

Conference Paper

In Vitro Fertilization Outcome after Intracytoplasmic Sperm Injection with Fresh and with Frozen-Thawed Epididymal Spermatozoa

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Abstract

Introduction. Testicular epididymal sperm aspiration (TESA) is one of the method to retrieve sperm from the testes in men with azoospermia. The aim of the study is to compare the In vitro fertilization (IVF) outcome of intracytoplasmic sperm injection (ICSI)-ET cycles with fresh testicular epididymal spermatozoa obtained on the same day with oocyte retrieval and with frozen-thawed testicular epididymal spermatozoa.

Material and Methods. A retrospective comparative analysis of patients who underwent fresh TESA and frozen-thawed TESA in ICSI-ET cycles from January 2012 to December 2014 in Halim Fertility Center was done. Fresh testicular epididymal sperm aspiration (fresh TESA) was performed on the same day with oocyte retrieval in 28 cycles and the frozen-thawed testicular epididymal sperm aspiration (frozen-thawed TESA) was used in 30 cycles. **Results.** The two groups were comparable in terms of the ages of male and female patients, etiology of infertility and duration of infertility. Fertilization rates in fresh TESA group were 53,5% and in frozen-thawed TESA group, fertilization rates were 50%. There was no statistically significant difference between the groups. Clinical pregnancy rates in fresh TESA group were 35,7% and in frozen-thawed TESA group, clinical pregnancy rates were 26,7% and statistically there was no significant difference between the groups. **Conclusion.** There is no significant difference in the in vitro fertilization outcome of intracytoplasmic sperm injection (ICSI)-ET cycles between fresh TESA and frozen-thawed TESA.

Keywords: fresh TESA, frozen-thawed TESA, IVF

1. Introduction

Some sperm retrieval method has been developed to collect epididymal and testicular sperm for ICSI procedures in men with azoospermia [1,2]. Testicular sperm retrieval was first introduced in 1993 and has been commonly used in patients with OA and NOA since then [1,3,4,5]. Although, some studies demonstrated lower rates to blastocyst stage and lower implantation rates with spermatozoa from men with NOA [6,7], testicular spermatozoa recovered from men with all types of azoospermia were found to be as effective as ejaculated spermatozoa in ICSI cycles [8,9]. Generally, either percutaneous epididymal sperm aspiration (PESA) or microepididymal sperm aspiration (MESA) can

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be successfully used to retrieve sperm from the epididymis in men with obstructive azoospermia (OA) [1,8,10]. Testicular epididymal sperm aspiration (TESA) can be used to retrieve sperm from the testes either in men with OA failed to do PESA as well as in men with non-obstructive azoospermia (NOA). Percutaneous epididymal sperm aspiration (PESA), Testicular Sperm Aspiration (TESA) and Testicular Sperm Extraction (TESE) is used when no sperm in the ejaculate (azoospermia). This can result from an obstruction in the reproductive tract (obstructive azoospermia) or due to lack of sperm production (non obstructive azoospermia) [10-12]. Obstructive azoospermia may be due to a failure to develop the vas deferens, the sperm carrying tube blockage (due to infection or due to a blockage of the vas following surgical sterilization of men) TESE (testicular sperm extraction) can be done on the day prior to oocyte retrieval to reduce stress on the couple [1,12,13]. Number fertilization is achieved by extracting spema before hCG administration. Testicular Sperm can be aspirated from epididmis or testicles, or extracted from the testicles [11]. Testicular Sperm can also be obtained before controlled ovarian stimulation and freezing for ICSI [1,14]. The aim of the study is to compare the In vitro fertilization (IVF) outcome of intracytoplasmic sperm injection (ICSI)-ET cycles with fresh testicular epididymal spermatozoa obtained on the same day with oocyte retrieval and with frozen-thawed testicular epididymal spermatozoa.

2. Materials and Methods

2.1. Patients

A retrospective comparative analysis of patients who underwent fresh TESA and frozen-thawed TESA in ICSI-ET cycles from January 2012 to December 2014 in Halim Fertility Center was done. Fresh testicular epididymal sperm aspiration (fresh TESA) was performed on the same day with oocyte retrieval in 28 cycles and the frozen-thawed testicular epididymal sperm aspiration (frozen-thawed TESA) was used in 30 cycles. Azoospermia was confirmed on at least two semen samples. All patients had a physical examination and assessment of FSH level before the procedure. Only the cycles in which motile spermatozoa had been used for ICSI were included in the study and the other cycles with immotile and/or immature spermatozoa were excluded. Twitching was accepted as a minimum criterion for motility.

