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Long-term clinical outcomes of testicular sperm extraction and intracytoplasmic sperm injection for infertile men

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Abstract

Purpose: To find the best methods to achieve the highest pregnancy and birth rates for couples needing testicular sperm extraction (TESE)-intracytoplasmic sperm injection (ICSI).

Methods: Retrospectively studied were 801 patients with male factor infertility who had undergone TESE-ICSI between April, 1996 and July, 2016 and who had been categorized into four groups: obstructive azoospermia (OA); non-obstructive azoospermia (NOA); Klinefelter syndrome (KS); and cryptozoospermia (Crypt). The sperm retrieval rate, hormone levels, fertilization rate (FR), pregnancy rate (PR), and birth rate (BR) after ICSI among three groups were compared: fresh testicular sperm (FS)-fresh oocytes (FO) (Group I); frozen-thawed testicular sperm-FO (Group II); and FS-vitrified-warmed oocytes (Group III).

Results: The testicular sperm recovery rate was 57.8% (463/801): 89.6% in the Crypt, 97.1% in the OA, 28.9% in the NOA, and 42.2% in the KS groups. The follicle-stimulating hormone levels were significantly higher in the NOA and KS groups and the testosterone levels were significantly lower in the KS group. The FR, PR, and BR were: 65.2%, 43.2%, and 28.5% in group I; 59.2%, 33.4%, and 18.7% in group II; and 56.4%, 33.8%, and 22.1% in group III.

Conclusion: Intracytoplasmic sperm injection with FS-FO achieved the best PR and BR. It should be considered what to do in cases with no testicular sperm by TESE. The authors hope that ICSI with donor sperm will be allowed in Japan in the near future.

KEYWORDS

azoospermia, fresh testicular sperm, frozen testicular sperm, intracytoplasmic sperm injection, testicular sperm extraction

1 | INTRODUCTION

Male infertility is observed in ~50% of all infertile couples. Azoospermia is defined as an absence of sperm in ejaculated semen. According to the World Health Organization, azoospermia is observed in 10%-15% of infertile men. An effective measure for azoospermia cases is testicular sperm extraction (TESE). The causes of azoospermia are obstructive azoospermia (OA), non-obstructive azoospermia (NOA), including a lack of the azoospermia factor (AZF) c region, and Klinefelter syndrome (KS) and the possibility of pregnancy and birth could be increased by using TESE-intracytoplasmic sperm injection (ICSI).[1-5] Men with male factor infertility have been treated successfully with ICSI and other additional methods, such as artificial oocyte activation, also have proven to be effective.[6,7] One effective technique for sperm retrieval that has been reported in a number of different studies is microdissection TESE (MD-TESE), which is performed for NOA, and simple TESE that is performed for OA.[8,9] According to recent reports, blood hormone levels, follicle-stimulating hormone (FSH), luteinizing hormone (LH), inhibin-B, testosterone (T), testicular volume, Y chromosome microdeletion, and chromosome inspection are used as predictors in sperm retrieval.[10-12] After retrieval, one study reported a comparison between fresh and vitrified oocytes that were used in ICSI with fresh testicular sperm. The fresh oocytes had a higher FR than the vitrified oocytes.[13] However, there are various reports that indicate that fresh or frozen-thawed sperm are useful to improve clinical outcomes.[14-17] Additionally, there are several reports on the influence of the maternal age factor.[15,18,19] This report is a retrospective, two-center study. The aim was to find the best methods to achieve the highest pregnancy rates (PRs) and birth rates (BRs) for couples who need TESE-ICSI.

2 | MATERIALS AND METHODS

2.1 | Patient population

From April, 1996 to July, 2016, 801 men with azoospermia or oligozoospermia underwent TESE in the authors' centers. Ultrasonography, clinical palpation, and clinical measurements of the testicular volume were performed. Hormonal assays, including serum FSH, LH, prolactin (PRL), and T, as well as karyotyping, were performed. The diagnoses were obtained from endocrine hormone levels (LH, FSH, PRL, and T) in the serum, chromosome karyotyping, clinical findings, and a semen analysis that was performed at least twice, which were confirmed by normal secondary sex characteristics. The cases with a testicular volume of both testes of >12 mL and FSH levels of <10 IU/mL were diagnosed as OA. The NOA cases included deletions of the AZFc region of the Y chromosome. The cases were classified into four groups: OA; NOA; KS; and Crypt.

