

CASE REPORT

Blastocyst development and pregnancies after IVF of mature oocytes retrieved from unstimulated patients with PCOS after in-vivo HCG priming

Weon-Young Son^{1,3}, San-Hyun Yoon^{1,2}, Suk-Won Lee¹, Yong Ko², Hye-Gyun Yoon¹ and Jin-Ho Lim¹

¹Maria Infertility Hospital and ²Department of Animal Science, Korea University, Seoul, Korea

³To whom correspondence should be addressed at: In Vitro Fertilization Laboratory, Maria Infertility Hospital, 103–11, Sinseol-dong, Dongdaemun-gu, Seoul, Korea. E-mail: sonyoung@mariababy.com

A major side-effect of controlled ovarian stimulation (COS) in patients with polycystic ovarian syndrome (PCOS) is the risk of ovarian hyperstimulation syndrome (OHSS). In-vitro maturation (IVM) of immature oocytes represents a potential alternative for the fertility treatment of these patients. Two patients at high risk of OHSS were primed with 10 000 IU HCG 36 h before oocyte retrieval. After retrieval, oocyte maturity was evaluated. Oocytes considered to be mature at the time of collection were inseminated by IVF or ICSI, and the resulting embryos were cultured to blastocysts. Transfer of these blastocysts resulted in pregnancy in both patients. Immature oocytes were cultured in YS medium supplemented with 30% human follicular fluid, 1 IU/ml rFSH, 10 IU/ml HCG and 10 ng/ml epidermal growth factor (rhEGF). After in-vitro maturation of the oocytes, ICSI was performed. Two and five expanded blastocysts were obtained after 5 day culture and were cryopreserved. This report indicates that mature oocytes can be collected at the time of retrieval using only in-vivo HCG priming in women with PCOS, and clinical pregnancy can be established by transfer of blastocysts derived from the mature oocytes. This approach opens a potential for a new dimension in the management of patients with PCOS.

Key words: blastocyst/HCG priming/IVM/PCOS/pregnancies

Introduction

Controlled ovarian stimulation (COS) is used to achieve multifollicular recruitment, enabling an increased number of embryos to be transferred. However, there are disadvantages associated with COS in the case of patients with polycystic ovarian syndrome (PCOS). The major side-effect of super-ovulation in patients with PCOS is ovarian hyperstimulation syndrome (OHSS) (Cobo *et al.*, 1999). Thus, in-vitro maturation (IVM) of immature oocytes from OHSS patients would be an attractive option to eliminate this problem.

Knowledge regarding the IVM of immature human oocytes and its clinical application has been accumulated during the past couple of years. Fertilization, embryo development and term pregnancies of IVM oocytes have been reported in stimulated cycles (Nagy *et al.*, 1996), natural cycles (Russell *et al.*, 1997) and PCO patients (Trounson *et al.*, 1994).

The efficiency of current IVM techniques is suboptimal in terms of obtaining the number of mature oocytes by natural cycle as compared with that by COS cycles (Barnes *et al.*,

1995; Russell *et al.*, 1997). In addition, the quality of oocytes is poor after IVM, showing frequently retarded cleavage and blockage of development (Barnes *et al.*, 1996; Trounson *et al.*, 1996), which may be a significant factor in the low pregnancy rates achieved. So far, only one case of blastocyst embryo transfer and pregnancy derived from immature oocytes collected from unstimulated ovaries has been reported (Barnes *et al.*, 1995). Although the number of IVM cycles was small, recent studies have shown improved pregnancy rates per embryo transfer (Mikkelsen *et al.*, 1999; Chain *et al.*, 2000).

Chian *et al.* reported that higher rates of oocyte maturation and pregnancies were achieved in patients with PCOS by HCG priming (Chian *et al.*, 2000). However, they reported that the pregnancies were achieved by transfer of day 2 or day 3 embryos derived from IVM of immature oocytes.

Here we report that mature oocytes can also be retrieved after HCG priming in women with PCOS in an IVM programme. The oocytes can undergo normal fertilization and blastocyst development, and pregnancies can be established by blastocyst transfer.

