

Selection of patients for natural cycle in vitro fertilization combined with in vitro maturation of immature oocytes

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Objective: To determine a successful method of selecting patients for natural cycle IVF combined with in vitro maturation (IVM) of immature oocytes and to determine treatment efficacy.

Design: Retrospective analysis of IVF treatments.

Setting: Hospital IVF clinic.

Patient(s): Women with infertility according to ovarian reserve and endocrine profile were selected for different treatments.

Interventions(s): According to screening criteria, infertile women were divided into three treatments: [1] natural cycle IVF combined with IVM (IVF/M), [2] IVM alone, and [3] controlled ovarian hyperstimulation (COH) with gonadotropin.

Main Outcome Measure(s): The distribution of completed IVF-ET cycles in each treatment; clinical pregnancy and implantation rates in each treatment.

Result(s): A total of 417 cycles were started, and 410 cycles (98.3%) were completed. Of 410 cycles, 151 (36.8%) were treated by natural cycle IVF/M, 63 (15.4%) underwent IVM alone, and 196 (47.8%) underwent COH. With increasing age fewer cycles can be treated by natural cycle IVF/M or IVM. Clinical pregnancy rates were 40.4% (61 of 151) for natural cycle IVF/M, 41.3% (26 of 63) for IVM alone, and 37.8% (74 of 196) for COH. There were no differences in implantation rate in the three groups (17.8% [82 of 462], 16.7% [35 of 210], and 20.1% [103 of 513]).

Conclusion(s): Natural cycle IVF/M together with IVM-alone treatment can offer more than 50% of infertile women with an acceptable pregnancy and implantations rates. (Fertil Steril® 2009;91:1050–5. ©2009 by American Society for Reproductive Medicine.)

Key Words: Natural cycle, IVF, IVM, oocytes, pregnancy

The first live birth after IVF was produced from natural cycle IVF (1). However, natural cycle IVF was slowly replaced by IVF with ovarian stimulation because it was believed that the number of oocytes retrieved relates to the embryos available for transfer, and this directly affects the probability of successful pregnancy (2–4). Initially the relatively inexpensive clomiphene citrate was used to stimulate ovaries to produce multiple follicles, but current ovarian stimulation protocols use the much more expensive GnRH agonist or antagonist in combination with gonadotropin to generate multiple follicles in the ovaries.

Some women are extremely sensitive to stimulation with exogenous gonadotropin and are at an increased risk of

developing ovarian hyperstimulation syndrome, a sometimes life-threatening condition (5). In addition, there is anxiety that the long-term side effects of repeated ovarian stimulation may increase the risk of ovarian, endometrial, and breast cancers (6). Although these problems are not encountered in natural cycle IVF, a number of other problems arise, including an increased risk of there being no oocyte retrieved during collection and no embryo available for transfer. Nevertheless, there is resurgence of interest in natural cycle IVF in recent years because the efficiency of IVF technology has been improved markedly (7, 8).

Immature oocyte retrieval followed by in vitro maturation (IVM) of these oocytes is an attractive infertility treatment for women with infertility. Compared with ovary-stimulated IVF, the major advantages of IVM include avoidance of the risk of ovarian hyperstimulation syndrome, reduced cost, and simplified treatment. Immature oocyte retrieval followed by IVM of these oocytes was initially shown to be a successful treatment for infertile women with polycystic ovary syndrome (PCOS) because there are numerous antral follicles within the ovaries in this patient group (9). Immature oocyte retrieval followed by IVM might be useful in up

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to approximately 30% of women undergoing IVF treatment who have large numbers of antral follicles, including patients with PCOS (10). Additionally, because of the low cost of therapy it is important to identify candidates for IVM among women with various causes of infertility. This strategy has been explored with natural IVF combined with IVM for women without PCOS (11–13). Therefore, it is important to evaluate the efficacy of natural cycle IVF combined with IVM as a clinical treatment for infertile women.

The objective of the present study was to establish screening criteria for patients who might consider natural cycle IVF combined with IVM and to determine the treatment's efficacy.

