Potential of testicular sperm to support embryonic development to the blastocyst stage is comparable to that of ejaculated sperm

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Abstract

Purpose Recent studies have shown that improved clinical outcomes can be achieved by transferring blastocysts rather than cleavage-stage embryos. However, blastocyst transfer is not performed in all patients. The aim of this study was to compare clinical outcomes of intracytoplasmic sperm injection (ICSI) cycles using testicular sperm (TE) with those of ICSI cycles using ejaculated sperm (EJ).

Methods ICSI was performed using EJ in 141 cycles and TE in 37 cycles. Embryos were cultured for 5 days. The quality of embryos was assessed on days 3 and 5 before embryo transfer.

Results Fertilization rate was 77.3% in the EJ group and 69.6% in the TE group ($p < 0.05$). The good-quality embryos on day 3 and 5 were not different between the EJ and TE groups. Embryos did not develop to blastocyst stage in 7 cycles of the EJ group (5.0%) and 2 cycles of the TE group (5.4%). There were no significant differences in blastocyst formation and blastocyst quality (46.1% vs. 47.5% and 5.7% vs 5.8%, respectively) on day 5 between both groups. Embryos were transferred in all cycles. Implantation (22.8 vs. 24.7%), clinical pregnancy (44.7 vs. 43.2%), miscarriage (21.7 vs. 33.3%), and delivery (76.5 vs. 66.7%) did not differ between EJ group and TE group. Clinical outcomes of ICSI were not different between the EJ and TE groups.

Conclusions In conclusion, the potential of testicular sperm supporting embryonic development to blastocysts is comparable to that of ejaculated sperm. Therefore, this study suggests that blastocyst transfer can be a very useful assisted reproductive technique in the ICSI cycles that require the use of testicular sperm, and the clinical outcomes of the cycles are comparable to those of ICSI cycles using ejaculated sperm.

Keywords Intracytoplasmic sperm injection · Testicular sperm extraction · Ejaculated sperm · Testicular sperm · Blastocyst

Introduction

At the beginning of its introduction, intracytoplasmic sperm injection (ICSI) was performed mainly in the treatment of

male factor infertility. Male factor infertility has been overcome successfully, and high pregnancy rates have been reported after treatment of male factor infertility by ICSI. The application of ICSI has gradually expanded since its introduction, and recently, it has been extensively applied not only in the treatment of male factor infertility, but also in the treatment of female factor infertility. Although ICSI is widely applied, it remains controversial whether the source or quality of sperm used to perform ICSI affect the clinical outcomes of ICSI, such as fertilization, development to blastocyst, implantation, and pregnancy.

The quality of sperm can be very different depending on the histopathological causes in men—even if the sperm are retrieved from same source. Because sperm qualities differ among men, the clinical outcomes of ICSI might be affected by sperm quality. A number of studies have reported that clinical outcomes of human in vitro fertilization (IVF) were better when the quality of sperm used to

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fertilize retrieved oocytes was good, rather than when sperm quality was poor [1, 2].

The sperm sources used for ICSI are various. ICSI can be performed using ejaculated sperm (EJ), epididymal sperm, or testicular sperm (TE) [3–5]. There are many studies comparing the clinical outcomes of ICSI according to sperm sources. However, there is still controversy about the clinical outcomes of ICSI according to sperm sources. Several studies have reported the improved clinical ICSI outcomes when EJ or epididymal sperm were used for ICSI than when TE were used. Compared to EJ or epididymal sperm, fertilization rates were significantly lower when ICSI was performed using TE. Blastocyst formation rate was also lower when ICSI was performed using TE, than when ICSI was performed using EJ [6, 7]. Moreover, implantation rates were significantly higher, and the rate of miscarriage was significantly lower in the latter than in the former. The number of embryos which developed to blastocyst stage by day 5 was the lowest in embryos originating from TE of men with azoospermia. Sperm retrieved from men with defective spermatogenesis have been expected to adversely affect the viability, quality, and development of embryos [8–10]. The effect of sperm on embryonic development, namely paternal effect, has been described in a number of recent studies. It has been suggested that sperm retrieved from men with defective spermatogenesis will have a negative paternal effect on embryonic development. However, to date, a negative paternal effect of such sperm on embryonic development is unclear and controversial.

