
Report

TREATMENT OUTCOME FOLLOWING INTRACYTOPLASMIC INJECTION OF SPERM RETRIEVED FROM EJACULATE, EPIDIDYMIS, OR TESTIS OF INFERTILE MEN

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Introduction of intracytoplasmic sperm injection (ICSI) offered an effective therapy for males with infertility including those with azoospermia. The novel techniques for sperm retrieval from the epididymis (percutaneous epididymal sperm aspiration [PESA]) and testis (testicular sperm aspiration [TESE]) have been successfully combined with ICSI. This is the first preliminary report on ICSI with ejaculated sperm (80 cycles), PESA/ICSI (40 cycles), and TESE/ICSI (40 cycles) in Iran. The study population comprised 160 consecutive young males with primary infertility admitted to Research and Clinical Center for Infertility in Yazd, Iran. Eighty subjects (mean \pm SD of age: 34.0 \pm 4.4 years) could produce ejaculates while in the rest who had obstructive azoospermia, the sperm was obtained by PESA or TESE. All wives were treated for ovarian stimulation and oocytes were retrieved 34 to 36 hours after human chorionic gonadotropin (HCG) injections. Sperm parameters of count, motility, and morphology were analyzed and ICSI was performed 2 to 3 hours after the incubation of the processed sperm. The rate of fertilization and embryo development and transfer were subsequently evaluated. The results showed that the fertilization rate for the groups whose sperms had been obtained by ejaculation, PESA, and TESE was 72.1%, 73.6%, and 51.3%, respectively. The mean number of embryo transfers for the aforementioned groups was 3.0, 3.8, and 2.6, respectively. The parameters of sperm count and morphology were similar in the ejaculate and PESA groups. They were, however, lower in TESE samples ($p < 0.01$). The results confirm that PESA and TESE are very effective for obtaining adequate numbers of sperms to successfully treat male infertility including obstructive azoospermia.

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Introduction

Introduction of the intracytoplasmic sperm injection (ICSI) has offered an effective therapy for male-factor infertility including azoospermia.^{1, 2} In this technique, a live spermatozoon is required to achieve fertilization of a metaphase II (MII) oocyte. ICSI, using ejaculate sperm, is generally considered as the gold standard

for the treatment of male infertility. However, in some infertile men, the sperm is not present in the ejaculate due to tubal obstruction, a condition called obstructive azoospermia. The causes of obstruction include congenital absence of vas deference and acquired causes like postinflammatory blocks along the semen carrying and failed reversal of vasectomy.^{3, 4} In azoospermic patients, the sperm may be effectively retrieved from the epididymis or testis.

In 1995, Craft et al were the first to introduce a novel technique for sperm retrieval from the epididymis, namely percutaneous epididymal sperm aspiration (PESA).⁵ PESA technique is based on the principle of minimal invasion, using needle aspiration to recover epididymal sperm. It is more acceptable to patients, the cost is low, and the risk of general anesthesia is avoided. However, if it

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is not possible to recover the sperm from the epididymis, testicular sperm extraction (TESE) is usually attempted next. This involves an open testicular biopsy, another minimally invasive technique.⁶ Although it is generally possible to recover live spermatozoa from TESE samples, their motility is usually very poor. Both PESA and TESE are now routine procedures in most assisted reproductive technology clinics including the infertility center in our university.

It is generally important to evaluate and compare the ICSI treatment cycles using ejaculate sperm obtained from infertile men with surgically retrieved sperm in azoospermic cases. Therefore, the main objective of this study was to evaluate and compare the outcome of ICSI program using ejaculate sperm in infertile men with that of PESA/ICSI and TESE/ICSI treatment cycles in men with obstructive azoospermia. This is the first preliminary report of such evaluation in a large series of infertile patients in Iran.

Patients and Methods

The study was performed over 3 years from 2001 to 2003. The research proposal was approved by the Ethics Committee of the institution. The population comprised 160 males with primary infertility ranging from 1 to 11 years who underwent ICSI cycles at Research and Clinical Center for Infertility in Yazd, Iran. Of these, 80 cycles were carried out with freshly obtained ejaculate spermatozoa. In the remaining 80 who had obstructive azoospermia, half (40 cases) were treated by PESA/ICSI and the other half by TESE/ICSI. Azoospermia was reconfirmed after two semen analyses with an interval of 1 to 2 months according to WHO definitions (1999). The incomplete ICSI cycles due to failures at the early stages of treatment such as lack of live spermatozoa in the retrieved samples, degenerated oocytes, fertilization failure, or failure during embryo formation were excluded from the study. The demographic details and the ICSI outcomes in the subjects are shown in Table 1. All wives were healthy and were treated for ovarian stimulation with intramuscular HCG injections. The oocytes were retrieved by ultrasound-guided puncture of the ovarian follicles 34 to 36 hours after HCG injections.² Then, the oocytes were kept in culture media in an incubator (Memorth, Germany) in 37°C and 5% CO₂.

