Pregnancies and deliveries after transfer of human blastocysts derived from in vitro matured oocytes in in vitro maturation cycles

A total of 82 patients underwent 106 cycles in which immature oocytes were recovered after hCG priming and transfered at blastocyst-stage. After transfer, the implantation rate was 26.8%, and the clinical pregnancy rate was 51.9%. We found that the oocytes retrieved after hCG priming in women with high risk of ovarian hyperstimulation syndrome in the in vitro maturation program can develop to blastocyst, and pregnancies can be established by transfer of the blastocysts. (Fertil Steril® 2007;xx:xxx. ©2007 by American Society for Reproductive Medicine.)

In vitro maturation (IVM) is an attractive option to eliminate several problems of controlled ovarian hyperstimulation (COH) used for conventional in vitro fertilization (IVF). Recent studies have shown improved pregnancy rates per embryo transfer in small numbers of cycles (1–3). In addition, several IVF centers have reported that acceptable rates of oocyte maturation and pregnancies were achieved in patients with polycystic ovary syndrome (PCOS) by hCG priming (3). However, they reported that the pregnancies were achieved by transfer of day 2 or day 3 embryos and that the implantation rate was less than 15%. Therefore, more embryos in an IVM program had to be transfered to obtain acceptable pregnancy rates than in COH cycles.

Only some cases of blastocyst ET and pregnancy derived from immature oocytes collected from unstimulated ovaries have been reported (4–7). Therefore, it is questionable whether the transfer of blastocyst-stage embryos is beneficial in an IVM program as much as in a COH program. We report the outcome experience with oocytes retrieved after hCG priming in women with high risk of ovarian hyperstimulation syndrome (OHSS) in the IVM program and allowing the embryos to develop to blastocysts.

Approval for the IVM program was obtained from the Institutional Review Board of the Maria Infertility Hospital. A total of 82 patients (mean age 32.2 ± 3.7 years) underwent 106 cycles in which immature oocytes were recovered. Patients with PCOS or PCO-like ovaries were recruited. The oocytes were collected between cycle days 9 and 18 based on the patient's cycle length and endometrial thickness. The patients were given 10,000 IU hCG (IVF-C; LG Chemical, Seoul, Korea) 36 hours before oocyte retrieval. Oocyte recovery was performed according to the protocol described previously (5–7). After collection, oocyte maturity was evaluated using a sliding method, and immature oocytes were cultured in maturation medium, consisting of YS medium with 30% human follicular fluid (hFF) supplemented with 1 IU/mL FSH, 10 IU/mL hCG, and 10 ng/mL recombinant human epiermal growth factor (rhEGF; Daewoong Pharmaceutical Co., Seoul, Korea) (5–7). The hFF was prepared using the method reported by Chi et al. (8). The oocytes were cultured in IVM medium at 37°C in 5% CO_2, 5% O_2, and 90% N_2. Intracytoplasmic sperm injection was used to fertilize the mature oocytes. The zygotes were cocultured with cumulus cells in 10μL YS medium supplemented with 10% hFF (9). Cumulus cells for coculture were prepared as described previously (9). Embryos were transfered at blastocyst stage on day 6 after oocyte retrieval (day 0). Blastocyst transfers were performed in patients who had more than 7 zygotes and 3 or more good-quality embryos on day 3 after oocyte collection. The remaining patients were allotted to day 4 transfers owing to the possibility of their not producing blastocyst-stage embryos in vitro. One to three blastocysts were transfered depending on quality.

For the preparation of the endometrium, 6 mg E_2 valerate (Progynova; Schering, Berlin, Germany) was administered daily from the day after oocyte retrieval. Progest, 100 mg, was administered daily starting on day of oocyte retrieval if MII was retrieved or starting 1 day after oocyte retrieval if there was no mature egg on day of retrieval. Both medications were continued until either a negative pregnancy urine test or a positive fetal heartbeat was observed.

Table 1 shows the clinical results of the human blastocyst transfer after culture to blastocysts from 2PN derived from IVM cycles. Out of 106 cycles, 79 cycles collected MII-stage eggs on the day of oocyte collection. A total of 2,563 oocytes were retrieved, and 2,004 oocytes (78.2%) became mature (15.4% on day of oocyte retrieval, 48.3% on day 1, 13.0% on day 2, and 1.4% on day 3 after culture).

Received March 22, 2006; revised and accepted November 9, 2006.
Reprint requests: Weon-Young Son, Ph.D., McGill Reproductive Center, Department of Obstetrics and Gynecology, Royal Victoria Hospital, McGill University, Montreal, Quebec H3A 1A1 (FAX: 514–843–1496; E-mail: weon-young.son@muhc.mcgill.ca).