MATERIALS AND METHODS

Patients

From April 2005 to May 2007 a total of 378 patients with a minimum 2-year history of infertility underwent 417 cycles. All patients had a normal basal FSH level (<10 IU/mL on day 3 of menstrual cycle) and normal uterus. The mean (\pm SD) age of patients was 32.4 ± 4.2 years. The treatment protocol was approved by the institutional review board of Maria Fertility Hospital, and written informed consent was obtained from all patients.

Baseline Ultrasound Scan

The treatment cycle was initiated on the basis of an ultrasound scan on day 3–5. According to the baseline ultrasound scan, patients were divided into three treatment groups. Group 1 patients had regular menstrual cycles and a total of ≥ 7 antral follicles in both ovaries and were treated with natural cycle IVF combined with IVM (IVF/M). Group 2 patients had irregular menstrual cycles and ≥ 7 antral follicles in both ovaries and were treated with IVM alone (IVM). Group 3 patients had <7 antral follicles in both ovaries with either regular or irregular menstrual cycles and were treated with controlled ovarian hyperstimulation (COH) by gonadotropin with a standard protocol and IVF.

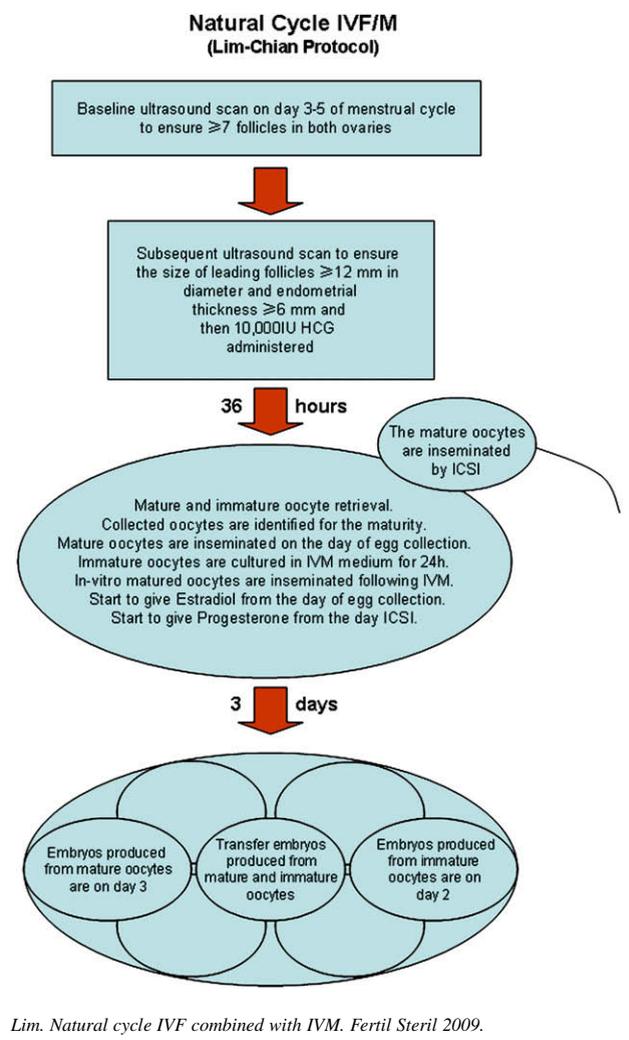
Treatments

For group 1 patients (IVF/M), transvaginal ultrasound scans were repeated on day 7–9 and repeated at 1–3-day intervals until the leading follicle reached 12–14 mm in diameter. At this point hCG was administered (10,000 IU IVF-C IM; LG Chem, Seoul, Korea), and oocyte retrieval was performed 36 hours later. The minimum endometrial thickness had to be ≥ 6 mm when hCG was administered. The brief protocol for natural cycle IVF/M is described in Figure 1.

For group 2 patients (IVM), on day 3–5 after the onset of menstrual bleeding either spontaneously or after administra-

FIGURE 1

Detailed protocol for natural cycle IVF combined with IVM.



tion of P to induce withdrawal bleeding, the patients underwent a baseline ultrasound scan, which was repeated on day 7–9. Human chorionic gonadotropin (10,000 IU) was administered when the endometrial thickness reached ≥ 6 mm, and immature oocyte retrieval was conducted 36 hours later.

