The First Pregnancies in Turkey Following in Vitro Ooctye Maturation

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OBJECTIVE: To report pregnancies that resulted from the intracytoplasmic sperm injection (ICSI) of in vitro matured oocytes derived from two unstimulated, anovulatory patients with polycystic ovary syndrome.
STUDY DESIGN: Two women with history of infertility and PCOS underwent in vitro maturation (IVM) program without controlled ovarian hyperstimulation. The patients were primed with 10.000 IU HCG 36 h before oocyte retrieval. Oocytes-cumulus masses were matured in IVM medium. The matured oocytes were fertilized by ICSI and embryo transfer was performed on day 3.
RESULTS: For the first patient thirty-four GV-stage oocytes were collected, twenty two of them reached MII stage following 24-48 hour in vitro maturation, 17 of mature oocytes were fertilized by ICSI and the embryos were cultured for 3 days. Four embryos were transferred on day 3. In the second patient, 7 of 9 GV-stage oocyte retrieved were matured in vitro, 4 of them were fertilized by ICSI and 4 embryos were transferred on day 3. Both of the patients achieved clinical pregnancy. The first pregnancy resulted in abortion in 6th gestational weeks while the other one is still ongoing singleton pregnancy (nine weeks of gestation)
CONCLUSIONS: Immature oocyte collection combined with IVM is a convenient option for infertile couple with PCOS compared to conventional assisted reproductive therapy.
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Key Words: In vitro maturation, Intracytoplasmic sperm injection, Clinical pregnancy, PCOS

There are many disadvantages of assisted reproductive technology (ART) treatment. First of them is the high cost.[1] The second one is that current ovarian stimulation protocols, though they last fewer days after the introduction of gonadotropin-releasing hormone (GnRH) antagonists, are still not patient-friendly. The other one is that the patients receive injections and undergo several blood samples and transvaginal ultrasound scans. Besides these, especially in patients with polycystic ovarian syndrome (PCOS) the development of several follicles is associated with a high risk of ovarian hyperstimulation syndrome.[2] In addition, gonadotropin therapy in IVF may have long term effects on children born and women health (such as cancer).[3,4] The high steroid hormone levels during ovarian stimulation may cause low implantation rate.[5]

In order to deal with these disadvantages, immature oocyte retrieval from unstimulated ovaries in combination with in vitro maturation (IVM) and fertilization is a feasible alternative for the management of infertile couples especially for PCOS patients. Benefits of IVM include simple and less time consuming protocols, no or minimal use of fertility drugs, and reduced cost of treatment. On the other hand, ovarian hyperstimulation syndrome (OHSS) risk is entirely avoided. The first IVM of human oocytes was reported in 1965, while the first fertilization of in vitro matured oocytes was reported in 1966 by the same authors.[6,7] Thereafter the first birth was achieved in 1983.[8] Since then, infertile women with polycystic ovaries or PCOS form the main category of patients in whom IVM applied for clinical purposes .[9]

The aim of the present study is to report our two successful clinical pregnancies occurring after IVM of oocytes derived from non-stimulated cycles in women PCOS.

Material and Methods

Patients

Two patients with PCOS managed at Clinic Women Health, Infertility and IVF Centre, Ankara, Turkey were enrolled in this report. Institutional review board approval was obtained for this study. All had signed consent form after receiving information on the goals and modalities of the IVM procedure.

The diagnosis of PCOS in our two patients was based on their history of anovulation and oligomenorrhea, the appearance of polycystic ovaries on ultrasonography and increased serum testosterone levels.[10]

Monitoring

Follicle development on the ovaries and determination of endometrial thickness were monitored by transvaginal ultrasonography beginning on Cycle Day 3. Transvaginal ultraso-

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nography scans were repeated around the sixth and eighth day of the cycle to exclude the development of dominant follicle. Human chorionic gonadotropin (10.000 IU IM, Pregnyl 5000 IU, Organon, Istanbul, Turkey) was administered when the follicle size reached 7 mm in diameter before the selection of dominant follicle and 36 h prior to oocyte collection.

