CHAPTER 27

Combination of natural cycle IVF with IVM as infertility treatment

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INTRODUCTION

The first live birth following in-vitro fertilization (IVF) resulted from natural cycle IVF[1]. However, this has been gradually replaced by ovarian stimulation combined with IVF, because it is believed that the number of oocytes retrieved relates to the embryos available for transfer, and that this directly affects the probability of successful pregnancy[2–4]. At the beginning, the relatively inexpensive clomiphene citrate was used to stimulate ovaries to produce multiple follicles, but currently ovarian stimulation protocols use the much more expensive gonadotropin-releasing hormone (GnRH) agonist or antagonist in combination with gonadotropins to generate multi-follicles in the ovaries. Some women are extremely sensitive to stimulation with exogenous gonadotropins and are at increased risk of developing ovarian hyperstimulation syndrome (OHSS), which, on rare occasions, can be a 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[6–8]. Although these problems are not encountered in natural cycle IVF treatment, a number of other problems arise, including an increased risk that no oocytes will be retrieved during oocyte collection and that no embryos will be available for transfer.

Literature reports for pregnancy rates per embryo transfer in natural cycle IVF vary between 0 and 30%[9–11]. However, there is an increasing interest in natural cycle IVF among patients, primarily because it is more comfortable and there are fewer side-effects, particularly the unknown long-term effects of repeated ovarian stimulation with GnRH and gonadotropins. Furthermore, in recent years, the efficiency of IVF technology has improved markedly[12]. It has been reported that, although the pregnancy rate was lower in natural cycle IVF treatment compared to ovarian stimulated IVF cycles, the implantation and birth rates achieved per started cycle were very similar[13]. Interestingly, Nargund et al.[12] indicated that when life-table analysis was performed to calculate the cumulative success rates after successive cycles of treatment, the cumulative probability of pregnancy was 46% with an associated live birth rate of 32% after four natural cycles of IVF treatment. Therefore, it is important to ask which infertility treatment we should offer primarily to our patients at the beginning.

In women, although only a single follicle usually grows to the preovulatory stage and releases its oocyte for potential fertilization, many small follicles also develop during the

same follicular phase of the menstrual cycle. It is believed that more than 20 antral follicles are selected and continue to the preovulatory stages of development during each cycle[14]. It has been documented that there are two or three waves of ovarian follicular development in women during each menstrual cycle based on daily transvaginal ultrasonography, challenging the traditional theory of a single cohort of antral follicles that grow only during the follicular phase of the menstrual cycle[15,16]. In addition, it seems that atresia does not occur in the non-dominant follicles even after the dominant follicle is selected in the ovary during folliculogenesis, because immature oocytes retrieved from non-dominant follicles have been successfully matured in vitro, fertilized, and have resulted in several pregnancies and healthy live births[10,17,18]. Therefore, one very attractive possibility for enhancement of the success of natural cycle IVF treatment is its combination with immature oocyte retrieval and in-vitro maturation (IVM)[19]. When we are successful in maturing the immature oocytes from small follicles that are collected along with the mature oocyte from the dominant follicle and producing several viable embryos, the chances of pregnancy are greatly increased.

PREGNANCY OUTCOME FROM IVM TREATMENT

Immature oocyte retrieval followed by IVM was shown to be a successful treatment for infertile women with polycystic ovaries (PCO) because there are numerous antral follicles within the ovaries in this group of patients. Immature oocyte retrieval followed by IVM might be useful in 20–37% of women undergoing IVF treatment who have polycystic ovaries as seen on ultrasound scan[20,21]. However, it is important to apply IVM technology for women with various causes of infertility. In general, clinical pregnancy and implantation rates for women who have polycystic ovaries and for hyper- and poor responders have reached 35–40% and 15–20%, respectively. These results demonstrate that IVM is an efficient clinical treatment for some infertile women. Thus, it is important to introduce this new approach, namely, the combination of natural cycle IVF and IVM, for all types of infertile women without any ovarian stimulation, if possible.

