CASE REPORT

Ongoing twin pregnancy after ICSI of PESA-retrieved spermatozoa into in-vitro matured oocytes

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The recovery of immature oocytes from unstimulated ovaries followed by in-vitro maturation (IVM) is an attractive alternative to conventional IVF in the treatment of female infertility. Similarly, surgical recovery of spermatozoa from the epididymis by percutaneous sperm aspiration (PESA) has simplified the retrieval of the male gamete in treatment of men with obstructive azoospermia. We report the first ongoing clinical twin pregnancy resulting from intracytoplasmic sperm injection (ICSI) of spermatozoa retrieved by PESA into IVM oocytes. In the treatment of a 24-year old woman, 12 immature oocytes were retrieved. Six oocytes matured (maturation rate 50%) after 24-hour incubation and were inseminated by ICSI. Four oocytes had two pronuclei (fertilization rate 67%) and 3 good quality embryos were transferred. A viable twin pregnancy was confirmed by ultrasound scan. This report illustrates the use of a combination of less invasive assisted reproductive techniques in overcoming barriers to infertility.

Key words: In-vitro maturation/human oocytes/polycystic ovaries/percutaneous sperm aspiration

Introduction

In in-vitro maturation (IVM) treatment cycles, immature oocytes retrieved from unstimulated ovaries are cultured overnight. Subsequently, matured oocytes are fertilized (Trounson et al., 1994; Barnes et al., 1995). IVM treatment has several advantages including lower treatment cost, reduced health risks and increased patient convenience compared with conventional IVF treatment. Recent reports of live births following IVM treatment of women during unstimulated cycles (Cha et al., 2000, Chian et al., 2000a) have emphasised the significant potential of IVM treatment, especially for women with polycystic ovaries. Similarly, the treatment of male infertility with the use of intracytoplasmic sperm injection (ICSI) has benefited many couples (Palermo et al., 1992). Both ejaculated spermatozoa and those retrieved from the testis and epididymis have been utilized in insemination of oocytes using ICSI (Temple-Smith et al., 1985; Craft and Shrivastav, 1994).

To the best of our knowledge, this is the first report of an ongoing clinical pregnancy resulting from ICSI of spermatozoa retrieved by percutaneous sperm aspiration (PESA), into IVM oocytes.

Case report

A 24-year old woman and her 30-year old husband presented to our clinic with a 3-year history of primary infertility. She had regular menstrual cycles and normal, early follicular and mid-luteal phase serum hormone concentrations. A transvaginal ultrasound examination performed during the early follicular phase diagnosed bilateral polycystic ovaries (Adams et al., 1985).

Her partner had normal sized testes and had previously been treated in another unit with surgical excision of a varicocele that was believed to be the cause of his severe oligozoospermia. Unfortunately, no spermatozoa were found on repeated post-operative semen analysis. His serum concentrations of FSH, LH, testosterone and prolactin were normal. The cause of his azoospermia was considered to be obstructive. The couple had no history of assisted reproduction treatment.

Treatment options including surgical sperm retrieval combined with either IVF or IVM, or donor insemination were discussed with the couple. They opted for IVM treatment with PESA.

