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ICSI outcome after microdissection testicular sperm extraction, testicular sperm aspiration and ejaculated sperm

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ABSTRACT

We conducted a case-controlled single-center cohort study to evaluate the intracytoplasmic sperm injection (ICSI) outcome in severe male infertility with different methods of sperm obtention. The data was compiled from a tertiary university hospital. The micro-TESE procedures were performed from 2008 to 2023, with a sperm recovery rate (SRR) of 45 %. The ICSI treatments were carried out between 2011 and 2023. The aim of the study was to compare the ICSI outcome using sperm obtained by microdissection testicular extraction (micro-TESE), testicular sperm aspiration (TESA), and ejaculated sperm with sperm concentration less than 15 million per milliliter. We included a total of 462 ICSI cycles, of which 340 ICSIs with ejaculated sperm of men with oligozoospermia, with or without asthenozoospermia or teratozoospermia (OAT group), 51 ICSIs with TESA sperm of men with obstructive azoospermia (OA, TESA group), and 71 ICSIs with micro-TESE sperm of men with non-obstructive azoospermia (NOA, micro-TESE group). The patient characteristics, fertilization rate, pregnancy rate, and pregnancy outcome data were similar between the groups. The fertilization rates were 66.0 % in the OAT group, 68.3 % in the TESA group and 62.8 % in the micro-TESE group and live birth rate per embryo transfer were 23.7 %, 28.9 %, and 25.0 %, respectively, without statistical difference. The obstetrical outcome was similar in all the groups. The overall clinical results in all ICSI cycles performed for treating severe male factor infertility were similar, independent of the method of collection of spermatozoa. The results also confirm the efficacy of micro-TESE in the treatment of severe male factor infertility.

1. Introduction

Azoospermia refers to the complete absence of spermatozoa in repeated semen analyses. Azoospermia affects 1–2 % of all males and 10–15 % of infertile males [1]. Non-obstructive azoospermia (NOA) is the most severe form of male infertility, where sperm production in the testes is absent or severely impaired. Intracytoplasmic sperm injection (ICSI) is an assisted reproductive technique that enables the oocytes to be fertilized with very small numbers of spermatozoa [2]. The first ICSI pregnancies using testicular sperm of men with NOA were reported in the late 90′s [3]. The most successful sperm extraction with the lowest complication rates seems to be microdissection testicular sperm extraction (micro-TESE), first described by Schlegel in 1999[4].

Clinical pregnancy rates between 20 % and 75 % have been reported after micro-TESE-ICSI [5,6]. However, there is significant variation in reporting standards, making it difficult to compare the data. ICSI outcomes after conventional TESE and micro-TESE have been analyzed in a 2019 meta-analysis, with 42 studies reporting ICSI outcomes. Based on this analysis, the cumulative pregnancy rate was 29 % (cPR) and live birth rate (LBR) was 24 %. No statistical difference was found in LBR between fresh and frozen sperm [7]. Scant data are available on the impact of different diagnoses on micro-TESE-ICSI outcomes. A Chinese study of 347 micro-TESE-ICSI cycles, reported the poorest results to in the treatments that used the spermatozoa of men with Y chromosome microdeletion AZFc [8]. In a study that compared ICSI using fresh and frozen sperm obtained by micro-TESE, the fertilization rates were 66 % and 60 %, respectively, with implantation rates from 51 % to 53 % [9].

The micro-TESE procedure was introduced in Finland in 2008 in Turku University Hospital [10]. By April 2023, the procedure had been performed in 360 men, with an overall sperm recovery rate (SRR) of 45 %. The SRR data on the micro-TESE surgeries at our unit have been described previously [10]. This case-cohort single-center real-life study

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was designed to provide data to counsel couples with severe male factor infertility more accurately.

2. Materials and methods

2.1. Patient selection and data collection

We collected data retrospectively from the Turku University Hospital database from January 2011 to June 2023. The micro-TESE patient criteria and preoperative assessment have been described elsewhere [11]. The patients who underwent micro-TESE were referred from various Finnish hospitals and IVF units. The clinical diagnoses and SRR of the men operated are shown in Table 1.