PATIENT SELECTION FOR IVF/IVM TREATMENT

All patients should be under 40 years of age and should have intact ovaries and regular menstrual cycles. The basal serum FSH level should be under 10 IU/l on day 2 or 3 of the menstrual cycle.

Baseline ultrasound scans

The treatment cycle is initiated by a baseline ultrasound scan on day 2 to 3 of the menstrual cycle to ensure that there are more than seven antral follicles present in the ovaries. Transvaginal ultrasound scans are repeated on day 6 or 8 of the menstrual cycle. At this point, the development of follicles and endometrial thickness are assessed.

hCG priming

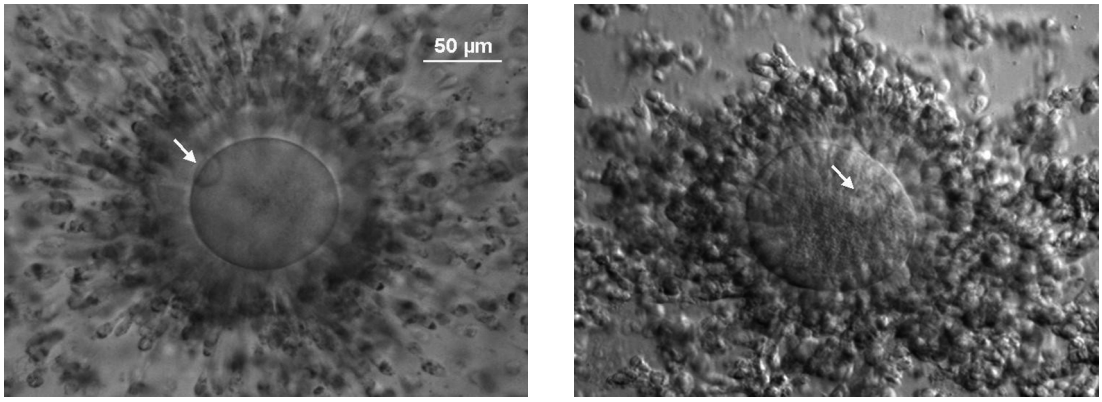
When a leading follicle has reached 12–14 mm in diameter and endometrial thickness is ≥6.0 mm, then 10 000 IU of human chorionic gonadotropin (hCG) will be administered intramuscularly and oocyte retrieval will be performed 36 h later. In cases where the leading follicle size is <12 mm in diameter, the patient can wait for 1 or 2 days for another ultrasound scan, and can then be given an hCG injection. Our experience indicates that the day of oocyte collection ranges between days 9 and 19 of the menstrual cycle, depending upon the individual patient.

MATURE AND IMMATURE OOCYTE RETRIEVAL

Transvaginal ultrasound-guided aspiration is performed using a 19G aspiration needle (Cook, Eight Mile Plains, Queensland, Australia). A portable aspiration pump is connected to the aspiration needle with a pressure between 80 and 100 mmHg. The aspirates are collected in tubes (10 ml) containing prewarmed heparinized flushing medium (Ham's F-10 medium) buffered with Hepes. Cumulus–oocyte complexes (COCs) are isolated by filtering the follicular aspirates through a mesh filter (diameter 70 μm, Falcon 1060, USA). In order to remove erythrocytes and small cellular debris, the filtrate is washed with Hepes-buffered Ham's F-10 medium. The retained COCs are then re-suspended in Ham's F-10 medium. The atretic and denuded COCs are discarded. The maturity of oocytes at the time of oocyte retrieval is evaluated under a stereomicroscope. Oocyte maturation is assessed by the presence of the first polar body (1PB) in the pelliviteline space (PVS). Mature and immature oocytes can be retrieved at the same time. As shown in Figure 27.1a, the mature oocyte was identified by extrusion of 1PB into PVS, and Figure 27.1b shows the immature oocyte assessed by containing germinal vesicle (GV) in the cytoplasm. Our experience indicates that the mature oocytes can be retrieved from follicles as small as 10 mm in diameter.