2.2. TESA Sperm Processing

In this procedure, sperm taken from the testes percutaneously by inserting a needle into the testis and perform aspiration of testicular tissue. This procedure is mostly done under local anesthesia. Depending on the case, one or more aspiration may be required. Discharge TESA aspirate to the outer-dish well. Under stereomicroscopy, identify seminiferous tubules and remove blood dots using the needled-tuberculin syringes. Transfer seminiferous tubules to the inner-dish well containing fresh sperm medium. Perform a mechanical dispersion of the tubules by mincing repeatedly using both needled-tuberculin syringes (use one to hold tubules in place at the bottom of

the dish and the other to squeeze and open them). Repeat this step until no intact tubules are seen. Examine the homogenate to confirm the presence of sperm using the inverted microscope at x400 magnification. This step should take no more than 10 minutes because the patient was kept under anesthesia until a decision of continuing or finishing the surgical retrieval is made. Aspirate and transfer the cell suspension from the inner-well dish to a sterile centrifuge tube. Dilute the aspirate with 3 mL of fresh sperm medium and wash it at x300 g for seven minutes. Discharge the supernatant and resuspend the pellet in 0.2 mL of sperm medium. When a processed TESA specimen is still contaminated with an excessive number of red blood cells, dilution and centrifugation with erythrocyte lysis buffer may be required. Prepare a petri dish containing a series of microdrops under mineral oil for sperm pick-up from a processed epididymal cell suspension. Load 1 to 2 μ L sperm suspension aliquot at each 10 μ L peripheral microdroplet of HEPES buffered culture medium to facilitate sperm search and pick-up. Proceed to sperm selection and ICSI. If progressive motility is low or absent and/or the sample is contaminated with cellular debris, load 1 to 4 μ L sperm suspension aliquot at each 10 μ L peripheral microdroplet of HEPES-buffered culture medium to facilitate search and selection of motile sperm. First aspirate a small volume of PVP into the injection micropipette to improve control during sperm pick-up and to avoid blowing air bubbles during ejection of selected sperm into the PVP droplet. After finishing to pick-up sperm wash the injection micropipette free of any debris in the PVP droplet and consider cryopreservation of left-over testicular aspirates. Dishes with microdroplets containing TESA processed sperm could be incubated up to 48 hours before ICSI at room temperature in an attempt to improve testicular sperm motility.

Sperm-containing suspensions were frozen for later use. Sample was allowed to liquefy at 37°C for 30 minutes, one vial of medium is thawed and brought to 37°C. The liquefied sample was transferred to a sterile, 15 mL, conical centrifuge tube, the volume determined and medium (Irvine) added dropwise until a 1:1 sample:medium ratio was achieved. The sample-medium mixture was placed in a beaker or other suitable container of 37°C water. The container was refrigerated at 2°C to 5°C to allow a slow cooling of the mixture (0.5°C/minute). After 90 minutes, the sample was ready to freeze in straws, vials or pellets using traditional procedures. For thawing, cryovials were placed in a 37°C water bath for 3–5 minutes. Then, the samples were washed in medium. The sperm were cultured at 37°C until used for ICSI.

2.3. Controlled Ovarian Hyperstimulation

Women with a normal uterine cavity, less than age 43 years and who produced at least three oocytes in response to Controlled ovarian hyperstimulation (COH) were included in the study. Controlled ovarian hyperstimulation was achieved by using either GnRH-antagonist or GnRH-agonist with recombinant FSH until at least three dominant follicles reached to 18 mm in diameter. Oocyte retrieval was performed 36 hours after the hCG administration with transvaginal ultrasound. Oocytes were cultured in medium. Cumulus-corona complex was removed by pipetting with hyaluronidase 3 hours after

Variables	Fresh TESA	Frozen-thawed TESA
No. Cycle	28	30
Male age	37,86±5,68	39,14±6,71
Female age	33,07±4,77	33,71±5,86
Duration of infertility	7,07±4,35	7,93±4,99
Baseline serum FSH level of women (IU/L)	4,68±2,00	4,80±1,97
FSH level of men (IU/L)	6,93±1,74	6,00±1,97
Etiology of infertility, n (%)		
Male factor	15(53,6)	14(46,7)
Mixed factor	13(46,4)	16(53,3)

TABLE 1: Table Distribution of Respondents.

incubation. The ICSI procedure was performed as described in detail elsewhere. Fertilization was assessed 16–18 hours after ICSI and cleavage rate was checked 48–72 hours after oocyte retrieval. Embryo transfer (ET) was performed on day 3 after oocyte retrieval. All couples had at least one embryo to be transferred. The luteal phase was supported by micronized progesterone and continued until a fetal heart beat was detected. Biochemical pregnancy was confirmed by assessing serum hCG level 14 days after ET. A clinical pregnancy was defined by the presence of a gestational sac with the fetal heart beat on a 6-7week ultrasound.