Many studies have reported that clinical outcomes of ICSI using TE are inferior to those of ICSI using EJ, but several studies have reported that clinical outcomes of ICSI were similar regardless of sperm sources [11, 12]. According to the studies, fertilization rate and embryonic development were not different between ICSI cycles using EJ and those using TE. And the ability of TE to support embryonic development is comparable to that of EJ or epididymal sperm. In single blastocyst transfer cycles, ongoing pregnancy rates did not differ between cycles in which epididymal or TE were used for ICSI and cycles in which EJ were used for ICSI. Furthermore, the rate was similar to that of IVF cycles in which conventional insemination was performed using EJ. And the rate of cycles in which one or more embryos developed to blastocysts did not differ among them. The implantation and pregnancy rates were not different when ICSI was performed using ejaculated, epididymal, or testicular sperm, and cleavage-stage embryos were transferred on day 3. There were also no significant differences in miscarriage rates, although the rate was slightly higher when ICSI was performed using TE than when EJ or epididymal sperm were used. In another study, implantation rates were significantly higher in

cycles in which ICSI was performed using TE than in cycles where EJ or epididymal sperm were used [13–16].

To date, it remains unclear whether the quality or source of sperm can affect the clinical outcomes of ICSI. As to whether the ability of TE to support embryonic development to blastocyst stage is comparable to that of EJ is currently under debate. To evaluate whether the sperm source can affect the clinical outcomes of ICSI, we analyzed the clinical outcomes in cycles in which ICSI was performed using EJ or TE and embryos were transferred on day 5. In addition, we compared the ability of sperm to support embryonic development to blastocyst stage between EJ and TE sperm by assessing blastocyst formation and quality in those cycles.

Materials and methods

Patients, controlled ovarian hyper-stimulation, and fertilization

A total of 172 couples underwent 178 IVF cycles from January 2006 to December 2016. The women were all under 34 years of age. ICSI was performed using EJ in 141 cycles for 135 couples (EJ group) and using TE in 37 cycles involving 35 couples. Embryos were transferred at day 5 in all cycles. In the EJ group, indications of infertility were male factor infertility (90 cycles in 84 couples), female factor infertility (27 cycles in 27 couples), and unexplained infertility (9 cycles involving 9 couples). Fifteen couples had female factor and male factor infertility (15 cycles). In the TE group, testicular sperm extraction (TESE) was performed due to non-obstructive azoospermia in 12 patients (12 cycles) and obstructive azoospermia in 25 patients (25 cycles). Among the patients with non-obstructive azoospermia, two were patients with retrograde ejaculation. TESE was performed in these individuals because no sperm were present in urine.

Controlled ovarian hyper-stimulation was carried out using GnRH agonist or GnRH antagonist, recombinant FSH, or hMG and hCG. Oocytes were retrieved transvaginally under ultrasound guidance 35 h after hCG injection. Cumulus cells were removed enzymatically and then mechanically approximately 2 h after oocyte retrieval. Cumulus cells were removed by brief exposure of cumulus-oocyte-complexes to medium containing 80 IU/ml hyaluronidase (SAGE, Trumbull, CT, USA). Then, cumulus cells that were not removed by exposure to hyaluronidase were removed completely by aspirating oocytes in and out of hand-drawn glass capillaries. The status of oocyte maturation was observed under an inverted microscope (Nikon, Tokyo, Japan). ICSI was performed only in metaphase II oocytes using EJ or TE sperm more than 1 h after removal of cumulus cells.

Testicular sperm extraction

Physical examination, hormone profiling, and semen analyses were performed in all men. Men were diagnosed as azoospermic when no sperm were present in three consecutive semen analyses. Non-obstructive azoospermia was diagnosed based on the histopathological results of testicular biopsy. TESE was performed on the day of, or the day before, oocyte retrieval. A small incision was made in the scrotum and tunica vaginalis. The tunica albuginea was opened and a small piece of exposed testicular tissue was excised. Testicular tissue was collected from men with obstructive azoospermia by conventional TESE and from men with non-obstructive azoospermia by microdissection TESE using microscope. The testicular tissue was gently dissected under a dissecting microscope after several rinses with Ham's F-10 medium supplemented with 0.4% human serum albumin. Seminiferous tubules were squeezed using two pairs of fine forceps after careful removal of blood vessels and connective and other tissues. The presence of spermatozoa was examined under a microscope. Testicular sperm were incubated at 37 °C, 6% CO₂ in air until ICSI was performed.

Assessment of fertilization and embryo quality

Fertilization was assessed 16 h after ICSI. Only oocytes with two pronuclei and the second polar body were considered normally fertilized oocytes. When more than ten normally-fertilized oocytes were observed after the fertilization status of individual oocytes was assessed, eight or nine fertilized oocytes were cultured to blastocysts. The others were frozen using slow freezing at the pronuclear stage. The zygotes transferred to medium were cultured to blastocyst stage in 50- μ l droplets of medium covered with paraffin oil and incubated in 6% CO₂ in a humidified atmosphere at 37 °C. The culture medium was changed from cleavage medium to blastocyst medium on day 3 after oocyte retrieval.