IVF AND IVM OF IMMATURE OOCYTES

The mature oocytes are subjected to insemination 2 or 3 h later by intracytoplasmic sperm injection (ICSI), and the remaining immature oocytes (at germinal vesicle or metaphase I stages) are further cultured in maturation medium[22]. The immature oocytes are cultured in maturation medium at 37°C in 5% CO_2, 5% O_2, and 90% N_2. After one day of culture, all COCs are denuded of the cumulus cells using 0.03% hyaluronidase (Sigma, St Louis, MO, USA) in Hepes-buffered Ham's F-10 medium and mechanical pipetting. At 24 and 48 h of culture, the mature oocytes are inseminated by ICSI. Fertilization is assessed 17–19 hours after ICSI in order to detect the appearance of two distinct pronuclei and two polar bodies. The zygotes are cultured in 10 μl of embryo development medium[23].



(a) **(b)**

Figure 27.1 Mature (a) and immature (b) oocytes were retrieved at the time of oocyte collection for combination of natural cycle IVF with IVM treatment. Arrows indicate first polar body and germinal vesicle respectively

ENDOMETRIUM PREPARATION AND EMBRYO TRANSFER

For endometrial preparation, 6 mg estradiol valerate (Progynova®, Schering, Korea) is administered daily, starting on the day of oocyte retrieval, and luteal support of 100 mg of progesterone in oil (Progest®; Samil Pharm Co, Ansan, Korea) is injected daily, starting on the day of ICSI. Embryo transfer (ET) is performed on day 3 or 4 after oocyte retrieval, depending on whether or not mature oocytes are retrieved. Before transfer, each patient's embryos are pooled together and selected for transfer. Pregnancy is determined by the level of serum β-hCG on day 15 or 16 after oocyte retrieval, and clinical pregnancy is established by the appearance of a gestational sac on ultrasound scan 6 weeks after ET.

OUTCOME FROM IVF/IVM TREATMENT

We recruited 129 patients who underwent natural cycle IVF combined with IVM treatment. The completion of natural cycle IVF combined with IVM treatment is outlined in Table 27.1; 95.4% (123/129) of patients completed the treatment cycles. The mean number of embryos available for transfer was 4.0 ± 1.6. The clinical pregnancy rate was 29.3% per embryo transfer, and the implantation rate 10.4%. Both mature and immature oocytes were retrieved from 74.0% (91/123) of the patients, and immature oocytes only from 26.0% (32/123).

A total of 123 patients completed 123 treatment cycles of natural cycle IVF combined with IVM (Table 27.2). Further analysis of these completed cycles for IVF/IVM treatment indicated that both mature and immature oocytes were retrieved from 91 patients, while immature oocytes only were collected from 32 patients. Out of the 32 patients in the latter group, 8 had already experienced premature ovulation at the time of egg collection. Regardless of whether or not mature oocytes were collected, the average number of oocytes retrieved from both groups was similar (8.1 ± 0.4 vs. 8.2 ± 0.3). There was no difference in terms of oocyte maturation, fertilization, and cleavage rates between the two groups. However, following embryo transfer, clinical pregnancy and implantation rates were significantly higher ($p < 0.05$) in the group from whom mature oocytes were retrieved (36.3% and 12.3%) than those of the group from whom immature oocytes only were collected (9.4% and 5.3%), although more embryos were transferred in the latter group (3.5 ± 1.6 versus 4.4

Table 27.1 Embryology and pregnancy outcome from natural cycle IVF combined with IVM treatment

Treatment cycles started (patients)	129 (129)
Treatment cycles completed (%)	123 (95.4)
No of mature oocytes retrieved (mean ± SD)	148 (1.2 ± 0.5)
No of immature oocytes retrieved (mean ± SD)	895 (6.9 ± 0.3)
No of oocytes fertilized (mean ± SD)	624 (4.9 ± 0.8)
No of oocytes cleaved (mean ± SD)	593 (4.6 ± 0.2)
No of embryos transferred (mean ± SD)	489 (4.0 ± 1.6)
No of clinical pregnancies (%)	36 (29.3)
No of embryos implanted (%)	51 (10.4)