TABLE 1

Clinical results for patients receiving blastocysts developed after IVM.

Variable	Value
No. of patients (age ± SD)	82 (32.2 ± 3.7)
No. of cycles	106
No. of oocytes (mean ± SD)	2,563 (24.4 ± 9.9)
No. of oocytes matured (%)	2,004 (78.2)
No. of oocytes fertilized (%)	1,614 (80.5)
No. of embryos developed to blastocyst (%)	671 (41.6)
No. of blastocyst transferred (mean ± SD)	313 (2.95 ± 0.2)
No. of implantation (%)	84 (26.8)
No. of clinical pregnancies (%)	55 (51.9)

Son. Pregnancies after blastocyst transfer in IVM cycles. Fertil Steril 2007.

Out of these, 1,614 were normally fertilized (80.5 %) and 671 (41.6 %) were developed to blastocysts. After transfer, the implantation rate was 26.8% (84 out of 313) and the pregnancy rate was 51.9% (55 our of 106).

The clinical pregnancy was the same between cycles with MII oocytes on the day of egg collection (51.9%, 41 out of 79) and cycles without MII (14 out of 27). The embryos in one cycle failed to develop to blastocyst stage even by day 7, resulting in the morulae stage of embryo transfer. Twenty-four male and 33 female infants (14 sets of twins and 29 singletons) from 43 patients were born, and 12 cycles had spontaneous abortion at 6–25 weeks of gestation. There was no triplet pregnancy. Birth weights of the infants were within the range of 1,540–4,200 g, and all delivered infants had a normal physical profile. Out of 521 IVM cycles, 415 cycles were transfered at cleavage stage during this period, and the clinical pregnancy and implantation rates were 28.4% (118 out of 415) and 9.7% (173 out of 1785), respectively (Table 2). The triplet rate was 9.3% (11 out of 118), because the mean number of embryos transfered was 4.3.

There are only some reports that blastocyst ET and pregnancies derived from immature oocytes collected from unstimulated ovaries are possible (4–7). There is no doubt that blastocyst transfer is a way to obtain a high implantation rate while eliminating triplets. However, the main problem of blastocyst-stage embryo transfer is the possibility of failure to produce blastocyst-stage embryos. The current culture systems may be good for culture to blastocysts from embryos generated from COH cycles, but they may still be suboptimal for supporting later development of the embryos generated in IVM cycles, even though new culture systems and media are currently being developed. Barnes et al. (4) were the first to report successful development to the blastocyst stage in sequential culture medium designed specifically to optimize blastocyst development. A pregnancy resulted from the transfer on day 6 of a single blastocyst after assisted hatching. However, only one of six embryos produced in that case report was competent to develop to the blastocyst stage. Hwu et al. (10) reported that embryos derived from IVM oocytes when cultured in human tubal fluid alone arrest at the cleavage stage. However, 30% of 2PN embryos developed to blastocyst when embryos were cocultured with human ampullary cells. Cobo et al. (11) similarly demonstrated 48.6% blastocyst development of embryos derived from IVM oocytes when embryos were cocultured with endometrial epithelial cells. These reports imply that the coculture is the best option for now to obtain blastocysts in an IVM program. Therefore, we applied the cumulus cells coculture system to produce blastocysts from oocytes retrieved from IVM cycles, and we obtained 41.6% blastocyst formation and a high implantation rate (26.8%) after ET. This indicates that the present IVM/in vitro culture system may be acceptable, even though the patients who had transfered blastocysts were included in this study.

To reduce the possibility of a triplet pregnancy and the failure of blastocyst development, we encouraged IVM

TABLE 2

Clinical results for patients receiving cleavage embryos after IVM.

Variable	Value
No. of patients (age ± SD)	311 (33.1 ± 4.1)
No. of cycles	415
No. of oocytes (mean ± SD)	5,865 (14.1 ± 5.8)
No. of oocytes matured (%)	4,340 (74.0)
No. of oocytes fertilized (%)	3,476 (80.1)
No. of embryos transfered (mean ± SD)	1,785 (4.3 ± 1.3)
No. of implantation (%)	173 (9.7)
No. of clinical pregnancies (%)	118 (28.4)

Son. Pregnancies after blastocyst transfer in IVM cycles. Fertil Steril 2007.

patients with more than seven zygotes and three or more good-quality embryos on day 3 to undergo blastocyst transfer and the remainder to undergo cleavage embryo transfer. Only 1 cycle out of 106 cycles failed to develop to blastocyst stage, but there was no triplet pregnancy after embryo transfer at blastocyst stage. Therefore, we propose that determination of the stage at which embryos should be transfered based on the number and quality of embryos on day 3 may help to obtain an acceptable pregnancy rate while minimizing failed blastocyst development and triplet multiple gestations.

