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

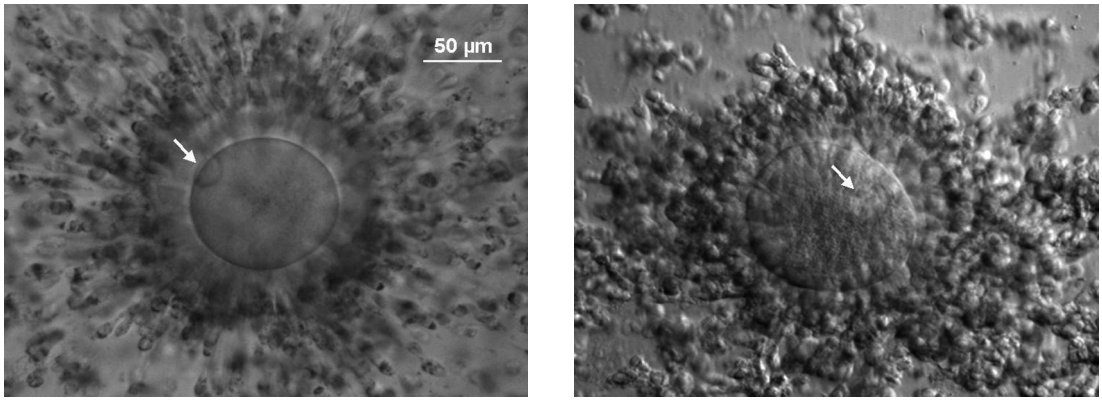
When a leading follicle has reached 12–14 mm in diameter and endometrial thickness is ≥6.0 mm, then 10 000 IU of human chorionic gonadotropin (hCG) will be administered intramuscularly and oocyte retrieval will be performed 36 h later. In cases where the leading follicle size is <12 mm in diameter, the 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