In Vitro Maturation of Human Immature Oocytes in Culture Medium Supplemented with Patient's Own Serum or Donor Follicular Fluid

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Abstract: *In this study, to avoid the virus contamination in the maturation medium by addition of donor follicular fluid (FF) from other in vitro fertilization (IVF) cycles, we examined the outcome of in vitro maturation (IVM) protocol using medium containing patient's own serum. Data were retrospectively collected from 68 irregularly cycling women (31 ± 3.6 years) who were diagnosed as having polycystic ovarian syndrome. Immature oocytes were retrieved transvaginally 36 h after an injection of 10,000 IU hCG. Immature oocytes were matured for 24–26 h in medium supplemented with 10% (v/v) heat-inactivated patient's own serum (group S) or with 20% (v/v) heat-inactivated FF from donors who had undergone IVF cycles (group FF). All mature oocytes were inseminated by intracytoplasmic sperm injection. There were no statistical differences in the rates of maturation (49.2% vs. 45.6%), fertilization (85.0% vs. 84.2%), and pregnancy (16.0% vs. 15.4%) between group S and group FF. The supplementation of patient's own serum to the maturation medium as a substitute for follicular fluid collected from other patients did not affect the outcome of fertility treatment. Thus, we are able to eliminate the risk of transferring infectious diseases by using the patient's own serum.*

Key words: *Human, In vitro maturation, Follicular fluid, Patient's own serum*

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Introduction

Trounson et al. reported a successful pregnancy following IVM of immature oocytes that were collected from a woman with anovulatory menstruation [1]. After this report, the number of IVM treatment cycles increased gradually. IVM of immature oocytes has potential an alternative to conventional IVF treatment. Especially, IVM-IVF has been used as an option in assisted reproductive technology (ART) for polycystic ovary (PCO) patients. However, IVM protocol has been performed in only a limited number of clinics.

Culture medium for IVM is usually supplemented with proteins. The commonly used protein sources in early IVM of human immature oocytes were fetal cord serum [2, 3] and fetal bovine serum [1, 4–8].

The presence of these additional factors during IVM has been shown to be important for the subsequent development of the oocytes after IVF [9]. However, there would be some possibilities of virus infections; HIV and HCV, and of prion contamination if FF from other stimulated patients were supplemented. Therefore, human serum albumin [10, 11] or synthetic serum substitute [12–14] has been used as a protein supplement instead of FF. However, there have been no comparative studies of FF and serum during *in vitro* maturation of human immature oocytes. Moreover, the collection of pure FF in stimulation cycles is laborious, and may also lead to ethical issues.

In this study, we assessed the outcome of IVM protocol using medium supplemented with 10% (v/v) patient's own serum or 20% (v/v) donor FF collected from other stimulated patients.

Table 1. Clinical outcome of oocytes matured in medium supplemented with patient's own serum (Group S) or with donor follicular fluid (Group FF) after IVM, ICSI and ET

Treatments (No. of patients)	Age of patients Mean ± SD (year)	Years of infertility Mean ± SD (year)	Number (%) of			Number (%) of embryo transfer trials	Number (%) of pregnancies after embryo transfer
			Collected oocytes	Matured oocytes	Zygotes with 2PN		
Group S (37)	32 ± 3.0	4.0 ± 3.0	433	213 (49.2)	181 (85.0[a])	25 (67.6)	4 (16.0)
Group FF (55)	31 ± 3.9	3.3 ± 2.7	651	297 (45.6)	250 (84.2[a])	39 (70.9)	6 (15.4)

[a] The percentages of zygotes per matured oocytes.

Materials and Methods

Sixty-eight polycystic ovarian syndrome (PCOS) patients who underwent 92 IVM cycles were included in this analysis. The patients (average age: 31 ± 3.6 years) had irregular menstrual cycles. PCOS was defined by ultrasound examination showing more than 10 follicles in one plane, and by hormone analysis showing elevated LH/FSH ratio (>1.0) or elevated free-testosterone levels (>3.0 pg/ml). There were no differences in the background of patients between group S and group FF in age (32 ± 3.0 years vs. 31 ± 3.9 years), or years of infertility (4.0 ± 3.0 years vs. 3.3 ± 2.7 years) (Table 1). Patients gave their informed consent before this study.

To initiate the treatment cycle, the patient received norethisterone-mestranol (Norluten-D®, Shionigi Pharmaceutical, Osaka, Japan). On the eighth day of withdrawal bleeding, small ovarian follicles were monitored by transvaginal ultrasonography (Toshiba, Tokyo, Japan), to confirm that there was no dominant follicle. Endometrial thickness was more than 8 mm. The patient was given 10,000 IU of hCG (Profasi, Serono Japan, Tokyo, Japan) 36 h before oocyte retrieval [15]. Immature oocytes were aspirated with 17–19 gauge double needles (FS-IVF OSAKA-IVM2; Kitazato supply, Shizuoka, Japan) under the guidance of transvaginal ultrasonography. A portable aspiration pump was used with a pressure of 300 mmHg. The aspirates were collected in 10 ml tubes containing pre-warmed heparinized human tubule fluid (HTF) medium, and the oocytes were isolated under a stereomicroscope. Degenerated or atretic oocytes were discarded. All oocyte handling procedures were conducted in a mini-chamber under 5% CO_2 in air at 37.5°C.

