EPIDIDYMAL AND TESTICULAR SPERM FOR INTRACYTOPLASMIC SPERM INJECTION IN THE TREATMENT OF AZOOSPERMIA

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Abstract: The technique of assisted reproduction have recently become the most effective methods of treatment of infertility: TESE (testicular sperm extraction) and MESA (microsurgical epididymal sperm aspiration). It has been increasingly successful, even above the average efficiency of classical IVF (in vitro fertilization). This study investigated that the sperm retrieval success rates, fertilization rates, and pregnancy rates of MESA and TESE in cases of azoospermia. Forty-six infertile couples with obstructive and non- obstructive azoospermia were included in this study. Twenty-three cycles each of MESA-ICSI and TESE-ICSI cycles were performed. A difference in the normal two-pronuclear (PN) fertilization rate was found between the two groups: 60% for epididymal spermatozoa and 33% for testicular biopsy spermatozoa. The cleavage rates were almost the same for epididymal 83% and testicular spermatozoa 90%. The ongoing pregnancy rate in this series were 35% and 19% respectively. Clinical data have confirmed that fertilization rate and pregnancy rate are not lower than conventional IVF. We conclude that epididymal spermatozoa and testicular spermatozoa gain different fertilization and ongoing pregnancy rates using ICSI. This technique could provide a useful alternative for the management of infertile men with azoospermia when compared with conventional IVF.

Key words: Azoospermia, TESE, MESA

INTRODUCTION

The use of testicular spermatozoa in the treatment of male infertility is a recent concept1~4). MESA (microsurgical epididymal sperm aspiration) and TESE (testicular sperm extraction) are the fundamental procedures in the case of non-reconstructable obstructive azoospermia or testicular azoospermia.

Silber et al demonstrated that high fertilization and pregnancy rates have been obtained after intracytoplasmic sperm injection (ICSI) with spermatozoa obtained from testicular biopsies in obstructive azoospermic males5. In non-obstructive azoospermia, the testicles are the source of sperm cells, and the only possible treatment is a combination of TESE and ICSI6~7). MESA comparing ICSI to conventional IVF has demonstrated completely normal fertilization and pregnancy rates with epididymal spermatozoa utilizing ICSI8). However, in the cases of non-obstructive azoospermic factors, MESA has been shown poor results1,8).

Recently, a number of clinical trials with conflicting results have been reported in which TESE and MESA are performed as a possible way to achieve pregnancy1,9). The technique of assisted reproduction has become the most effective method to treat infertility. The technique has been increasingly successful, even above the average efficiency of classical IVF1,8).
ICSI has offered high rates of fertilization and pregnancy even in the extreme cases of oligoasthenozoospermia or cases of azoospermia treated by MESA or TESE techniques. The purpose of the present study was to compare ICSI using testicular biopsy spermatozoa with ICSI using epididymal spermatozoa in the same time period and to evaluate our results of MESA and TESE.

**MATERIALS AND METHODS**

All patients were referred to the Department of Obstetrics and Gynecology Gunma University School of Medicine, as outpatients for evaluation and treatment by assisted reproductive technologies between January 1994 and December 2000. In this study 56 couples with azoospermia were treated in a total of 288 stimulated IVF cycles. Ages of women ranged from 23 and 36 (mean 30) years. Their partners, 23-45 (mean 34) years of age were treated according to the routine IVF procedures. The patients have given their consent to participate this study.

After down regulation and suppression of pituitary gland using a gonadotropin releasing hormone analogue (GnRHa), human menopausal gonadotropin (HMG Pergogreen; Serono Japan, Tokyo, Japan) 150–300IU/day i.m was administered.

A transvaginal ultrasound showed two follicles diameter excess 16mm mean, human chorionic gonadotropin (HCG Pregnyl; Organon Japan, Tokyo, Japan) 10000IU s.c. was administered. A transvaginal ultrasound guided oocyte retrieval was performed 34 h after HCG administration. Fertilization was obtained by ICSI and 2-4 embryos were transferred on day 2 after ovum pick up. Sperms were retrieved by MESA in 26 cycles and by TESE in 36 cycles.

**Oocyte preparation**

After incubating in Modified HTF medium (Santz Ana, California), containing 60IU/ml hyaluronidase for 30s, the cumulus cells were removed and the oocytes pick out. The oocytes were assessed under an inverted microscope at ×200 magnification for nuclear maturity and cytoplasmic evaluation. Oocytes were incubated in Menezos B2 medium at 37°C in the atmosphere of 5% CO₂, 5% O₂ and N₂ with air covered by paraffin oil. Only metaphase II oocytes were used for microinjection.