Ejaculate sperm

The semen was first collected in a sterile container and incubated for 15 min. The specific parameters of concentration, motility, and morphology of spermatozoa were evaluated as described before.⁷ The samples were processed by swim-up method using Ham's F10+6% serum substitute substance (SSS) (culture medium) kept in an incubator in 37°C and 5% CO₂. Following incubation, 10 µL of the swim-up supernatant was removed and analyzed again. The sample was incubated until microinjection which was performed 2 hours after incubation.

PESA

All epididymal sperm aspirations were performed by a urologist under local anesthesia.⁸ A 21-gauge needle was carefully directed through the skin of the scrotum into the caput of the epididymis. While applying negative pressure, the tip of the needle was pushed within the substance of the epididymis until a cloudy fluid was observed in the needle tubing. The aspirate was then flushed with culture medium and was examined under a microscope. The sample was centrifuged twice at 600 g for 5 min each time. The supernatant fluid was removed to leave just 0.3 mL of the fluid above the pellet. Finally, the tube was incubated for 45 min in 37°C and 5% CO₂.^{3, 8}

TESE

In some cases of obstructive azoospermia, it is impossible to recover epididymal sperm. Therefore, retrieving testicular sperm is attempted by TESE.^{8, 9} Following local anesthesia with 1% lidocaine, an incision was made in the scrotal skin and the tunica albuginea. Next, a small piece of testicular tissue was excised and placed in a Falcon tube containing 1 mL of the culture medium (Ham's F10+SSS). The tissue was then shredded into small pieces with needles and examined under a microscope for the presence of live sperms. The TESE sample was then processed as mentioned for PESA.

ICSI procedure

ICSI was performed with microinjection equipment (Narishege, Japan) and an inverted microscope (Nikon, Japan) as previously described.^{2, 10, 11} The microinjection dish was prepared by adding several 5-µL droplets of Ham's F10+ 6% SSS. The prepared sperms (1-2 µL) were added to the drops of 10% polyvinylpyrrolidone

Table 1. Patient characteristics according to the source of spermatozoa.

Variable	Ejaculate	PESA	TESE
Male, age (yr)	34.0 ± 4.4	33.9 ± 2.1	34.2 ± 5.6
Female, age (yr)	29.0 ± 1.8	26.2 ± 3.2	29.2 ± 4.8
Infertility duration (yr)	7.5 ± 3.0	6.2 ± 1.7	7.7 ± 3.9
No. of MII oocyte retrieved	7.3 ± 3.3	8.5 ± 2.7	6.6 ± 2.9 (3)
No. of fertilized oocyte	5.2 ± 1.2	6.25 ± 3.1	3.35 ± 1.0 (1)
No. of embryo	5.0 ± 1.3	5.6 ± 0.9	3.1 ± 0.6 (1)
No. of embryo transferred	3.0 ± 0.9	3.8 ± 1.35	2.6 ± 0.65 (3)
Rate of fertilization (%)	72.1	73.6	51.3 (4)
Rate of embryo/ fertilization (%)	97.2	89.5	92.2

Values are mean ± SD

- (1) Ejaculate versus TESE ($p < 0.01$)
 (2) Ejaculate versus PESA ($p < 0.01$)
 (3) PESA versus TESE ($p > 0.05$)
 (4) PESA versus TESE ($p < 0.05$)

(PVP; Sigma, USA) and the oocytes were placed into the droplets of the medium. The oocytes were already denuded from the cumulus cells using hyaluronidase 80 IU/mL (Sigma, USA) and mechanical aid of fine Pasteur pipettes. Then, each oocyte was rinsed a couple of times in the culture media. After oocyte microinjection, they were rinsed and placed in equilibrated culture medium with 10% SSS. The oocytes were incubated and observed for signs of fertilization 16 to 18 hours after injection. The cleavage of fertilized oocytes was assessed about 24 hours after fertilization. Around 48 hours after injection, adequate numbers of embryos were transferred into the uterine cavity.

Statistical analysis

For analysis, SPSS software (version 9, SPSS Inc., Chicago, USA) was used. The results are expressed as the mean ± SD. The p values less than 0.05 were considered significant. Chi-square test was used to test the frequencies and Student's t -test was used for comparing the means.

Results

In the 80 ICSI cycles with ejaculate sperms, 583 MII oocytes were retrieved. In addition, a total of 339 and 258 MII oocytes were retrieved from 40 PESA/ICSI and 40 TESE/ICSI cycles, respectively. A total of 424 (72.1%) oocytes in the ejaculate group, 250 (73.6%) in PESA/ICSI, and

134 (51.3%) in TESE/ICSI group were fertilized. This shows that the rates of fertilization with ejaculated and PESA sperms were significantly higher than TESE ones ($p < 0.05$). The highest rate of embryo formation was achieved in ICSI with ejaculate sperm (Table 1). Only one embryo was available for transfer in 12 women using ejaculate, 3 using PESA, and 7 using TESE spermatozoa. The highest numbers of embryos (mean 3.8) were transferred in the women undergoing PESA/ICSI compared to those undergoing TESE/ICSI (mean 2.6).

