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Case Report

Successful Birth After Transfer of Blastocysts Derived from Oocytes of Unstimulated Woman with Regular Menstrual Cycle After IVM Approach

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***Purpose*:** To report a delivery after transfer of blastocysts derived from eggs collected following in vivo HCG priming in a patient with regular menstrual cycles undergoing in vitro maturation (IVM) program.

***Methods*:** A woman had regular menstrual cycle and had experience of ovarian hyperstimulation syndrome (OHSS) during a previous conventional IVF-ET cycle. The patient was primed with 10,000 IU HCG 36 h before egg retrieval. After oocyte collection, the maturity of oocytes was evaluated and immature oocytes were cultured in IVM medium. The matured oocytes were fertilized with husband sperm, and normal fertilized eggs were cultured to blastocysts stage until embryo transfer in uteri.

***Results*:** Three MII-stage and 13 GV-stage oocytes were collected from the patient. Three mature oocytes were fertilized by conventional IVF. All three fertilized oocytes were developed to blastocysts. Immature oocytes were matured in vitro and insemination was carried out by ICSI. Out of eight fertilized zygotes, two developed to blastocyst stage. Transfer of three expanded blastocysts on Day 6 resulted in pregnancy in the patient and one healthy baby was born.

***Conclusions*:** This report provides an approach to treat infertile women with regular menstrual cycle and high risk of OHSS.

KEY WORDS: Blastocyst; IVM; pregnancy.

INTRODUCTION

Controlled ovarian hyperstimulation (COH) is used to achieve multifollicular recruitment, enabling an increased number of embryos to be transferred. In comparison with COH, the major benefits of in vitro maturation (IVM) treatment include avoidance of the risk of ovarian hyperstimulation syndrome (OHSS), reduced cost, and less complicated treatment. Although it is possible to mature and fertilize human oocytes obtained from unstimulated cycles, the pregnancy rate after IVM is low. Chian *et al.* (1) reported that a higher rate of oocyte maturation and pregnancy was achieved in patients with polycystic ovarian syndrome (PCOS) by HCG priming. Also, we reported that mature oocytes can be collected by only in vivo HCG priming in women with PCOS undergoing IVM program. Clinical pregnancy can be established by transfer of blastocysts derived from the mature oocytes (2).

Here we report a case in which the oocytes were collected after one HCG priming in a woman with regular menstrual cycles and several oocytes were able to undergo maturation, fertilization, and blastocyst development. Blastocyst transfer was able to establish pregnancy.

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CASE REPORT

Before the study, approval was obtained from the Institutional Review Board of the Maria Infertility Hospital.

A 32-year-old woman with normal regular cycles (30 days) referred for IVF because of tubal disease, failed to become pregnant after four cycles of conventional IVF treatment and three cycles of cryopreservation over the past 6 years. The patient had an experience of OHSS in previous conventional IVF cycles. The patient presented with some polycystic-like ovaries with about 10 small follicles visible on ultrasound, but she had normal serum testosterone and LH concentrations on menstrual cycle day 2.

Ovarian follicle development was monitored by transvaginal ultrasonography (Aloka, Tokyo, Japan) beginning on cycle day 3 to exclude the development of a dominant follicle. Oocyte collection was performed on Day 8 in the first IVM cycle and on Day 9 in the second IVM cycle on the basis of the cycle length and the endometrium thickness. The patient was administered 10,000 IU of HCG (IVF-C, LG Chemical, Korea) subcutaneously, 36 h prior to oocyte collection. Transvaginal ultrasonographically guided oocyte collection was done using a 19-gauge aspiration needle (Cook, Eight Mile Plains, Queensland, Australia) with an aspiration pressure between 80 and 100 mmHg. Nineteen oocytes were collected from both ovaries in the first IVM cycle and 16 oocytes were collected in the second IVM cycle.

The aspirates were collected in tubes containing prewarmed heparinized Ham's F-10 medium. Follicular aspirates were filtered (70-μm mesh size, Falcon 1060; Life Technologies) and washed with addition of more medium to filtrate. The filtrate was further washed with medium by vigorous pipetting using 10 mL serological pipette (Becton Dickinson & Company, NJ) to remove erythrocytes and small cellular debris. The retained cells were then resuspended in the medium. The oocytes were isolated under a stereomicroscope and washed twice in the same medium.

After oocyte collection, immature oocytes with a germinal vesicle (GV) were transferred to maturation medium for culture and oocytes without GV were denuded of cumulus cells with 0.003% hyaluronidase (Sigma, St. Louis, MO) and mechanical pipetting. Oocytes without an intact GV were defined as Metaphase I (MI) and Metaphase II (MII). All oocyte handling procedures were conducted in a 37°C minichamber (Iljin, Seoul, Korea).

The IVM medium consisted of YS medium supplemented with 30% human follicular fluid (hFF), 1 IU/mL rFSH, 10 IU/mL HCG, and 10 ng/mL rhEGF (2). Immature oocytes were cultured in IVM medium at 37°C in an atmosphere of 5% CO_2, 5% O_2, and 90% N_2. Nuclear maturation was assessed at 24 and 48 h after culture under the dissecting microscope.

