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SHORT COMMUNICATION

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Pregnancies and births resulting from in vitro matured oocytes fertilized with testicular spermatozoa

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***Purpose*:** In vitro maturation (IVM) of immature human oocytes is an attractive option for the treatment of infertility. Similarly, intracytoplasmic sperm injection (ICSI) followed by testicular fine needle aspiration (TEFNA) is an important treatment for primarily male-factor infertility. This report highlights the combination of these two advanced assisted reproduction techniques, namely IVM and fertilization with TEFNA-retrieved spermatozoa by ICSI to overcome both of male and female infertility problems.
***Methods*:** Before immature oocyte retrieval (IOR), gonadotropin stimulation was given for 3 or 5 days. Following IVM, and mature oocytes were inseminated by ICSI followed by TEFNA.
***Results*:** Four couples with five completed treatment cycles were performed, and total of 36 immature oocytes were retrieved. Following 36 to 48 h of culture, 32 (88.89%, 32/36) oocytes became mature. The mature oocytes were inseminated with TEFNA-retrieved sperm, and 18 (56.25%, 18/32) oocytes were fertilized normally following ICSI. Eleven embryos were transferred in five cycles and two pregnancies and two singleton births were achieved in two patients.
***Conclusions*:** This result demonstrates that the successful pregnancies and live births can be established from embryos produced from in vitro matured oocytes that fertilized with testicular sperm.

KEY WORDS: ICSI; immature human oocytes; in vitro maturation; testicular fine needle sperm aspirations.

INTRODUCTION

Recently it has been demonstrated that immature oocyte retrieval followed by in vitro maturation (IVM) is a successful treatment especially for women with polycystic ovary syndrome (PCOS) (1). The major benefits of IVM treatment include avoiding the side effects of hormone stimulation, reducing direct and indirect costs and simplifying the treatment. Subsequently, it has been reported that a live birth from in vitro matured oocytes following intracytoplasmic sperm injection (ICSI) of spermatozoa retrieved by percutaneous sperm aspiration (PESA) (2). However, there is no report indicating whether the pregnancy and live birth can be established with testicular aspirated spermatozoa or not.

Since ICSI was successfully introduced as a means to alleviate primarily male-factor infertility, the use of ICSI has benefited many couples, especially for males with obstructive and non-obstructive azoospermia (3,4). High pregnancy and live birth rates have been reported by ICSI using spermatozoa retrieved from the ejaculates, epididymis and testes (5). To the best of our knowledge, this is the first report of pregnancies and live births resulting from in vitro matured oocytes fertilized with spermatozoa retrieved by testicular fine needle aspiration (TEFNA) following ICSI.

CASE REPORTS

IVM of immature oocytes is a standard infertility treatment in our IVF center (6), the First Affiliated Hospital of Nanjing Medical University. A total of five completed cycles in four women with PCOS, who experienced the risk of ovarian hyperstimulation syndrome (OHSS), were included in this IVM treatment. Their husbands were all diagnosed as azoospermia. Following immature oocyte retrieval and IVM, the in vitro matured oocytes were inseminated with TEFNA-retrieved spermatozoa. Out of five completed cycles, two patients became pregnant and delivered two healthy infants (Table I). The details of two patients who achieved pregnancy are described below.

Case 1

The female patient with 5 years of primary infertility history was aged 30 years. Her menstrual cycle was regular, and her hormone profile on day 3 was normal (FSH, 11.8 IU/L; LH, 2.0 IU/L; E_2, 110.5 pmol/L; Prolactin, 5.7 μg/L). The patient had failed to become pregnant after a controlled ovarian hormone (COH) stimulated cycle of conventional IVF treatment, and had experienced with high risk of OHSS. Her partner was aged 30 years,

Table I. Results of In Vitro Maturation and Fertilization of Oocytes Fertilized with Testicular Sperm Following ICSI

