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Natural-cycle in vitro fertilization combined with in vitro maturation of immature oocytes is a potential approach in infertility treatment

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Objective: To describe pregnancies and live births that resulted from IVF of mature oocytes retrieved from dominant follicles in a natural cycle combined with in vitro maturation (IVM) of immature oocytes retrieved from small follicles.

Design: Case reports.

Setting: McGill Reproductive Center, Royal Victoria Hospital, McGill University.

Patient(s): Three women with normal ovaries or polycystic ovaries who underwent infertility treatment.

Intervention(s): Administration of SC hCG (10,000 IU) 36 hours before oocyte retrieval in a natural cycle. After aspiration of all follicles, mature oocytes were inseminated immediately; immature oocytes were matured in vitro, inseminated by intracytoplasmic sperm injection (ICSI), and then the embryos transferred.

Main Outcome Measure(s): Pregnancy and live birth.

Result(s): Three pregnancies (two live births and one ongoing at time of writing) were achieved after the combination of natural-cycle IVF with IVM after transfer of the resulting embryos.

Conclusion(s): Natural-cycle IVF combined with IVM might be a new approach to IVF treatment for women with various causes of infertility. (Fertil Steril® 2004;82:1675–8. ©2004 by American Society for Reproductive Medicine.)

Key Words: Natural cycle, IVF, hCG, immature oocytes, in vivo maturation, pregnancy

The first successful pregnancy and live birth resulting from IVF occurred during an unstimulated natural menstrual cycle (1). Soon thereafter, natural-cycle IVF treatment was replaced by IVF cycles in which ovarian stimulation was applied because the success rate of IVF treatment has been found to be related to the number of embryos available for transfer. The side effects of ovarian stimulation with gonadotropins, including ovarian hyperstimulation syndrome, have been well described. However, the long-term side effects of ovarian stimulation with protocols involving a GnRH agonist or antagonist in combination with gonadotropins are largely unknown. Although these problems are not encountered in natural-cycle IVF treatment, a number of other problems arise, including an increased risk of no oocyte retrieved during oocyte retrieval and no embryo available for transfer. Nevertheless, there has been a resurgence of interest in natural-cycle IVF treatment in recent years because of the increased efficiency of IVF technology.

It has been demonstrated that immature oocyte retrieval followed by in vitro maturation (IVM) of these oocytes is a successful treatment for infertile women with polycystic ovaries (PCO) and that the administration of hCG 36 hours before the harvesting of immature oocytes improves the maturational and developmental competence of the oocytes, resulting in higher pregnancy rates (2). In our standard protocol for IVM treatment, the treatment cycle

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is canceled if a dominant follicle is seen in the ovaries on the day-8 transvaginal ultrasound scan, and the sizes of all follicles have to be <10 mm in diameter. This is because we initially believed that the dominant follicle would suppress development of other follicles and would induce follicular and oocyte atresia. However, recent studies in the animal model indicate that the maturational and developmental competence of immature oocytes derived from the small antral follicles is not affected by the presence of a dominant follicle or phase of folliculogenesis (3).

We describe herein three successful pregnancies and subsequent live births achieved in patients who developed a dominant follicle during IVM treatment. Our results indicate that mature and immature oocyte retrieval followed by IVF combined with IVM might be a potential treatment for women with various causes of infertility.

CASE REPORTS

The initial IVM study was approved by the research institute of the hospital. The IVM treatment was explained to the patients, and informed consent was obtained. Natural-cycle IVF treatment combined with IVM was performed in three patients who were initially scheduled for IVM treatment only. The details of three patients who became pregnant are described below.

Case 1

A 40-year-old woman presented with regular menstrual cycles and a 5-year history of infertility. She had failed to become pregnant after six cycles of conventional IVF treatment over the preceding 3 years. On day 3 of her menstrual cycle a baseline ultrasound scan was performed, and she was noted to have a total of four follicles (the diameter of all follicles was ≤4 mm). On day 8 of her cycle, transvaginal ultrasound revealed that a dominant follicle (13 mm in diameter) had developed. The patient was given SC hCG (10,000 IU) (Profasi; Serono, Oakville, Ontario, Canada) on day 10, and oocyte retrieval was scheduled for 36 hours later, on day 12 of her menstrual cycle.

