Successful Delivery Following Cryopreservation of Zygotes Produced by In Vitro Matured Oocytes Retrieved from a Woman with Polycystic Ovarian Syndrome-Like Disease: A Case Report

Koichi Kyono,^{1,3} Noritaka Fukunaga,¹ Kosuke Haigo,¹ Setsuyo Chiba,¹ Chisako Ohira,¹ Yukiko Nakajo,¹ and Yasuhisa Araki²

Submitted March 21, 2002; accepted March 22, 2002

Purpose: To estimate frozen zygotes, which developed from in vitro matured oocytes retrieved from polycystic ovarian syndrome-like disease.

Methods: Oocyte retrieval was performed on Day 15 following withdrawal bleeding. The oocytes were incubated for 24 h in TCM-199 maturation medium supplemented with follicle fluid, E2, FSH, and hCG.

Results: A total of 12 immature oocytes were collected. Seven of the 12 oocytes (58.3%) developed to the metaphase-II stage, and subsequently, all seven fertilized oocytes were frozen at the pronuclear stage. The remaining five oocytes failed to develop to the metaphase-II stage after an additional 24 h of incubation. Three of seven cryopreserved oocytes were thawed and developed to 2–8-cell cleaved stage embryos. The first pregnancy failed. However, the second frozen-thawed embryo transfer resulted in the delivery of healthy twins.

Conclusions: Successful delivery using frozen zygotes from an anovulatory woman with polycystic ovarian syndrome-like disease.

KEY WORDS: Cryopreservation; ICSI; mature oocyte; PCOS.

INTRODUCTION

In general, women with polycystic ovarian syndrome (PCOS) are characterized by abnormal endocrine parameters: anovulation; numerous antral follicles within the ovaries; and frequently, infertility. Furthermore, a group of patients has been designated as PCOS-like; they are extremely sensitive to stimulation with exogenous gonadotropins and are at increased risk of developing ovarian hyperstimulation syndrome (OHSS) when treated with gonadotropins for assisted reproduction. Recovery of immature

oocytes followed by in vitro maturation (IVM) of the oocytes represents a new technique for the treatment of patients with infertility secondary to PCOS or PCOS-L. In vitro fertilization after IVM would offer a patient access to assisted reproductive technology (ART) without any risks of OHSS. Trouson *et al.* described methods for transvaginal ultrasonographically guided recovery of immature oocytes from the ovaries of patients with PCOS and introduced IVM as a clinical treatment option (1). It has been noted that most of the follicles from patients with PCOS are not atretic, and that these oocytes have developmental capability (2–6). However, the maturation rate of immature oocytes retrieved from women with PCOS is lower than that of those retrieved from women with normal menstrual cycles (7,8). It has been reported that priming with human chorionic

¹ Kyono Reproduction Research Center and Lady's Clinic Kyono, 3-8-6 Omiya, Furukawa, Miyagi 989-6221, Japan.

² The Institute for ARMT, Ishii, Fujimi, Setagun, Gunma, Japan.

³ To whom correspondence should be addressed; e-mail: info@ivf-kyono.or.jp.

gonadotrophin (hCG) before immature oocyte retrieval in PCOS patients improves oocyte maturation, fertilization, embryo development, and pregnancy rates (9,10). Furthermore those IVM embryos can be frozen for further embryo transfer (6). However, few reports discuss cryopreservation of oocytes obtained from an unstimulated patient with PCOS or PCOS-L. In this case report, we present a successful pregnancy and delivery outcome after the transfer of frozen-thawed embryos produced from IVM oocytes, which were cryopreserved at the pronuclear stage (2PN) following intracytoplasmic sperm injection (ICSI). The cryopreserved embryos (zygotes) developed from IVM oocytes, which were obtained from an unstimulated patient with PCOS-L.

CASE REPORT

A 35-year-old woman with PCOS-L presented with irregular menstrual cycles, anovulation, and 4 years of infertility; she did not exhibit hirsutism. Serum endocrine levels were normal except for the prolactin (PRL); laboratory findings were LH 3.5 mIU/L, FSH 6.0 mIU/L, PRL 47.6 ng/L and E2 42.2 pg/mL. The diagnosis of PCOS-L was made by ultrasound. Her husband's semen analysis was within normal limits: volume 4.0 mL; sperm count 152×10^6 /mL; motility 43.4%; and deformed sperm morphology 18.4%.