Table 27.2 Embryology and pregnancy outcome from natural cycle IVF combined with IVM based on whether or not mature oocytes were retrieved from the leading follicles for women with regular menstrual cycles

	With mature oocytes retrieved from the leading follicles	*Without mature oocytes retrieved from the leading follicles*	*Total*
No of cycles completed (patients)	91 (91)	32 (32)	123 (123)
Age (mean ± SD)	35.5 ± 3.2	36.4 ± 2.1	36.9 ± 2.5
No of oocytes collected (mean ± SD)	773 (8.1 ± 0.4)	270 (8.2 ± 0.3)	1043 (8.1 ± 0.6)
No of oocytes matured in vivo (mean ± SD)	148 (1.6 ± 0.6)	0 (0.0 ± 0.0)	148 (1.2 ± 0.5)
No of in-vivo matured oocytes fertilized (%)	114 (77.0)	0 (0.0)	114 (77.0)
No of oocytes matured in vitro (%)	455 (72.8)	204 (75.6)	659 (73.6)
No of in-vitro matured oocytes fertilized (%)	346 (76.0)	164 (80.4)	510 (77.4)
No of embryos cleaved (%)	442 (96.1)	151 (92.1)	593 (95.1)
No of embryos transferred (mean ± SD)	358 (3.5 ± 1.6)	131 (4.4 ± 1.7)	489 (4.0 ± 1.6)
No of clinical pregnancies (%)	33 (36.3)[a]	3 (9.4)[b]	36 (29.3)
No of embryos implanted (%)	44 (12.3)[a]	7 (5.3)[b]	51 (10.4)

[a,b] At least $p < 0.05$ between columns

± 1.7). Importantly, it must be mentioned here again that 8 out of 32 patients in the group from which only immature oocytes were collected had already experienced premature ovulation at the time of egg collection.

When hCG was administered at the time of the leading follicles had reached <12 mm in diameter, 34.8% of patients (8/23), no mature oocytes were retrieved from the leading follicles (Table 27.3). Accordingly, failure rates for the mature oocytes retrieved were 25.6% (12/47), 18.2% (8/44), and 44.4% (4/9), respectively, when the size of the leading follicles reached 12–14 mm, 15–17 mm, and >17 mm in diameter, at the time of hCG injection. The pregnancy rate (20.0%) was significantly lower in the group from whom mature oocytes were retrieved where the size of the leading follicles had reached >17 mm in diameter at the time of hCG injection compared to others (33.3%, 40.0%, and 36.1%). However, there is no significant difference in the pregnancy rates (12.5%, 8.3%, 12.5%, and 0.0%, respectively) among patients from whom only immature oocytes were retrieved when the size of leading follicles reached <12.0 mm, 12–14 mm, 15–17 mm, and >17 mm in diameter respectively, at the time of hCG injection. These are our preliminary data, and it is necessary to increase the number of patients in order to corroborate this finding.

POTENTIAL PROBLEMS DURING IVF/IVM TREATMENT

A number of problems arise in natural cycle IVF treatment alone, including an increased risk of empty retrieval during oocyte collection leading to cancellation of the treatment cycle. Although this problem does not occur in natural cycle IVF

Table 27.3 Analysis of mature and immature oocyte retrieval in terms of the size of leading follicles related to pregnancy outcome