For group 3 patients (COH), treatment cycles started with exogenous gonadotropins after a desensitization protocol with GnRH analogues according to a long or a short protocol, depending on the patient's previous history of gonadotropin response and other factors. An ultrasound scan was performed on day 7–9 and subsequently until one follicle reached 18 mm and two reached 16 mm in diameter, and then hCG (10,000 IU) was administered. Oocyte retrieval was performed approximately 36 hours after hCG administration.

Mature and Immature Oocyte Retrieval

Transvaginal ultrasound-guided aspiration was performed with a 17-gauge double-lumen needle (Cook, Eight Mile Plains, Queensland, Australia) for aspiration of the leading follicles and then with a 19-gauge single-lumen needle (Cook) for aspiration of small follicles. A portable aspiration pump was connected to the aspiration needle with a pressure <100 mm Hg. The aspirates were collected in tubes (10 mL) containing prewarmed heparinized Ham's F-10 medium buffered with *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES). Cumulus–oocyte complexes (COCs) from the leading follicle verified its maturity. The maturity of oocytes at the time of oocyte retrieval was evaluated under a stereomicroscope with a “sliding” technique (9). Briefly, as a COC slowly slides from one side to another on the bottom of a tissue culture dish (60 × 15 mm BD Falcon; BD, Franklin Lakes, NJ) it is observed under a microscope. The oocyte cytoplasm will be clearly observed to contain (or not) a germinal vesicle (GV) during COC sliding. If an immature oocyte did not contain a GV, the oocyte was defined as GV breakdown. Oocyte maturation was assessed by the presence of the first polar body in the perivitelline space.

IVF and IVM

The mature oocytes collected at the time of oocyte retrieval were subjected to insemination 2 or 3 hours later by intracytoplasmic sperm injection (ICSI). The immature oocytes (metaphase I and GV stage) were transferred into an organ culture dish (60 × 15 mm BD Falcon) containing 1 mL of maturation medium (Maria Fertility Hospital, Seoul, South Korea) supplemented with the final concentrations of 75 mIU/mL of FSH and LH at 37°C in 5% CO₂, 5% O₂, and 90% N₂ with high humidity for maturation in culture. After 1 day of culture, all COCs were denuded of the cumulus cells with 0.03% hyaluronidase (Sigma, St Louis, MO) in HEPES buffered Ham's F-10 medium and mechanical pipetting. In vitro matured oocytes were also inseminated by ICSI. Fertilization was assessed 17–19 hours after ICSI to detect the appearance of two distinct pronuclei and two polar bodies. The zygotes were cultured in 20 μL of embryo culture medium (Maria Fertility Hospital) for further developmental culture.

ET and Endometrial Preparation

Embryo transfer was performed on day 3 after oocyte retrieval. Before transfer all embryos for each patient were pooled together and selected for transfer. For endometrial preparation, estradiol valerate (6 mg Progynova; Schering, Korea) was administered daily starting on the day of oocyte retrieval, and daily luteal support (injection of 100 mg P in oil [Progest]; Samil Pharmaceutical, Ansan, Korea) was started on the day of initial ICSI. Pregnancy was determined by the level of serum β-hCG on day 15 or 16 after oocyte retrieval, and clinical pregnancy was determined by visualization of a gestational sac on ultrasound scan 6 weeks after ET.

Statistical Analysis

The data were analyzed for agreement with κ statistics using StatsDirect 1.9.14 for Windows (StatsDirect, Cheshire, United Kingdom). For evaluation of the differences among groups, clinical pregnancy, implantation, and miscarriage rates were analyzed by the χ^2 test. Results are expressed as mean differences were considered statistically significant at $P < .05$.

RESULTS

At total of 378 patients started 417 treatment cycles, and 371 patients completed 410 treatment cycles (Table 1). Of 410 cycles, 36.8% (151) were treated by natural cycle IVF/M, 15.4% (63) by IVM alone, and 47.8% (196) by COH.

Figure 2 shows the completed cycles for each treatment group according to age. In patients aged <30 years, 50.0% of cycles (46 of 92) were treated by natural cycle IVF/M, 20.7% (19 of 92) were treated by IVM alone, and 29.3% (27 of 92) were treated by COH. With increasing patient age there was a tendency for fewer cycles to be treated by natural cycle IVF/M or IVM alone. In patients aged ≥40 years, only 5.7% of cycles (2 of 35) were treated by natural cycle IVF/M and 5.7% (2 of 35) by IVM alone, whereas 88.6% (31 of 35) were treated by COH.