Oocyte collection

Transvaginal oocyte retrieval was scheduled 36 hours after HCG injection between Cycle Days 7 and 13 after the follicle had reached 10 mm in diameter. During the collection, patients recieved a mild i.v. sedation with propofol. A transvaginal ultrasound machine with 19-gauge aspiration needle, special for IVM pick-up (Cook, Eight Mile Plains, Queensland, Australia) was used to aspirate follicles. A portable aspiration pump was used with a pressure of 7.5 kPa. The aspirates were collected in tubes containing IVM flushing medium (MediCult, Mollehaven, Denmark). Follicular aspirates were isolated under a stereomicroscope and washed in the culture medium (MediCult, Mollehaven, Denmark).

IVM-ICSI and sperm preparation

The cumulus-oocyte complexes were preincubated in the LAG medium (MediCult, Mollehaven, Denmark) than placed in a central wells of a Falcon 3037 culture dish (Becton, Dickinson, USA) containing 1 ml maturation culture medium(IVM medium) (MediCult, Mollehaven, Denmark) supplemented with 20% inactivated maternal serum at 56 ^{0}C and 0.75 IU FSH and 0.75 IU LH (Menogon, Ferring, Turkey).[11] Immature oocytes were cultured in IVM medium at 37^{0}C in an atmosphere of 5% CO_2, 5% O_2, and 90% N_2. Nuclear maturation was assessed at 24-48h after culture under the dissecting microscope.

Ejaculate semen samples were collected at the day following follicular puncture. After liquefaction at 370C, semen samples were analyzed according to the World Health Organization guidelines (1999). Spermatozoa were prepared by sperm preparation medium (MediCult, Mollehaven, Denmark)

ICSI was performed in MII-stage oocytes after 24-48h of culture. Fertilization was assessed 18 h after ICSI to detect the appearance of two distinct pronuclei and two polar bodies. ISM I (MediCult, Mollehaven, Denmark) was used for embryo culture medium and UTM (MediCult, Mollehaven, Denmark) was used for embryo transfer.

Endometrial preparation and embryo transfer

For endometrial preparation, Estradiol valerate (6 mg) (Cyclo-progynova, Schering, Istanbul, Turkey) was administered daily from the day of oocyte retrieval. Progesterone (100 mg) (Progestan, Kocak, Istanbul, Turkey) was administered daily from day 1 after oocyte retrieval. Both medications were continued until a fetal heartbeat was positively identified.

Embryos were transferred on day 3 after ICSI by the transcervical route in standard fashion under ultrasound guidance. A transvaginal ultrasound scan was carried out on the day scheduled for the transfer to ensure that the endometrial thickness was >7 mm.

Clinical pregnancy was defined as an intrauterine gestation with a fetal heartbeat seen by transvaginal ultrasound scan.

Results

For the first patient thirty-four GV-stage oocytes were collected, twenty two of them reached MII stage following 24 hour in vitro maturation, 17 of mature oocytes were fertilized by ICSI and the embryos were cultured for 3 days. Four embryos were transferred on day 3. In this patient, four of thirty-four GV-oocytes reached MII stage following 48 h maturation but none of them fertilized. In the second patient, 7 of 9 GV-stage oocyte retrieved were matured in vitro following 24 h, 4 of them were fertilized by ICSI and 4 embryos were transferred on day 3. Both of the patients achieved clinical pregnancy. The first pregnancy resulted in abortion in 6th gestational weeks while the other one is still ongoing singleton pregnancy (nine weeks of gestation)

Clinical and laboratory characteristics of patients are given in Table 1.

Table 1. Patients' clinical and laboratory characteristics.

Characteristics	Case 1	Case 2
Women age	29	26
Infertility duration (year)	5	6
HCG injections day	7	8
Number of oocytes retrieved	34	9
24h maturation		
Oocyte reaching MII	22	7
Oocyte fertilized (2 PN)	17	4
48h maturation		
Oocyte reaching MII	4	-
Oocyte fertilized (2 PN)	-	-
Embryo transfer		
Total number of embryo	16	4
Number of embryo transfer	4	4
Quality of embryo (number of cleaved cells)	8, 6, 6,5	5, 4, 4, 3
Clinical pregnancy	Yes	Yes
Outcome of pregnancy		
Abortus	Yes (6 wks)	-
Ongoing pregnancy	-	Yes (9 wks)

* All pregnancies were singleton. Data are presented as numbers. 2PN; two pronuclei.