		No of patients (%)		*No of patients (%)*	
Size of leading follicle (mm) at hCG injection	*No of patients*	*Who had mature oocytes collected*	*Who become pregnant*	*Who had immature oocytes collected only*	*Who become pregnant*
<12	23	15 (65.2)[a]	5 (33.3)[a]	8 (34.8)[a]	1 (12.5)[a]
12–14	47	35 (74.5)[b]	14 (40.0)[a]	12 (25.6)[b]	1 (8.3)[a]
15–17	44	36 (81.8)[b]	13 (36.1)[a]	8 (18.2)[b]	1 (12.5)[a]
>17	9	5 (55.6)[c]	1 (20.0)[b]	4 (44.4)[c]	0 (0.0)[a]
Total	123	91 (74.0)	33 (36.3)	32 (26.0)	3 (9.4)

[a,b,c] $p < 0.05$ in the same column

combined with IVM, there is the risk of premature ovulation, if we wait until the size of the follicles reaches more than 15 mm in diameter before administering an hCG injection; 15.1% (8/53) of patients had premature ovulation at the time of egg collection when the size of follicles had reached ≥15 mm in diameter (Table 27.3). However, the recovery rate of mature oocytes (81.8%) from the leading follicles was highest in the group where the size of the leading follicles reached 15–16 mm in diameter at the time of hCG injection. In addition, our experience indicates that although the quality of mature oocytes retrieved from the leading follicles was not different based on their morphology and fertilization rate as well as early embryonic development, the quality of immature oocytes was poor when the leading follicles reached >17 mm in diameter at the time of hCG injection. Furthermore, there was a higher risk of premature ovulation when the leading follicles reached >17 mm in diameter at the time of hCG administration. Therefore, the optimum size of follicles seems to be 12–16 mm in diameter when hCG is administered in order to maximize its efficiency of treatment, and at the same time to reduce the risk of premature ovulation from the leading follicles.

In addition, the recovery rate from the leading follicles at the time of egg collection is an important issue. Our recovery rate of mature oocytes from the leading follicles using a single-lumen aspiration needle (19G, Cook, Australia) was 74.0% (91/123). However, based on our experience, we recommend using a double-lumen needle first to aspirate the leading follicles, followed by flushing until the mature oocytes are removed from the leading follicles, and then changing to a single-lumen IVM aspiration needle to aspirate the small follicles. By following this procedure, the recovery rate of mature oocytes from the leading follicles will be significantly improved.

EMBRYO QUALITY FROM EGGS MATURED AT DIFFERENT TIMES

It has been demonstrated that the administration of hCG 36 h before harvesting immature oocytes improves the maturational and development competence of the oocytes, resulting in higher pregnancy rates[24,25]. This has been confirmed by several reports[22,26,27]. Interestingly, recent findings indicate that the time course and maturation rates are different when

germinal vesicle (GV)-stage oocytes are divided into different groups based on the morphology of cumulus cells after hCG priming[28]. Cleavage and embryonic development rates are significantly different based on the maturation timing of oocytes in vitro, although there is no difference in the fertilization rate regardless of the maturation time of the oocytes (Table 27.2), suggesting that oocytes reaching metaphase II (MII) faster and have better embryonic developmental competence[29]. From the results of our study, it is hard to know which of the embryos implanted originated from in-vivo matured versus in-vitro matured oocytes. However, results from the present study clearly indicate that embryos produced from oocytes cultured 24 h for maturation are of better quality, as observed by morphology, and thus may have a higher implantation potential than those generated from the oocytes cultured 48 h for maturation. In addition, it is impossible to determine which of the embryos produced from in-vivo or in-vitro matured oocytes are implanted in natural cycle IVF combined with IVM. However, the results from natural cycle IVF combined with IVM treatment clearly indicate that there are embryos generated from implanted in-vitro matured oocytes.

CONCLUSIONS

The preliminary results from our study confirm that mature and immature oocyte retrieval followed by IVF and IVM is an efficient treatment for women with various causes of infertility. The results from this study also indicate that hCG should be given when the size of the leading follicles has reached 12–14 mm in diameter in order to maximize the mature oocyte recovery rate, which may relate directly to the pregnancy rate. In summary, the results from our study demonstrate that natural cycle IVF combined with IVM is an effective treatment in a selected group of women.

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