Oocyte retrieval was performed under IV sedation (2 mg midazolam and 175 μg fentanyl) and a paracervical block with 10 mL of 1% lidocaine. At the time of oocyte retrieval, the mean diameter of the dominant follicle was 19 mm, and the mean diameters of the other three follicles were 9 mm, 8 mm, and 6.5 mm, respectively. Transvaginal ultrasound–guided oocyte collection was performed with a specially designed 19-gauge single-lumen aspiration needle (K-OPS-7035-RWH-ET, length 35 cm, lot 547865; Cook, Queensland, Australia) with an aspiration pressure of 85 mm Hg for both the dominant follicle and the small follicles. Four follicles were aspirated from the two ovaries. Aspirates were collected in 10-mL culture tubes (Falcon, Franklin Lakes, NJ) containing 2 mL of warm 0.9% saline solution with 2 IU/mL of heparin (Baxter, Toronto, Ontario, Canada).

After oocyte collection, the maturity of the oocytes was determined by whether the oocytes contained germinal vesicle (GV) in their cytoplasm or if the first polar body had been extruded into the perivitelline space, as previously described (2). No oocyte was collected from the dominant follicle. However, a total of two immature oocytes (GV stage) were retrieved from the three small follicles. These immature oocytes were incubated in an organ tissue culture dish (60 × 15 mm; Falcon) containing 1 mL of modified IVM medium (4) supplemented with 20% patient's own serum (inactivated at 56°C for 30 minutes) with the final concentrations of 75 mIU/mL of FSH and LH (Humegon; Organon, Scarborough, Ontario, Canada) at 37°C in an atmosphere of 5% CO_2 in air and high humidity.

After maturation in culture for 24 hours, the oocytes were denuded from cumulus cells with finely drawn glass pipettes after 1 minute of exposure to 0.1% hyaluronidase solution (Medi-Cult, Hopkinton, MA). At this point, two oocytes were still immature (at metaphase I stage). After 48 hours of culture, two oocytes were noted to be at the metaphase II stage. Intracytoplasmic sperm injection (ICSI) was performed for insemination because we believe that ICSI can result in a higher fertilization rate than conventional IVF. Two oocytes were confirmed to be fertilized 16 hours after ICSI, and two embryos (one grade 2, four-cell-stage embryo and one grade 3, two-cell-stage embryo) were produced; they were transferred 48 hours after ICSI.

For the preparation of the endometrium, the patient was given 6 mg of E_2 (Estrace; Roberts Pharmaceutical, Mississauga, Ontario, Canada) in divided doses starting on the day of oocyte retrieval. Luteal support was provided in the form of intravaginal P (Prometrium; Schering, Pointe-Claire, Quebec, Canada) in a dose of 400 mg three times daily starting on the day of ICSI. On the day of ET, the endometrial thickness was 12.2 mm at transvaginal ultrasonography. Two weeks after ET, the serum β-hCG level was 374.23 IU/L, and 6 weeks after ET, an intrauterine singleton pregnancy with a fetal heartbeat was seen on transvaginal ultrasonography. An uneventful delivery at 39 weeks resulted in the birth of a normal healthy male infant (3,740 g).

Case 2

A 39-year-old woman presented with regular menstrual cycles and polycystic ovary–like morphology noted on pelvic ultrasonography. She had conceived and delivered a healthy female infant with IVM treatment 4 years earlier. In the preceding 2 years, she had failed to become pregnant after undertaking two cycles of conventional IVF treatment outside Canada. On day-3 baseline ultrasound scan, a total of 16 small follicles (<4 mm) were observed in both ovaries, with no cysts seen. On the day-8 transvaginal ultrasound scan, a dominant follicle (14 mm in diameter) was noted. The patient was given SC hCG (10,000 IU) (Profasi) 36 hours before oocyte retrieval.