Crypt was diagnosed in 58 cases; 272 were OA; and 388 were NOA. The NOA cases included 28 deletions of the AZFc region. There were 83 KS cases. Of the 737 ICSI cycles, 63 ICSI cycles were diagnosed as Crypt, 418 ICSI cycles as OA, 201 ICSI cycles as NOA, and 55 ICSI cycles were diagnosed as KS.

The various risks were explained in interviews and counseling. Written informed consent was obtained from all the patients who were involved in this study. Institutional review board approval was obtained from the local ethical committee.

2.2 | Ovarian stimulation

Ovarian stimulation was performed by using a combination of gonadotrophin-releasing hormone (GnRH) agonist, FSH, and human menopausal gonadotrophin (hMG) or GnRH antagonist, FSH, hMG, and clomiphene citrate. An injection of 5000 IU of human chorionic gonadotrophin (hCG) and/or GnRH agonist was administered when the dominant follicle reached a mean diameter of 18 mm. A vaginal ultrasound-guided follicular puncture was performed 36 hours after the hCG and/or GnRH agonist injection.

2.3 | Sperm cryopreservation and thawing

The testicular sperm were mixed with sperm cryopreservation fluid (Sperm Freeze; Medi-Con, Osaka, Japan) at a ratio of 1:0.7 before standing for 10 min. This then was exposed to liquid nitrogen (LN_2) vapor for 15 min before being immersed in LN_2 for storage.

When the sperm needed to be thawed, the frozen tube was warmed at room temperature for 10 s and placed directly into a 37°C water bath. Next, it was centrifuged with a hydroxyethyl piperazineethanesulfonic acid (HEPES)-buffered medium. The pellet was suspended in 0.2 mL of the same medium (G-MOPS; Vitrolife, Göteborg, Sweden) and then incubated at 37°C under 5% CO_2 in air for 2-3 hours.

2.4 | Testicular sperm extraction and intracytoplasmic sperm injection

Basically, TESE was timed to coincide with oocyte retrieval during controlled ovarian stimulation in each case. As long as there was a prospect of positive sperm retrieval, fresh TESE-ICSI was performed in the first cycles and MD-TESE was performed for NOA and simple TESE was performed for OA. In the OA cases, a simple TESE was performed at first. If no sperm were found, the switch was made to MD-TESE. The biopsy was testicular tissue that had been placed in a dish filled with HEPES-buffered medium (G-MOPS; Vitrolife). The MD-TESE was used, in which seminiferous tubules are directly examined throughout the testes by using an operating microscope and selectively biopsying for all of the patients as follows, in a modification of Schlegel's method.[20] The testicular tissue was minced with scissors in a dish and passed through a 21 gauge needle. After mincing, the tissue suspension was placed in a dish and covered with mineral oil. A microscope (×400) was used to check for sperm morphological quality and motility. An injection pipette was used to pick out the testicular sperm from the tissue suspension. The selected sperm were stored in a medium (Universal IVF; Origio, Copenhagen, Denmark).

The retrieved oocytes were cultured for 2-3 hours in a medium (Universal IVF; Origio) at 37°C in an atmosphere of 6% CO_2, 5% O_2, and 89% N_2 under humidified conditions and all the oocytes were denuded

enzymatically with 40 IU/mL of hyaluronidase (Origio), followed by mechanical denudation. Subsequently, the oocytes were rinsed two-to-three times and cultured in fresh culture medium until ICSI.

The ICSI was performed by using fresh testicular sperm or frozen-thawed testicular sperm. The injected oocytes were incubated in a culture medium under 6% CO_2 and 5% O_2 in air at 37°C. Normally, fertilized oocytes are defined as zygotes with two pronuclei (2PN) after ICSI. The clinical results using artificial oocyte activation were not included in this study.