Case reports

Two patients with PCOS presented with anovulation, polycystic ovaries visible on ultrasound, elevated serum testosterone concentrations, >10 IU/ml LH in serum on menstrual cycle day 2 and a minimum 3 year history of infertility. The patients had a high risk of OHSS. Before the study, approval was obtained from the Institutional Review Board of the Maria Infertility Hospital.

Case 1

A 27 year old woman with PCOS failed to become pregnant after three cycles of conventional IVF treatment over the past 3 years. To initiate each treatment cycle, the patient received i.m. injections of progesterone (Progestin; Samil Pharmacology, Seoul, Korea). Withdrawal bleeding occurred on 3 days after the last dose. Ovarian follicle development was monitored by transvaginal ultrasonography (Aloka, Tokyo, Japan) beginning on cycle day 3. The patient was given 10 000 IU of HCG (IVF-C; LG Chemical, Seoul, Korea) subcutaneously on cycle day 9 based on the cycle length and the endometrial thickness. After 36 h, oocytes were aspirated with a 19 gauge aspiration needle (Cook, Eight Mile Plains, Queensland, Australia) under the guidance of transvaginal ultrasound. A portable aspiration pump was used with a pressure between 80 and 100 mmHg.

The aspirates were collected in tubes containing prewarmed heparinized Ham's F-10 medium supplemented with bicarbonate, HEPES and 0.3% bovine serum albumin. Oocytes were isolated by washing the follicular aspirate through a filter (70 mm mesh, Falcon 1060; Becton Dickinson, NJ, USA). In order to remove erythrocytes and small cellular debris, the filtrate was further washed with Ham's F-10 medium by vigorous pipetting using 10 ml serological pipette. The retained cells were then resuspended in the medium and the oocytes were isolated under a stereomicroscope. All oocyte handling procedures were conducted in a mini-chamber under 5% CO₂ atmosphere at 37°C. At the time of oocyte collection, 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 Chemical Co., St Louis, MO, USA) and mechanical pipetting. Oocytes without an intact GV were defined as metaphase I (MI) and oocytes with a first polar body extrusion were identified as metaphase II (MII).

The IVM medium consisted of YS medium (Yoon *et al.*, 2001a,b) supplemented with 30% human follicular fluid (hFF), 1 IU/ml rFSH, 10 IU/ml HCG and 10 ng/ml recombinant human epidermal growth factor (rhEGF). The hFF was prepared as the method reported by Chi *et al.* (Chi *et al.*, 1998). Oocytes were cultured in IVM medium at 37°C in an atmosphere of 5% CO₂, 5% O₂ and 90% N₂. Nuclear maturation was assessed under the dissecting microscope.

Conventional IVF or intracytoplasmic sperm injection (ICSI) was used to fertilize the mature oocytes. Fertilization was assessed 19 h after insemination to detect the appearance of two distinct pronuclei and two polar bodies. Zygotes were co-cultured with cumulus cells in 10 µl YS medium supplemented with 10% hFF. The cumulus cells for co-culture were prepared

using the method reported by Yoon *et al.* (Yoon *et al.*, 2001a). Blastocysts on day 5 were transferred to the patients, and the remaining embryos were cultured until day 7. Those embryos that developed to expanded blastocyst stage were cryopreserved by Ménéz *et al.*'s method (Ménéz *et al.*, 1992).

For the preparation of the endometrium, the patients were 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. Progestin 100 mg was administered daily from 1 day after oocyte retrieval. Both medications were continued until a fetal heartbeat was positively identified.

A total of 36 oocytes were retrieved; 18 oocytes were at MII stage, six were MI stage and 12 were GV stage. MII stage oocytes were inseminated with conventional IVF ($n = 11$) or ICSI ($n = 7$). Thirteen oocytes [7/11 (64%) from IVF and 6/7 (86%) from ICSI] were fertilized. Three blastocysts (one middle expanding blastocyst and two early blastocysts) were observed at day 5 and were transferred to the patient. On the day of embryo transfer, the endometrial thickness was 10 mm on transvaginal ultrasonogram. Two weeks after embryo transfer, serum β-HCG concentration was 649.3 IU/ml, and 4 weeks after embryo transfer, an ongoing intrauterine twin pregnancy was observed using transvaginal ultrasonography. Immature oocytes (MI = 6, GV=12) at the time of oocyte retrieval were transferred to maturation medium for culture, 11 oocytes reached metaphase II stage 24 h later. After ICSI, eight oocytes (72.7%) were fertilized. Two expanding blastocysts observed at day 5 after insemination were cryopreserved.