Oocyte retrieval was performed on day 10 of the menstrual cycle. Transvaginal ultrasonographically guided oocyte collection was performed under local anesthesia, as described previously. At the time of oocyte retrieval, the mean diameter of the dominant follicle was 18 mm; three other follicles were 14 mm, 13 mm, and 12 mm in diameter respectively, whereas all the remaining follicles were <8 mm in diameter. Sixteen follicles were aspirated from both ovaries. A total of 10 oocytes were retrieved. At oocyte retrieval, 2 oocytes were mature (M-II stage), 2 oocytes were at metaphase I stage, and the remaining 6 oocytes were at GV stage.

The two mature oocytes were inseminated by ICSI 3 hours after collection. The two metaphase-I oocytes were matured 6 hours after culture in the modified IVM medium and inseminated by ICSI 8 hours after collection. The remaining six GV-stage oocytes were cultured for a further 48 hours, and two matured to the M-II stage and were inseminated by ICSI. After 18 hours, one oocyte had fertilized (1 of 2) from the first ICSI batch, two oocytes had fertilized (2 of 2) from the second ICSI batch, and one oocyte fertilized (1 of 2) from the third ICSI batch.

On day 2 after oocyte retrieval, three embryos chosen from the first two ICSI batches (one five-cell, grade 2 embryo, one four-cell, grade 2 embryo, and one four-cell, grade 3 embryo) were transferred. The remaining one embryo was discarded because it was of poor quality and not suitable for cryopreservation. The endometrial thickness was 11.5 mm on the day of ET. Two weeks after ET, the serum β-hCG level was 258.89 IU/L, and 6 weeks after ET, an intrauterine singleton pregnancy with a fetal heartbeat was seen at transvaginal ultrasonography. An uneventful delivery at 38 weeks resulted in the birth of a normal healthy female infant (3,659 g).

Case 3

A 32-year-old anovulatory woman with polycystic ovary syndrome (PCOS) presented with irregular menstrual cycles and a 3-year history of infertility. She had failed to become pregnant after six cycles of IUI with her husband's sperm combined with ovarian stimulation with clomiphene citrate and gonadotropins. To initiate the treatment cycle, the patient received intravaginal P (Prometrium) in a dose of 300 mg each night for 10 days to induce a withdrawal bleeding. No ovarian cysts were observed on day-3 baseline ultrasound scan, and a total of 25 follicles were seen in the two ovaries (all <4 mm in diameter). The largest follicle was 12 mm in diameter on the day-8 ultrasound scan. The endometrial thickness was 6.5 mm on the day-8 ultrasound scan, and SC hCG (10,000 IU) (Profasi) was administered.

Oocyte retrieval was performed on day 10, 36 hours after hCG administration. Transvaginal ultrasonographically guided oocyte collection was done under local anesthesia, as described previously. On the day of oocyte retrieval, the mean diameter of the largest follicle was 14 mm, and the mean diameters of the other four follicles were 13 mm, 12 mm, 12 mm, and 11.5 mm, respectively. The diameters of the remaining follicles were <8 mm. A total of 17 oocytes were retrieved (5 oocytes were at M-II stage, and 12 oocytes were at GV stage at oocyte collection).

Five mature oocytes were inseminated by ICSI 3 hours after collection. Twelve immature oocytes were incubated in the modified IVM medium. Seven oocytes became mature after 24 hours of culture, and a further three became mature after 48 hours. All metaphase-II oocytes were inseminated by ICSI.

After ICSI, four oocytes (4 of 5) from the first batch (day of oocyte collection), six oocytes (6 of 7) from the second batch (24 hours after collection), and none (0 of 3) from the third batch (48 hours after collection) were observed to have fertilized. A total of four embryos (all grade 3, six-cell-stage embryos) were transferred 72 hours after the first-batch ICSI. Five embryos from the remaining six were cryopreserved 48 hours after the second ICSI. The endometrial thickness was 9.4 mm on the day of ET. Two weeks after ET, the serum β-hCG level was 315.69 IU/L, and 6 weeks after ET, an intrauterine singleton pregnancy with a fetal heartbeat was seen at transvaginal ultrasonography. At the time of submission of this report, the patient had a 17-week ongoing pregnancy.