Embryo quality was assessed immediately before changing the culture medium on day 3 and before embryo transfer on day 5. On day 3, embryos were classified as good, fair, or poor-quality embryos according to the number of blastomeres, the symmetry of blastomeres and the degree of fragmentation. Embryos with more than seven even-sized blastomeres and less than 10% fragmentation were classified as good-quality embryos. Embryos with more than six uneven-sized blastomeres and 11–40% fragmentation were classified as fair-quality embryos. Embryos with more than 40% fragmentation, regardless of the blastomere number and symmetry, or embryos with less than five blastomeres were classified as poor-quality embryos. Blastocysts were classified into six grades based on the degree of expansion and the status of hatching [17]: early blastocyst (grade 1), middle blastocyst (grade 2), blastocyst (grade 3), expanded blastocyst (grade

4), hatching (grade 5), or hatched blastocyst (grade 6). Subsequently, the quality of the inner cell mass (ICM) and trophectoderm were assessed in blastocysts, except early and middle blastocysts. ICM was classified into three grades: ICM with many tightly packed cells (grade A), several loosely packed cells (grade B), or very few cells (grade C). Trophectoderm was also classified into three grades: trophectoderm with many cohesive cells (grade A), several loose cells (grade B), or very few large cells (grade C). Fully expanded blastocysts ($\geq$ grade 4) with grade A ICM and grade A or B trophectoderm were classified as good-quality embryos. Blastocysts ($\geq$ grade 3) with grade B ICM and grade A or B trophectoderm were classified as fair-quality embryos. Embryos that did not develop to blastocyst, early, middle blastocyst, or blastocysts with grade C ICM and trophectoderm, regardless of the degree of expansion and hatching status, were assigned as poor-quality embryos. Embryo quality was compared between EJ and TE groups.

Statistical analysis

Continuous data are expressed as means $\pm$ SD. Fertilization rate was calculated as the percentage of normally fertilized oocytes per sperm-injected oocytes at each cycle. Blastocyst formation rate was calculated as the percentage of blastocysts per cultured embryos at each cycle. And mean $\pm$ SD of the fertilization rates or blastocyst formation rates were presented in tables. Categorical data are presented as percent frequency. Implantation rate was the percentage of the number of gestational sacs to the number of transferred embryos. Pregnancy and delivery outcomes, such as clinical pregnancy, miscarriage, ectopic pregnancy, delivery, follow-up loss, were calculated per cycle. For statistical analysis, the Statistics Package for Social Sciences, version 12.0 for Windows (SPSS Inc., Chicago, IL, USA), was used. Independent samples *t* test or Mann-Whitney *U* test was performed to analyze the significant differences of continuous data between EJ and TE groups. Chi-square test was performed to analyze significant differences in categorical data. A *p* value of <0.05 was considered significantly different between two groups.

Results

Fertilization and embryo transfer after ICSI using ejaculated sperm or testicular sperm

Characteristics of patients and variables of cycles were compared between both groups (Table 1). Body mass indexes (BMI) of female partners were significantly higher in the TE group than in the EJ group (22.0 ± 2.9 vs. 20.9 ± 2.9 kg/m², $p < 0.05$). Baseline characteristics of patients except for BMI, such as age of female (31.2 ± 2.5 vs. 30.2 ± 2.9 years old)

Table 1 Comparison of patients' characteristics between two groups

	ICSI using ejaculated sperm	ICSI using testicular sperm	<i>p</i> value
No. of cycles	141	37	
Age of female partner (year) ^a	31.2 ± 2.5	30.2 ± 2.9	0.057
BMI of female partner (kg/m ²) ^a	20.9 ± 2.7	22.0 ± 2.9	0.025
Age of male partner (year) ^a	34.7 ± 3.7	35.5 ± 5.4	0.794
Duration of infertility (year) ^a	3.6 ± 2.3	2.9 ± 2.3	0.070
FSH level on day 2 or 3 of the menstrual cycle (mIU/ml) ^a	7.4 ± 2.5	7.0 ± 1.7	0.428
Duration of controlled ovarian hyper-stimulation (days) ^a	9.9 ± 1.7	10.2 ± 1.7	0.344
Dosage of FSH used to stimulation (IU) ^a	2317.6 ± 942.9	2457.8 ± 909.5	0.353
E ₂ level on hCG administration day (pg/ml) ^a	3097.1 ± 1995.8	3578.9 ± 1759.1	0.075
No. of retrieved oocytes	2672	797	0.014
No. of injected oocytes	2116 (79.2)	620 (77.8)	0.026

