Comparison of the Fertilization Rate after Intracytoplasmic Sperm Injection (ICSI) Using Ejaculated Sperms, Epididymal Sperms and Testicular Sperms

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Abstract

Intracytoplasmic sperm injection (ICSI) has been successfully used to achieve fertilization and pregnancies for patients with extreme oligoastheno-zoospermia using ejaculated sperms or patients with azoospermia using epididymal or testicular sperms. The aim of this study was to compare the fertilization rate after ICSI using ejaculated, epididymal and testicular sperms. Between January and September 1997, 10 azoospermic men underwent percutaneous epididymal sperm aspiration (PESA) or testicular sperm extraction (TESE) to recover sperm for ICSI. A total of 5 PESA cases and 5 TESE cases were performed at the Center for Assisted Reproduction & Embryology. Thirty-one patients performed ICSI using ejaculated sperms during the same period of time were used as a control group. ICSI using ejaculated sperms, epididymal sperms from PESA and testicular sperms from TESE was a highly successful technique, achieving fertilization rates of 78.5 per cent, 83.3 per cent and 80.8 per cent, respectively. Good fertilization rates were achieved without significant differences among the various sperm sources.

Previously, patients with obstructive azoospermia due to congenital absence of the vas deferens or those who failed reconstructive surgery were considered hopelessly infertile. The fertilization and pregnancy rates after epididymal sperm retrieval and *in vitro* fertilization (IVF) were low and epididymal sperm appeared to have impaired fertilizing ability with conventional IVF(1). The introduction of intracytoplasmic sperm injection (ICSI) to clinical practice has revolutionized the treatment of male factor infertility in recent years (2). This procedure uses only a few sperms from the ejaculate in order to achieve high fertilization and pregnancy rates along with the concept of

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using epididymal sperms[3] or testicular sperms[4] to achieve fertilization and pregnancies has revolutionized the potential to treat patients with azoospermia. ICSI could achieve better results than conventional IVF with epididymal or testicular sperms[5]. In a previous study, the normal fertilization rate was significantly higher with ejaculated sperms than with epididymal or testicular sperms [6]. However, other studies demonstrated that the fertilization rate *in vitro* using ejaculated, epididymal and testicular sperms were similar[7,8]. The aim of this study was to compare the fertilization rates after ICSI using ejaculated sperms, epididymal sperms and testicular sperms.

MATERIAL AND METHOD

Study population

Between January and September 1997, 10 azoospermic men underwent percutaneous sperm aspiration (PESA) or testicular sperm extraction (TESE) to recover sperm for ICSI. A total of 5 cycles using epididymal sperms and 5 cycles using testicular sperms were performed at the Center for Assisted Reproduction & Embryology. At the initial consultation, both partners were assessed fully and counselled concerning the procedures involved.

PESA was performed due to obstructive azoospermia. One patient had bilateral congenital absence of the vas deferens due to cystic fibrosis. One patient had acquired obstructive azoospermia. Four patients had a previous vasectomy operation for sterilization and failed reconstructive surgery but PESA was impossible in one patient because of totally destroyed epididymis.

TESE was performed due to non-obstructive azoospermia and obstructive azoospermia. One patient had obstructive azoospermia which failed PESA. The remaining four cases were non-obstructive azoospermic patients with severe spermatogenic defect where the testicles were the only source of sperm cells. The control group consisted of a cohort of 31 patients using ejaculated sperms for ICSI treatment at the same period of time. The indications for ICSI were severe male infertility and poor fertilization rate in previous IVF cycles. The mean age of the female partners in each group using ejaculated sperms, epididymal sperms and testicular sperms were 36.4 $\pm$ 0.7, 37.0 $\pm$ 3.3 and 34.2 $\pm$ 3.6 years, respectively and the mean age of the male partners in each group were 40.2 $\pm$ 1.0, 47.6 $\pm$ 11.6, 38.2 $\pm$ 3.1 years, respectively.