The study group (micro-TESE group) consisted of the 71 micro-TESE-ICSI that were treated in our unit. The control groups comprised other ICSI treatments carried out in the same period at the Turku University Hospital. The first control group (OAT group) consisted of 340 ICSI treatments with male factor infertility (oligozoospermia with sperm concentration less than 15 million per milliliter, with or without asthenozoospermia and/or teratozoospermia). The second control group (TESA group) of 51 TESA-ICSI consisted of ICSI treatments with mainly frozen sperm collected by testicular needle biopsy (TESA). All these men had obstructive azoospermia (OA) due to a history of trauma, infection, surgical trauma, hereditary causes, or unexplained. None of the men in the TESA group were vasectomized. None of the men in any of the groups were tobacco smokers at the time of the sperm collection.

2.2. Micro-TESE procedure

The micro-TESE operations were performed in Turku University Hospital between 2008 and 2023. Until 2020, the surgery was performed under general anesthesia. However, from 2021 onward local anesthesia by spermatic cord block combined with intravenous alfentanil, began to be used.

The skin was incised in the scrotal midline using a scalpel. The larger testis was chosen for incision through the tunica vaginalis using a monopolar instrument and the testis was then lifted out of the scrotum. The tunica albuginea was then incised using a scalpel under an operating microscope. Mosquito clamps were placed on both sides of the tunical incision. The most dilated seminiferous tubules were identified to take biopsies of the most potential loci for spermatogenesis. Microsurgical forceps and scissors were used to remove the biopsies, which were placed in four well cell culture plates containing sperm transport buffer (G-Gamete™, Vitrolife Sweden AB, Frölunda, Sweden) and immediately taken to the laboratory for examination by higher magnification. If no sperm were recovered, operating on the first testis was continued until the entire testis had been examined. Hemostasis was then ensured using bipolar electrocauterization, the tunica albuginea closed in running suture using 5–0 monofilament suture, and the testis was placed back in the scrotum. The contralateral testis was operated on, if necessary. The tunica vaginalis and the skin were closed using 4–0 absorbable running intracutaneous suture.

In the fertility laboratory, the biopsies were transferred to a one well dish containing 0.5 ml of cell culture media (G-Gamete™, Vitrolife Sweden AB, Frölunda, Sweden). A few biopsies were quickly screened to inform the surgeon whether sperm were seen. If no sperm were detected, the embryologist continued by mechanically dispersing the tissue by squeezing out the intratubular cell mass using fine needles (e.g., 27 G) to produce a suspension. After the suspension settled down for a few minutes, it examined in more detail using an inverted phase contrast microscope at 400-fold magnification.

Samples containing a larger number of sperms were pooled and divided into small batches for freezing and future use. The samples with low amounts of sperms were pooled and frozen separately from those containing abundant sperm. Regardless of the quality and amount of observed sperms, the samples were divided into at least eight straws (CBS™ High Security sperm straw 0.5 ml, CryoBio System, Saint Ouen Sur Iton, France).

Table 1
Micro-TESE results.

Diagnosis	n	%	SRR
Unexplained	190	52.8 %	34 %
Klinefelter syndrome	70	19.4 %	47 %
Y-deletion (AZFc)	22	6.1 %	77 %
Cryptorchidism	27	7.5 %	89 %
Cytotoxic medication and/or radiation	22	6.1 %	45 %
TOTAL	360		45 %

SRR= sperm recovery rate

2.3. TESA procedure

TESA was performed in local anesthesia, with spermatic cord block and skin anesthesia combined with intravenous alfentanil, if necessary. A preliminary skin incision was made, and a spring-loaded biopsy needle (Biopty™, Bard Urological, Covington, GA, USA) was used to obtain one or more samples, which were then placed in sperm transport buffer and microscopically examined to ensure good sperm yield. If no additional biopsies were needed, the biopsy locus was compressed to minimize the risk of a hematoma. The sperm samples were frozen for future use according to the standard sperm freezing protocol (SpermFreeze Solution™, Vitrolife Sweden AB, Frölunda, Sweden).

