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Pregnancies from obstructive azoospermia patients after intracytoplasmic sperm injection (ICSI) with testicular spermatozoa

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SUMMARY

The success rate of ICSI in terms of fertilization rate and resulting pregnancies using testicular sperm was evaluated. Testicular biopsy was performed on the husbands and the tissue was dissected followed by washing twice in modified human tubal fluid medium. Ovarian stimulation was done by injection of hMG after down-regulation using GnRH analogue. The ICSI procedure was carried out with fresh testicular spermatozoa following by cultured in vitro of the zygote. The average age of the wives treated was 34 years old. The number of oocytes retrieved was 140 oocytes of which 115 (82%) oocytes in metaphase II stage. Fertilization rate of ICSI with testicular sperm was 61% (70/115) with four zygotes showing three pronuclei. One to three of the best developed embryos were transferred to the mothers on day two or three and resulted on 9 out off 17 (53%) patients were pregnant. ICSI of testicular sperm is a useful treatment for patient with obstructive azoospermia in whom standard microsurgical procedures are non feasible.

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INTRODUCTION

In couples suffering from extreme oligoasthenoteratozoospermia, intracytoplasmic sperm injection (ICSI) has been established as a treatment that resulted in high fertilization (Palermo et al., Lancet, 1992, 340:17-18). In cases of congenital absence of the vas deferens (CAVD), or irreparable obstructive azoospermia, high fertilization and pregnancy rate have been obtained after microsurgical epididymal sperm aspiration (MESA) or testicular sperm extraction (TESE) followed by ICSI (Silber et al., 1995). The aim of this study was to evaluate a clinical potential of ICSI with testicular sperm in whom the male partners had the obstructive azoospermia.

MATERIALS AND METHODS

Ovarian stimulation and Oocytes Collection:
Ovarian stimulation was achieved by treatment of gonadotropin-releasing hormone (GnRH) analogue buserelin acetat (Spreeur: Hoechst Marion Roussel, Tokyo, Japan), pure follicle-stimulating hormone (FSH, Fertinorm P; Serono, Tokyo, Japan) and the human menopausal gonadotropin (hMG, Humegon; Organon, Holland).. Oocytes were collected by vaginal ultrasound guided 35 to 36 hours after human chorionic gonadotropin (hCG. Profasi; Serono, Tokyo, Japan) was administered. The cumulus-corona cells initially were removed by exposure to flushing medium (modified HTF; Irvine Scientific, Santa Ana, California, USA) and 80 IU of hyaluronidase (Hyaluronidase type VIII, Sigma, St. Louis, MO).

Testicular biopsy:
The pieces of testicular tissue were finely minced with sterile glass microscope slides in modified human tubal fluid, following by washing two times by centrifugation 600G for 10 min. The presence of motile free spermatozoa was then assessed on the inverted microscope at x200 or x400 magnification.

Intracytoplasmic Sperm Injection (ICSI) procedure:
ICSI was carried out on intact oocytes that developed to metaphase II stage which was identified by the presence of a single polar body. Oocytes were injected with single, living, and immobilized spermatozaon. The oocyte was held securely on the holding pipette in such away that the polar body was situated at the 6 o'clock or at the 12 o'clock position while the injection pipette was pushed through the zona pellucida at the 3 o'clock position into the cytoplasm, where the sperm was delivered. The injected oocytes were then washed in HTF medium and transferred into 60 ul droplets of HTF medium supplemented with 10% PPF covered by lightweight paraffin oil. The Petri dishes with the oocytes were incubated at 37°C and 5% CO_2, 90% N_2 and 5% O_2 (Astec, Fukuoka, Japan) for further culture.

RESULTS AND CONCLUSIONS

The fertilization, cleavage and pregnancy rate after ICSI with testicular spermatozoa are summarized in Table 1 and Table 2. The results of ICSI with spermatozoa obtained from testicular biopsy were remarkably similar in patients with ejaculated spermatozoa. The completely immature spermatozoa, which had only recently been released from the Sertoli cells, could fertilize and result in a normal pregnancy if freed from the need to penetrate the zona pellucida and to fuse with the oolemma

Table 1. Fertilization and cleavage rate after intracytoplasmic sperm injection with testicular spermatozoa.

Age	No. of cycles	Oocytes retrieved	MT-II (%)	0 PN (%)	1 PN (%)	2 PN (%)	>2 PN (%)	Cleavage (%)
≤ 35 y.o.	9	88	73(83)	14(19)	10(14)	46(63)	1(1)	44(96)
> 35 y.o.	8	49	42(86)	11(26)	3(7)	24(57)	3(7)	24(100)
Total	17	140	115(82)	25(22)	13(11)	70(61)	4(3)	68(97)

Table 2. Pregnancy rate after intracytoplasmic sperm injection with testicular spermatozoa.

Age	No. of cycles	No. of ET	No. of embryo replaced	No. of pregnancy (%) Chemical	Clinical
≤ 35 y.o.	9	9	27	5(56)	3(33)
> 35 y.o.	8	8	20	4(50)	4(50)
Total	17	17	47	9(53)	7(41)

membrane. ICSI of testicular sperm is a useful treatment for patient with obstructive azoospermia in whom standard micro surgical procedures are non feasible.

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