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The First Report from Bosnia and Herzegovina on Micro-tese Results in Azoospermic Patients

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ABSTRACT

Introduction: To present results of MicroTese procedure in treatment of male infertility in patients with azoospermia at Bahçeci BIH IVF Center during two-year period of 2013-2014. **Methods:** In the stated time-period, 55 MicroTese surgeries were performed. In 52.7% of the cases, sperm cells were isolated after MicroTese surgery, and in 47.3% of the cases, there was a negative outcome of the procedure. Obtained sperm cells were subjected to cryopreservation. Furthermore, ICSI procedure was performed by use of the obtained sperm cells. **Results:** Of 29 positive MicroTese surgeries, 21 (72.4%) resulted in clinical pregnancies. Biggest percentage of negative MicroTese procedures happened in patients with cryptorchidism and orchidopexy. **Conclusion:** MicroTese is the most precise and successful method of retrieving sperm cells surgically in men with azoospermia. Our results are within scope of results in referent world centers.

Key words: Azoospermia, MicroTese, ICSI.

1. INTRODUCTION

Total absence of sperm cells in the ejaculate is defined as Azoospermia. Incidence of azoospermia is approximately 1% of all men and 10 to 15% of infertile males. In order to confirm the diagnosis of azoopermia, at least two semen samples obtained should be examined according to World Health Organization guidelines from 2010. Azoospermia is divided into two groups: as obstructive and non-obstructive (inadequate hormonal stimulation and impaired spermatogenesis) (1). The etiologies that lead to azoospermia are categorized into three groups: pre-testicular, testicular and post-testicular. Pre-testicular causes of azoospermia represent endocrine abnormalities that affect spermatogenesis. Testicular causes encompass intrinsic disorders of spermatogenesis inside the testicles. Post-testicular causes of azoospermia include ductal obstruction in any part of the male reproductive tract. Evaluation of patients with diagnosis of azoospermia includes:

* Medical history (previous surgery, illnesses and disorders such as mumps, orchitis, undescended testicle, trauma and infections, and exposures gonadotoxins)

* Physical examination (local finding on testicles, resistance of epididymis, inspection of vasa, presence of varicocele or undescended testis, as well digital rectal examination and use of ultrasound)

* Endocrine and genetic evaluation (hormone serum levels of FSH, LH, testosterone and prolactin, karyogram, Y deletion) and,

* Semen analysis as the most important parameter (2).

After all of these tests are performed along with semen analysis, patients with azoospermia will need one of Sperm Retrieval Procedures. Only men with azoospermia need to have sperm cells retrieved directly from the testis or epididymis. This includes a simple aspiration or extraction from different locations of spermatogenesis, and includes Testicular Sperm Aspiration (TESA), Percutaneous Sperm Aspiration (PESA), and Testicular Sperm Extraction (TESE) all performed under local anesthesia. Testicular sperm extraction (TESE) involves making a small incision in the testis and examining the tubules for the presence of sperm. Microdissection TESE has replaced above mentioned procedure as the optimal method of sperm cell retrieval for men with azoospermia due to problematic sperm production. Microepididymal Sperm Aspiration (MESA) is a procedure performed in men who have vasal or epididymal obstruction (vasectomy, congenital bilateral absence of the vas deferens) and is performed in the operating room under general anesthesia utilizing surgical microscope (3). Micro TESE (microdissection testicular sperm extraction) presents as

well surgery that is being performed under general anesthesia through a small midline incision in the scrotum through which one or both testicles may be approached. The surgeon examines the testicles under the microscope, with magnification up to 20x, in search of areas where the seminiferous tubules – small areas of testicular tissue – are dilated and therefore more likely to contain sperm. The surgeon then removes these areas, and we examine them in our laboratory for the presence of sperm. We continue examining different areas of the testicles until we find sperm, or until we examine and biopsy all sites of the testicles without finding any sperm. Micro TESE has significantly improved sperm retrieval rates in azoospermic men, which represents a safer procedure since less testicular tissue is removed. Patients cryopreserve sperm during this procedure for future IVF/ICSI.

2. PATIENTS AND METHODS

Prospective-retrospective study was performed, spanning a period of two years (January 1st 2013 – December 31st2014). In this study, patients with azoospermia diagnosis who contacted the BahceciBiH IVF center were analyzed. Each patient underwent clinical examination (FSH, testosterone, hormonal status), as well as karyotype and y-deletion. Upon completed examinations and on basis of obtained findings, the patients were subjected to treatment, i.e. surgical treatment of retrieving sperm cells by means of Micro-TESE surgical technique. Out of 55 patients who were treated with Micro-TESE, successful treatment was achieved in 29 patients.