As shown in Table 1, the clinical pregnancy rates was 40.4% (61 of 151) with natural cycle IVF/M, 41.3% (26 of 63) with IVM alone, and 37.8% (74 of 196) with COH. There were no significant differences among groups. The implantation rate was also not significantly different among the three groups (17.8% [82 of 462], 16.7% [35 of 210], and 20.1% [103 of 513]). However, the miscarriage rate was significantly higher in the IVM group (38.5% [10 of 26]) compared with the other two groups (27.9% [17 of 61] and 24.3% [18 of 74]).

DISCUSSION

Recently considerable changes are afoot regarding “routine” IVF. As mentioned by Edwards (14, 15), two modified forms of treatment are attracting attention. One is minimal-stimulation IVF using low-dose gonadotropin, and another is IVM. Apart from these attractive treatments, there is an increasing interest in natural cycle IVF among patients, primarily because it is more comfortable and has fewer side effects, particularly the unknown long-term effects of repeated ovarian stimulation with GnRH and gonadotropin.

Published pregnancy rates per ET in natural cycle IVF vary between 0 and 30% (16–18). A number of problems arise in natural cycle IVF, including an increased risk of empty retrieval during oocyte collection, leading to cancellation of the treatment cycle. This disadvantage seems not to be present with natural cycle IVF/M. We have demonstrated that natural cycle IVF/M is an efficient treatment with an acceptable pregnancy rate (11–13). However, to

TABLE 1**Clinical outcomes in patients treated by IVF/M, IVM alone, or COH.**

Variable	IVF/M	IVM	COH
No. of cycles started (patients)	153 (140)	63 (56)	201 (182)
No. of cycles completed (patients)	151 (138)	63 (56)	196 (177)
Age (y), mean \pm SD	31.4 \pm 3.7	31.4 \pm 4.0	34.4 \pm 4.8
No. of oocytes retrieved (mean \pm SD)	1,826 (12.1 \pm 5.6)	1,017 (16.1 \pm 10.3)	2,079 (10.6 \pm 6.6)
Oocytes matured	1,216 (66.6)	658 (64.7)	1,875 (90.2)
Oocytes fertilized	996 (81.9)	519 (78.9)	1,485 (79.2)
Zygotes cleaved	928 (93.2)	469 (90.4)	1,429 (96.2)
No. of embryos transferred (mean \pm SD)	462 (3.1 \pm 0.8)	210 (3.3 \pm 0.6)	513 (2.5 \pm 0.4)
Clinical pregnancies obtained	61 (40.4)	26 (41.3)	74 (37.8)
Embryos implanted	82 (17.8)	35 (16.7)	103 (20.1)
Live births	21 (34.4)	8 (30.8)	35 (47.3)
Singleton	18	7	21
Twin	3	1	14
Ongoing pregnancies	23 (37.7)	8 (30.8)	21 (28.4)
Miscarriages	17 (27.9)	10 (38.5) ^a	18 (24.3)

Note: Values are number (percentage) unless otherwise noted.

^a Significantly different compared with the other two groups ($P < .05$).

Lim. Natural cycle IVF combined with IVM. Fertil Steril 2009.

date it is unknown how many patients can be treated by natural cycle IVF/M. The results of the present study indicate that more than a half of infertile women receiving IVF can be treated with natural cycle IVF/M or IVM alone when

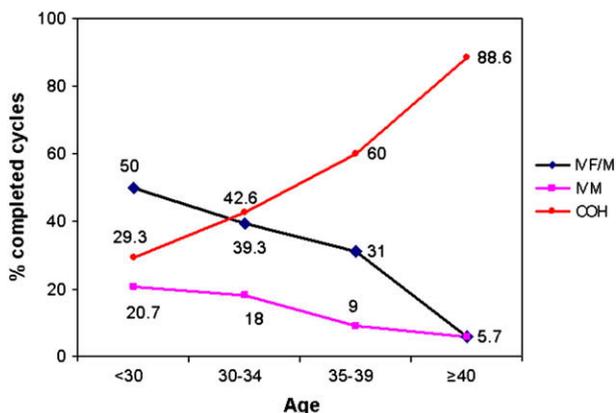
these treatments have been chosen primarily and that natural cycle IVF/M is an efficient treatment, especially for women aged <35 years (Fig. 2).