2.4. Data Analysis

Processing and analyzing data were using SPSS 17 (Statistic Package for Social Science) software. The statistical analysis was performed using Chi-Square test.

3. Results

From January 2012 to December 2014, we included 58 total cycles ICSI-ET, consisted 28 cycles with fresh TESA and 30 cycles with frozen-thawed TESA. The patient demographics, duration of infertility, FSH level, etiology of infertility were depicted in Table 1. Table 1 showed that the mean (SD) age of male in frozen-thawed TESA (39,14±6,71) was higher than fresh TESA (37,86±5,682). It also showed that age of woman in fresh TESA (33,07±4,77) was similar with frozen-thawed TESA (33,71±5,86). From Table 1, duration of infertility in fresh TESA (7,07±4,35) was similar with frozen-thawed TESA (7,93±4,99). Serum FSH level of women was similar between fresh TESA (4,68±2,00) and frozen-thawed TESA (4,80±1,97) (Table 1). FSH level of men was also similar between fresh TESA (6,93±1,74) and frozen-thawed TESA (6,00±1,97) (Table 1). Table 1 showed that the majority of etiology infertility in fresh TESA was male factor as many as 15 patients (53,6%) and mixed factor as many as 13 patients (46,4%). Then in frozen-thawed TESA, the etiology of infertility was mixed factor as many as 16 patients (53,3%) and male factor as many as 14 patients (46,7%).

Variables	Fresh TESA	Frozen-thawed TESA	p
No.of oocytes collected	13,07±8,81	13,93±13,4	0,84*
Fertilization rate n (%)	15 (53,5)	15 (50)	0,72**
Clinical pregnancy rates n (%) Negative Positif	18 (64,3) 10 (35,7)	22 (73,3) 8 (26,7)	0,83**
*students t-test			
**chi-square			

TABLE 2: Outcome of ICSI-ET cycles.

Table 2 showed that the mean (SD) oocytes collected in fresh TESA 13,07 (8,81) was similar with frozen-thawed TESA 13,93 (13,4) and there was no statistically significant difference between the groups ($p > 0,05$). The fertilization rate in fresh TESA was 53,5% similar with frozen-thawed TESA and there was no statistically significant difference between the groups ($p > 0,05$) (Table 2). Table 2 also showed that In fresh TESA, clinical pregnancy rates were 35,7% and in frozen-thawed TESA, clinical pregnancy rates were 26.7% with no significant difference between the groups ($p > 0,05$).

4. Discussion

Since the initial reports of pregnancies following ICSI, a dramatic improvement in the treatment of cases of severe male factor infertility has been witnessed. Initially, ICSI was performed in patients with repeated failure fertilization following conventional IVF. It soon became clear that ICSI could also be used for couples in which the male partner has a limited number of spermatozoa in the ejaculate, or for epididymal and testicular spermatozoa [1]. Several sperm retrieval methods have been developed to collect epididymal and testicular sperm for ICSI in azoospermic men. Testicular epididymal sperm aspiration (TESA) can be used to retrieve sperm from the testes either in men with obstructive azoospermia (OA) who fail percutaneous epididymal sperm aspiration (PESA) as well as in those with non-obstructive azoospermia (NOA) [10-12]. From our study, we found that there was no statistically significant difference in no.of oocytes collected, fertilization rates and clinical pregnancy rates between fresh TESA and frozen-thawed TESA. Karacan M et al. (2013) also stated that fresh testicular sperm obtained on the day of or the day before oocyte retrieval and frozen-thawed testicular spermatozoa yield similar clinical PRs, miscarriage rates, and delivery rates once motile spermatozoa are found with microdissection TESE. They found that the etiology of azoospermia does not have any influence on the outcome with the timing of the microdissection TESE procedure for ICSI [1]. Wald et al. (2006) reported that a PR with cryopreserved testicular sperm did not differ from that achieved with fresh sperm (27.3% vs. 27%) with ICSI [12]. Cayan et al. (2001) compared the outcome of ICSI with fresh and cryopreserved epididymal spermatozoa from the same couple and reported the clinical PRs as 31.6% with fresh and 36.8% with frozen-thawed spermatozoa [13]. These discrepancies in the previous studies may be due to the assorted cryopreservation and thawing methods among the centers. Friedler et al. (1997) stated that there was no statistically significant differences in all parameters

examined between ICSI cycles with fresh or cryopreserved testicular spermatozoa from the same nine patients and comparing all ICSI cycles performed. They found that testicular sperm cryopreservation using a simple freezing protocol was promising in patients with nonobstructive azoospermia (NOA) augmenting the overall success achieved after surgical sperm retrieval [2].