Retrieved immature oocytes were cultured for 24–26 h in the maturation medium (IVM®; MediCult) with 75 IU/ml FSH (Fertinorm-P, Serono Japan, Tokyo, Japan) and 100 IU/ml hCG (Profasi, Serono Japan, Tokyo, Japan) at 37.5°C in an atmosphere of 5% CO_2, 5% O_2 and 90% N_2 as previously reported [16]. The medium was supplemented with 10% (v/v) patient's own serum (group S) or 20% (v/v) donor follicular fluid (group FF). The oocytes were denuded with 10 units/ml hyaluronidase (Sigma Chemical, St. Louis, MO, USA) and mechanical pipetting after maturation culture. Mature oocytes were identified by the presence of the first polar body.

To prepare the serum, blood was collected in a sterilized glass tube from the patient within 12 h after hCG administration. It was allowed to coagulate at room temperature, and then centrifuged at 1,600 g for 5 min. Supernatant was collected and inactivated for 30 min at 56°C in a water bath, then added to the maturation medium [10% (v/v), group S]. The FF was collected from a donor without HIV, HBV or HCV, who had undergone a stimulation cycle at an age of less than 30 years old, and had become pregnant in that cycle. The FF was collected in a sterilized glass tube and immediately centrifuged at 1,600 g for 5 min. Supernatant was collected and inactivated for 30 min at 56°C in a water bath. After cooling, the FF was kept at –80°C until use. Frozen-thawed supernatant FF was added to the medium [20% (v/v), group FF].

Sperm were prepared by 90% Percoll separation at 300 g for 20 min. After Percoll separation, motile sperm were collected using the swim-up method with 10 ml of culture medium (universal IVF®; MediCult). All MII oocytes were inseminated by ICSI. The presumed zygotes were then cultured in the culture medium (Universal IVF®; MediCult), and fertilization was assessed 16-18 h after ICSI by the appearance of two distinct pronuclei and two polar bodies. Fertilized zygotes were transferred into culture medium (IVC-1®, In Vitro Care, MD, Frederick, USA). Day 3 embryos were transferred on the fourth day after oocyte retrieval. Pregnancy was determined as the detection of more than 25 IU/ml hCG in urine 14 days after embryo transfer (ET).

Statistical Analysis

The statistical significance of the results was evaluated by the χ^2 test ($p<0.05$ was considered to be statistically significant).

Results and Discussion

In this study, we assessed the outcome of IVM protocol using medium supplemented with 10% (v/v) patient's own serum or 20% (v/v) donor FF collected from another stimulated patient. Our data show there were no differences in the rates of maturation (49.2% vs. 45.6%), fertilization (85.0% vs. 84.2%), ET cancellation (32.4% vs. 29.1%), and pregnancy (16.0% vs. 15.4%) (Table 1) between groups S and FF.

Fetal bovine serum [1, 4–8], serum from the patient [17, 18], synthetic serum substitute [12–14] and human serum albumin (HSA) [10, 11] have been used as protein supplements in the maturation medium of human immature oocytes. The presence of these additional factors in IVM medium has been shown to be important for the subsequent development of oocytes after IVF [9]. However, there are possibilities of virus infections; HIV and HCV, and of prion contamination if donor FF or animal sources are used. Thus, patient's own serum is preferable as a protein supplementation in the IVM medium. However, there have been no comparative studies of serum and FF during IVM of human immature oocytes. Thus, we compared the outcome of fertility treatment using oocytes that were matured with own serum or with donor FF.

Patient's own serum is easily available and has the theoretical advantage of no contaminations of foreign viruses. However, the use of patient's own serum results in a wide variation of serum components that depend on health conditions, sampling at endocrinological timings, and so on.

Cha *et al.* has shown that the rates of maturation and fertilization were increased by addition of mature follicular fluid into Ham's F-10, compared with addition of fetal cord serum [2]. The difference of the preparation and the origin of serum might explain the contradictory results between Cha *et al.* [2] and our present study. Moreover, the births of several healthy babies were obtained in our recent study using patient's own serum as a protein source [16, 19, 20]. Thus, we believe that patient's own serum is beneficial for supporting the developmental competence of human immature oocytes during IVM.

The results of the present study suggest that supplementation of patient's own serum to maturation medium for IVM-IVF had a similar effect to the supplementation of FF from stimulated donors. In conclusion, IVM protocol supplemented with patient's own serum avoids the risk of transferring infectious diseases without decrease in outcomes of fertility treatment.