2.4. Ovarian stimulation

The clinical characteristics of the female partners determined the treatment protocols in the ICSI cycles, with more agonist cycles performed in the earlier years and more antagonist cycles in the later years of the study. Confounding female factor infertility was not excluded in this study.

2.5. Preparing the frozen testicular sperm and ICSI

On the oocyte retrieval day of a micro-TESE-ICSI cycle and TESA-ICSI cycle, a single sperm straw was thawed shortly before the ICSI, according to our standard sperm thawing protocol, avoiding osmotic shock. Due to the low number of sperm cells in the frozen samples, no gradient wash was performed to avoid the loss of spermatozoa. Only a short centrifugation wash (2000 – 2500 x g in a conical or round bottom tube) with 3–5 ml of equilibrated cell culture media (G-IVF PLUS™ Vitrolife Sweden AB, Frölunda) was performed. After centrifugation, the supernatant was carefully removed, and 50–75 μl of the cell culture media was added and mixed with the pellet to reach a final volume of approximate 150 μl.

Theophylline (GM501 SpermMobil™, Gynemed GmbH&co, Lensahn, Germany) was used on several occasions to enhance the identification of viable sperm cells for the ICSI. The cell suspension was pipetted to form multiple (4−6) long and narrow droplets on a Petri dish, covered in oil, without the use of polyvinylpyrrolidone (PVP) solution. The droplet was then thoroughly searched through, and viable sperm cells were collected and placed in a PVP solution (ICSI™, Vitrolife Sweden AB, Frölunda, Sweden) droplet on the dish to immobilize them. In the event of a very low number of spermatozoa, the ICSI was performed after searching all the droplets, avoiding the attaching of sperm cells to the debris. If the amount of sperm cells was still not sufficient, another straw was thawed.

On the oocyte retrieval day of the ICSI cycle with ejaculated sperm, the semen samples were processed with a gradient wash according to the standard protocol described by the manufacturer (PureSperm™, Nidacon International, Mölndal, Sweden). Samples that contained very few sperm were not processed with a gradient wash to avoid the loss of sperm but processed in the same manner as the thawed micro-TESE and

TESA samples.

The ICSI procedure has been described elsewhere [2]. The fertilization rate was assessed by calculating the number of 2pn zygotes of all mature oocytes fertilized by ICSI. The embryo transfer was performed on day 2–5 after oocyte retrieval, according to clinical and practical considerations.

2.6. Statistical analyses

Statistical analyses were performed using JMP® Pro 17.0.0 for Macintosh (JMP Statistical Discovery LLC, Cary, NC, USA 27513). Data were expressed in mean (±SD) or percentages. Between-group comparisons were performed using nonparametric tests, and P values < 0.05 were considered statistically significant.

2.7. Ethical approval

The study protocol was approved by the clinical research center of the University of Turku. No ethical committee approval or patient consent was required for collecting and analyzing the data retrospectively.

3. Results

3.1. Study populations

The mean (±SD) age of the men in this study was 36.2 (±6.8) years in the OAT group, 35.4 (±6.4) in the TESA group, and 34.3 (±6.0) in the micro-TESE group, with no statistically significant difference. The mean (±SD) BMI was similar in all the groups, 27.5 kg/m^2 (±4.8) in the OAT group, 26.8 kg/m^2 (±3.8) in the TESA group, and 27.3 (±5.0) kg/m^2 in the micro-TESE group. Primary infertility was most prevalent in the micro-TESE group, 89.5 %, 75 % in the OAT group, and 64.4 % in the TESA group ($p = 0.001$). The patients' characteristics are shown in Table 2.