MicroTESE surgery

In concurrence of urologist, gynecologist and embryologist, MicroTese procedure is performed at a set date. The procedure is performed under general anesthesia. Firstly, disinfection of surgical area has to be performed. Then, 4-5 cm incision of scrotal skin and tunics in medial line is made. Tunica albuginea is shown. After that, incision of tunica albuginea of one testicle is made, and areas of spermatogenesis are searched for under microscope. Obtained material is sent to embryology room. If the sperm cells are isolated, the procedure ends, but if they are not, the procedure is also performed on the other testicle. The surgery ends with wound closure by layers, and individual resorptive sutures are applied to the skin.

Isolation of sperm cells from testicular tissue

Received material of testicular tissue is transferred to Petri dish with incubated medium. Received material is first mechanically treated (macerated) with needles on a heated plate, and then the search for sperm cells is performed under microscope (magnification x 400). This procedure is followed by the procedure of centrifuging the obtained macerated material and isolation of sperm cells. All positive samples were frozen.

(Micro TESE, surgical technique with use of dfv operating microscope allows identification of the seminiferous tubulest that are most likely to contain sperm)

Protocol for controlled stimulation of ovulation and ICSI method

Patients were subjected to brief antagonist protocol for controlled stimulation of ovulation. The stimulation started on the second day of menstrual cycle. Stimulation of ovulation was initiated with application of human menopausal gonadotropin (Menopur, Merional) or with recombinant folliculostimulating gonadotropins (Gonal G, Puregon), with the use of Cetrotide or Orgalutran as antagonist. Growth of follicles was monitored by means of ultrasonic folliculometry, and when diameter of follicles was greater than 18mm, recombinant human chorionic gonadotropin was introduced (Choriomon, Ovitrelle).

Follicle puncture procedure was performed under general anesthesia in sterile conditions of the operating room. Obtained material was immediately transported to embryology room where oocytes were isolated, their maturity assessed and ICSI procedure performed. The dynamics of embryo development was monitored 3 to 5 days; FRESH embryo transfer was performed in 19 cases, while embryos were frozen in 10 cases so embryo transfer could be performed at a later date.

3. RESULTS

In the two-year period (2013-2014), 55 micro-TESE surgeries were performed at BahceciBiH IVF center. Of the total number of performed surgical procedures, sperm cells were found in 52.7% (n=29) patients, while in 47.3% of the patients (n=26) sperm cells were not found. Average age of patients with azoospermia was 37.72 ± 6.27 years (28-60). With use of ANOVA test between patients with positive and negative finding of micro-TESE surgery, there was no statistically significant difference in age of patients, F=0,807; p=0,373.

Of the total number of patients with azoospermia, the largest number had the azoospermia due to inflammation (23.6%), followed by cryptorchidism (20%), orhidopexy (20%) and trauma (18.2%). In the examined sample, 4 patients had Klinefelter syndrome, and one patient had azoospermia due to cystic fibrosis. Mean FSH level in pa-

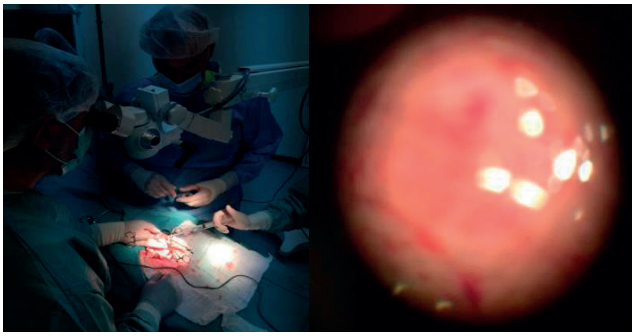

Figure 1. MicroTESE surgery treatment in Bahceci IVF Centre. Sarajevo

Cause of azoospermia	Number of patients	FSH (IU/l)	Testosterone (nmol/L)	Karyotype	Y-deletion
Cystic fibrosis	1 (1.8%)	5.19	5,25	Normal	Not detected
Genetics	4 (7.3%)	23.35	7.11	Klinefelter syndrome	Not detected
Cryptorchidism	11 (20.0%)	13.48	10.18	Normal	Not detected
Inguinal hernia surgery	2 (3.6%)	26.29	22.18	Normal	Not detected
Orhidopexy	11 (20.0%)	22.37	7.35	Normal	Not detected
Trauma	10 (18.2%)	26.54	10.35	Normal	Not detected
Inflammation	13 (23.6%)	14.60	13.41	Normal	Not detected
Varicocele	3 (5.5%)	10.17	2.20	Normal	Not detected
Total	55 (100.0%)	25.19	10.48		