References

1) Trounson, A., Wood, C. and Kaunsche, A. (1994): In vitro maturation and fertilization and developmental competence of oocytes recovered from untreated polycystic ovarian patients. Fertil. Steril., 62, 353–362.
2) Cha, K.Y., Koo, J.J., Ko, J.J., Choi, D.H., Han, S.Y. and Yoon, T.K. (1991): Pregnancy after in vitro fertilization of human follicular oocytes collected from nonstimulated cycles, their culture *in vitro* and their transfer in a donor oocyte program. Fertil. Steril., 55, 109–113.
3) Hwu, Y-M., Lee, R.K., Chen, C-P., Su, J.T., Chen Y.W. and Lin, S.P. (1998): Development of hatching blastocysts from immature human oocytes following in-vitro maturation and fertilization using a co-culture system. Hum. Reprod., 13, 1916–1921.
4) Barnes, F.L., Crombie, A., Gardner, D.K., Kausche, A., Lacham-Kaplan, O., Suikkari, A.M., Tiglias, J., Wood, C., and Trounson, A.O. (1995): Blastocyst development and birth after in-vitro maturation of human primary oocytes, intracytoplasmic sperm injection and assisted hatching. Hum. Reprod., 10, 3243–3247.
5) Barnes, F.L., Kausche, A., Tiglias, J., Wood, C., Wilton, L. and Trounson, A. (1996): Production of embryos from in vitro-matured primary oocytes. Fertil. Steril., 65, 1151–1156.
6) Cha, K.Y., Han, S.Y., Chung, H.M., Choi, D.H., Lim, J.M., Lee, W.S., Ko, J.J. and Yoon, T.K. (2000): 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., 73, 978–983.
7) Chian, R.C., Buckett, W.M., Tulandi, T. and Tan, S.L. (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.
8) Suikkari, A-M., Tulppala, M., Tuuri, T., Hovatta, O. and Barnes, F. (2000): Luteal phase start of low-dose FSH priming of follicles results in an efficient recovery, maturation and fertilization of immature human oocytes. Hum. Reprod., 15, 747–751.
9) Pinyopumminter, T. and Bavister, B.D. (1991): In virto matured/in vitro fertilized bovine oocytes can develop into morulae/blastocysts in chemically defined, protein-free culture media. Biol. Reprod., 45, 736–742.
10) Wynn, P., Picton, H.M., Krapez, J.A., Rutherford, A.J., Balen, A.H. and Gosden, R.G. (1998): Randomized study of oocytes matured after collection from unstimulated or

mildly stimulated patients. Hum. Reprod., 13, 3132–3138.
11) Cobo, A.C., Requena, A., Neuspiller, F., Aragon, S.M., Mercader, A., Navarro, J., Simon, C., Remohi, J. and Pellicer, A. (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.
12) Jaroudi, K.A., Hollanders, J.M.G., Sieck, U.V., Roca, G.L., EL-Nour, A.M. and Coskun, S. (1997): Pregnancy after transfer of embryos which were generated from in-vitro matured oocytes. Hum. Reprod., 12, 857–859.
13) Russell, J.B., Knezevich, K.M., Fabian, K.F. and Dickson, J.A., (1997): Unstimulated immature oocyte retrieval: early versus midfollicular endometrial priming. Fertil. Steril., 67, 616–620.
14) Whitacre, K.S., Seifer, D.B., Friedman, C.I., Coskun, S., Kennard, E.A., Kim, M.H. and Alak, B.M. (1998): Effects of ovarian source, patients age, and menstrual cycle phase on *in vitro* maturation of immature human oocytes. Fertil. Steril., 70, 1015–1021.
15) Chian, R.C., Gulekli, B., Buckett, W.M. and Tan, S.L. (1999): Priming with human chorionic gonadotropin before retrieval of immature oocytes in women with infertility due to the polycystic ovary syndrome. N. Engl. J. Med., 341, 624–1626.
16) Hashimoto, S., Fukuda, A., Murata, Y., Kikkawa, M., Oku,H., Kanaya, H., Sonoda, M., Sugihara, K., Murata, T., Nagata, F., Nakaoaka, Y. and Morimoto, Y. (2007): Effect of aspiration vacuum on the developmental competence of immature human oocytes retrieved using a 20-gauge needle. RBMOnline, 14, 444–449.
17) Mikkelsen, A.L., Smith, S. 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.
18) Mikkelsen, A.L., Smith, S. and Lindenberg, S. (2000): Impact of oestradiol and inhibin A concentrations on pregnancy rate in in-vitro oocyte maturation. Hum. Reprod., 15, 1685–1690.
19) Kanaya, H., Murata, Y., Oku, H., Hashimoto, S., Morimoto, Y., Murata, T., Sugihara, K., Nagata, F., Nakaoka, Y. and Fukuda, A. (2006): Successful monozygotic twin delivery following in-vitro maturation of oocytes retrieved from a woman with polycystic ovary syndrome: a case report. Hum. Reprod., 21, 1777–1780.
20) Hashimoto, S., Murata, Y., Kikkawa, M., Sonoda, M., Oku, H., Murata, T., Sugihara, K., Nagata, F., Nakaoka, Y., Fukuda, A. and Morimoto, Y. (2007): Successful delivery after the transfer of twice-vitrified embryos derived from in vitro matured oocytes: a case report. Hum. Reprod., 22, 221–223.