On day 3 of a spontaneous menstrual cycle the woman
underwent a baseline transvaginal ultrasound scan to exclude ovarian cysts and to estimate the number of retrievable oocytes. A total of 35 ovarian follicles between 4–6 mm diameter were counted. A second ultrasound scan was performed on day 7 to exclude the development of a dominant follicle and to measure the endometrial thickness. A total of 10 000 IU HCG (Profasi; Serono, Oakville, Ontario, Canada) was administered 36 hours before oocyte retrieval (Chian et al., 2000a). On cycle day 9, transvaginal ultrasound-guided oocyte collection was performed using a specially designed 17G single-lumen aspiration needle (K-OPS-1235-Wood, Cook, Queensland, Australia) with a reduced aspiration pressure of 7.5 kPa. Aspiration of all small follicles was performed without flushing. Oocyte retrieval was performed under i.v. sedation (2 mg Midazolam and 175 µg Fentanyl) and a paracervical block with 10 ml of 1% lidocaine. Oocytes were collected in 10 ml culture tubes containing 2 ml warm 0.9% saline with 2 IU/ml heparin (Baxter, Toronto, Ontario, Canada). Cumulus-oocyte complex (COC) were identified, washed and incubated in an organ tissue culture dish (60×15 mm; Falcon, Becton Dickinson Labware, Franklin Lakes, NJ, USA) containing 1 ml of maturation medium. The IVM medium consisted of TCM-199 (Sigma Chemical Co., St Louis, MO, USA) supplemented with 75 mIU/ml FSH and LH (Humegon; Organon, Scarborough, Ontario, Canada), 25 mol/l pyruvic acid (Sigma Chemical Co.) and 20% heat-inactivated maternal serum. The oocytes were incubated in an incubator at 37°C in an atmosphere of 5% carbon dioxide and 95% air with high humidity.

Twenty-four hours post-collection, the oocytes were denuded with hyaluronidase (Scandinavia IVF Science, Gothenburg, Sweden) and mechanical pipetting. Mature (metaphase II) oocytes were identified by the presence of the first polar body. Immature oocytes were further cultured in IVM medium, and examined prior to ICSI and 48 hours post-collection.

On the day of ICSI, the husband produced two ejaculated semen samples, but no spermatozoa were found in either specimen. A percutaneous epididymal sperm aspiration (PESA) procedure was performed. Epididymal fluid was collected with a syringe connected to a 21G butterfly needle, which was inserted directly through the skin and the epididymal tubule. The aspirated fluid from the right testis was examined under the microscope. Motile spermatozoa were observed, isolated and then washed (200 g for 12 min) and resuspended in IVF-20 medium (Scandinavia IVF Science). The spermatozoa were incubated (37°C, 5% carbon dioxide) until use. Mature oocytes were inseminated by ICSI and cultured in organ tissue culture dish (60×15 mm; Falcon). After the ICSI procedure, surplus spermatozoa were cryopreserved.

For endometrial preparation, the patient was given 6 mg of oestradiol valerate (Estrace; Roberts Pharmaceutical, Mississauga, Ontario, Canada) in divided doses starting on the day of oocyte retrieval. Endometrial thickness on the day of oocyte collection was 7.5 mm. Luteal support was provided in the form of 400 mg of progesterone twice a day for 16 days starting on the day of ICSI until 12 weeks gestation.

Twelve immature oocytes were retrieved at oocyte collection. Six mature oocytes (50% maturity rate) were obtained after 24 h culture in maturation medium and were inseminated by ICSI. Upon examination of the injected oocytes 18 h later, four had two pronuclei (fertilization rate 67%). No additional mature oocytes were obtained after 48 h culture. Three good quality embryos (one embryo 4 cells, grade 1; 2 embryos 4 cells, grade 2) were transferred 48 hours after ICSI. The serum β-HCG concentration 14 days after transfer was 1824 IU/ml. Two weeks later, a clinical twin pregnancy was confirmed with two fetal hearts seen at transvaginal ultrasonography. A subsequent scan at 12 weeks gestation confirmed viability of both fetuses.

**Discussion**

The collection of immature oocytes from unstimulated ovaries, followed by in-vitro oocyte maturation and subsequent insemination, has received increasing attention as an alternative to conventional IVF treatment (Cha and Chian, 1998; Chian et al., 2000a). The benefits of IVM compared with IVF include reduced cost [less drugs and monitoring], reduced health risks (no gonadotrophin associated ovarian hyperstimulation syndrome (OHSS) or putative future ovarian cancer risk], and increased patient acceptability (reduced blood test and ultrasonographic monitoring and no daily injections). IVM is particularly useful for patients with polycystic ovaries, who have an increased risk of developing OHSS (MacDougall et al. 1993) and who produce a significant number of immature oocytes following ovarian stimulation (Dor et al., 1990) resulting in lower fertilization rates (MacDougall et al., 1993; Kodama et al., 1995, Aboulghar et al., 1997).