2.5 | Embryo transfer

The observed 2PN and the top- or second-quality embryos were selected. The morphological quality of the embryos was measured according to Veeck's and Gardner's criteria.[21,22] A single fresh embryo transfer was performed at day 2 or day 3 after oocyte retrieval. Fresh embryo transfer was avoided in cases with a risk of ovarian hyperstimulation syndrome or when the endometrial thickness was <8 mm. In such cases, the blastocysts were vitrified and vitrified-warmed embryo transfer was scheduled for a subsequent cycle. A single vitrified-warmed blastocyst was transferred in either natural or hormone replacement therapy cycles. A clinical pregnancy was defined as pregnancy by observation of a gestational sac by a transvaginal ultrasound examination. The PRs were calculated per embryo transfer.

2.6 | Fertilization rate, pregnancy rate, and birth rate

The fertilization rate (FR), PR, and BR after ICSI were studied among three groups: fresh testicular sperm (FS)-fresh oocytes (FO) (Group I); frozen-thawed testicular sperm-FO (Group II); and FS-vitrified-warmed oocytes (Group III).

2.7 | Statistical analysis

The FRs were analyzed by the chi-square test and the hormonal levels and age by the t test. All the statistical analyses were performed with State Mate v. 5.01 for Windows (ATMS, Inc., Tokyo, Japan). All *P*-values of ≤.05 were considered to be statistically significant.

3 | RESULTS

3.1 | Sperm retrieval

Of the patients who underwent TESE, testicular sperm was retrieved successfully from 57.8% (463/801) of the patients. The retrieval rates in each group were as follows: 89.6% (52/58) for Crypt; 97.1% (264/272) for OA; 28.9% (112/388) for NOA; and 42.2% (35/83) for KS. The FSH levels were significantly higher in the NOA and KS groups, but the T levels were significantly lower in the KS group (Table 1).

3.2 | Hormonal levels

The FSH and LH levels were significantly higher in the NOA and KS groups than in the OA group. The T levels were significantly lower in the KS group than in the OA group (Table 1). The FSH and LH levels were significantly higher in the negative sperm retrieval group than in the positive group (*P*<.001). The T level was lower in the negative sperm retrieval group than in the positive group (*P* = .009) (Table 2).

TABLE 1 Clinical results of testicular sperm extraction (TESE)-intracytoplasmic sperm injection (ICSI)

Group	Crypt	OA	NOA	KS	Total
Patients (N)	58	272	388	83	801
Male age: y (mean ± SD)	38.7 ± 6.7	35.1 ± 6.5	35.5 ± 6.4	34.1 ± 4.9	35.5 ± 6.1
Positive retrieval (%)[a,b,c]	52 (89.6)	264 (97.1)	112 (28.9)	35 (42.2)	463 (57.8)
LH (mIU/mL)[a,b,c]	6.1 ± 3.8	4.1 ± 6.5	9.4 ± 7.4	18.6 ± 8.7	8.4 ± 7.5
FSH (mIU/mL)[a,b,c]	13.0 ± 10.1	6.1 ± 2.3	25.3 ± 15.9	36.8 ± 14.8	19.3 ± 16.5
PRL (mIU/mL)	12.9 ± 8.3	10.6 ± 5.7	14.1 ± 9.9	10.1 ± 5.5	12.4 ± 8.3
T (ng/mL) [a,b]	4.9 ± 1.8	5.1 ± 4.3	4.5 ± 2.5	2.5 ± 1.6	4.5 ± 2.4
No. of ICSI cycles	63	418	201	55	737
Female age, y	37.2 ± 4.5	34.2 ± 6.3	34.1 ± 4.9	32.8 ± 4.0	34.6 ± 4.9
No. of oocytes	367	2794	899	240	4300
No. of zygotes (%)[a,b,c]	218 (59.4)	1802 (64.5)	501 (55.7)	126 (52.5)	2647 (61.6)
No. of transfers	85	589	197	50	921
No. of pregnancies (%)	22 (25.9)	220 (37.3)	82 (41.6)	20 (40.0)	344 (37.4)
No. of miscarriages (%)	3 (3.5)	44 (7.5)	21 (10.7)	3 (6.0)	71 (7.7)
No. of live births (%)	14 (16.5)	147 (25.0)	43 (21.8)	14 (28.4)	218 (23.7)