The mean (±SD) age of the female partner was significantly higher in the OAT group (32.8 ± 4.4 years) than in the TESA group (31.4 ± 4.7 years) and in the micro-TESE group (31.7 ± 4.4, $P = 0.022$). The mean (±SD) female BMI was similar in the OAT group, the TESA group, and the micro-TESE group (25.4 kg/m^2 (±4.7), 26.4 kg/m^2 (±4.8) and 25.1 kg/m^2 (±4.6), respectively; NS). There was no statistical difference in the primary vs. secondary female infertility rate between the groups, although the primary infertility rate seemed higher in the micro-TESE group (82.5 %), compared to 62.1 % in the TESA group and 73.8 % in the OAT group. Antagonist protocol was used more frequently in the OAT group, 61.9 %, and the micro-TESE group 51.1 % than in the TESA group, 28.9 % ($p = 0.0001$). The total dose of gonadotropins administered did not show any statistical difference between the three groups. The female data are shown in Table 2.

Table 2
Patient characteristics.

PARAMETERS	OAT	TESA	MicroTESE	p-value
Age of women, mean (SD)	32,8 (4,4)	31,4 (4,7)	31,7 (4,4)	0.022
Age of men, mean (SD)	36,2 (6,8)	35,4 (6,4)	34,3 (6,0)	NS
BMI of women, mean (SD)	25,4 (4,7)	26,4 (4,8)	25,1 (4,6)	NS
BMI of men, mean (SD)	27,5 (4,8)	26,8 (3,8)	27,3 (5,0)	NS
Primary infertility (women)	73.8 %	62.2 %	82.5 %	NS
Primary infertility (men)	75.0 %	64.4 %	89.5 %	0.01
Antagonist protocol	61,9 %	28,9 %	51.1 %	0.0001
Total dose gn (mean)	1900 (711)	1844 (602)	1913 (624	NS
Days of stimulation (mean)	9,6 (1,8)	10,0 (1,9)	9,6 (1,3)	NS

3.2. ICSI results

The mean number of oocytes collected was similar in all the groups, 11.9 in the micro-TESE and TESA groups and 11.8 in the OAT group, with a mean number 9.1 mature oocytes in micro-TESE and TESA groups and 8.5 mature oocytes in OAT group (NS). The fertilization rate was 66.0 % in the OAT group, 68.3 % in the TESA group and 62.8 % in the micro-TESE group (NS, Table 3). The mean number of viable embryos was similar in the groups, 3.9 in the OAT, 3.7 in the TESA, and 4.1 in the micro-TESE group (NS).

There were significantly more canceled embryo transfers in the micro-TESE group (32.4 %) compared to the other groups, 18.2 % in the OAT group and 13.5 % in the TESA group ($p = 0.02$). All groups' most common reasons for cancellations were a high ovarian stimulation response, preplanned freeze-all and COVID-19 shutdown (Table 4).

The single embryo transfer rate was similar in the groups, 96 % in the OAT group, 93.3 % in the TESA group, and 93.5 % in the micro-TESE group. No more than two embryos were transferred in any cycle. The positive pregnancy test rate was 41.4 % in the OAT group, 44.4 % in the TESA group, and 39.6 % in the micro-TESE group. Clinical pregnancy rates per embryo transfer were 33.1 %, 33.3 %, and 27.1 %, respectively, leading to live birth rates of 23.7 %, 28.9 % and 25.0 %. None of these differences between the groups were statistically significant.

3.3. Obstetric outcomes

The obstetric outcomes between the three groups were similar. There was one twin pregnancy in the OAT group, while all the others were singleton pregnancies. In the OAT group, there were 6 children below 1500 g and 7 above 4000 g in birthweight. The birthweights of all the children in the micro-TESE and TESA groups were over 2500 g, with one child more than 4000 g in the micro-TESE group. In the OAT group, 3 children were born in gestational week 33–36 and 8 children in gestational week 42 + . All the children in the TESA group were born between 37 and 41 gestational weeks, while in the micro-TESE group, 1 child was born between gestational weeks 33–36 and one child over 42 gestational weeks. The obstetric data are shown in Table 6.

