Short Communication

In Vitro Oocyte Maturation in Polycystic Ovarian Syndrome Patients

Fatemeh Ramazanzadeh, M.D., Mansur Malek Aksa, M.D., Said Amanpour, Ph.D., Maryam Bagheri, M.Sc., Mina Jafarabadi, M.D., Maryam Nazm Bojnordi, M.Sc.*

Vali-e-asr Reproductive Health Research Center, Tehran University of Medical Science

Abstract

Prevalence of Polycystic Ovarian Syndrome(PCOS) in Iran is more than 6%. Therefore we encounter with many PCOS patients. In Vitro Maturation (IVM) of oocytes as an attractive method in ART is considered. Studies show that changes in culture conditions should be administered to make IVM protocol more successful .For this purpose in this study we have set up the beneficial cultures for IVM procedure. Fourteen PCOS patients received FSH, 75 IU or 150 IU per day for 3 days initiating on day 3 of menstruation. Oocyte retrieval was performed transvaginally using an ultrasound-guided 17-gauge single lumen needle and filtered through a 70 micron gauge filter. Viable oocytes were put to maturation in TCM-199 supplemented with 10% Patient serum, recombinant FSH, pyruvate, penicillin, streptomycin sulphate and human chorionic gonadotropin..Oocytes were then inseminated by ICSI. The results indicated that 43.4% of oocytes matured to metaphase II. After 48 hours 47.5 % of M II oocytes fertilized by ICSI and cleaved to 2- and 4-cell stage. No pregnancy observed in PCOS patients. The oocytes maturation rate (43.4%) and embryo formation (47.5 %) from immature oocytes obtained in our IVM and ICSI culture system indicate that the present system may be nearly good, even though the number of patients were too small to draw significant conclusions.

Keywords: IVM, PCOS Patients, FSH, hCG

Patients with polycystic ovarian syndrome (PCOS) are characterized by abnormal endocrine parameters, anovulation, numerous antral follicles within their ovaries and frequently infertility (1-3). PCOS patients may be extremely sensitive to exogenous gonadotrophin and at risk of ovarian hyperstimulation syndrome when treated with gonadotrophins for assisted reproduction (4-6). In vitro maturation (IVM) of immature oocytes from PCOS patients would be an attractive option to eliminate this problem and immature oocyte recovery could be developed as a new method for treatment of patients with infertility due to PCOS, because the oocytes of these patients retain their maturational and developmental competence (6-9). In fact in this method oocytes are typically retrieved from cycles which are nearly similar to natural cycles, then matured in vitro and fertilized by intracytoplasmic sperm injection (10-13). But reported implantation and pregnancy rates of this method are much lower than what achieved by IVF after controlled ovarian hyperstimulation (COH) (13). The benefit of this method is that low hormonal stimulation is required, and less physical risks are encountered (14). Because IVM works best in patients with many antral follicles in the minimally stimulated ovaries, the procedure might have an even greater benefit for women with PCOS (15). Polycystic ovary syndrome patients, who have also the risk of recruitment of poor-quality embryos after COH/ICSI might benefit IVM in another way (16).

Studies show that alterations in culture conditions must occur to make IVM protocol more successful (17, 18). For this purpose in this study, we set up the beneficial cultures for IVM procedure. In our center the cases were selected from PCOS patients who were at high risk of OHSS during stimulation process for IVM/ICSI. PCOS was detected using ultrasonograghic imaging or clinical situation such as dismenorrhea with obesity, oily skin or hirsutism. Patients received FSH, 75 IU or 150 IU daily for 3 days initiating on day 3 of menstruation being continued according to Transvaginal ultrasonograghy monitoring. Oocyte retrieval was performed when at least one leading follicle of 10-14mm

Received: 12 May 2007, Accepted: 10 Nov 2007
* Corresponding Address: P.O.Box: 14194, Vali-e-asr Reproductive Health Research Center, Tehran University of Medical Science, Tehran, Iran
Email: bojnordim@yahoo.com

Royan Institue
Iranian Journal of Fertility and Sterility
Vol 1, No 3, Nov-Dec 2007, Pages: 131-134

was observed. Oocyte retrieval was performed transvaginally using an ultrasound-guided 17-gauge single lumen needle connected to a syringe in order to induce an aspiration pressure of with 60-180 mm Hg. The follicular aspirates were collected into tubes containing pre-warmed Ham's F10 with l0IU/L heparin. Follicular aspirates were filtered through a 70 micron gauge filter to remove erythrocytes and small cellular debris. Viable oocytes were put to maturation in TCM-199 supplemented with 10% patient serum, recombinant FSH, pyruvate, penicillin, streptomycin sulphate and human chorionic gonadotrophin.

For the last sixth cases Midcult culture medium which had the same ingredient was used. All oocytes and embryos cultures were performed under paraffin oil at 37°C and 5% CO_2. Oocytes were matured 32-36 hours after aspiration when the presence of the first polar body (Metaphase II oocytes) will be considered as sign of maturation. Oocytes were then inseminated by ICSI. Embryonic development to 8 cell stage usually takes place at 3 days after insemination. During this period of time the speed and morphological parameters were evaluated and grades of embryos were determined.

Embryo transfer was performed 2 or 3 days after insemination. Hormonal supplementation included estradiol valerate 6 mg daily plus progesterone vaginal suppositories 400 mg BD (Cyclogest). Pregnancy test was performed by serum hCG measurement two weeks after embryo transfer. Clinical pregnancies was confirmed by ultrasound examination and observing gestational sac. The results indicated that 43.4% of oocytes cultured in vitro matured to metaphase II. After 48 hours 47.5% of M-II oocytes fertilized by ICSI (Table 1 and 2). Except for one chemical pregnancy, no clinical pregnancy has been achieved from PCOS patients in our center. These indicate that immature oocyte recovery from women with infertility due to PCOS can be used for assisted reproduction.