Crypt, cryptozoospermia; FSH, follicle-stimulating hormone; KS, Klinefelter syndrome; LH, luteinizing hormone; NOA, non-obstructive azoospermia; OA, obstructive azoospermia; PRL, prolactin; SD, standard deviation; T, testosterone. [a]*P* < .01: OA vs NOA; [b]*P* < .01: OA vs KS; [c]*P* < .05: OA vs Crypt.

TABLE 2 Comparison of positive sperm retrieval and negative sperm retrieval

Sperm retrieval	Positive	Negative	*P*-value
Patients (N)	463	337	—
Male age, years (mean ± SD)	35.9 ± 7.3	34.5 ± 5.3	.006
LH (mIU/mL)	6.7 ± 5.5	10.7 ± 9.1	<.001
FSH (mIU/mL)	13.8 ± 13.7	26.5 ± 17.2	<.001
PRL (mIU/mL)	11.8 ± 7.8	13.2 ± 8.9	.406
T (ng/mL)	4.7 ± 2.4	4.3 ± 2.4	.009

FSH, follicle-stimulating hormone; LH, luteinizing hormone; PRL, prolactin; SD, standard deviation; T, testosterone.

3.3 | Fertilization rate, pregnancy rate, and birth rate in cryptozoospermia, obstructive azoospermia, non-obstructive azoospermia, and Klinefelter syndrome

The 424 couples who underwent 737 TESE-ICSI cycles were studied. Their FR, PR, and BR levels were respectively 61.6%, 37.4%, and 23.7% in all patients; 59.4%, 25.9%, and 16.5% in the Crypt patients; 64.5%, 37.3%, and 25.0% in the OA patients; 55.7%, 41.6%, and 21.8% in the NOA patients; and 52.5%, 40.0%, and 28.4% in the KS patients (Table 1).

3.4 | Fertilization rate, pregnancy rate, and birth rate in Groups I-III

The FR, PR, and BR were respectively 65.2%, 43.2%, and 28.5% in group I, 59.2%, 33.4%, and 18.7% in group II, and 56.4%, 33.8%, and 22.1% in group III. The PR and BR in group I was significantly higher than those of group II ($P = .004$ and $P < .001$, respectively) (Table 3).

4 | DISCUSSION

This study analyzed the clinical results of TESE since 1996. First were investigated the sex and pituitary hormone levels in the male positive sperm retrieval and negative sperm retrieval groups. The sperm retrieval rate was 57.8% of the total; however, the result that FSH and LH were higher in the sperm-negative cases simply reflects the fact that they are usually higher in a NOA cohort, compared to an OA cohort and OA cases were a large proportion of all the cases in this study. This is related to an accidental bias and there is no locality. In the current results, the FSH and LH hormone levels were significantly higher in the negative sperm retrieval group than in the positive group. The T levels were lower in the negative sperm retrieval group than in the positive group. Spermatogenic function of the testis needs to be suitable in various sex hormone parameters. Recent reports of the predictive factor of sperm retrieval investigated FSH, LH, inhibin-B, and T.[10,11] In many cases of high FSH levels, there is a high possibility that sperm retrieval will fail. The FSH levels are considered to be useful as a predictor. However, according to one report, men with maturation arrest have normal serum FSH levels (≤ 7.6 IU/mL). These patients with spermatozoa with maturation arrest had a higher frequency of genetic anomalies, compared to NOA patients.[23] Male age also is considered to be a predictive factor in KS cases.[5,24] The hormonal level indication is not intended to set the criteria for the positive or negative result of sperm retrieval; it is important to comprehend the patient's background by receiving adequate patient information prior to surgery.