DISCUSSION

The results from these cases indicate that mature and immature oocyte retrieval followed by IVF combined with IVM is a potential treatment for women with various causes of infertility. Although it is a conventional belief that the development of a dominant follicle suppresses the remaining small follicles and induces atresia of these small follicles, the results of these cases demonstrate that developmental competence of the oocytes retrieved from these small follicles are not necessarily adversely affected by the presence of a dominant follicle during folliculogenesis. Therefore, it is appropriate to modify the current protocol of IVM treatment for women with ovulatory PCO-like ovaries or anovulatory PCOS and avoid canceling cycles if a dominant follicle develops.

Although immature oocytes are frequently retrieved in conventional IVF cycles after ovarian stimulation from follicles >14 mm in diameter, the results of the present study indicate that maturation can also occur in oocytes retrieved from follicles as small as 11.5 mm in diameter (case 3) 36 hours after hCG administration. This suggests that some small follicles can respond to an LH surge to trigger oocyte maturation, which might become fully mature in vivo. In a previous study of women with PCOS undergoing IVM treatment, when immature oocyte retrieval was performed 36 hours after hCG administration with follicles <12 mm diameter (2), it has been reported that approximately 46% of

oocytes had initiated the process of oocyte maturation and undergone germinal vesicle breakdown. This suggests the presence of LH receptors in these follicles, which could respond to hCG priming.

There is an increasing interest in natural-cycle IVF among patients, because it is simple, quick, and avoids the side effects associated with ovarian stimulation (5). However, natural-cycle IVF is also associated with a relatively high incidence of failure of oocyte retrieval, so that the pregnancy rate per cycle started is relatively low. Nevertheless, because natural-cycle IVF can be undertaken in successive cycles, the cumulative pregnancy and live birth rates might be as high as 46% and 32%, respectively, after four cycles of treatment (6). Some patients might prefer to undergo a number of successive natural IVF treatment cycles instead of having a single stimulated IVF cycle, which can only be repeated once every few months.

In women, although only a single follicle generally develops to the preovulatory stage and releases its oocyte for potential fertilization in each menstrual cycle, a number of small follicles often develop during the follicular phase. It has been estimated that approximately 20 antral follicles are selected and continue to the preovulatory stage of development during each menstrual cycle (7). Therefore, an attractive possibility to enhance the success of natural-cycle IVF treatment is to combine it with immature oocyte retrieval and IVM. If the mature oocyte from the dominant follicle together with immature oocytes from the small follicles were pooled, the chance of a pregnancy would be greatly increased if one managed to mature these immature oocytes and produce several viable embryos. This notion is supported by the results of the present case reports.

Recently, it has been reported that the clinical pregnancy and implantation rates of IVM treatment have reached approximately 30%–35% and 10%–15%, respectively, in infertile women with ovulatory PCO-like ovaries or anovulatory PCOS, on the basis of more than 1,000 cycles of IVM treatment in a multicenter study (8). Therefore, natural-cycle IVF combined with IVM might provide more efficient treatment for women with ovulatory PCO-like ovaries or anovulatory PCOS. Furthermore, it is possible to extend both the already-existing natural-cycle IVF and IVM treatment for women with various causes of infertility.

In addition, it has been proved that the pregnancy rate is not affected when the different developmental stages of embryos produced from IVM are pooled together and transferred in individual women (2). The results of the present case reports also indicate that it is possible to synchronize uterine endometrial receptivity and embryonic development for embryo implantation (case 1), although it is unclear which embryo implanted that the embryo was produced from either the dominant follicle or the smaller follicles (case 2). However, further research is required to achieve the correct synchronization between endometrium and embryo developmental stage to optimize the implantation rate of the embryos produced from natural-cycle IVF combined with IVM.

In conclusion, these case reports demonstrate that maturational and developmental competence of immature oocytes is not detrimentally affected by the presence of a dominant follicle during the follicular phase and that natural-cycle IVF combined with IVM might be a practical treatment for women with various causes of infertility undergoing treatment with assisted reproductive technologies.

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