4. Discussion

The fertilization rate of 62.6 % in micro-TESE-ICSI in our clinic was similar compared to previous reports, showing fertilization rates of 44–65.7 % [6,9,12–14]. The fertilization seemed slightly lower in micro-TESE-ICSI in comparison to other, less severe male factor ICSI treatments, without statistical significance. There were also slightly more failed fertilizations in the micro-TESE-ICSI group, which is not a surprise, since in some micro-TESE samples there were only very few sperms retrieved. No difference was observed in the clinical pregnancy rate or live birth rate between the groups. In some of the previous studies comparing the ICSI treatments of the men with OA and the men with NOA, the ICSI treatments of the men with NOA have been less successful

Table 3
Laboratory parameters.

PARAMETERS	OAT	TESA	microTESE
OPU	340	52	71
Oocytes	4018	619	843
Oocytes mean	**11.8**	**11.9**	**11.9**
Mature	3104	442	643
Mature mean	**9.1**	**8.5**	**9.1**
Normal fertilized	2048	302	404
Fertilization rate	**66.0 %**	**68.3 %**	**62.8 %**
ET			
No. of emb transferred	289	48	51
No. of frozen emb	1043	143	239
Mean no. of viable embryos	**3.9**	**3.7**	**4.1**

Table 4
Reason of cancellations.

Reason of cancellations	OAT (sperm concentration <15 mill/ml)	TESA	MicroTESE
Preservation of fertility	1	0	0
Polyp, Myoma, Fluid in endom	2	0	1
High response, Covid, freeze all	46	5	13
No fertilization	10	0	4
Emb. Development	1	1	0
Abnormal embryos	2	0	0
Other reason	0	0	1
TOTAL	**62**	**6**	**19**

Table 5
ICSI treatments by method of obtention of Sperm.

PARAMETERS	Sperm conc <15 mill/ml (OAT)	Testicular Sperm by TESA	Testicular Sperm by Micro TESE	p-value
OPU	**340**	**51**	**71**	**462**
ET	278	45	48	
Cancelled %	62	6	23	
Cancelled %	18.2 %	11.8 %	32.4 %	0.02
Pregnancy tests Pos	118	20	19	
Rate of Pregnancy tests Pos/ET	**42.4 %**	**44.4 %**	**39.6 %**	NS
Clinical Pregnancies	92	15	13	
Rate of Clinical Pregnancies/ET	**33.1 %**	**33.3 %**	**27.1 %**	NS
Live births/Ongoing pregnancy	66	13	12	
Rate of Live births/ET	**23.7 %**	**28.9 %**	**25.0 %**	NS
Rate of Live births/preg test	**55.9 %**	**65.0 %**	**63.2 %**	
Biochemical preg	25	5	6	
Rate biochemical preg/preg test	**21.2 %**	**25.0 %**	**31.6 %**	
Pregnancy losses	27	2	1	
Rate pregnancy losses/preg test	**22.9 %**	**10.0 %**	**5.3 %**	NS

Table 6
Obstetric parameters.

PARAMETERS	OAT	TESA	Micro TESE	p-value
No. of births	66	13	12	
Singletons N (%)	65	13	12	NS
Birthweight				
• 1500 gr or Less	6	0	0	NS
• 1501–2500 gr	0	0	0	NS
• 2501–4000 gr	48	13	11	NS
• More 4000 gr	7	0	1	NS
	61	13	12	
Gestational Age				NS
• 22–27 wk	0	0	0	
• 28–32 wk	0	0	0	
• 33–36 wk	3	0	1	
• 37–41 wk	50	13	10	
• 42+ wk	8	0	1	
	61	13	12	

in terms of fertilization [15,16]. Our sample size is limited, but being a pure micro-TESE vs. TESA setting, this may offer some reassuring new data for clinical decision-making and patient counseling.

The obstetric data showed that most of the children born after severe male factor infertility treatments reached full term and the birth weight was within the normal range. These results are similar to a previous study with conventional TESE and ICSI in NOA and OA diagnoses, where no differences were found between the treatment outcome or the obstetric outcome in these groups [17]. Previous reports on the health of the children born after ICSI with testicular sperm have also been reassuring [18–21]. However, conclusive data on the health of the children born after micro-TESE-ICSI are lacking.