To initiate the treatment cycle the patient was injected with estrogen depot 10 mg and progesterone depot 125 mg to induce a withdrawal bleeding. Oocyte retrieval was performed on Day 15 without a stimulating cycle. Transvaginal ultrasound guided oocyte retrieval was done using a specially designed aspiration needle (Cook, Australia, K-MIN-1720-VUB) with an aspiration pressure of 300 mmHg. Aspiration of all small follicles (5–8 mm diameter) was done under intravenous anesthesia. 23 follicles were aspirated from both ovaries. Twelve immature oocytes were collected in a 10 mL culture tube containing 2 mL modified human tubal fluid (m-HTF) with 20 IU/mL heparin. Following oocyte collection, the maturity of the oocytes was microscopically determined and the immature oocytes were transferred to maturation media for culture. All oocyte handling procedures were conducted on warm stages and plates at 37°C. Cumulus oocyte complexes were washed in m-HTF with 10% synthetic serum substance (SSS; Irvine Scientific, CA) and then incubated for 24 h in TCM-199 maturation medium supplemented with 20% patient's follicle fluid, 10 IU/mL FSH, 10 ng/mL E2, and

20 IU/mL hCG at 37°C in an atmosphere of 5% CO₂, 5% O₂, 90% N₂. After incubation, mature oocytes were denuded of cumulus cells using finely drawn glass pipettes following a 1-min exposure to 60 IU/mL hyaluronidase solution. Spermatozoa for ICSI were prepared by 80% percoll gradient separation at 750g for 20 min. Following gradient separation, the sperm pellet was washed twice with 5 mL of HTF medium with 10% SSS. After ICSI, each oocyte was transferred to 40 µL of HTF with 10% SSS under mineral oil for culture. Luteal support was provided by the insertion of a 200 mg progesterone suppository twice a day for 16 days beginning 48 h after embryo transfer.

Following maturation in culture media for 24 h, 12 oocytes were noted to be at metaphase-II stage. Five oocytes were incubated an additional 24 h; however, development arrested prior to maturation. The seven oocytes, which matured, were used for ICSI. After ICSI all of the oocytes were found to have 2PN on the second day. However, embryo transfer was not performed because the endometrial thickness was less than 8 mm. Therefore, those seven fertilized oocytes were cryopreserved immediately after fertilization assessment. The freezing and thawing of embryos (2PN-stage embryo) was performed according to the standard technique that involves 1,2-propanediol (PROH) and sucrose as cryoprotectants for slow freezing. The solutions were prepared using m-HTF supplemented with 10% SSS. For freezing, the embryos were equilibrated for 10 min at room temperature in 0.5, 1.0, 1.5 mol/L PROH, and for 10 min in 1.5 mol/L PROH containing 0.1 mol/L sucrose. The embryos were loaded into straws, and cooled from –7°C to –30°C at a rate of –0.3°C/min, after which the straws were plunged into liquid nitrogen (LN₂). The thawing procedure consisted of warming at room temperature for 10 s, then placement in a 27°C water bath, where thawing was completed; cryoprotectants were removed by exposing the embryos to stepwise decreasing PROH concentrations (1.0 mol/L PROH + 0.1 mol/L sucrose, then 0.5 mol/L PROH + 0.1 mol/L, 0.1 mol/L sucrose). After thawing, the embryos developed to Day 3 cleavage stage in HTF with 10% SSS.

Approximately 1 month after cryopreservation, the patient was injected with estrogen 10 mg and depot progesterone 125 mg to induce withdrawal bleeding. On the third menstrual day, the patient was given Premarin (conjugated estrogen) 2.5 mg daily until pregnancy test. On the 16th day the cryopreserved embryos were thawed and cultured. Embryo transfer

took place 1 day later, and the patient was given progesterone 50 mg/day from the 15th day for 2 weeks.

A total of three embryos (one 2-cell cleavage and two 3-cell cleavage with 20–30% fragmentation) were transferred 24 h after thawing. Two weeks later the serum β hCG level was 242 mIU/mL. At 6 weeks of gestation, an ongoing intrauterine singleton pregnancy with a fetal heartbeat was confirmed by transvaginal ultrasound. Unfortunately, this pregnancy spontaneously aborted at 8 weeks of gestation.

Six months after cryopreservation, four fertilized oocytes were thawed for a scheduled embryo transfer. Following thawing, all four fertilized oocytes survived and all four cleaved. Three embryos were transferred (one 8-cell cleavage with no fragmentation and two 5-cell cleavage with no fragmentation). The endometrial thickness measured 9.9 mm on the day of embryo transfer. Two weeks later, the serum β hCG concentration was 1027 mIU/mL, and 6 weeks after embryo transfer an ongoing intrauterine twin pregnancy with fetal heartbeat was confirmed by transvaginal ultrasound. An uneventful delivery at 36 weeks resulted in the birth of two normal healthy infants, (1820 g male (46, XY) and a 2360 g female (46, XX)).

DISCUSSION

Our results demonstrate that embryos, which develop from IVM oocytes that were retrieved from women with PCOS-L, can be cryopreserved; following embryo transfer, these embryos can develop to term. PCOS and PCOS-L are the most common reproductive disorders in women of childbearing age. The syndromes encompass a heterogeneous group of symptoms, including hyperandrogenism and anovulation. The exact mechanism that produces anovulation in PCOS and PCOS-L is unknown. There is consensus that the general findings in women with PCOS and PCOS-L include arrested follicular development at the stage when selection of the dominant follicle should normally occur (11). The initial steps of folliculogenesis, recruitment, and growth to the small antral stage are functional in PCOS/PCOS-L, but the terminal step, selection of dominant follicles that are capable of ovulation, does not occur regularly (12). It is well known that in patients with PCOS/PCOS-L the cyclical pattern of FSH and LH secretion is typically absent, and there is a disproportionately high secretion of LH with a relatively constant low rate of FSH secretion.