^a Values represent means ± standard deviation

and male partners (34.7 ± 3.7 vs. 35.5 ± 5.4 years old) and duration of infertility (3.6 ± 2.3 vs. 2.9 ± 2.3 years), were not different between both groups. And cycle variables, such as FSH level on day 2 or 3 of the menstrual cycles (7.4 ± 2.5 vs. 7.0 ± 1.7 mIU/ml), duration of controlled ovarian stimulation (COH) (9.9 ± 1.7 vs. 10.2 ± 1.7 days), total dosage of FSH used for COH (2317.6 ± 942.9 vs. 2457.8 ± 909.5 IU), and E₂ level on day of hCG administration (3097.1 ± 1995.8 vs. 3297.5 ± 1669.7 pg/ml), were comparable between the both groups.

Normal fertilization (2PN and the second polar body) rate was compared between the EJ and TE groups (Table 2). Ejaculated sperm were injected into oocytes in 141 cycles and testicular sperm in 37 cycles. A total of 2672 oocytes (19.0 ± 8.1 per cycle) were retrieved, and ejaculated sperm were injected into 2116 oocytes (15.0 ± 6.8 per cycle) in the EJ group. Among the injected oocytes, normal fertilization was observed in 1617 oocytes (11.5 ± 5.3 per cycle). Seven hundred and ninety-seven oocytes (21.5 ± 6.1 per cycle) were retrieved, and testicular sperm were injected into 620 oocytes (16.8 ± 4.7 per cycle) in the TE group. Normal fertilization was observed in 437 oocytes (11.8 ± 4.8 per cycle). Significantly more oocytes were retrieved in the TE group than in the EJ group (*p* < 0.05). There was no significant

difference in the rate of metaphase II oocytes (80.0 ± 12.7% vs. 79.5 ± 14.3%, respectively). Normal fertilization rate was significantly higher (*p* < 0.05) in the EJ group (77.3 ± 15.0%) than in the TE group (69.6 ± 17.0%). Embryos were transferred on day 5 in all cycles. An average of 2.5 ± 0.8 embryos was transferred in the EJ group and 2.3 ± 0.8 embryos in the TE group. The number of transferred embryos was not different between the two groups. Implantation was confirmed by examining the number of gestational sacs. Implantation rate was 22.8% in the EJ group and 24.7% in the TE-group. Implantation rate was not different between two groups.

In the TE group, fertilization was compared between patients with obstructive azoospermia and patients with non-obstructive azoospermia (Supplemental Table 1). Clinical outcomes of patients with retrograde ejaculation were excluded in comparison between obstructive and non-obstructive azoospermia patients. Female age was not different between patients with obstructive azoospermia and patients with non-obstructive azoospermia (30.7 ± 2.7 vs. 29.2 ± 3.4). In total, 544 oocytes (21.8 ± 6.3 per cycle) were retrieved, and testicular sperm were injected into 427 oocytes (17.1 ± 5.3 per cycle) in patients with obstructive azoospermia. Three hundred and eighteen oocytes were normally fertilized (12.7 ± 5.3 per cycle). In patients with non-obstructive azoospermia, 219

Table 2 Fertilization after intracytoplasmic sperm injection (ICSI) using ejaculated or testicular sperm

	ICSI using ejaculated sperm	ICSI using testicular sperm	<i>p</i> value
No. of cycles	141	37	
No. of retrieved oocytes ^a	2672 (19.0 ± 8.1)	797 (21.5 ± 6.1)	0.014
No. of injected oocytes ^a	2116 (15.0 ± 6.8)	620 (16.8 ± 4.1)	0.026
No. of normally fertilized oocytes (2PN) ^a	1617 (11.5 ± 5.3)	437 (11.8 ± 4.8)	0.688
Rate of normally fertilized oocytes (%) ^b	77.3 ± 15.0	69.6 ± 17.0	0.017
No. of cultured embryos ^a	1171 (8.3 ± 2.3)	326 (8.8 ± 2.0)	0.103
No. of transferred embryos ^a	346 (2.5 ± 0.8)	85 (2.3 ± 0.8)	0.297
No. of gestational sacs (%) ^c	79 (22.8)	21 (24.7)	0.716

^a Values in parentheses represent mean number of oocytes or embryos per cycle. Values are expressed as mean ± standard deviation^b Values are the average of the fertilization rate after the fertilization rate is calculated for each cycle. Values are expressed as mean ± standard deviation^c Values are the percentage of gestational sacs to transferred embryos

oocytes (21.9 ± 6.0 per cycle) were retrieved, and testicular sperm were injected into 160 oocytes (10 ± 3.1 per cycle). Normal fertilization was observed in 101 oocytes (10.1 ± 2.7 per cycle). The rate of normal fertilization was not different between patients with obstructive azoospermia and patients with non-obstructive azoospermia (73.3 ± 16.3 vs $63.9 \pm 15.7\%$, respectively).