Ovarian stimulation

Ovarian stimulation was carried out by two protocols (down-regulation and flare-up) using the GnRH agonist (GnRH-a), buserelin acetate in combination with hMG and FSH. In the down-regulation protocol, buserelin acetate (Superfact nasal spray; Hoechst AG, Frankfurt, Germany) was administered 600 µg/d, divided into six daily doses, beginning from midluteal phase until pituitary desensitization was achieved (E2 <50 pg/mL) before the administration of FSH (Metrodin; Serono Laboratories, Aubourne, Switzerland) and/or hMG (Pergonal; Serono Laboratories or Humegon; Organon Laboratories, Netherlands). The dosage of buserelin acetate was decreased to 400 µg/d, divided into four daily doses on the first day of menses and continued until the day of hCG administration. In the flare-up protocol, 200 µg of buserelin acetate (Suprefact injectables; Hoechst AG) was daily injected subcutaneously on cycle day 2 until the day of hCG administration, followed by FSH and/or hMG from day 3. Initial dosage was individualized based on day 3 FSH, or response to prior stimulations. Changes in gonadotrophin dosage were based on follicular development as reflected by changes in follicular diameter and number assessed by serial transvaginal ultrasonography. Five thousand international units of hCG (Profasi; Serono Laboratories) were administered intramuscularly when leading follicles were $\geq$ 20 mm. Transvaginal follicular aspiration was performed 36 hours later.

Ejaculated sperm processing

One hour before the scheduled time of oocyte retrieval, a semen sample was collected by masturbation into sterile containers. After liquefaction, the semen sample was mixed with a sterile pipette. Sperm was then prepared by two-layer (40% and 80%) discontinuous Percoll (Pharmacia, Uppsala, Sweden) gradient centrifugation for 20 minutes at 300 x g. The pellets were removed carefully, washed twice by centrifugation (5 minutes at 600 x g), and resuspended at a concentration of $1x10^6$/mL in IVF medium (cat. no. 10310060; Medicult, Copenhagen, Denmark) and incubated until the time of injection.

Percutaneous sperm aspiration (PESA)

PESA took place in the operating room using intravenous sedation anesthesia with Pro-

pofol and local anesthesia with Bupivacaine injected at the skin and underneath the epididymal caput. The epididymal caput was identified and held firmly between thumb and index finger. A small needle (26 gauge) was connected to a 1 ml disposable syringe. The proximal part of the epididymal caput was punctured. Suction was applied to the syringe and the needle was withdrawn gradually to the point where segments of fluid from the epididymis were seen entering the syringe. The aspirate was then flushed out of the needle and syringe into a sterile Falcon petri dish (Becton Dickinson Ltd, Plymouth, UK) using HEPES-buffered Ham's F10 supplemented with 10 per cent patient serum. This procedure can be performed as many times as necessary until sufficient sperms are recovered. Testicular sperm retrieval was prepared as a back up procedure in case percutaneous sperm aspiration failed.

Epididymal sperm processing

The epididymal sample was washed with HEPES and centrifuged for 7 minutes at 400 x g. The pellet was resuspended in 0.2 ml media and incubated until the time of injection.

Testicular sperm extraction (TESE)

Open excision testicular biopsies were taken under general and local anesthesia as described above. The testicular tissue was placed in a Petri dish containing HEPES-buffered Ham's F 10 medium supplemented with 10 per cent patient serum and then was teased apart with two needles. Under an inverted microscope (x 400 magnification) the minced tissue was then checked for the presence of sperms. If no sperms were observed, another biopsy specimen was taken. Surgery was stopped when sperms were found. All couples were offered matched donor sperm 'back-up' in case sperm retrieval was not successful.

Testicular biopsy processing

The morselized tissues were incubated in a Petri dish containing HEPES-buffered Ham's F 10 medium supplemented with 10 per cent patient serum for approximately 2 hours. The contents then were mixed and allowed to settle for 1 minute and the deposited pieces of testicular tissue were removed. Two or three microdroplets were used from this suspension to be put near the PVP microdroplet in the injection dish. Using the injection micropipette, a search was done for a motile sperm, which was aspirated from among Sertoli cells, red blood cells, and debris and transferred to the polyvinylpyrrolidone (PVP) droplet and used for microinjection later.

