RESEARCH ARTICLE

AN AZOOSPERMIC MALE CAN FATHER A CHILD
Mohammed Kouser, Farrukh Jehan, Shaheen Panjwani, Kousar Kazmi, Shaheen Zafar, Zahida Baqai

ABSTRACT
This study included 184 azoospermic men who visited Baqai Institute of Reproduction and Developmental Sciences (BIRDS) during a period of 20 months (October 1997 to July 1999). Sperms were retrieved in 65 men by Percutaneous Epididymal Sperm Aspiration (PESA) and in 28 men by Testicular Sperm Excision (TESE). Motile sperms were obtained in 42 men by PESA (65%) whereas in 9 men by TESE (32%). Intra Cytoplasmic Sperm Injection (ICSI) was used in 28 cases where sperms were obtained by PESA. 252 oocytes were injected by ICSI and fertilization was noted to be 67.5%. Sperms obtained through TESE were used in 25 cases of ICSI. 289 oocytes were injected and fertilization was 61.6%. Patients were selected after full investigation and counseling. Patients having high serum FSH level and atrophied testis were not included in the study. Four pregnancies were obtained in PESA cases (14.2%) and 6 pregnancies in TESE cases (24%). Thus confirming an azoospermic man can become a father of a child.

Keywords: Intra cytoplasmic sperm injection, percutaneous epididymal sperm aspiration, testicular sperm excision, azoospermia.

1. INTRODUCTION
Men having azoospermia can father a child, and latest developments in Assisted Reproductive Techniques (ART) have given a ray of hope to them. For azoospermic patients sufficient number of sperms can be collected by Surgical Sperm Collection (SSC)\(^1\). The advent of Intra Cytoplasmic Sperm Injection (ICSI) by Palermo et al.\(^2\) in 1992 as one of the ART techniques has revolutionized the treatment for male infertility. Since then the ICSI has become an indispensable tool for the male having azoospermia or severe oligoasthenoteratozoospermia. As only one viable sperm oocyte is required for ICSI, men with few sperms can father a child. SSC includes Per Epididymal Sperm Aspiration (PESA), Microsurgical Epididymal Sperm Aspiration (MESA) and Testicular Sperm Excision (TESE)\(^1,2\).

ICSI has been applied to the cases of azoospermia at Baqai Institute of Reproduction and Developmental Sciences (BIRDS). Both motile and immotile sperms were collected from the epididymus or testes in obstructive azoospermia cases and in the cases of atrophic azoospermia with multiple biopsies from different parts of testes. Fertilization rate was found to be more than 60% in cases where sperm were obtained through PESA and TESE. Pregnancy percentage was found lower with PESA sperms than that of TESE sperm.

2. MATERIALS AND METHODS
The present survey includes the results of 51 consecutive ICSI treatment cycles from October 1997 to July 1999. It also presents updated results on the outcome of pregnancies after ICSI. One hundred and eighty five infertile couples with a history of azoospermia visiting O.P.D. of BIRDS were included in this study.

2.1. Patients Selection
Male patients falling under the age of group of 20 to 60 years were investigated. All patients selected for SSC underwent diagnostic procedures. Patients having high FSH (>25) were not included in the study. Patients having atrophied testes or having a history of mumps due to radiation therapy were also excluded. ICSI is now performed with spermatozoa collected through one of the three types of procedures, i.e. PESA, MESA, TESA. In our lab, sperms were retrieved by PESA and TESE only.

2.2. Epididymal Sperm
In PESA procedure, sperms were aspirated from the epididymus under local anesthesia using 21 gauge

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needle. As it is a blind procedure, expertise counts a lot for successful aspiration of sperm from the duct. In 65% cases motile sperms were retrieved (Table 1).

Table 1. Retrieval of motile sperms by PESA and TESE procedures.

<table>
<thead>
<tr>
<th></th>
<th>PESA</th>
<th>TESE</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of cases</td>
<td>119</td>
<td>49</td>
<td>168</td>
</tr>
<tr>
<td>Motile sperms observed</td>
<td>77 (65%)</td>
<td>16 (33%)</td>
<td>93 (55%)</td>
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2.3. Testicular Spermatozoa

Spermatozoa are isolated from testicular biopsy specimen obtained by open surgery under local or general anesthesia from patients with obstructive or non obstructive azoospermia. The biopsy specimen is transferred into a Falcon Petri dish containing HEPES [(4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid] buffered medium. The testicular tissue is macerated with the help of two insulin syringes and the fluid is separated and examined for the presence of sperms. In 32% cases motile sperms were obtained. If the sperms were to be used for ICSI, then the sample was hanged on density gradient medium (pure sperm 90% and 45%). The pellet obtained after centrifugation was washed and the sperms were diluted and used for injecting the oocytes.

2.4. Clinical Procedure

All the female patients were super ovulated using gonadotrophin releasing hormone analogue (GnRH) suppression protocol using Buserelin (Sustenfact® nasal spray, Hoechst Roussel Pharmaceuticals, Paris, France). Oocytes were recovered by trans vaginal ultrasound guided needle aspiration, 36 hours after administration of 1000 i.u. of Human Chorionic Gonadotrophin (HCG).