We have reported that the oocytes matured in vivo in hCG-primed IVM cycles have better embryonic developmental competence, and in vitro matured oocytes in these cycles having matured oocytes on retrieval day could also implant in the same cycle (7). In the present study, the four out of seven patients who had transfered embryos derived from only IVM oocytes because in vivo matured oocytes were not fertilized had pregnancy. These results imply that the immature oocytes retrieved from cohort follicles have viability, even those mature oocytes collected from follicles with a diameter of ~14 mm. Therefore, we think that the developmental competence of immature oocytes is not compromised by the presence of MII-stage oocytes at the time of egg retrieval. However, further studies are necessary to see when hCG priming may be critical, allowing as many follicles as possible to reach sufficient size to obtain MII-stage oocytes on the day of collection while avoiding a prolonged negative effect on the developing dominant follicles.

In conclusion, the present study suggests that transfer of human blastocysts in an IVM program is a clinically useful method to select the more viable embryos without risk of triplet gestations. This approach potentiality opens a new dimension in the management of patients who had many good quality embryos generated from IVM cycles, and the patients should be offered blastocyst culture to give them the best chance of a successful implantation. To our knowledge, this is the largest number of pregnancies achieved by transfer of blastocysts derived from IVM cycles. Subsequent studies on clinical and laboratory procedures should be undertaken for further optimizing IVM programs.

Weon-Young Son, Ph.D.[a]
Seok-Yoon Lee, M.Sc.[b]
San-Huyn Yoon, Ph.D.[b]
Jin-Ho Lim, M.D.[b]
[a] *McGill Reproductive Center, Department of Obstetrics and Gynecology, Royal Victoria Hospital, McGill University, Montreal, Canada; and* [b] *Maria Infertility Hospital, Seoul, Korea.*

REFERENCES

1. Mikkelsen AL, Smith SD, Lindenberg S. In-vitro maturation of human oocytes from regularly menstruating women may be successful without follicle stimulating hormone priming. Hum Reprod 1999; 14:1847–51.
2. 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;15:165–70.
3. Chian RC. In-vitro maturation of immature oocytes for infertile women with PCOS. RBM Online 2004;8:547–52.
4. Barnes FL, Crombie A, Gardner DK, Kausche A, Lacham-Kaplan O, Suikkari AM, et al. Blastocyst development and birth after in-vitro maturation of human primary oocytes, intracytoplasmic sperm injection and assisted hatching. Hum Reprod 1995;10:3243–7.
5. Son WY, Yoon SH, Lee SW, Ko Y, Yoon HG, Lim JH. Blastocyst development and pregnancies after in vitro fertilization of mature oocytes retrieved from unstimulated patients with PCOS after in vivo hCG priming. Hum Reprod 2002;17:134–6.
6. Son WY, Park SJ, Hyun CS, Lee WD, Yoon SH, Lim JH. Successful birth after transfer of blastocysts derived from oocytes of unstimulated woman with regular menstrual cycle after IVM approach. J Assist Reprod Genet 2002;19:541–3.
7. Son WY, Lee SY, Lim JH. Fertilization, cleavage and blastocyst development according to the maturation timing of oocytes in in vitro maturation cycles. Hum Reprod 2005;20:3204–7.
8. Chi HJ, Kim DH, Koo JJ, Chang SS. The suitability and efficiency of human follicular fluid as a protein supplement in human in vitro fertilization programs. Fertil Steril 1998;70:871–7.
9. Yoon HG, Yoon SH, Son WY, Kim JG, Im KS, Lim JH. Alternative embryo transfer of day 3 or day 5 for reducing the risk of multiple gestations. J Assist Reprod Genet 2001;18:188–93.
10. Hwu YM, Lee RK, Chen CP, Su JT, Chen YW, Lin SP. Development of hatching blastocysts from immature human oocytes following in-vitro maturation and fertilization using a co-culture system. Hum Reprod 1998;13:1916–21.
11. Cobo AC, Requena A, Neuspiller F, Aragonés M, Mercader A, Navarro J, et al. Maturation in vitro of human oocytes from unstimulated cycles: selection of the optimal day for ovum retrieval based on follicular size. Hum Reprod 1999;14:1864–8.