The higher cancellation rate found in the micro-TESE-ICSI group in our study was unexpected. The most common reasons were COVID-19 infection, COVID-19 shutdown, preplanned freeze-all, and high ovarian response. The high number of COVID-19-related cancellations in the micro-TESE group compared to the other groups is most likely purely coincidental. Due to the absolute male factor infertility and the slightly younger female age in the micro-TESE group, the clinicians may have been more cautious when facing the risk of ovarian hyperstimulation syndrome and resorted to freezing all the embryos to minimize this risk. This is also the most likely explanation for the significant differences between the stimulation protocols of the groups. The couples in the OAT group were a little older than the couples with testicular sperm, and their rate of secondary infertility was also higher. These differences need to be considered when comparing the results between the groups. The data on the effect of female age on ICSI results with testicular sperm are conflicting. For example, although Bocca et al. found that female age was a limiting factor [5], Corona et al. did not in their meta-analysis comparing the results of micro-TESE and conventional TESE [14].

Most of the men with NOA in Finland are referred to Turku University Hospital for the micro-TESE. Turku University Hospital performs 200–300 fresh IVF and ICSI cycles in total per year. Thus, the OAT and the TESA groups are representative of a general Finnish ICSI population. The micro-TESE group, however, contains the most demanding micro-TESE-ICSI procedures; the men with the best sperm yield were referred for the ICSI treatment to their closest fertility clinics, where their frozen micro-TESE samples were shipped. This difference in the treatment groups may lower the pregnancy results in the micro-TESE group.

The use of frozen sperm in the groups with testicular sperm but mainly fresh sperm in the OAT group is a major limitation to consider when interpreting our results. The use of fresh versus frozen testicular sperm in micro-TESE-ICSI treatment has been a long debate. While many have reported excellent results with frozen testicular sperm [6,22–24], others have conflicting results showing that using fresh sperm synchronized with the oocyte retrieval produces better clinical results [9,25]. In a meta-analysis of 17 studies and 1261 ICSI cycles with fresh and frozen testicular sperm, no difference was found in the ICSI outcome [26]. In our retrospective data, the ejaculated sperm was fresh while the testicular sperm of both TESA and micro-TESE-group was frozen. As there was only mild tendency favoring the ejaculated sperm group, we see no reason to change our current practice of freezing the micro-TESE and TESA sperm. This allows more informed planning for the ovarian stimulation.

There is large variation in existing data on the micro-TESE-ICSI results showing clinical pregnancy rates from 20 % to 68 %, which may be explained by differences in clinical practices and study protocols [5,6,12,14]. In our study, the clinical pregnancy rate per embryo transfer was 27.1 % in the micro-TESE group, leading to a live birth rate of 25.0 %. Our study represents real-life data of the male factor ICSI treatments performed in a single, tertiary fertility center. This is the main strength, but also a weakness of the study; it consolidates the existing data on the clinical outcome of micro-TESE but fails to detect statistical significance between the clinical results of different groups of patients going through ICSI treatments for severe male infertility. In a previous study analyzing micro-TESE-ICSI treatments in different NOA subgroups, the clinical pregnancy rates varied between 20.3 % (Y chromosome microdeletion AZFc) and 78.3 % (previous bilateral orchitis) [8]. Due to the insufficient numbers, in our study, we could not make reliable comparisons between different male infertility diagnoses or histopathological groups in micro-TESE. A well-designed prospective, preferably multicenter

study with a larger sample size is needed to verify these results.

To conclude; this study confirms that the sperm of men with NOA obtained by micro-TESE are able to fertilize oocytes with ICSI similarly to the sperm of men with OA obtained by TESA, or the ejaculated sperm of men with severe oligozoospermia. Although this treatment of the most severe form of male infertility appears to be effective in assisting men with NOA to father biological children, the health of the resulting offspring needs to be verified in larger follow-up studies. Our findings provide practical help in counseling infertile couples with severe male factor.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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