Oocyte preparation and handling

The cumulus-oocyte complexes were collected and washed in HEPES-buffered Ham's F-10 medium and incubated at 37°C in 5 per cent CO_2 in air for 2 hours before removal of the cumulus and corona radiata. Cells of the cumulus and corona radiata were removed by incubation for approximately 30 seconds in HEPES-buffered Ham's F-10 medium supplemented with 10 per cent patient serum containing 80IU hyaluronidase/mL (type VIII; Sigma Chemical Co., St Louis, MO). The oocytes then were transferred to HEPES-buffered Ham's F-10 medium supplemented with 10 per cent patient serum for complete removal of the corona cells by repeated aspiration in a finely pulled pipette. The oocytes then were rinsed and incubated in HEPES-buffered Ham's F10 supplemented with 10 per cent patient serum under Liquid paraffin (Medicult, Copenhagen, Denmark) until the time of injection, which was done only for the mature oocytes that extruded their first polar bodies. Oocytes were assessed for nuclear maturity by examination for the first polar body and the presence or absence of a germinal vesicle, and any cytoplasmic abnormalities were also examined.

Intracytoplasmic sperm injection procedure

A 4 μL-microdroplet of 10 per cent polyvinylpyrolidone (PVP) (cat.no.10890001; Medicult) was put in the middle of a plastic dish (Falcon; Becton Dickinson, NJ) and approximately 1 μL of the prepared sperm suspension was added. Eight 4 μL-microdroplets of HEPES buffered Ham's F-10 were placed around the middle microdroplet under oil. The oocytes were put individually in each droplet. The microinjection procedure was done using an inverted-phase microscope (Nikon diaphot 300; Nikon, Tokyo, Japan) with an incubator and equipped with Hoffman modulation contrast system (Modulation Optics Inc. Greenvale, NY) and micromanipulation set (Narishige Inc., Tokyo, Japan). The procedure was allowed to be followed on a monitor (model no.KV-14 VM2MT, Sony, Tokyo, Japan) connected to a video camera (Panasonic model no. WVCL 120E; Matsushita Communica-

tion Industrial Co., Tokyo, Japan) that was attached to the microscope. The injecting micropipettes (Humagen Fertility Diagnostics, Charlottesville, VA, U.S.A.) and the holding pipettes (cat. no. K-HPIP-1030; Cook, Eight Mile Plains, Queensland, Australia) were ready made. The microinjection pipette was lowered in the PVP microdroplet and one sperm was chosen (the best available motile and morphologically well-formed sperm) and was immobilized by touching its tail near the midpiece with the injecting micropipette. The immobilized sperm was aspirated, tail first, into the injecting pipette. The Petri dish was then moved in order to visualize an oocyte in one of the droplets surrounding the sperm suspension. The oocyte was immobilized by slight negative pressure exerted on the holding pipette. After securing the oocyte in position (polar body at 6 or 12 o'clock position), the injecting pipette was introduced at 3 o'clock position through the zona pellucida, oolemma, deeply into the cytoplasm and then oocyte cytoplasm was aspirated. The sperm then was injected slowly and the pipette was withdrawn. After injection, the oocytes were rinsed and incubated under oil in IVF medium. To provide a reliable assessment of the effect of sperm morphology on fertilization rate in vitro, only cycles that had three or more MII oocytes collected were included in this study. After completing the microinjection, the rest of the sperm was cryopreserved in all cases for possible future use.

Assessment of fertilization

About 16-18 h after the microinjection, the oocytes were observed under the inverted microscope (x 200 or x 400 magnification) for any sign of damage which may have been due to the microinjection and for the presence of pronuclei and polar bodies. Fertilization was considered normal when two clearly distinct pronuclei containing nucleoli were present.