**Sperm sampling and preparation**

Testicular tissue samples were obtained by open biopsy on the same day that the oocytes were retrieved. The tissue samples were taken under spinal anesthesia through a 2-3cm scrotal incision and testicular sperm extraction was performed. The testicular tissues were placed in a falcon tube (FALCON 2057, USA) containing Modified HTF medium (Santz Ana, California). The testicular tissue which was crushed by slide glass and progressively divided into small segments. A suspension of spermatozoa was then transferred into a Falcon tube and centrifuged at 2000/h 20min into the 40 and 80% percoll for isolation of spermatozoa. Isolation of a single spermatozoon was considered for ICIS. The testicular tissue solution was kept in an incubator (5% CO₂ and 5% O₂ in air) at 37°C until the ICSI procedure.

**Assessment of fertilization-embryo cleavage and establishment of pregnancy.**

The oocytes were observed for the presence of pronuclei 16-18h after ICSI. Fertilization was assessed as normal when two clearly distinct pronuclei containing nucleoli were present. The state of embryo cleavage and quality were assessed after an additional 24h of in vitro culture. The embryos were evaluated according to the blastomere size equality and the relative proportion of anucleate fragments. A maximum of 3 embryos were transferred in exceptional cases where all the available embryos had ≥50% fragmentation. Pregnancy was confirmed by detecting increasing serum HCG concentrations 14days after embryo transfer. Clinical pregnancy was diagnosed by ultrasonography at 7 weeks of pregnancy. Statistical analysis was performed by Fisher’s exact test. The statistical significance was defined as P<0.05.

**RESULTS**

Table 1 shows results of in vitro fertilization performed between January, 1994 and December, 2000. The total number of in vitro fertilization increased during six years and exceeded 230 cases per year in 2000. The pregnancy rate by in vitro fertilization increased during six years and exceeded 230 cases per year in 2000. The pregnancy rate by in vitro fertilization had been hovering at about 20%, but it rose to almost 30% or so for the first time last year. Table 2 represents the result of

| Table 1 The outcome of IVF-ET between January 1994 and December 2000. |
|---|---|---|---|
| Years | No. of cycles | No. of transfers (% of cycles) | No. of pregnancies/Transfer : positive HCG (% of transfers) |
| 1994 | 173 | 134 (77.4) | 30 (22.3) |
| 1995 | 180 | 142 (77.8) | 17 (12.0) |
| 1996 | 189 | 132 (69.8) | 28 (21.2) |
| 1997 | 205 | 168 (82.0) | 32 (19.0) |
| 1998 | 217 | 167 (77.0) | 38 (22.7) |
| 1999 | 224 | 184 (82.1) | 37 (20.1) |
| 2000 | 231 | 188 (81.4) | 55 (29.2) |

HCG = human chorionic gonadotrophin
The treatment of azoospermia by ICSI

The cases treated by the conventional technique and ICSI. In our hospital, ICSI rate was almost 40% of IVF cycles, and this rate remains almost unchanged. A total of 23 MESA and ICSI cycles were performed, 343 oocytes were obtained and were injected (Table 3). The number of injected metaphase II oocytes with two pronuclei (2PN) was 162 (60%). Cleavage rates were 83% and 5 pregnancies were achieved in total (35%) and one pregnancy resulted in clinical abortion (Table 4). One set of twins and 3 singletone pregnancies were achieved. A total of 23 TESE and ICSI cycles were performed, 273 oocytes were obtained and were injected. The number of injected metaphase II oocytes with two pronuclei (2PN) was 92 (33%). Cleavage rates were 90% and 3 pregnancies were achieved in total (19%). One set of twins and 2 singletone pregnancies were achieved.