No. of cycles (couples)	5 (4)
Age	27.2 ± 2.2
No. of oocytes retrieved	
Total	36
Mean	7.2 ± 6.5
No. of oocytes matured (%)	32 (88.9)
No. of oocytes fertilized (%)	18 (56.3)
No. of embryos cleaved (%)	15 (83.3)
No. of embryos transferred	
Total	11
Mean	2.2 ± 0.8
No. of clinical pregnancy (%)	2 (40.0)
No. of implantation (%)	2 (18.2)

Note. Values with the plus/minus sign are means ± SD.

had normal size of testes with normal karyotype of chromosomes. Screening for Y-chromosome showed no any microdeletions. His serum concentrations of FSH, LH, testosterone and prolactin were with normal range. Repeat semen analysis presented no spermatozoa at all from the ejaculate. The spermatozoa could be only found in testicular tissue fragment that was extracted from the testes by a fine needle (K-TESA-20-3.0-MARIBOR, Cook, Queensland, Australia). Therefore, the cause of his azoospermia was considered to be obstructive.

Before immature oocyte retrieval, mild ovulation stimulation was started on day 3 of the cycle by administering 150 IU of gonadotropins daily (Metrodin HP; Serono, Switzerland) for 3 or 5 days. On day 7 of the cycle, the serum estradiol level was 433.4 pmol/L, and there were more than 10 follicles (8 to 9 mm in diameter) in each ovary. The thickness of endometrium was 8.1 mm. Immature oocyte retrieval was performed on day 7 of the treatment cycle with a transvaginal ultrasonographically guided probe, using a specially designed 17-gauge single lumen aspiration needle (K-OPS-1235-Wood, Cook, Queensland, Australia) at an aspiration pressure of 7.5 kPa. Three immature oocytes were obtained. The individual immature oocytes were cultured in 20 μL droplet of maturation medium, tissue culture medium 199 (TCM-199; Sigma) supplemented with 0.075 IU/mL FSH (Metrodin HP; Serono), 0.075 IU/mL HCG (Profasi; Serono), 0.5 μg/mL 17β-estradiol (Sigma) and 10% (v/v) human follicular fluid (HFF), under mineral oil (Sigma) at 37°C in an atmosphere of 5% CO_2 in air for 36 h. HFF was collected previously from the patient undergoing conventional IVF cycle. For preparation of HFF, the HFF was filtered with 0.22 μm filters, and then inactivated at 56°C for 30 min, stored at −20°C for further use.

After 36 h of culture, the oocytes were denuded from cumulus cells by using a fine-drawn glass pipette following exposure to 0.1% hyaluronidase (Sigma) in modified human tubal fluid (mHTF) medium (Irvine Scientific, Santa Ana, CA, USA). Mature oocytes were determined by the presence of a polar body in the perivitelline space (PVC). All three oocytes were mature. Three spermatozoa were found from the testicular tissue fragment on the day of oocytes become mature. ICSI was performed for these three mature oocytes. Following ICSI, each oocyte was transferred into 20 μL droplet of P-1 medium (Irvine Scientific) for further culture. Fertilization was assessed 18 h after ICSI for the appearance of two distinct pronuclei (2PN) and two polar bodies in the PVC. All 3 oocytes were fertilized with 2PN. Two embryos were transferred 72 h after ICSI. Two weeks later, serum β-hCG test was positive, and an intrauterine singleton pregnancy with a fetal heartbeat was confirmed by ultrasonography after 5 weeks of embryo transfer (ET). For endometrium preparation and luteal support, the patient was given 80 mg progesterone and 4.0 mg estrodial valerate (Progyova, Shering 5A, France) daily, starting from the day of immature oocyte collection until 70 days of gestation. The dose of both steroids was reduced gradually, and stopped on day 90 of gestation. The patient delivered a healthy baby boy at term, with a birth weight of 3700 g.

Case 2

A 28-year old woman had a 3-year history of infertility. She had tubal factor diagnosed by hysterosalpingography. She underwent two unsuccessful IVF cycles at other IVF clinics. Previous IVF treatment records indicated that her follicular development was slow and less in number. After 15 days of ovarian stimulation, only two mature oocytes were collected in the previous cycle. Therefore, she was considered as a poor responder. Her hormone profile on day 3 of the cycle was 19.1 IU/L FSH, 12 IU/L LH, and 18.71 pg/mL estrodial.