Table 1. Analysis of patients with azoospermia diagnosis

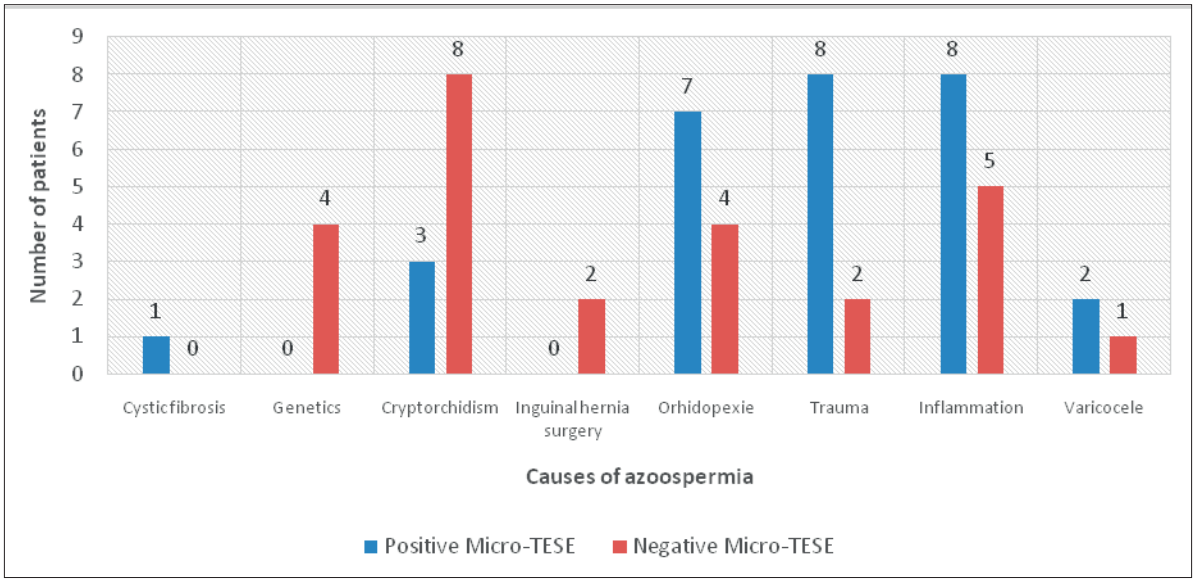

Figure 2. Analysis of patients with azoospermia

tients with azoospermia was 25.19 IU/I, and testosterone level was 10.48 nmol/L. Y-deletions were not detected. (Table 1).

The analysis of the cause of azoospermia in relation to the outcome of micro-Tese surgery showed that regarding negative outcome, the most common cause of azoospermia is cryptorchidism (n=8), inflammation (n=5),then genetics (n=4) and orhidopexy (n=4). Regarding positive outcome of surgery, the most common cause of azoospermia is trauma/inflammation (n=8), followed by orhidopexy (n=7). (Figure 2).

Following the micro-TESE procedure, the obtained sample was subjected to cryopreservation, and then IVF-ICSI procedure was performed using the obtained sample. Conception occurred in 21 cases (72.4%) after performed procedure of in vitro fertilization, while negative outcome occurred in 8 cases (27.6%). Of total number of procedures performed, embryo transfer with frozen embryos was done in 9 cases, and with fresh embryos in 19 cases. Of total number of patients with fresh embryo transfer, 68.4% got pregnant (n=13), while the percentage of pregnancies with frozen embryos was 80% (n=8). With use of chi-square test, statistically significant difference in the outcome of IVF treatment was not determined in relation to use of fresh or frozen embryos, p=0.571. (Table 2).

Positive Micro-Tese surgery			ET		Total
			FRESH	FET	
Pregnancy	No	Number	6	2	8
		%	31.6%	20.0%	27.6%
	Yes	Number	13	8	21
		%	68.4%	80.0%	72.4%
Total		Number	19	10	29
		%	100.0%	100.0%	100.0%
X^2=0,093; p=0,571					

Table 2. Outcome of IVF procedure with use of frozen and fresh embryos

Biggest percentage of successful fertilization was achieved with use of sperm cells retrieved from patients in which azoospermia was caused by varicocele (75%), followed by inflammation/infection (65.5%) and trauma (53.15%). Smallest percentage of successful fertilization was recorded in patients with cryptorchidism (39.08%) and orhidopexy (43.67%). (Figure 3.)