**DISCUSSION**

Approaches at medical therapy include stimulation of spermatogenesis at testicular levels, improvement of epididymal function (sperm maturation), influence on sperm transport and activation of sperm metabolism with improvement of sperm motility10,11). But, the possibilities of treating male infertility are still limited. In cases of severe male sterility factor, ICSI has been a breakthrough in the therapy of childless because the collection of spermatozoa from MESA and TESE12). With the development of ART in recent years, infertile patients, particularly patients for whom in vitro fertilization is indicated have increased in number13). In our hospital too, this tendency has become increasing in recent years since in vitro fertilization-embryo transfer was performed in 1984. In 1997, the number of such patients exceeded 200 cases per year for the first time, and even now it keeps on increasing (Table 1). The weight on diagnosis and treatment of male infertility has increased too. We have started to perform MESA and TESE for male patients with severe infertility from 1996; they have begun showing the pregnancy rate of 20–30% compared with the pregnancy rate by in vitro fertilization in our hospital (Table 1) from 1998 (Table 2). Patients with azoospermia are roughly divided into those with spermatogenic dysfunction and those with obstructed passage of sperm (obstructive azoospermia). Effective therapy for spermatogenic dysfunction due to there being Sertoli cell only has yet to be established, while in obstructive azoospermia surgery is expected to be effective and collection of motile spermatozoa can also be expected. With improvement in surgery for reopening the obstructed passage of sperm and progress of microsurgical fertilization in the domain of

<table>
<thead>
<tr>
<th>Source of spermatozoa</th>
<th>No. of cycles</th>
<th>No. of eggs at M II injected</th>
<th>No. of 2PN oocytes (% of M II eggs)</th>
<th>No. of cleaved embryos (% of 2PN oocytes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epididymal (MESA)</td>
<td>23</td>
<td>343</td>
<td>206 (60)*</td>
<td>170 (83)</td>
</tr>
<tr>
<td>Testicular biopsy (TESE)</td>
<td>23</td>
<td>273</td>
<td>92 (33)</td>
<td>83 (90)</td>
</tr>
</tbody>
</table>

*P<0.05.  PN=pronuclear  M II=metaphase2

<table>
<thead>
<tr>
<th>Source of spermatozoa</th>
<th>No. of cycles</th>
<th>No. of transfers</th>
<th>No. of pregnancies/Transfer: positive HCG (% of transfers)</th>
<th>No. of pregnancies Clinical Abortion Ongoing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epididymal (MESA)</td>
<td>23</td>
<td>17</td>
<td>6 (35)</td>
<td>4 1 1</td>
</tr>
<tr>
<td>Testicular biopsy (TESE)</td>
<td>23</td>
<td>16</td>
<td>3 (19)</td>
<td>1 0 2</td>
</tr>
</tbody>
</table>
obstetrics-gynecology such as MESA, many cases of pregnancy have been reported\textsuperscript{14,18}. In our present study too, the fertilization rate for metaphase 2 ovum was 66\%, the embryo cleavage rate was 88\%, and the pregnancy rate was 35\%, indicating the effectiveness of these techniques (Table 3). Zumbo et al have shown that MESA in combination with ICSI was performed in same cases, the embryo cleavage rate was 80\%, and the pregnancy rate was 26\%\textsuperscript{9}. Good results were obtained with ICSI using MESA when compared with other reports. In the cases of azoosper-

mia in which sperms cannot be collected from the deferent duct or epididymis, however, spermatozoa have to be collected directly from testicular tissues. If spermatozoa can be collected by centrifuging sliced testicular tissues and examining them under a microscope, it is possible to obtain a live baby by TESE-ICSI. Our results have shown that the rates of fertilization and pregnancy rates were 33\% and 19\% in the TESE-ICSI (Table 3, 4). The fertilization and pregnancy rates after TESE-ICSI are significantly lower than MESA-ICSI. One hypothesis is that abnormal spermatogenesis and complete fertilization failure may occur more frequently\textsuperscript{16,19}. Also, it has been reported that the incidence of abnormal fertilized ova is high in TESE-ICSI compared with ICSI using ejected spermatozoa\textsuperscript{16,17}. In addition, satisfactory results cannot be obtained with TESE in which it is difficult to collect motile spermatozoa compared with MESA as shown in Figs 3 and 4. In recent years, it has been reported that the defect of the gene (AZF ; azoospermia factor) said to be concerned with the spermatogenic function including the interval 6 area in chromosome leads to the development of various disturbances such as azoospermia and severe oligospermia, which can possibly be inherited over generations\textsuperscript{18-21}. There are many problems including studies of the presence or absence of a gene associated with the spermatogenic function. Many reports have demonstrated that microsurgical fertilization is effective for severe male infertility in which fertilized sperm cannot be obtained by in vitro fertilization\textsuperscript{22}. Further studies are needed to raise the pregnancy rate. In practice, it is also necessary to give counseling to husband and wife and to obtain their informed consent.

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