Embryo quality and development to blastocyst stage after ICSI using ejaculated sperm or testicular sperm

Among the fertilized oocytes, some zygotes were frozen at the pronuclear stage, and the remaining zygotes were cultured for 5 days. Embryo quality was observed on day 3 and just before embryo transfer on day 5 (Table 3). In total, 1171 zygotes were cultured for 5 days in the EJ group. Of these, 298 embryos (2.1 ± 1.8 per cycle) were good-quality embryos on day 3. In the TE group, 326 zygotes were cultured for 5 days. Of these, 66 embryos (1.8 ± 1.9 per cycle) were assessed as good quality. There was no difference in good-quality embryos on day 3 between the EJ and TE groups. In the EJ group, 58 embryos (0.4 ± 0.7 per cycle) were good-quality embryos on day 5 after oocyte retrieval. Eighteen embryos (0.5 ± 0.7 per cycle) showed good quality in the TE group on day 5. Similar to embryo quality on day 3, there were no differences in good-quality embryos on day 5 between both groups. In the TE group, embryo quality was not different between patients with

obstructive azoospermia and those with non-obstructive azoospermia patients on days 3 and 5 (Supplemental Table 2).

Blastocyst formation rate was compared between the EJ and TE group (Table 4). In the EJ group, embryos did not develop to blastocysts in 7 of 141 cycles (5.0%). In total, 1171 embryos were cultured for 5 days, and 532 embryos (3.8 ± 2.1 per cycle) developed to blastocyst stage ($46.1 \pm 24.7\%$). Among the blastocysts, 188 embryos (1.3 ± 1.5 per cycle) were expanded or hatching blastocysts. In the TE group, embryos did not develop to blastocysts in 2 of 37 cycles (5.4%). A total of 326 embryos were cultured for 5 days, and 154 embryos (4.2 ± 2.2 per cycle) developed to blastocyst stage. Sixty-one embryos (1.6 ± 1.5 per cycle) were expanded or hatching blastocysts. The blastocyst formation rate was not different between the EJ and TE groups (46.1 ± 24.7 vs. $47.5 \pm 21.6\%$, respectively). In the TE group, the blastocyst formation rate and the rate of embryos that were developed to expanded or hatching blastocysts were not different between patients with obstructive azoospermia and patients with non-obstructive azoospermia (Supplemental Table 3).

Pregnancy outcomes after ICSI using ejaculated sperm or testicular sperm

Clinical pregnancy was confirmed in 63 cycles of the EJ group (44.7%) and in 16 cycles of the TE group (43.2%) (Table 5). There was no significant difference in clinical pregnancy rate between both groups. Pregnancy was not established in cycles

Table 3 Embryo quality on day 3 and day 5 after intracytoplasmic sperm injection (ICSI) using ejaculated or testicular sperm

		ICSI using ejaculated sperm	ICSI using testicular sperm	<i>p</i> value
Day 3	Good-quality embryos ^a (%)	2.1 ± 1.8 (27.7 ± 24.0)	1.8 ± 1.9 (19.5 ± 19.9)	0.184
	Fair-quality embryos ^b (%)	3.9 ± 2.2 (45.9 ± 23.1)	4.7 ± 1.9 (53.6 ± 21.6)	0.025
	Poor-quality embryos ^c (%)	2.3 ± 2.1 (26.7 ± 20.3)	2.4 ± 1.9 (26.8 ± 21.1)	0.678
Day 5	Good-quality embryos ^d (%)	0.4 ± 0.7 (5.7 ± 9.8)	0.5 ± 0.7 (5.5 ± 8.8)	0.523
	Fair-quality embryos ^e (%)	1.2 ± 1.1 (15.2 ± 14.5)	1.5 ± 1.3 (17.4 ± 14.2)	0.142
	Poor-quality embryos ^f (%)	6.7 ± 2.7 (79.3 ± 19.5)	6.8 ± 2.0 (77.1 ± 15.3)	0.654