Her husband was aged 30-years, and had normal size of testes. Screening for karyotype and sex chromosome also showed no microdeletions. His hormone profiles for FSH, LH, testosterone and prolactin were normal. However, spermatozoa could not be observed in the ejaculates, and the sperms could only can be found in testicular tissue fragment extracted from testes.

The treatment cycle was started with GnRH agonist (Decapeptyl, 0.1 mg/day) on day 22 of the previous menstrual cycle and continued for 5 days followed by administration of gonadotropins (Metrodin-HP, 225 IU/day plus hMG, 150 IU/day) for 7 days. On day 5 and day 8 of stimulation, the serum estradiol levels were 47.95 pg/mL and 132.84 pg/mL respectively. Sixteen follicles (10–12 mm in diameter) were observed from bilateral ovaries and the thickness of the endometrium was 8.5 mm. Immature oocyte retrieval was performed on day 8, and 12 immature oocytes were collected. Eight oocytes matured after 36 h of culture, and four oocytes were fertilized by ICSI with the testicular spermatozoa freshly retrieved on the day of insemination. After transfer of three embryos on day 3 following ICSI, an ongoing clinical singleton pregnancy was confirmed by the presence of a fetal heartbeat on 7 weeks of gestation. The patient had a safe Caesarian-section delivery of a healthy boy (birth weights of 3700 g) at 38 weeks of gestation.

DISCUSSION

Recent progress on IVM treatment demonstrated that priming with FSH or HCG prior to immature oocyte retrieval improved oocyte maturation rates and embryo quality as well as pregnancy rates in infertile women with PCOS (1). It has been indicated that FSH priming with a fixed dose (150 IU/day) for 3 days from day 3 of the menstrual cycle does not increase the number of oocytes obtained per aspiration and does not improve oocyte maturation, cleavage rates or embryo development when women with normal cycling ovaries are treated (7). However, it has been reported that priming with recombinant FSH during the follicular phase before harvesting of immature oocytes from women with PCOS improves the maturational potential of the oocytes and the implantation rate of the cleaved embryos (8). Furthermore, Lin *et al.* (9) reported that FSH priming with 75 IU/day for 6 days in combination with 10,000 IU HCG priming for 36 h before immature oocyte retrieval has no additional benefit in women with PCOS. Although these results are controversial on the benefits of using FSH priming in women with regular ovaries or irregular menstrual cycles associated with PCOS, our previous results indicated that the use of FSH priming at the beginning of follicular phase enhances more follicular development for IVM treatment in women with the poor responders (6). Therefore, further confirmation of the beneficial effect of priming with FSH before immature oocyte retrieval from women with poor responders seems important.

Since Palermo *et al.* (3) reported the first successful pregnancy and live birth with ICSI, the management of infertility due to azoospermia has dramatically changed. Now the epididymal and testicular sperm aspirations are routinely used to obtain spermatozoa in the cases of obstructive and non-obstructive azoospermia. Recently, FEFNA, as a technique, is introduced, and it is a simpler and less invasive procedure compared to microsurgical intervention on the testes (10). In the present five completed cycles, the spermatozoa could not be found from the epididymal aspirates, but the spermatozoa were found finally from the testicular extracts.

The fertilization rate obtained from these cases were comparable to those achieved by ICSI from the mature oocytes collected after COH stimulation for conventional IVF, using spermatozoa from the ejaculates, epididymis or testis (11). Although the pregnancy and live birth from IVM oocytes fertilized with PESA-retrieved spermatozoa have been reported (2), there has been no report of pregnancies and live births of IVM-matured oocytes fertilized with testicular spermatozoa. This report highlights the use of two advanced assisted reproduction techniques, namely IVM and ICSI with TEFNA-retrieved spermatozoa to overcome male and female infertility problems. As far as we are aware, this is the first report in the literature of successful pregnancies and live births resulting from in vitro matured oocytes fertilized with TEFNA-retrieved spermatozoa following ICSI. Thus, from the present information, it appears that IVM combined with ICSI using testicular spermatozoa can produce healthy infants.

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