If these women are given hormonal therapy, their ovaries are markedly stimulated; thus, they are

difficult subjects for infertility therapy. However, in 1994, a pregnancy was first reported in a woman with anovulatory infertility following IVM of immature oocytes and IVF (1). Recently, it has been demonstrated that recovery of immature oocytes followed by IVM of these immature oocytes is a promising treatment modality for women with PCOS (8–10). The IVM of oocytes without anomalies yields higher quality embryos, with higher cleavage rates, than those with anomalies (5). To date, several infertility centers have reported clinical pregnancy rates of approximately 25–30% from immature oocytes retrieved from patients with PCOS following IVM (4). Therefore, this data suggest that women with PCOS-related infertility can be treated with immature oocyte retrieval followed by IVM procedure and fertilization, as well as cryopreservation.

It was essential to use a cryopreservation procedure for ART; embryos survive and implant at high rate following freezing either at the pronuclear stage or cleavage stage. Recent reports also show that embryo cryopreservation at the pronuclear stage optimizes the possibility of achieving a live-born infant from a single oocyte retrieval (13,14). This case demonstrated a high embryo survival rate following freeze/thawing at the 2PN stage (7/7, 100.0%).

CONCLUSION

The embryos produced from in vitro matured oocytes retrieved from unstimulated women with PCOS-L can be safely cryopreserved and pregnancies can develop to term following embryo transfer. This case study is encouraging for PCOS and PCOS-L patients. Furthermore, patients desirous of pregnancy who must undergo chemotherapy can have their oocytes retrieved and cryopreserved. Further development of IVM techniques will increase its usefulness for in vitro fertilization of infertile patients.

REFERENCES

1. Trounson AO, Wood C, Kausche A: In-vitro maturation and the fertilization and developmental competence of oocytes recovered from untreated polycystic ovarian patients. *Fertil Steril* 1994;62:353–362
2. Almabbobi G, Anderiesz C, Hutchinson P: Functional integrity of granulosa cells from the polycystic ovaries. *Clin Endocrinol* 1996;44:571–580
3. Barnes FL, Kausche A, Tiglias J, Wood C, Wilton L, Trounson A: Production of embryos from in vitro-matured primary human oocytes. *Fertil Steril* 1996;65:1151–1156

4. Cha KY, Han SY, Chung HM, Choi DH, Lim JM, Lee WS, Ko JJ, Yoon TK: Pregnancies and deliveries after in-vitro maturation culture followed by in-vitro fertilization and embryo transfer without stimulation in women with polycystic ovary syndrome. *Fertil Steril* 2000;73: 978–983
5. Mikkelsen AL, Lindenberg S: Morphology of in vitro matured oocytes: Impact on fertility potential and embryo quality. *Hum Reprod* 2001;16:1714–1718
6. Chian RC, Gülekli B, Buckett WM, Tan SL: Pregnancy and delivery after cryopreservation of zygotes produced by in-vitro matured oocytes retrieved from a women with polycystic ovarian syndrome. *Hum Reprod* 2001;16:1700–1702
7. Cha KY, Chian RC: Maturation in vitro of immature human oocytes for clinical use. *Hum Reprod Update* 1998;4: 103–120
8. Fukuda A, Kawata A, Tohnaka M, Yamazaki K, Iwamoto H, Nakaoka Y, Morimoto Y, Kanzaki H: Successful pregnancies by intracytoplasmic sperm injection (ICSI) of in vitro matured oocytes from non-stimulated women (in Japanese). *Jpn J Fertil Implant* 2001;18:1–4
9. Chian RC, Gülekli B, Buckett WM, Tan SL: Priming with human chorionic gonadotrophin before retrieval of immature oocytes in women with infertility due to the polycystic ovary syndrome. *N Eng J Med* 1999;341:1624–1626
10. Chian RC, Buckett WM, Tulandi T, Tan SL: Prospective randomized study of human chorionic gonadotrophin priming before immature oocyte retrieval from unstimulated women with polycystic ovarian syndrome. *Hum Reprod* 2000;15:165–170
11. Erickson GF, Yen SSC: New data on follicle cells in polycystic ovaries: A proposed mechanism for the genesis of cystic follicles. *Semin Reprod Endocrinol* 1984;42:39–43
12. Jakimiuk AJ, Jakowicki JA, Magoffin DA: Follicular development in polycystic ovary syndrome. *Assist Reprod Rev* 1997;7:54–57
13. Damario MA, Hammitt DG, Session DR, Dumesic DA: Embryo crypreservation at the pronuclear stage and efficient embryo use optimizes the chance for a liveborn infant from a single oocyte retrieval. *Fertil Steril* 2000;73:767–773
14. Senn A, Vozzi C, Chanson A, De Grandi P, Germond M: Prospective randomized study of two cryopreservation policies avoiding embryo selection: The pronucleate stage leads to a higher cumulative delivery rate than the early cleavage stage. *Fertil Steril* 2000;74: 946–952