Values are mean number of embryos per cycle and expressed as means $\pm$ standard deviation

Values in parentheses are the average of the rates after the rates of good-, fair-, or poor-quality embryos are calculated for each cycle. Values are expressed means $\pm$ standard deviation

^a Embryos with seven or more even-sized blastomeres and less than 10% cytoplasmic fragmentation on day 3 after oocyte retrieval

^b Embryos with six or more uneven-sized blastomeres and 10 to 40% cytoplasmic fragmentation on day 3 after oocyte retrieval

^c Embryos with five or less blastomeres or more than 40% cytoplasmic fragmentation on day 3 after oocyte retrieval

^d Expanded, hatching, or hatched blastocysts with ICM consisted of tightly packed cells on day 5 after oocyte retrieval

^e Blastocysts with ICM consisted of several loose cells and trophoctoderm consisted of several cells on day 5 after oocyte retrieval

^f Embryos that did not develop to blastocysts, early, middle blastocysts, or blastocysts with ICM consisted of very few cells and trophoctoderm with very few large cells on day 5 after oocyte retrieval

Table 4 Development to blastocyst stage after intracytoplasmic sperm injection (ICSI) using ejaculated or testicular sperm

	ICSI using ejaculated sperm	ICSI using testicular sperm	<i>p</i> value
No. cultured embryos ^a	1171 (8.3 ± 2.3)	326 (8.8 ± 2.0)	0.103
No. of blastocysts ^a	532 (3.8 ± 2.1)	154 (4.2 ± 2.2)	0.348
Rate of blastocysts (%) ^b	46.1 ± 24.7	47.5 ± 21.6	0.118
No. of expanded or hatching blastocysts ^a	188 (1.3 ± 1.5)	61 (1.6 ± 1.5)	0.994
Rate of expanded or hatching blastocysts (%) ^a	16.8 ± 19.0	18.4 ± 16.1	0.407

^a Values in parentheses represent mean number of embryos or blastocysts per cycle. Values are expressed as mean ± standard deviation

^b Values are the average of the blastocyst formation rate after the blastocyst formation rate is calculated for each cycle. Values are expressed as mean ± standard deviation

in which embryos did not develop to blastocyst stage (7 cycles in the EJ group and 2 cycles in the TE group). In the EJ group, 47 babies were delivered in 46 cycles (76.7%). One pregnancy was twin pregnancy (1.7%), and 45 were singleton pregnancies (75.0%). Thirteen pregnancies resulted in miscarriages within the first trimester of pregnancy (21.7%). One pregnancy was ectopic, and follow-ups of three pregnancies were unsuccessful because contact with the patients was lost. In the TE group, 11 babies were delivered in 10 cycles (66.7%). One pregnancy was twin pregnancy (6.7%), and nine were singleton pregnancies (60.0%). Five pregnancies ended in miscarriage (33.3%), and follow-up was failed in one pregnancy. Live-birth rate and miscarriage rate were not different between the EJ and TE groups.

Pregnancy outcomes were compared between patients with obstructive azoospermia and patients with non-obstructive azoospermia (Supplemental Table 4). Clinical pregnancies were confirmed in 11 cycles, and implantation rate was 24.1% in patients with obstructive azoospermia. Five babies were delivered in 5 cycles, five pregnancies resulted in miscarriages, and follow-up of one pregnancy was failed. In patients with non-obstructive azoospermia, five clinical pregnancies were established. The implantation rate was 32.0%, and six babies were delivered in those cycles. Live-birth rate and miscarriage rate were not different between patients with

obstructive azoospermia and patients with non-obstructive azoospermia.

Discussion

For pregnancy in infertile couples due to azoospermia, ICSI is widely performed using TE. However, in these couples, blastocyst transfer is not widely performed compared to couples for whom ICSI is performed using EJ. This may be due to the fact that poor quality of TE may affect embryonic development to blastocyst stage [18]. Recently, it has been reported that clinical outcomes of ICSI using TE are comparable to those of ICSI using EJ [13–16]. Furthermore, these studies also suggest that the ability of TE to support embryonic development to blastocyst stage is comparable to that of EJ, and clinical outcomes of blastocyst transfer in cycles in which ICSI is performed using TE are not different to those in cycles where ICSI is performed using EJ.