2.5. Laboratory Procedure

Cumulus-oocyte complexes were maintained in fertilization medium (Cook, Australia), equilibrated in 6% CO₂. In embryology laboratory, cumulus cells were removed before performing ICSI. The final corona cells were sheared off until the cytoplasm was easily observed. The denuded oocyte was then placed in fresh culture medium and allowed to further mature for 2 to 4 hours prior to injection. Oocytes at maturation stage of metaphase II were selected for injection. Fertilization was confirmed after 18 to 20 hours, when two distinct pronuclei were observed under dissecting microscope.

The oocytes were rotated to indentify the total number of pronuclei present. All the pronucleated zygotes were cultured in 10 μl droplets of medium under oil for 24 hours and examined for cleavage. Zygotes were co-inhabited with up to five other zygotes so the maximum co-culture conditions could be achieved resulting from sharing of growth factors. Two to three embryos were transferred to uterine cavity 40 to 72 hours after sperm injection depending on the quality of embryo. Luteal support was given using progestrone pessaries (cyclogest).

3. RESULTS AND DISCUSSION

3.1. Comparison of PESA-ICSI and TESE-ICSI

The object of this study was to compare and investigate the fertilization capacity of sperms obtained by PESA and TESE procedures, and to ascertain capacity of an azoospermic man to become a father.

During a period of twenty months (i.e. October 1997 to July 1999), a total of 168 azoospermic patients with irreparable obstructive azoospermia were selected. The sperms obtained by PESA were used for ICSI which showed the fertilization percentage as 67.5%. The sperms obtained by TESE showed the fertilization to be 61.6%. This indicates a rise of 5.9% in fertilization rate with sperms obtained by PESA (Table 2). These results are in agreement with the results of Silber et al. indicating that the life span in epididymus is not a contributing factor in fertilization. The percentage of pregnancy with PESA-ICSI was obtained as 14.2% whereas with that of TESE-ICSI, it was found to be 24%, indicating a rise of 9.8.

Table 2. Comparison of PESA-ICSI and TESE-ICSI in a similar patient population.

<table>
<thead>
<tr>
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<th>PESA-ICSI</th>
<th>TESE-ICSI</th>
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<tbody>
<tr>
<td>No of cycles</td>
<td>28</td>
<td>25</td>
</tr>
<tr>
<td>No. of matured oocytes injected</td>
<td>252</td>
<td>289</td>
</tr>
<tr>
<td>No. of oocytes fertilized (2pn)</td>
<td>170</td>
<td>178</td>
</tr>
<tr>
<td>Fertilization percentage</td>
<td>67.5</td>
<td>61.6</td>
</tr>
<tr>
<td>No. of embryo transfers</td>
<td>26</td>
<td>24</td>
</tr>
<tr>
<td>No. of pregnancies (ongoing or delivered)</td>
<td>4 (14.2%)</td>
<td>6 (24%)</td>
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The standard PESA procedure was found to be more effective because a large number of sperms could be obtained without the need of surgery. However, in many cases, sperm could not be obtained by this procedure as it is a blind procedure and surgery is necessary to get sperm by TESE.

The patients in each of the two groups did not represent mixture of clinical conditions or technique. For example, if fresh epididymal sperms were used, then no eggs were injected in those patients with testicular sperm and vice versa. It is, therefore, concluded that although complex mechanism involving epididymal transport may be beneficial for conventional fertilization of human oocyte (in vivo or in vitro) none of these mechanisms are required for fertilization after ICSI.

3.2. Implantation rate after PESA-ICSI and TESE-ICSI

A comparison of implantation rates after PESA-ICSI and TESE-ICSI has been presented in Table 3. In a series of 28 PESA-ICSI and 25 TESE-ICSI cases, it was decided to determine whether implantation rate was different for the embryos derived from ICSI using epididymal (PESA) and testicular (TESE) sperms. There was failure of fertilization in 2 (7.1%) of the PESA cycles and 1 (4%) in TESE cycles. The ongoing implantation rate per embryo was higher in TESE-ICSI cycle (24%) than in PESA-ICSI (14.2%). These results are in agreement with the results of Silber et al.\(^5\)

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Ongoing implantation rates per embryo after ICSI with epididymal and testicular sperms.</th>
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<tbody>
<tr>
<td>No. of patient cycles</td>
<td>28</td>
</tr>
<tr>
<td>No. of embryo transfers</td>
<td>26</td>
</tr>
<tr>
<td>No. of embryos transferred</td>
<td>63</td>
</tr>
<tr>
<td>No. of fetal heart</td>
<td>4</td>
</tr>
<tr>
<td>Ongoing implantation rate</td>
<td>14.2%</td>
</tr>
</tbody>
</table>

4. CONCLUSION

The patients suffering from azoospermia showed excellent fertilization and pregnancy rates by ICSI when their sperms were retrieved by PESA and TESE. This study documents the solution to the problems of obstructive azoospermia. If micro surgery is impractical or unsuccessful, PESA-ICSI and TESE-ICSI could provide the most successful treatment. Thus it is concluded that an azoospermic man can father a child.

ACKNOWLEDGMENT

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REFERENCES