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There is no conflict of interests in this manuscript.

References

- [1] M. Karacan, F. Alwaeely, S. Erkan, Z. Çebi, M. Berberoğlulugil, M. Batukan, M. Uluğ, A. Arvas, and T. Çamlıbel, Outcome of intracytoplasmic sperm injection cycles with fresh testicular spermatozoa obtained on the day of or the day before oocyte collection and with cryopreserved testicular sperm in patients with azoospermia, *Fertility and Sterility*, **100**, no. 4, 975–980, (2013).
- [2] S. Friedler, D. Strassburger, A. Raziel, D. Komarovsky, Y. Soffer, and R. Ron-El, Intracytoplasmic injection of fresh and cryopreserved testicular spermatozoa in patients with nonobstructive azoospermia - A comparative study, *Fertility and Sterility*, **68**, no. 5, 892–897, (1997).
- [3] R. Schoysman, P. Vanderzwalmen, M. Nijs, L. Segal, G. Segal-Bertin, L. Geerts, E. van Roosendaal, and D. Schoysman, Pregnancy after fertilisation with human testicular spermatozoa, *The Lancet*, **342**, no. 8881, p. 1237, (1993).
- [4] S. J. Silber, A. C. Van Steirteghem, J. Liu, Z. Nagy, H. Tournaye, and P. Devroey, High fertilization and pregnancy rate after intracytoplasmic sperm injection with spermatozoa obtained from testicle biopsy, *Human Reproduction*, **10**, no. 1, 148–152, (1995).
- [5] I. De Croo, J. Van Der Elst, K. Everaert, P. De Sutter, and M. Dhont, Fertilization, pregnancy and embryo implantation rates after ICSI in cases of obstructive and non-obstructive azoospermia, *Human Reproduction*, **15**, no. 6, 1383–1388, (2000).
- [6] B. Balaban, B. Urman, A. Isiklar, C. Alatas, R. Mercan, S. Aksoy, and A. Nuhoglu, Blastocyst transfer following intracytoplasmic injection of ejaculated, epididymal or testicular spermatozoa, *Human Reproduction*, **16**, no. 1, 125–129, (2001).
- [7] M. A. Aboulghar, A. Kamal, R. T. Mansour, N. A. Tawab, G. I. Serour, Y. M. Amin, and I. Fahmy, Fertilization and pregnancy rates after intracytoplasmic sperm injection using ejaculate semen and surgically retrieved sperm, *Fertility and Sterility*, **68**, no. 1, 108–111, (1997).
- [8] J. D. M. Nicopoulos, C. Gilling-Smith, P. A. Almeida, J. Norman-Taylor, I. Grace, and J. W. A. Ramsay, Use of surgical sperm retrieval in azoospermic men: A meta-analysis, *Fertility and Sterility*, **82**, no. 3, 691–701, (2004).

- [9] A. Hourvitz, A. Shulman, I. Madjar, J. Levron, D. Levran, S. Mashiach, and J. Dor, In vitro fertilization treatment for severe male factor: A comparative study of intracytoplasmic sperm injection with testicular sperm extraction and with spermatozoa from ejaculate, *Journal of Assisted Reproduction and Genetics*, **15**, no. 6, 386–389, (1998).
- [10] H. Tournaye, T. Merdad, S. Silber, H. Joris, G. Verheyen, P. Devroey, and A. Van Steirteghem, No differences in outcome after intracytoplasmic sperm injection with fresh or with frozen-thawed epididymal spermatozoa, *Human Reproduction*, **14**, no. 1, 90–95, (1999).
- [11] I. Craft and M. Tsirigotis, Simplified recovery, preparation and cryopreservation of testicular spermatozoa, *Human Reproduction*, **10**, no. 7, 1623–1626, (1995).
- [12] M. Wald, L. S. Ross, G. S. Prins, J. Cieslak-Janzen, G. Wolf, and C. S. Niederberger, Analysis of outcomes of cryopreserved surgically retrieved sperm for IVF/ICSI, *Journal of Andrology*, **27**, no. 1, 60–65, (2006).
- [13] S. Cayan, D. Lee, J. Conaghan, C. A. Givens, I. P. Ryan, E. D. Schriock, and P. J. Turek, A comparison of ICSI outcomes with fresh and cryopreserved epididymal spermatozoa from the same couples, *Human Reproduction*, **16**, no. 3, 495–499, (2001).
- [14] E. N. Tavmergen Göker, F. Sendag, R. Levi, H. Sendag, and E. Tavmergen, Comparison of the ICSI outcome of ejaculated sperm with normal, abnormal parameters and testicular sperm, *European Journal of Obstetrics Gynecology and Reproductive Biology*, **104**, no. 2, 129–136, (2002).