Same as pervious researches, this study demonstrates that abnormally matured oocytes retrieved from women with PCOS can undergo fertilization and development, and that the transfer of resulting embryoes can establish pregnancies (16).

One of the important factors regulating the number and quality of oocytes maturing ***in vitro*** is the culture conditions used for IVM (19). The composition of most media used for human IVM is based on experiences with other mammalian species (18). Researches show a higher maturation rate of immature oocytes, in TCM-199 supplemented with 10% patient serum, recombinant FSH, pyruvate, penicillin, streptomycin sulphate and human chorionic gonadotrophin (18, 19). We supplemented gonadotrophins into IVM medium to improve embryonic developmental competence. Clinical studies on non stimulated IVM, report implantation rate of approximately 8% and from this mature oocytes pregnancy rate of approximately 25% (13-15).

Table 1: The number of oocyte and maturation state after OPU (day 0)

M1	GV	Follicle Nnumber	Patient number
4	4	8	1
1	2	3	2
1	1	2	3
-	3	3	4
-	-	-	5
4	1	5	6
5	2	7	7
2	1	3	8
3	1	4	9
3	2	5	10
10	2	12	11
17	3	20	12
3	1	4	13
5	2	7	14
5	4	9	15

Regardless of whether exogenous ovarian stimulation occurs, one can assume that crucial events are occurring within the follicle during maturation that are necessary for oocytes to obtain their full developmental potential (17) . More studies are required to determine what changes in culture conditions need to occur to make IVM more successful (18). In the meantime, IVM does offer certain patients the chance of pregnancy (16). Results of studies show that 65% of oocytes cultured in medium with gonadotrophins, oestrogen and fetal calf serum matured to metaphase II by 43-47 h, and

81 % were matured at 48-54 h of culture (17-19). Of the inseminated oocytes, 34% fertilized and 56% of the cultured pronuclear oocytes cleaved to eight cells or more.

Table 2: In vitro maturation and fertilization of the oocytes

ET Day 5	PN (After 72 hours)	M2 (After 48 hours)	Patient Number
2	2	4	1
-	-	1	2
-	-	1	3
-	-	-	5
-	-	-	6
1	1	2	6
1	1	1	7
-	-	2	8
-	-	3	9
3	5	2	10
2	4	5	11
3	3	8	12
1	1	2	13
2	4	4	14
2	4	5	15

The oocytes maturation rate (43.4%) and embryo formation (47.5%) from immature oocytes obtained in our IVM and ICSI culture system indicate that the present system maybe nearly good, even though the number of patients was too small to draw significant conclusions.

References

1. Piquette GN. The in vitro maturation (IVM) of human oocytes for in vitro fertilization (IVF): is it time yet to switch to IVM-IVF. Fertil Steril 2006; 85(4): 833-835
2. 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: 188-193
3. 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: 3243-3247
4. 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
5. Nagy ZP, Cecile J, Liu J. Pregnancy and birth after intracytoplasmic sperm injection of in vitro matured germinal-vesicle stage oocytes: case report. Fertil Steril, 1996; 65: 1047-1050
6. Cekleniak NA, Combelles CM, Ganz DA, Fung J, Albertini DF and Racowsky C. A novel system which improves in vitro maturation of human oocytes. Fertil Steril. 2001; 74: 532
7. Menezo Y, Nicollet B, Herbaut N, Andre D. Freezing cocultured human blastocysts. Fertil Steril, 1992; 58: 977-980
8. 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
9. Russell JB, Knezevich KM, Fabian KF, Dickson JA. Unstimulated immature oocyte retrieval: early vs. mid follicular endometrial priming. Fertil Steril. 1997; 67: 616-620
10. Chi HJ, Kim DH, Koo JJ, Anderiesz C, Ferraretti A, Magli C. Effect of recombinant human gonadotrophins on human, bovine and murine oocyte meiosis, fertilization and embryonic development in vitro. Hum Reprod. 2000; 15: 1140-1148
11. Cobo AC, Requena A, Neuspiller F, Aragones 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: 1864-1868
12. Trounson A, 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
13. Yoon HG, Yoon SH, Son WY, Kim JG, Im KS, Lim JH. High implantation and pregnancy rates with transfer of human hatching blastocysts developed on day 6. Fertil Steril, 2001; 75: 832-833
14. Lanzendorf Susan E. Developmental potential of in vitro– and in vivo–matured human oocytes collected from stimulated and unstimulated ovaries. Fertil Steril. 2006; 85(4): 836-837
15. Trounson A, Wood C, Kauche A. In vitro maturation and fertilization and developmental competence of oocytes recovered from untreated polycystic ovarian patients. Fertil Steril 1994; 62: 353-362
16. Weon S, Yong K, Hye Jin L. Blastocyst development and pregnancies after IVF of mature oocytes retrieved from unstimulated patients with PCOS after in-vivo HCG priming: Case report,Human Reproduction. 2002; 17(1): 134-136
17. Roberts R, Franks S, Hardy K. Culture environment modulates maturation and metabolism of human oocytes. Hum Reprod 2002; 17: 2950–2956
18. Li Y, Feng HL, Cao YJ, Zheng GJ, Yang Y, Mullen S, et al. Confocal microscopic analysis of the spindle and chromosome configurations of human oocytes matured in vitro. Fertil Steril 2006; 85: 827-832

19. Trounson A, Bongso A, Szell A. Maturation of human and bovine primary oocytes in vitro for fertilization and embryo production. Singapore J. Obstet Gynaecol. 1996; 27: 78-84