The relationship between the clinical outcomes of ICSI and the sperm sources has long been controversial and unclear. Recent studies have shown that clinical outcomes of ICSI were better when ICSI was performed using EJ than when ICSI was performed using TE [6, 7, 19, 20]. According to these studies, fertilization, blastocyst formation, and

Table 5 Pregnancy outcomes after intracytoplasmic sperm injection (ICSI) using ejaculated or testicular sperm

	ICSI using ejaculated sperm	ICSI using testicular sperm	<i>p</i> value
Transfer cycles	141	37	
Transfer cycles with blastocysts	134 (95.0)	35 (94.6)	1.000
Clinical pregnancies (%)	63 (44.7)	16 (43.2)	0.876
Follow-up loss	3	1	
Miscarriages (%) ^a	13 (21.7)	5 (33.3)	0.335
Ectopic pregnancies	1	0	
Deliveries (%) ^a	46 (76.7)	10 (66.7)	0.537
Singleton pregnancy (%) ^a	45 (75.0)	9 (60.0)	
Twin pregnancy (%) ^a	1 (1.7)	1 (6.7)	
Delivered babies	47	11	

^a Values are the percentage of miscarriages or deliveries to clinical pregnancy excluding follow-up loss

implantation rates were higher, qualities of embryos were better, and miscarriage rate was lower when ICSI was performed using EJ than when it was performed using TE. More recently, however, conflicting studies have been reported. Nilsson et al. [13] reported that clinical outcomes of single blastocyst transfer after ICSI using epididymal or TE were similar to those of standard IVF or ICSI using EJ. However, the authors did not distinguish between the clinical outcomes of epididymal sperm and those of TE. Therefore, if the clinical outcomes of TE were distinct to those of epididymal sperm, it might also be different from those of EJ. Naru et al. [14] observed no difference in pregnancy and miscarriage rates between ICSI cycles using EJ and those using TE. Furthermore, Braga et al. [15] reported significantly higher implantation rate in cycles using TE than in cycles using EJ or epididymal sperm. Xie et al. [16] also reported higher implantation rates in cycles using TE than in cycles using EJ. Although the reasons are unclear, there have been conflicting studies on the relationship between clinical outcomes of ICSI and sperm sources. These conflicting results may be caused by the different procedures of TE retrieval or different characteristics of patients who underwent TE retrieval. Testicular sperm can be retrieved through a variety of medical procedures. However, two procedures are mainly performed: needle biopsy (testicular sperm aspiration (TESA)) or open testicular biopsy (testicular sperm extraction (TESE)). Retrieval of TE and the quantity and quality of retrieved TE may be affected by retrieval procedures. Several studies have reported that the clinical outcomes of ICSI using EJ were better than those of using TE. In those studies, testicular sperm was recovered by TESA rather than TESE. However, in studies that reported the outcomes of ICSI using TE comparable to those using EJ, the frequency of studies using TESE were similar to that using TESA. Hauser et al. [21] conducted TESE and TESA simultaneously in men with non-obstructive azoospermia and reported that TESE was more advantageous than TESA in retrieving TE. Although the results of the study were limited to men with non-obstructive azoospermia, the retrieval rate of TE, the retrieval rate of motile sperm, and the quantity of retrieved sperm were higher in TESE than in TESA. Therefore, medical procedures involving testicular sperm retrieval may affect the clinical outcome of ICSI. Testicular histopathology of men with azoospermia was not described in most of the studies comparing clinical outcomes of ICSI dependent on sperm sources. The sperm quantity may be different in patients with obstructive azoospermia and those with non-obstructive azoospermia. The clinical outcomes of ICSI can also be affected by the sperm quality. Fertilization rates after

ICSI were higher when motile sperm were injected into oocytes than when immotile sperm were injected [22, 23]. The more sperm available to ICSI, the more appropriate sperm for ICSI will be selected. Balaban et al. [6] compared the clinical outcomes of ICSI between men with obstructive azoospermia and men with non-obstructive azoospermia. According to their study, the clinical outcomes of ICSI using TE retrieved from men with non-obstructive azoospermia were decreased compared to those using TE retrieved from men with obstructive azoospermia. When ICSI was performed using TE retrieved from men with obstructive azoospermia, the clinical outcomes of ICSI were comparable to those of ICSI using either ejaculated or epididymal sperm. Taken together, these results indicate that the histopathology of men with azoospermia may have significant impacts on the clinical outcomes of ICSI using TE. Therefore, clinical outcomes of men with obstructive azoospermia should be analyzed separately from those of men with non-obstructive azoospermia. In the present study, clinical outcomes of men with obstructive azoospermia and those of men with non-obstructive azoospermia were compared but no differences were observed between them. This could be attributed to the low number of men with non-obstructive azoospermia in this study.