Statistical analysis

Data are generally presented as mean ± SEM. Comparison of the fertilization rate after intracytoplasmic sperm injection (ICSI) using ejaculated sperms, epididymal sperms and testicular sperms were made using Chi-square test, respectively. All statistical analyses were performed using the SPSS for Windows v 6.0 (SPSS Inc., Chicago, IL) on an IBM compatible microcomputer. $P<0.05$ was considered to be significant.

RESULT

There have been no complications, i.e. infection or hematoma in the patients undergoing PESA or TESE. Adequate sperms for ICSI were retrieved from all groups of patients.

Table 1 summarizes the results of ICSI using ejaculated sperms, sperms from PESA and sperms from TESE, respectively. The fertilization rate after ICSI using ejaculated sperms, epididymal sperms and testicular sperms were 78.5 per cent, 83.3 per cent and 80.8 per cent, respectively. There was no significant difference in the fertilization rate *in vitro* between these three groups ($p>0.05$). There are 3 ongoing pregnancies, 1 from PESA and 2 from TESE. Therefore, the pregnancy per patient using sperm from PESA and sperms from TESE were 20 per cent and 40 per cent, respectively.

DISCUSSION

In cases of severe male infertility, intracytoplasmic sperm injection (ICSI) has been a breakthrough in the therapy of childlessness. A further progress is the collection of sperms from the epididymis (PESA=Percutaneous sperm aspiration) or testis (TESE=Testicular sperm extraction). In previous studies, surgically retrieved

Table 1. Comparison of overall fertilization rate of ICSI cycles using ejaculated sperms (n=31), epididymal sperms (n=5) and testicular sperms (n=5).

Outcome	Ejaculated sperm	Epididymal sperm	Testicular sperm
Numbers of cycles (oocyte retrievals)	31	5	5
Number of oocytes	366	44	83
Number of injected oocytes (MII oocytes)	312	42	73
Number of fertilized oocytes (2PN)	245	35	59
In vitro fertilization rate (%)	(78.5%)	(83.3%)	(80.8%)

sperms were used for conventional IVF[9-11]. However, the mean fertilization rate was low due to sperm parameters which were suboptimal for conventional IVF in terms of sperm density and motility. With micromanipulation, the *in vitro* fertilization rate after ICSI using epididymal sperms and testicular sperms increases dramatically[12,13].

Maturational changes occur during the transport of the sperm in the epididymis. Sperms are immotile or ineffectively motile at the time they enter the caput. In ICSI treatment, it is no longer valid that sperm need to pass through the full length of the male genital tract. Using this technique, the fertilizing capacity of the sperm aspirated from the caput or sperm retrieved from the testis is favourable. Therefore, it is mandatory that ICSI should be used with the retrieved sperm to maximize the prospect of fertilization, as some of the sperm samples may have extremely poor parameters. This present study demonstrated that ICSI, using ejaculated sperms, epididymal sperms from PESA and testicular sperms from TESE, is a highly successful technique, achieving fertilization rates of 78.5 per cent, 83.3 per cent and 80.8 per cent, respectively. Although complex mechanisms involving epididymal transport may be beneficial for conventional fertilization of human oocytes (*in vivo* or *in vitro*), none of these mechanisms are required for fertilization after ICSI. In conclusion, there were no differences in fertilizing ability among ejaculated sperms, sperms retrieved from PESA and sperms retrieved from TESE in conjunction with ICSI treatment. Percutaneous sperm aspiration and testicular sperm extraction can be considered standard procedures to treat male factor infertility.

Percutaneous epididymal sperm aspiration (PESA) can be used successfully to retrieve sperms in men with azoospermia due to obstructive disorders. The technique is simple, cost-effective and associated with no complications observed. In this study, the fertilization rate from epididymal sperms was also higher than the results from previous studies which ranged from 32.7-67 per cent[12-17]. In some cases, no epididymal sperms were available and so testicular sperm extraction (TESE) was used for sperm retrieval. Because of the consistent results obtained using epididymal sperms or testicular sperms, it appears that all cases of obstructive azoospermia can now be successfully treated.