Though Cha et al. (1991) were first to describe the successful use of IVM in a donor oocyte programme (Cha et al., 1991), Trounson and colleagues (1994) placed IVM in the clinical realm when they first reported a pregnancy using oocytes retrieved by transvaginal ultrasound-guided follicle aspiration from a patient with polycystic ovaries (Trounson et al., 1994). Since then, there have been a number of reports of the successful use of IVM, but pregnancy rates and live births associated with IVM have remained relatively low (Barnes et al., 1995; Cha and Chain, 1998). Recently, our group showed that the maturation rate can be improved by the addition of HCG priming before immature oocyte retrieval in women with polycystic ovaries (Chian et al., 1999, 2000a). High maturation, fertilization and cleavage rates were obtained, resulting in a clinical pregnancy rate of 39% per cycle commenced. Several live births following IVM treatment of women during unstimulated cycles have been reported (Cha et al., 2000, Chian et al., 2000a).

It was appropriate that the patient selected IVM as her treatment option since she had a large number of follicles in both ovaries. Her prognosis during the treatment cycle was excellent as she did not develop a dominant follicle (Cobo et al., 1999) by the time of HCG administration and she had a good endometrial thickness of 7.5 mm on the day of oocyte collection (unpublished observation). The thickness of the endometrial lining was achieved by priming with exogenous oestradiol to assist in the proper synchronization of embryo and endometrium development.
The introduction of ICSI (Palermo et al., 1992) has revolutionized the treatment of male infertility. Furthermore, modern sperm recovery techniques have made it possible to help men with obstructive or non-obstructiveazoospermia to achieve fatherhood. For men with obstructive azoospermia, high sperm recovery rates from the epididymis have been obtained using microsurgical epididymal sperm aspiration (MESA) (Temple-Smith et al., 1985; Silber et al., 1994) or PESA (Craft and Shrivastav, 1994; Shrivastav et al., 1994).

Before the introduction of ICSI in IVM treatment, fertilization and pregnancy were reported using standard insemination of in-vitro matured oocytes with ejaculated spermatozoa (Cha et al., 1991, Trounson et al., 1994). In IVM treatment, ICSI may be beneficial because of zona changes due to longer in-vitro culture prior to insemination (Nagy et al., 1996; Hwang et al., 2000). ICSI may not, however, always be necessary if ejaculated semen shows normal characteristics and there is a large number of IVM oocytes (Chian et al., 2000b).

The fertilization rate obtained in this case was comparable with those achieved by ICSI of mature oocytes retrieved after ovarian stimulation for conventional IVF, using ejaculated spermatozoa (Tournaye et al., 1995; Nagy et al., 1996). This is consistent with the observations of Ghazzawi et al. (1998) who reported, in a prospective study, that there were no significant differences in fertilization and pregnancy rates following ICSI using spermatozoa from ejaculates, epididymis or testis (Ghazzawi et al., 1998).

Although pregnancies and live births from IVM oocytes have been reported, there has been no report of ongoing clinical pregnancies of IVM-matured oocytes from unstimulated cycles using PESA-retrieved spermatozoa. This report highlights the use of two advanced assisted reproduction techniques to overcome infertility problems faced by the couple, which are relatively less invasive compared with conventional IVF or MESA.

The number of births from IVM is still relatively few. However, the technique has significant potential in the treatment of infertility for women with polycystic ovaries and, with further advances in culture techniques, its use will hopefully be extended to women with normal ovaries.

Note added in proof

The patient had a safe Caesarian-section delivery of healthy twin girls (birthweights of 2200 and 2150 g) at 36 weeks gestation.

References


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