In addition, it was reported that the cumulative probability of pregnancy was 46%, with an associated live birth rate of 32%, after four natural cycles of IVF treatment when life-table analysis was performed to calculate the cumulative success rates after successive cycles of treatment (7). Although the pregnancy rate is lower in natural cycle IVF compared with ovary-stimulated IVF cycles, the implantation and birth rates per started cycle seem almost similar in both treatments (8). The results from the present study clearly demonstrate that natural cycle IVF/M is a more efficient treatment than natural cycle IVF alone. Interestingly, the pregnancy rate obtained from natural cycle IVF/M was 40.4%, and there was no significant difference compared with the other two treatment groups (Table 1).

In vitro maturation is offered as a successful option to infertile women with PCOS, because there are many antral follicles in the ovaries in this patient group. In general the clinical pregnancy and implantation rates have reached 30%–35% and 10%–15%, respectively, in infertile women with PCOS (19). Polycystic ovary syndrome is the most widespread endocrinologic disorder among women of reproductive age, as well as the most common cause of anovulatory infertility, and has been shown to exist in approximately 10% of the general population (20). The results

FIGURE 2

Percentages of completed treatment cycles in the natural cycle IVF/M, IVM alone, or COH groups, according to age. In patients aged <30 years and 30–34 years, 70.7% and 57.3% of cycles were treated by natural cycle IVF/M and IVM, respectively.



Lim. Natural cycle IVF combined with IVM. Fertil Steril 2009.

of the present study further confirm that 15.4% of infertile women have PCOS and can be treated with IVM alone, with pregnancy and implantation rates reaching as high as 41.3% and 16.6%, respectively (Table 1). However, this group of patients, as predicted, has a higher miscarriage rate compared with other two treatment groups. Although it has been reported that IVM may have deleterious effects on the organization of the meiotic spindle and chromosome alignment of human oocytes, indicating that this is one possible explanation for the higher miscarriage rate with IVM oocytes (21), the exact reason needs to be further verified. Nevertheless, we believe that one of the reasons could be the endocrine disorder in this group of patients with PCOS.

It is a common belief that recruiting the dominant follicle in the ovaries would suppress the development of other follicles and would induce other follicular and oocyte atresia. Recent studies in animal models indicate that the maturational and developmental competence of immature oocytes derived from the small antral follicles is not adversely affected by the presence of a leading follicle or phase of folliculogenesis (22). Furthermore, this notion has been confirmed in humans, that the maturational and developmental competence of immature oocytes is not detrimentally affected by the presence of a dominant follicle during the follicular phase (11).

It has been demonstrated that mature oocytes can be retrieved from follicles as small as 11.5 mm in diameter (20). Therefore, it seems better to wait for the size of the leading follicles to increase more to improve the recovery rate of mature oocytes from the leading follicles. However, when the size of leading follicles reaches >16 mm in diameter after hCG administration, there is risk of premature LH surge and premature ovulation (12). The optimal time for the patient to receive an hCG injection to trigger oocyte maturation in vivo seems to be when the leading follicles reach 12–14 mm in diameter (13).

Pelinc et al. (23) reported that minimal-stimulation IVF is suitable for all indications, with acceptable ongoing pregnancy rates. Recently it has been speculated that natural cycle IVF has specific application in poor responders and in those in whom stimulation with gonadotropin can be avoided (24). Therefore, further modified natural cycle/mild stimulation with antagonist seems to be the future IVF treatment for all indications (25). On the basis of vast changes in the practice of IVF, Edwards et al. (26) have recently been defining IVF terminology. However, the terminology did not include a definition of the technology applied in the present study. The present study is the first to propose the new concept, namely natural cycle IVF/M, as an efficient infertility treatment for all indications, especially for women age <35 years.

In conclusion, the results from this study demonstrate that natural cycle IVF/M together with IVM alone can be used to treat more than a half of infertile women, with an acceptable pregnancy rate.

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