Case 2

A 32 year old woman with PCOS and a 4 year history of infertility had failed to become pregnant after six cycles of conventional IVF. To initiate each treatment cycle, the patient received IM injections of progesterone (Progestin; Samil Pharmacology, Seoul, Korea). Withdrawal bleeding occurred on 2 days after the last dose. The patient was given 10 000 IU of HCG (IVF-C; LG Chemical) s.c. on cycle day 13 based on the cycle length and the endometrial thickness. Oocyte retrieval was performed on day 15. Three MII stage and 29 GV stage oocytes were retrieved from both ovaries. Three MII oocytes were inseminated with conventional IVF using the husband's sperm. Three oocytes were fertilized (100%). All embryos developed to blastocysts on day 5 (one middle expanding blastocyst and two early blastocysts) and were transferred to the patient. The endometrial thickness was 10 mm on the day of embryo transfer. Two weeks after embryo transfer, serum β-HCG concentration was 165 IU/ml, and 4 weeks after embryo transfer, a pregnancy with single fetal heartbeat was confirmed by ultrasonography. Out of 29 GV oocytes, 12 and 11 (total 23) reached metaphase II stage after 24 and 48 h of incubation in the IVM medium respectively. Twenty-two oocytes (96%) were fertilized after ICSI, and five expanding blastocysts were observed at day 5 after insemination, and were cryopreserved.

Discussion

This study demonstrates that mature oocytes retrieved from women with PCOS following only HCG priming can undergo fertilization and development, and that the transfer of resulting blastocysts can establish pregnancies.

YS medium was designed to support the development of the human zygote to the blastocyst stage under the cumulus cell co-culture system (Yoon *et al.*, 2001a,b). The medium is without glucose and contains vitamins, amino acids and taurine (Yoon *et al.*, 2001a,b). We routinely use YS medium for blastocyst culture and have achieved an acceptable pregnancy rate for 6 years (Yoon *et al.*, 2001a; Yoon *et al.*, 2001b). Therefore, the zygotes derived from mature oocytes at the time of oocyte collection in IVM/F-embryo transfer cycles were also cultured to blastocysts in YS medium under the cumulus cell co-culture. In addition, we obtained 23.3% (7/30) of expanded blastocyst in embryos derived from IVM/IVF of immature oocytes collected from two patients. This strongly suggests that our IVM culture system is acceptable.

One of the important factors regulating the number and quality of oocytes maturing *in vitro* is the culture conditions used for IVM. The composition of most media used for human IVM is based on experiences with other mammalian species. Cekleniak *et al.* observed a higher maturation rate of immature oocytes in P1 medium without glucose than with TCM-199 containing glucose (Cekleniak *et al.*, 2001). We used YS medium without glucose for culture of the human immature oocytes. We supplemented gonadotrophins into IVM medium at a 1:10 ratio of rFSH:HCG, as this ratio of gonadotrophins was found to improve embryonic developmental competence (Anderiez *et al.*, 2000). In addition, EGF, a growth factor that demonstrated increasing maturation of immature oocytes, was added to the IVM medium. In our IVM/IVC culture system, we obtained 72.3% (34/47) of maturation and 23.3% (7/30) expanded blastocyst formation in two patients. High blastocyst rate (23.3%) from embryos derived from immature oocytes was obtained in this report. This indicates that the present IVM and IVC system may be very good, even though the numbers of patients were too small to draw significant conclusions.

For preparation of endometrium, we induced luteinization with HCG potentially to enhance the uterine and embryo synchrony, as suggested previously (Barnes *et al.*, 1995). Two administrations of HCG were given to the patient at 36 h before and at the time of oocyte recovery. This may aid the collection of metaphase II oocytes and the synchronizing of embryo development with the endometrium.

