CHAPTER 26

IVM as an alternative for over-responders

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INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most common reproductive disorders in women of childbearing age. It has a heterogeneous presentation, which is clinically characterized by anovulation and hyperandrogenism, and pelvic ultrasound examination shows numerous antral follicles within the ovaries[1]. Women with PCOS often present with anovulatory infertility with a significant proportion being resistant to induction of ovulation by clomiphene citrate. Although ovulation can be induced successfully in 75% of clomiphene-citrate non-responders with human menopausal gonadotropin (HMG), gonadotropin use requires intensive monitoring. In addition, many patients ovulate but do not achieve pregnancy even after their anovulation has been corrected. For these women, in-vitro fertilization (IVF) is the standard treatment but there is a significantly higher risk of ovarian hyperstimulation syndrome (OHSS) compared with IVF treatment in women with normal ovaries[2].

Some women are extremely sensitive to stimulation with exogenous gonadotropins and are at increased risk of developing OHSS that, sometimes, is a potentially life-threatening complication[3]. In general, the incidence of OHSS ranges from 0.6% to 14% in women undergoing ovarian stimulation for IVF[4]. Several preventive strategies have been proposed to reduce the incidence and severity of OHSS, including cancellation of treatment cycle, cryopreservation of all embryos, or intravenous administration of albumin or other plasma-expanding agents[5–7]. However, these methods are not efficient in preventing OHSS. Another popular strategy is withholding gonadotropin stimulation, the 'coasting' method. The advantage of coasting is that the treatment cycle is not necessarily cancelled and that no additional procedure is needed[8–10]. But coasting cannot be applied when the signs and symptoms predictive of OHSS are observed early in the stimulation phase of the cycle, because premature withholding of gonadotropin may result in arrest of follicular growth and atresia of oocytes. In addition, with coasting, frequent estimations of serum estradiol level and ultrasound scans are needed in order to determine the time of hCG administration[11]. Furthermore, the crucial timing of hCG administration has not been well defined.

Following the first successful live birth from in-vitro maturation (IVM) of immature oocytes in women with PCOS-related infertility[12], immature oocyte retrieval followed by IVM has been applied as a clinical treatment,

often in women with PCOS[13,14]. In comparison with conventional IVF treatment, the major advantages of IVM treatment include reduced cost, simplified treatment, and eliminated side-effects from gonadotropins, particularly avoidance of the risk of OHSS. Furthermore, recently it has been reported that mature oocytes were collected from infertile women with PCOS when the leading follicle reached a mean diameter of 12 to 14 mm following administration of hCG[15,16]. This finding suggests that limited ovarian stimulation can result in retrieval of mature oocytes and may prevent the recurrence of severe forms of OHSS without reducing the clinical pregnancy rate from that of conventional IVF treatment. We report a new strategy for OHSS prevention during conventional IVF treatment that results in efficient elimination of OHSS among infertile women with PCOS.

OVARIAN STIMULATION

Women with PCOS receive the oral contraceptive pill, Mercilon (Organon, Netherlands), 1 tablet a day for 21 days, in order to induce menstrual bleeding. After withdrawal menstrual bleeding, the patients underwent either the 'long protocol' of downregulation with a GnRH-agonist for IVF or the GnRH-antagonist protocol.

For women undergoing the 'long protocol', pituitary downregulation was confirmed by demonstrating an endometrial thickness of <5.0 mm with a transvaginal ultrasound scan after at least 2 weeks of GnRH-agonist and the absence of any ovarian cysts. The initial dose of gonadotropin for ovarian stimulation is individualized according to the age of patients, day 3 serum FSH level, body mass index, and previous response to ovarian stimulation. The GnRH-antagonist protocol involved starting gonadotropins on day 3 of the menstrual cycle. Transvaginal ultrasound monitoring was commenced on day 5 of ovarian stimulation and repeated every 2–3 days. Serum estradiol concentrations were not measured routinely in our IVF program.

IDENTIFYING THE OVER-RESPONDER PATIENTS

A woman is considered an 'over-responder' when there are more than 20 follicles with a mean diameter >10 mm in both ovaries following gonadotropin stimulation for at least 5 days. In our study, a total of 123 patients, who were undergoing conventional IVF treatment and who over-responded with this sign, were included in this alternative treatment. The mean age of the patients was 28.1 ± 3.4 years.