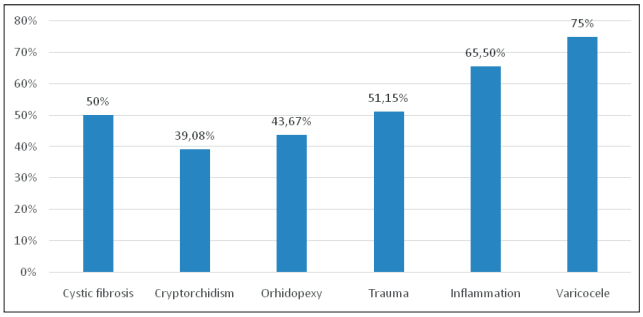

Figure 3. Percentage of successful fertilization using the sperm cells retrieved by Micro TESE procedure in relation to the cause of azoospermia

Of the total number of ET done during procedure of in-vitro fertilization, ET happened on 3rd day in 21 cases, and in 8 cases, the embryos returned to blastocyst stage. 66.7% of fresh embryos were returned on the 3rd day of embryonic development, and 62.5% in blastocyst phase. Approximately equal number of frozen embryos was returned on 3rd day of development (33.3%) and in the blastocyst phase (37.5%). (Table 3).

			Day ET		Total
			Day 3	Blastocyst	
ET	FRESH	Number	14	5	19
		%	66.7%	62.5%	65.5%
	FET	Number	7	3	10
		%	33.3%	37.5%	34.5%
Total		Number	21	8	29
		%	100.0%	100.0%	100.0%
X^2=0,093; p=0,571					

Table 3. Outcome of IVF procedure with use of frozen and fresh embryos

With application of Pearson Correlation, positive statistically significant correlation was established regarding

percentage of fertilization and number of frozen embryos after completed embryo transfer. (p=0.015). (Table 4.)

Of the total number of accomplished pregnancies, 11 were carried to term and ended in delivery of live child. In two cases, pregnancies were terminated in 8th week because they were ectopic. 6 twin pregnancies that were carried to term were recorded, while 2 pregnancies are ongoing. It must be noted that in one case, pregnancy with three babies was achieved and it was successfully carried to term in 33rd week.

Groups			Fertilization percent	Number of IVF attempts	Number of frozen embryos after ET
Positive MICRO TESE	Fertilization percent	Pearson Correlation	1	-.277	.448*
		Sig. (2-tailed)		.145	.015
		N	29	29	29
	Number of IVF attempts	Pearson Correlation	-.277	1	-.108
		Sig. (2-tailed)	.145		.579
		N	29	29	29
	Number of frozen embryos after ET	Pearson Correlation	.448*	-.108	1
		Sig. (2-tailed)	.015	.579	
		N	29	29	29

Table 4. Correlations between number of IVF attempts, number of frozen embryos after procedure and degree of fertilization.

4. DISCUSSION

Due to various methods of retrieving sperm cells from testicular tissue–primarily MicroTESE surgery–and with application of ICSI method of fertilization of oocyte, infertility of married couples caused by azoospermia can be treated successfully (4). The type of surgical method to be applied to a male patient with azoospermia largely depends on the cause of azoospermia, clinical and laboratory parameters, as well as the judgment of experienced urologist who is performing the procedure. Most authors agree that MicroTESE surgery is the best and most precise surgical procedure to retrieve sperm cells, especially in patients with non-obstructive azoospermia (5,6). In our practice, we have demonstrated the successfulness of MicroTESE surgery and the outcome of ICSCI procedure with retrieved sperm cells in the two-year period 2013/2014. In the period analyzed, there were 55 MicroTESE surgeries performed and sperm cell retrieval was successful in 52.7% of the cases (n=29). The most common cause of azoospermia in patients with negative outcome of surgical retrieval of sperm cells was cryptorchidism and orhidopexy and chromosomal anomalies (Klinefelter Sy.). It must be noted that we subjected all obtained positive samples to cryopreservation and that the obtained spermatozoids were later used in ICSI procedure, after controlled stimulation of ovulations and performed puncture procedure. The percentage of successful fertilization was 55.28%, the largest percentage of successful fertilization was accomplished with sperm cells from patients with azoospermia caused by varicocele (75%), and the smallest percentage was in case of cryptorchidism as the cause (39.08%). Out of the total number of performed procedures, ICSI with frozen sperm cells from positive Micro-TESE surgeries was performed in 72.4% of cases (FRESH-68.4%; FET-80%). Numerous clinical studies dealt with issue of health of babies born by use of surgically obtained sperm cells. Although there are conflicting views on this subject, our research shows that all the babies were born healthy and that all pregnancies were carried to term except in two cases where the pregnancies were terminated due to ectopic gravidity.

5. CONCLUSION

Procedure for surgical retrieval of sperm cells (MicroTESE) is being used with success in the entire Bahceci group as a method of treating male infertility caused by azoospermia. Results achieved are in line with results of leading centers. Since the existing method and modern medical technology are getting perfected, the outcome of the treatment will get even better, while unfortunately, the problem of male infertility is escalating.

CONFLICTS OF INTEREST: NONE DECLARED.

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