To the best of our knowledge, only one case report has demonstrated thus far that early blastocyst stage and pregnancy can be obtained from immature oocytes recovered from a

PCOS patient (Barnes *et al.*, 1995). This is the first report to obtain mature oocytes by HCG priming and also establish a pregnancy by transfer of blastocysts derived from mature oocytes. This approach potentiality opens a new dimension in the management of patients with PCOS.

Acknowledgements

The authors are grateful to Wei-Hua Wang and Li Meng, Division of Reproductive Medicine and Infertility, Department of Obstetrics and Gynecology, Brown University School of Medicine, Rhode Island, USA, for their critical review of the manuscript.

References

- Anderiesz, C., Ferraretti, A., Magli, C. *et al.* (2000) Effect of recombinant human gonadotrophins on human, bovine and murine oocyte meiosis, fertilization and embryonic development *in vitro*. *Hum. Reprod.*, **15**, 1140–1148.
- Barnes, F.L., Crombie, A., Gardner, D.K. *et al.* (1995) Blastocyst development and birth after *in vitro* maturation of human primary oocytes, intracytoplasmic sperm injection and assisted hatching. *Hum. Reprod.*, **10**, 3243–3247.
- Barnes, F.L., Kausche, A., Tiglias, J. *et al.* (1996) Production of embryos from *in vitro*-matured primary human oocytes. *Fertil. Steril.*, **65**, 1151–1156.
- Cekleniak, N.A., Combelles, C.M.H., Ganz, D.A. *et al.* (2001) A novel system which improves *in vitro* maturation of human oocytes. *Fertil. Steril.*, **74**, S32.
- Chain, R.C., Buckett, W.M., Tulandi, T. *et al.* (2000) Prospective randomized study of human chorionic gonadotrophin priming before immature oocyte retrieval from unstimulated women with polycystic ovarian syndrome. *Hum. Reprod.*, **15**, 165–170.
- Chi, H.J., Kim, D.H., Koo, J.J. *et al.* (1998) The suitability and efficiency of human follicular fluid as a protein supplement in human *in vitro* fertilization programs. *Fertil. Steril.*, **70**, 871–877.
- Cobo, A.C., Requena, A., Neuspiller, F. *et al.* (1999) Maturation *in vitro* of human oocytes from unstimulated cycles: selection of the optimal day for ovum retrieval based on follicular size. *Hum. Reprod.*, **14**, 1864–1868.
- Ménézo, Y., Nicollet, B., Herbaut, N. *et al.* (1992) Freezing cocultured human blastocysts. *Fertil. Steril.*, **58**, 977–980.
- Mikkelsen, A.L., Smith, S.D. and Lindenberg, S. (1999) *In vitro* maturation of human oocytes from regularly menstruating women may be successful without follicle stimulating hormone priming. *Hum. Reprod.*, **14**, 1847–1851.
- Nagy, Z.P., Cecile, J., Liu, J. *et al.* (1996) Pregnancy and birth after intracytoplasmic sperm injection of *in vitro* matured germinal-vesicle stage oocytes: case report. *Fertil. Steril.*, **65**, 1047–1050.
- Russell, J.B., Knezevich, K.M., Fabian, K.F. *et al.* (1997) Unstimulated immature oocyte retrieval: early vs. mid follicular endometrial priming. *Fertil. Steril.*, **67**, 616–620.
- Trounson, A., Wood, C. and Kausche, A. (1994) *In vitro* maturation and the fertilization and developmental competence of oocytes recovered from untreated polycystic ovarian patients. *Fertil. Steril.*, **62**, 353–362.
- Trounson, A., Bongso, A. and Szell, A. (1996) Maturation of human and bovine primary oocytes *in vitro* for fertilization and embryo production. *Singapore J. Obstet. Gynaecol.*, **27**, 78–84.
- Yoon, H.G., Yoon, S.H., Son, W.Y. *et al.* (2001a) Alternative embryo transfer of day 3 or day 5 for reducing the risk of multiple gestations. *J. Assist. Reprod. Genet.*, **18**, 188–193.
- Yoon, H.G., Yoon, S.H., Son, W.Y. *et al.* (2001b) High implantation and pregnancy rates with transfer of human hatching blastocysts developed on day 6. *Fertil. Steril.*, **75**, 832–833

Received on June 8, 2001; accepted on September 17, 2001