Discussion

It is well known that no more than 400 follicles reach ovulation throughout women's reproductive ages. Almost 99 percent of oocytes are destroyed through atresia. In in vivo folliculogenesis, the primordial follicles enter the growth phase progressively and the diameter of the oocyte increases from 30 to 120 µm by granulosa cell proliferation and theca cell differentiation. During growth both active transcription and translation occur in order to accumulate proteins that are essential for later stages of oocyte maturation. Oocyte maturation includes nuclear (meiotic resumption and progression to MII stage) and cytoplasmic (production and secretion of proteins and gylcoproteins, increase in cytoplasmic organels, formation of new structures) events.[12]

Recovery of immature oocytes from women with unstimulated cycles, followed by in vitro maturation of these oocytes is a possible treatment modality for women with infertility and has been known since 1965.[6] Although it has been known for forty years, no more than 300 healthy infants have been born following immature oocytes retrieval and in vitro maturation.[13] The reasons for decreased popularity of IVM by reproductive endocrinologist may the technical difficulty of puncture of immature oocytes that are <9mm, low cost, need for experienced clinician and laboratory team, and also availability of IVM culture mediums. However, low rate of implantation and clinical pregnancy rates compared to controlled ovarian hyperstimulation and ICSI/ET cycles may be another reason of decreased popularity until 1990s.[13,14]

Although recent studies have shown increased pregnancy and implantation rates, the pregnancy rate after IVM of oocytes is low. The implantation and clinical pregnancy rates in patients with PCO or PCOS were reported in between 6.9-27.2%, and 20.0-53.8%, respectively.[14,15] The possible reasons may be suboptimal culture conditions during IVM or inadequate cytoplasmic maturation of the oocytes themselves or low number of immature oocyte retrieval.

The best results are obtained in patients with PCO/PCOS in whom the presence of numerous antral follicles are easily assessed by transvaginal sonography.[16] Another group of patients that may benefit from IVM are the higher responder during ovarian stimulation for in vitro fertilization..[9] However, IVM technology may be expanded for regular cycling women with normal ovaries (natural cycle in vitro maturation), poor responders and also for patients whom use of gonadotropins is contraindicated.[13] In the current report, all patient underwent IVM had PCOS.

There have been many efforts to improve the implantation and pregnancy rates. One of them is to optimize the quality of oocytes by mild ovarian stimulation with FSH or human menopausal gonadotropin. The rationale of FSH priming is the hope of obtaining more immature oocytes or enhancing oocytes maturation.[17] The second one is administering HCG before retrieval of immature oocytes for IVM. In the first prospective study, it was reported as oocytes maturation was hastened in the HCG priming group compared with non- HCG priming group.[18] Although the value FSH priming is still unclear, the HCG priming prior to immature oocytes retrieval has several positive effect on clinical pregnancy rates of IVM. In our report, all pregnant cases had HCG priming while only one case had FSH priming.

Another concern about IVM success is the timing of oocytes retrieval. Although there have been conflicting results, current practice suggests oocytes retrieval timing in a follicular range between 8-12 mm.[9,19] In our practice, oocytes retrieval was performed in a follicular diameter >10 mm. There have been still controversies on oocytes aspiration technique. However, ultrasound guided transvaginal aspiration of immature oocytes with reduced vacuum, under propofol sedation is still recommended by many authorities.[17,18]

The most important factor affecting IVM pregnancy rates may be the composition of culture medium. FSH and LH are usually added to the culture medium. Cultured medium for human IVM is also supplemented with serum. Significantly increased maturation and implantation/pregnancy rates have been reported in culture medium with serum supplementation.[20] In our study serum supplemented culture medium was also used as suggested in the literature. In addition to other features in order to improve the implantation rates, endometrial preparation by administration of exogenous estrogen and progesterone after oocytes retrieval is necessary.[9]

This is the first report in Turkey demonstrates that human immature oocytes retrieved from women with PCOS can undergo maturation and fertilization, and that the resulting embryos can establish clinical pregnancies.

In conclusion, immature oocytes retrieved from women with PCOS are good candidates for in vitro maturation. The immature oocytes obtained from women with unstimulated cycles can fertilize and cleave and the resulting embryos can also establish pregnancies. To increase the pregancy and implantation rates, further prospective studies are needed to define the best conditions for both clinical and laboratory procedure.

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