In addition, there were no significant differences in the sperm count and morphology between ejaculate and PESA groups. However, significant differences were noticed between PESA and TESE groups on one hand and the ejaculate group on the other hand. The highest rate of sperm progressive motility was observed in the ejaculate specimens compared with surgically retrieved ones ($p < 0.01$, Table 2).

Discussion

The data presented here demonstrate that both PESA and TESE are the methods of choice for obtaining adequate numbers of spermatozoa from patients with obstructive azoospermia. These sperm samples can be successfully used in ICSI treatment cycles. However, it should be emphasized that in comparison with the results generated from ejaculate specimens, the

Table 2. Summary of spermatozoa parameters.

Variable	Ejaculate	PESA	TESE
Sperm count ($\times 10^6$ /mL)	38	36 (3)	1 (1)
Normal morphology (%)	26.9	26.4 (3)	10.5 (1)
Progressive motility (%)	21.2	9.7 (2)	3.1 (1)
Nonprogressive motility (%)	9.5	9.25 (3)	4.75 (1)

- (1) Ejaculate versus TESE ($p < 0.01$)
 (2) Ejaculate versus PESA ($p < 0.01$)
 (3) Ejaculate versus PESA ($p > 0.05$)

progressive motility of the sperms was significantly lower in both PESA and TESE samples. This is in agreement with the studies conducted by Aboulghar et al and Meniru et al.^{3, 12} In addition, while the parameters of sperm concentration and normal morphology in PESA samples from our study were comparable to the ejaculate samples, these parameters were significantly lower in the TESE samples. This is most probably related to the fact that spermatozoa reach their maturity in the epididymis rather than testis.

The technique of ICSI has been applied worldwide for the treatment of male infertility. Satisfactory results have been achieved with ejaculate as well as surgically retrieved spermatozoa.¹⁻³ It has been well documented that the results from ICSI treatment with either epididymal or testicular sperms are broadly similar though fertilization rates are usually lower than those obtained following ICSI with ejaculate sperm. Both PESA and TESE are now considered as simple techniques requiring minimal equipment.³⁻⁶ However, the majority of TESE samples show poor sperm motility which may affect treatment outcome. The motility of sperm retrieved from testis may be enhanced with the *in vitro* addition of pentoxifylline.^{8, 13}

Meniru et al also compared the outcome of ICSI cycles using ejaculates with 54 PESA/ICSI treatment cycles.¹² There were no significant differences in men's age between the groups. Fertilization rates in the ejaculate group were significantly higher than in PESA group ($p < 0.01$). However, the difference in embryo development and cleavage rates of the groups were insignificant (92% and 91%). Similarly, Ubaldi et al noticed lower fertilization rates in the patients undergoing PESA/ICSI compared to those using ejaculate sperm in ICSI though the rate of embryo implantation was higher.⁴ Unfortunately, we could not compare the rates of embryo implantations among the three patient groups. This was due to the fact that the patients attended the ICSI programs from all over the country, and we did not have any access to them for follow-up. All the patients are usually discharged one day following the embryo transfer, and they return home soon after. Therefore, it is very difficult to follow up their clinical pregnancy outcomes. Our results showed that the rate of fertilization in both ejaculate and PESA samples were similar. While fertilization was significantly reduced in TESE

samples ($p < 0.05$). The rates of embryo formation were similar in all 3 groups (ejaculate, PESA, and TESE) in our study. It is difficult to explain this fact, but whatever sperm factor responsible for the lower fertilization of testicular sperm, may soon cease operation so that it will no longer affect embryo formation or cleavage rates.

In addition, Aboulghar et al compared the ICSI outcomes with ejaculate, epididymal, and testicular sperms in cases with severe male infertility.³ There were no significant differences in fertilization or pregnancy rates between ejaculate and surgically retrieved sperms in obstructive azoospermia. It was also found that the normal semen had an important role in causing a higher fertilization rate compared with other groups. Therefore, normal sperm motility and morphology are associated with higher success rates following ICSI.¹⁴ In this clinical setting, the fertilization rates observed for PESA/ICSI (73.6%) and TESE/ICSI (51.3%) were higher than the rates in our previous reports. It can possibly be due to the fact that extensive experience and better knowledge of sperm selection for microinjection has been gained. A possible explanation for the poor fertilization rates after ICSI in TESE group compared with ejaculate or PESA groups in this study might be the lower concentration of motile TESE sperms which could reduce the probability of choosing a normal mature and live sperm.

In conclusion, the findings show that the minimally invasive techniques of PESA and TESE can be successfully performed to retrieve adequate numbers of spermatozoa for ICSI. Our results also demonstrated that although the fertilization rates were significantly lower in TESE/ICSI than in groups using ejaculate or PESA/ICSI, once fertilization was achieved, embryonic development and cleavage rates were similar among all 3 groups. It should be emphasized that ICSI for the treatment of severe male-factor infertility is especially important in Muslim countries because the use of donor spermatozoa is usually forbidden. At last, with continued analysis of sperm parameters, embryo development, and implantation rates in a large multicenter study, the treatment outcome of ICSI could be advanced even more.

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