Comparing fresh TESE-ICSI and frozen-thawed TESE-ICSI injected with fresh oocytes, the factors of female age, FRs, and clinical PRs of the fresh TESE-ICSI group were significantly higher than in the frozen-thawed TESE-ICSI group. A couple of reports found that female age factors did not influence TESE-ICSI in each case.[15,19] In previous reports, FRs were lower in older women.[18] However, they did not compare fresh sperm with frozen-thawed sperm.[16,17]

There are various reports on the aneuploidy of sperm.[25,26] The assessment of testicular function and sperm quality is related to varicocele,[27-30] aging,[31-35] and freezing-thawing. Male aging does not influence testicular sperm specifically.[35] However, the sperm recovery rate decreases according to men's age in KS, so men's age should be considered.[36] Freezing-thawing of sperm in assisted reproductive technology (ART) is an important technique.

Freezing and thawing is an important technique in ART, although it is difficult to avoid damage to cryopreserved sperm in the freezing and thawing process. It is known to result in sperm DNA fragmentation[37,38] and decreased motility and mitochondrial function.[39] DNA damage is correlated with the period of gestation after embryo transfer. The abnormality of sperm chromatin also affects the growth after implantation and has a negative effect on pregnancy loss.[40,41] Sperm cryopreservation and in vitro incubation are related to DNA integrity of the testicular sperm.[42] The PR has been shown to be significantly lower in the frozen-thawed TESE-ICSI group than in the fresh TESE-ICSI group.[43] This study found that fresh TESE-ICSI in the OA group showed significantly higher FRs and PRs than cryopreserved testicular sperm, though this was not the case in the NOA group. The current authors could not identify the causes. There might be cases that are sensitive to freezing damage. The authors would like to investigate this as a subject of analysis. In ICSI, morphologically normal sperm are selected as much as possible, focusing on the lack of observable DNA and the structural integrity of normal sperm under a bright field. Therefore, it is desirable to avoid sperm damage.

There is no guarantee, depending on the case, that TESE can provide sufficient sperm retrieval after surgery. In cases of low sperm counts, other methods, such as the cryoloop and empty zona methods could be used for cryopreservation. However, these methods are not useful for cryopreserving a few sperm in clinical practice.[44] In this study, the FRs and PRs were not significantly different. However, a study also reported cases with small numbers of recovered sperm in non-mosaic KS cases, with the result that non-pregnant couples obtained only a few immotile sperm following the freezing-thawing procedure. For such cases, fresh TESE-ICSI should be considered first.[5] In particular, it is very difficult to cryopreserve in NOA and KS cases with few sperm that lack motility and have severe DNA damage (eg,

TABLE 3 Results after fresh oocytes were injected with fresh and frozen-thawed testicular sperm