High fertilization rates can be achieved using testicular sperms in combination with ICSI in both obstructive and non-obstructive azoospermic groups. In this study, the fertilization rate from testicular sperms was higher than the results from previous studies which ranged from 38.6-57 per cent[13,14,18-21]. Using testicular sperms in combination with ICSI in both obstructive and non-obstructive azoospermic groups, high fertilization rates can be achieved. Testicular sperm extraction of sperm for intracytoplasmic sperm injection is a simple, inexpensive method of sperm retrieval in cases of azoospermia resulting from genital tract obstruction or severe spermatogenic disorder.

In conclusion, recovery of epididymal or testicular sperms from azoospermic patients for ICSI is a recent advance in the treatment of male infertility. Percutaneous sperm aspiration (PESA) and testicular sperm extraction (TESE) yields successful results. Good fertilization rates are achieved without significant differences among the various sperm sources.

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การศึกษาเปรียบเทียบอัตราการปฏิสนธิภายหลังการฉีดอสุจิเข้าไปในไข่ โดยการใช้อสุจิจากน้ำอสุจิ อสุจิที่ได้จากท่อนำอสุจิ (Epididymis) และอสุจิที่ได้จากลูกอัณฑะ

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การฉีดอสุจิเข้าไปในไข่ (Intracytoplasmic sperm injection :ICSI) มีความสำเร็จสูงโดยทำให้มีการปฏิสนธิและการตั้งครรภ์ ในการรักษาผู้ป่วยที่มีจำนวนอสุจิน้อยและมีการเคลื่อนไหวน้อย หรือในรายที่ตรวจไม่พบอสุจิในน้ำอสุจิ แต่ใช้อสุจิที่ได้จากท่อนำอสุจิ (Epididymis) และลูกอัณฑะ จุดประสงค์ของการศึกษานี้ต้องการเปรียบเทียบอัตราการปฏิสนธิภายหลังการฉีดอสุจิเข้าไปในไข่ โดยการใช้อสุจิธรรมดา อสุจิที่ได้จากท่อนำอสุจิ (Epididymis) และอสุจิที่ได้จากลูกอัณฑะ ในระหว่างเดือนมกราคมถึงกันยายน 2540 มีผู้ป่วยจำนวน 10 รายที่ตรวจไม่พบอสุจิในน้ำอสุจิมาเข้ารับการดูดอสุจิจากท่อนำอสุจิ (Epididymis) ผ่านทางผิวหนัง (Percutaneous epididymal sperm aspiration : PESA) หรือ การผ่าตัดชิ้นเนื้อลูกอัณฑะเพื่อแยกอสุจิ (Testicular sperm extraction : TESE) เพื่อนำอสุจิมาทำ ICSI รวมทั้งสิ้นทำ PESA ทั้งหมด 5 ราย และทำ TESE ทั้งหมด 5 ราย ที่ศูนย์การแพทย์นวบุตร โดยใช้ผู้ป่วยที่มารักษาด้วยการทำ ICSI โดยใช้อสุจิที่ได้จากน้ำอสุจิในช่วงเวลาเดียวกันจำนวน 31 รายเป็นกลุ่มควบคุม พบว่าอัตราการปฏิสนธิจากการทำ ICSI โดยใช้อสุจิที่ได้จากน้ำอสุจิ อสุจิที่ได้จากท่อนำอสุจิ (Epididymis) และอสุจิที่ได้จากลูกอัณฑะมีอัตราการปฏิสนธิเป็น 78.5%, 83.3% และ 80.8% ตามลำดับ จะเห็นได้ว่าไม่มีความแตกต่างกันอย่างมีนัยสำคัญของอัตราการปฏิสนธิไม่ว่าจะใช้ตัวอสุจิจากแหล่งใด

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