Not all clinical outcomes of ICSI using TE were comparable to those of ICSI using EJ. Recent studies have reported decreased fertilization rates in ICSI cycles using TE [16, 20, 24, 25]. In this study, fertilization rates were significantly lower in ICSI cycles using TE than in cycles using EJ. However, fertilization rates were decreased only in cycles involving men with non-obstructive azoospermia when clinical outcomes were analyzed separately in men with obstructive and non-obstructive azoospermia. In ICSI cycles of men with obstructive azoospermia, fertilization rates were comparable to those of ICSI cycles using EJ. Consistent results were also reported in other studies [6, 26, 27]. Fertilization rate was significantly lower in ICSI cycles of men with non-obstructive azoospermia than in cycles of men with obstructive azoospermia. These decreased fertilization rate in men with non-obstructive azoospermia may be consequent on centrosome defects of TE retrieved from men with non-obstructive azoospermia [28, 29].

In this study, we analyzed the clinical outcomes of blastocyst transfer performed for quite a long time, blastocyst transfer cycles of 11 years. During this period, we performed blastocyst transfer very carefully, especially in ICSI cycles using TE, because the age of female partners was increased (from 34.7 ± 4.4 to 37.3 ± 4.6 years old), and the number of retrieved oocytes was decreased (from 11.6 ± 9.5 to 9.0 ± 8.4 per cycle). Therefore, such a long time was required until enough data of blastocyst transfer were collected in ICSI cycles using TE.

Although many techniques were changed in IVF and ICSI during this period, we performed ICSI in the same manner in all ICSI cycles using EJ or TE. The medical equipment used to ICSI, such as micromanipulators and microscopes, were also developed and improved during the period, but we performed ICSI using the same equipment. And three embryologists performed ICSI mainly during this period. Their experience of performing ICSI is more than 10 years. They have also performed various assisted reproductive techniques but mainly ICSI. The preparation method of EJ or TE for ICSI was not changed. EJ was prepared for ICSI by swim-up methods and TE by squeezing seminiferous tubules using two pairs of fine forceps under dissecting microscope. Surgical method for collecting testicular tissue has not changed. Testicular tissue was collected from men with obstructive azoospermia by conventional testicular biopsy. Microdissection TESE using microscope (micro-TESE) was performed for collecting testicular tissue in men with non-obstructive azoospermia. We have maintained many factors that can affect the clinical outcomes of ICSI, such as the manner of ICSI, equipment, embryologists, sperm preparation methods, and surgical method for collecting testicular tissue, largely unchanged and constant during this period. Therefore, in our opinion, the long data collection period will have little impact on the results of this study.

In this study, the embryo quality was analyzed and compared between two groups, which was not performed in other studies. The rate of good-quality embryos was low on day 3 or day 5. The low rate of good-quality embryos seems to be due to our strict evaluation of embryo quality. The rates of good or poor-quality embryos were similar between two groups on 3 or 5 days after oocyte retrieval. The rate of fair-quality embryos was higher in TE group than in EJ group on day 3. However, the rate was not different between two groups on day 5. We failed to analyze and compare the correlation between cleavage-stage embryo quality and blastocyst quality since the number of embryos was too small to analyze and compare it. In order to perform blastocyst transfer, the number of cultured embryos and the blastocyst formation rate seem to be more important rather than embryo quality on day 3 or the correlation of between cleavage-stage embryo quality and blastocyst quality. This study showed that TE had the potential similar to that of EJ in supporting embryonic development to blastocysts. Our results are consistent with several studies reported recently [13–16]. Blastocyst transfer should be actively considered in cycles using TE as well as in cycles using EJ. It is already well-known that pregnancy rate can be improved by performing blastocyst transfer [30, 31]. Recently, blastocyst transfer has been extensively performed, and its implementation is increasing. However, until now, it appears that this is not the case in the cycles using TE. The improvement of pregnancy rates was also reported in cycles using TE when blastocysts were transferred [32]. In cycles using TE,

blastocyst transfer should be performed more extensively than now. Of course, the appropriate number of oocytes should be retrieved although it is not easy since the number of the retrieved oocyte is decreasing due to the rise in female age and other causes.

Conclusion

The present study showed that the potential of TE supporting embryonic development to blastocyst stage was comparable to that of EJ. The clinical outcomes of blastocyst transfer in ICSI cycles using TE were similar to those in ICSI cycles using EJ. Therefore, the present study suggests that blastocyst transfer may be considered when the appropriate number of oocytes is retrieved, and motile sperm are identified in ICSI cycles using TE.

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