Condition	Group		
Cryptozoospermia	**Group I: Fresh oocytes and fresh sperm**	**Group II: Fresh oocytes and frozen-thawed sperm**	**Group III: Frozen-thawed oocytes and fresh sperm**
Patients (N)	19	17	–
No. of ICSI cycles	19	33	10
Female age, years (mean ± SD)	35.5 ± 4.6	38.5 ± 4.0	36.5 ± 5.0
No. of oocytes	159	169	33
N (%) of zygotes	102 (64.2)	94 (55.6)	22 (66.7)
No. of transfers	32	38	8
N (%) of pregnancies	8 (25.0)	11 (28.9)	3 (37.5)
N (%) of miscarriages	1 (3.1)	0 (.0)	1 (12.5)
N (%) of births	1 (3.1)[a]	9 (23.7)[a]	1 (12.5)
[a]$P < .05$	–		
Obstructive azoospermia	**Group I: Fresh oocytes and fresh sperm**	**Group II: Fresh oocytes and frozen-thawed sperm**	**Group III: Frozen-thawed oocytes and fresh sperm**
Patients (N)	130	113	–
No. of ICSI cycles	139	237	38
Female age, years (mean ± SD)	32.1 ± 4.7	36.1 ± 4.6	33.9 ± 4.8
No. of oocytes	1299	1241	250
N (%) of zygotes	878 (67.6)[a]	775 (62.4)[a]	148 (59.2)[b]
No. of transfers	260	280	73
N (%) of pregnancies	115 (44.2)[a,b]	92 (32.9)[a]	21 (28.8)[b]
N (%) of miscarriages	25 (9.6)	17 (6.1)	4 (5.5)
N (%) of births	82 (31.5)[a]	55 (19.6)[a]	14 (19.1)[b]
[a]$P<.01$ and [b]$P<.05$	–	–	–
Non-obstructive azoospermia	**Group I: Fresh oocytes and fresh sperm**	**Group II: Fresh oocytes and frozen-thawed sperm**	**Group III: Frozen-thawed oocytes and fresh sperm**
Patients (N)	68	38	–
No. of ICSI cycles	71	78	50
Female age, years (mean ± SD)	32.7 ± 4.6	35.7 ± 5.1	33.8 ± 4.2
No. of oocytes	438	367	94
N (%) of zygotes	260 (59.4)	193 (52.6)	48 (51.1)
No. of transfers	73	96	40
N (%) of pregnancies	36 (49.3)	33 (34.3)	15 (37.5)
N (%) of miscarriages	8 (11.0)	10 (10.4)	3 (7.5)
N (%) of births	23 (31.5)[a]	12 (12.5)[a]	9 (22.5)
[a]$P < .01$	–	–	–
Klinefelter syndrome	**Group I: Fresh oocytes and fresh sperm**	**Group II: Fresh oocytes and frozen-thawed sperm**	**Group III: Frozen-thawed oocytes and fresh sperm**
Patients (N)	19	14	–
No. of ICSI cycles	19	18	18
Female age, years (mean ± SD)	32.1 ± 3.5	33.4 ± 3.9	32.8 ± 4.3
No. of oocytes	77	100	63
No. of zygotes (%)	46 (59.7)	50 (50.0)	30 (47.6)
No. of transfers	17	14	18
N (%) of pregnancies	5 (29.4)	7 (50.0)	8 (44.4)
N (%) of miscarriages	2 (11.8)	0 (.0)	1 (12.5)
N (%) of births	3 (17.6)	4 (28.6)	7 (38.9)

(Continues)

TABLE 3 (Continued)

Total	Group I: Fresh oocytes and fresh sperm	Group II: Fresh oocytes and frozen-thawed sperm	Group III: Frozen-thawed oocytes and fresh sperm
Patients (N)	236	182	—
No. of ICSI cycles	248	366	116
Female age, years (mean ± SD)	32.5 ± 4.7	36.1 ± 4.7	33.9 ± 4.6
No. of oocytes	1973	1877	440
N (%) of zygotes (%)	1286 (65.2)[a]	1112 (59.2)[a]	248 (56.4)
No. of transfers	382	428	139
N (%) of pregnancies (%)	165 (43.2)[a]	143 (33.4)[a]	47 (33.8)
N (%) of miscarriages	36 (9.4)	27 (6.3)	9 (6.5)
N (%) of births	109 (28.5)[a]	80 (18.6)[a]	31 (22.1)

SD, standard deviation.
[a]$P<.01$.
[b]$P<.05$.

hypospermatogenesis cases). In Crypt cases, semen cryopreservation before TESE also might be an alternative for cases that are expected to have low probabilities of obtaining fresh sperm at the time of egg retrieval.

As there are ~50 urology specialists in Japan, it is very difficult for full-time special urologists to perform TESE in ~600 ART centers every day. Thus, in the authors' two centers, two part-time special urologists perform TESE one day per week realistically. There have been good results following ICSI with fresh testicular sperm and vitrified warmed oocytes.[13] In conclusion, this study's results suggest that fresh testicular sperm have a high developmental potential, compared to frozen-thawed testicular sperm.

The authors recommend the use of fresh oocytes with fresh testicular sperm as the first-choice treatment. The second-choice treatment would be vitrified-warmed oocytes with fresh testicular sperm or fresh oocytes with frozen-thawed testicular sperm, depending on the couple's opinion. It is important to understand the background of each patient and to select the treatment strategies accordingly.

DISCLOSURES

Conflict of interest: The authors declare no conflict of interest. *Human and Animal Rights*: This study was approved by an institutional ethics committee. This article does not contain any studies with human or animal subjects performed by any of the authors.

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