KS, was relatively small. Moreover, selection bias might have existed due to the retrospective nature of the current study, thereby resulting in age differences between the non-mosaic and mosaic KS groups. Therefore, the IVF outcomes between the two groups were simply not comparable. Hence, future studies based on large databases should be performed to identify a more detailed pathology of mosaic KS.

In conclusion, compared with the non-mosaic KS group, the mosaic KS group had significantly better ICSI outcomes. This was possibly due to the higher likelihood of obtaining motile sperm in this group.

CONFLICT OF INTEREST STATEMENT

Koji Chiba is an Editorial Board member of *Reproductive Medicine and Biology* and a co-author of this article. To minimize bias, he was excluded from all editorial decision-making related to the acceptance of this article for publication.

ETHICS STATEMENT

This study was approved by the Ethical Committee of Hanabusa Women's Clinic, which comprises members selected by our institution and a third-party medical institution (approval number: 2024-02).

HUMAN RIGHTS STATEMENT AND INFORMED CONSENT

All patients were well-informed about the study, and a written informed consent was obtained before the treatment period.

ANIMAL RIGHTS

This article does not contain any studies with animal subjects performed by any of the authors.

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