Spermatozoa for insemination were prepared at the day of oocyte collection. Conventional IVF was performed in MII-stage oocytes collected at the time of oocytes collection and ICSI was performed in MII-stage oocytes matured in vitro. Spermatozoa were prepared by 90% Percoll separation at $300 \times g$ for 20 min. After Percoll separation, the sperm pellet was washed twice ($300 \times g$) with 3 mL of Ham's F10 medium containing 10% hFF and the motile spermatozoa were collected by swim-up method. Fertilization was assessed 19 h after insemination to detect the appearance of two distinct pronuclei and two polar bodies.

Zygotes were cocultured with cumulus cells in 10 μL YS medium supplemented with 10% hFF (3). Embryo transfer (ET) was performed on Day 4 or Day 6 after oocyte collection, and the remaining embryos were cultured until Day 7. Those embryos that developed to expanded blastocyst stage were cryopreserved. The endometrium was prepared by using the method reported by Son *et al.* (2). Briefly, the patient was also given 10,000 IU HCG at the time of oocyte recovery. Oestrogen valerate (6 mg) (Progynova; Schering, Berlin, Germany) was administered daily from the day of oocyte retrieval and progesterone (100 mg) (Progest; Samil Pharmacology, Seoul, South Korea) was administered daily from Day 1 after oocyte retrieval. Both medications were continued until a fetal heartbeat was positively identified.

For the first IVM attempt, a total of 3 MII oocytes and 16 immature oocytes (1 at MI stage and 15 at GV stage) were obtained. Three MII oocytes were inseminated with conventional IVF, all fertilized and cleaved. Sixteen immature oocytes were cultured in IVM medium, 11 and 2 reached to MII after 24 and 48 h of culture, respectively. Twelve oocytes were fertilized after ICSI, and 10 cleaved. A total of four embryos (three compacted embryos of Grade 2 and one eight-cell embryo of Grade 2) were transferred 4 days after oocyte collection. On the day of ET, the endometrial thickness was 10 mm at transvaginal ultrasonogram. No pregnancy occurred after ET. Out of remaining nine embryos, three were developed to blastocysts of Grade 3 and the blastocysts were cryopreserved.

For the second IVM attempt, a total of 16 oocytes were retrieved: 3 oocytes were at MII stage and 13 were at GV stage. MII-stage oocytes were inseminated with conventional IVF ($n = 3$), and all oocytes fertilized and cleaved. GV-stage oocytes were cultured in IVM medium, eight and two reached to MII stage after 24 and 48 h of incubation, respectively. Eight oocytes were fertilized after ICSI. All fertilized oocytes cleaved. After coculture with cumulus cells, three blastocysts from MII oocytes and two blastocysts from maturation of GV oocytes were obtained, respectively. Three expanded blastocysts (two formed on Day 6 and one formed on Day 5) were transferred to the patients on Day 6 after oocyte collection. On the day of ET, the endometrial thickness was 10 mm on transvaginal ultrasonogram. Two weeks after ET, serum β-hCG level was 863.1 IU/mL, and 4 weeks after ET, an ongoing intrauterine single pregnancy combined with a left tubal pregnancy was observed using transvaginal ultrasonography. The left salpingoectomy was performed 5 weeks after ET. The patient delivered a healthy boy at 36 weeks of gestation. The remaining two blastocysts derived from GV-stage oocytes were cryopreserved.

DISCUSSION

This study demonstrates that the oocytes retrieved from women with regular menstrual cycles following only HCG priming can develop to blastocyst stage in culture after maturation, fertilization, and development, and that the transfer of resulting blastocyst can establish pregnancy and delivery.

Although recent studies have shown increased pregnancy rates, the pregnancy rate after IVM of oocytes is low. The possible reasons are suboptimal culture conditions during IVM and/or inadequate cytoplasmic maturation of the oocytes themselves.

To compensate for these problems, we modified hormone supplementation of our IVM medium which consisted of a 1:10 ratio of rFSH/HCG and EGF (2). We also performed HCG priming before oocyte collection in women with regular menstrual cycles and in IVM cycles of patients with PCOS (2). In this case report, we obtained 82.8% (29/35) of maturation rate, 89.7% (26/29) of fertilization rate, and 92.3% (24/26) of cleavage rate. From 2PN 36.4% (8/22) developed to expanded blastocyst, without counting four embryos transferred at Day 4 in the first IVM attempt. This indicates that the present stimulation and IVM protocols can produce high quality of human embryos.

To our knowledge, a pregnancy has been reported by transfer of early blastocyst produced by IVM, ICSI, and culture in G1/G2 medium from a PCOS patient (4). Recently, we reported that mature oocytes can be collected by one in vivo HCG priming in PCOS patients receiving IVM program. Clinical pregnancy can be established by transfer of blastocysts derived from the mature oocytes at the time of oocyte collection (2). Then present case is the first report to establish the pregnancy and delivery by transfer of blastocysts from a woman with regular menstrual cycles.

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