MATURE AND IMMATURE OOCYTE RETRIEVAL

When the leading follicle reached 12–14 mm in diameter, 10 000 IU of human chorionic gonadotropin (hCG) was administered, and oocyte collection was performed 36 hours later. Transvaginal ultrasound-guided aspiration was conducted with a 19G aspiration needle (Cook, Eight Mile Plains, Queensland, Australia). A portable aspiration pump was connected to the aspiration needle with a pressure between 80 and 100 mmHg. The aspirates were collected in tubes (10 ml) containing prewarmed heparinized Ham's F-10 medium buffered with Hepes. Cumulus–oocyte complexes (COCs) were 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 filtrates were washed with Hepes-buffered Ham's F-10 medium. The retained COCs were then re-suspended in the medium. The maturity of oocytes at the time of oocyte retrieval was evaluated under a stereomicroscope. Oocyte maturation was assessed by the presence of the first polar body (1PB) in the pelliviteline space (PVS).

OOCYTE IN-VITRO FERTILIZATION AND IN-VITRO MATURATION

The collected mature oocytes (metaphase II) were subjected to insemination 2 or 3 h later using intracytoplasmic sperm injection (ICSI). The remaining immature oocytes (at germinal vesicle or metaphase I stages) were further cultured in oocyte IVM medium. We used YS-medium as oocyte IVM medium, containing 30% human follicular fluid (HFF) supplemented with 1 IU/ml FSH, 10 IU/ml hCG, and 10 ng/ml rhEGF (Daewoong Pharmaceutical Co, Korea)[17,18]. The HFF was prepared using the method reported by Chi et al.[19]. The immature oocytes were cultured in maturation medium at 37°C in 5% CO_2, 5% O_2, and 90% N_2. After 1 day of culture, COCs were 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 were inseminated by ICSI, respectively. Fertilization was assessed 17–19 h after ICSI to detect the appearance of two distinct pronuclei and two polar bodies. The zygotes were co-cultured with the cumulus cells prepared on the day of oocyte retrieval in 10 μl of YS medium supplemented with 10% HFF[20].

EMBRYO TRANSFER AND ENDOMETRIAL PREPARATION

Embryo transfer (ET) was performed on day 4 or 6 after oocyte retrieval. Blastocyst transfer was performed in some patients if they had more than three good-quality embryos examined on day 2 after insemination. Before transfer, all embryos for each patient were pooled together and selected for transfer. For endometrial preparation, 6 mg estradiol valerate (Progynova®, Schering, Korea) was administered daily, starting on the day of oocyte retrieval, and luteal support in the form of 100 mg progesterone in oil (Progest®, Samil Pharm Co, Ansan, Korea) was injected daily, starting on the day after oocyte collection. A pregnancy test by measuring the level of serum β-hCG was performed on day 16 after oocyte retrieval, and clinical pregnancy was determined by visualization of the fetal heartbeat using an ultrasound scan. Differences between fertilization and cleavage in each group were compared using the χ^2-test (statistical analysis system, SAS Institute, Cary, NC, USA).

PREGNANCY OUTCOME

For each patient, the average dose of gonadotropins used for controlled ovarian hyperstimulation (COH) was 1228.6 ± 655.6 IU, and the mean duration of COH was 7 ± 2.8 days (Table 26.1). The average thickness of endometrium on the day of hCG administration was 10.5 ± 1.8 mm. As shown in Table 26.2, a total of 1554 oocytes were retrieved from 123 patients (12.6 ± 6.9); 293 (18.9%) oocytes were mature at the time of oocyte retrieval and the remaining 1261 oocytes were at either metaphase I (MI) or germinal vesicle (GV)-stages. Following culture, 764 (60.6%) oocytes matured at 24 h and another 188 (14.9%) oocytes were matured at 48 h. A total of 952 (75.5%) oocytes were matured following IVM. Together with 293 in-vivo mature oocytes, a total of 1245 (80.1%) mature oocytes were obtained from those 123 patients (10.7 ± 6.9).

A total of 967 (77.7%) oocytes were fertilized following ICSI (Table 26.2), and the cleavage rate was 93.5% (904/967). Following transfer, a mean of 4.2 ± 1.5 embryos per patient, the clinical pregnancy and implantation rates were 36.6% (45/123) and 11.3% (58/514); respectively. As shown in Table 26.2, 17 patients underwent blastocyst transfer and 8 (47.1%) of them became pregnant. The implantation rate was 23.6% (13/55). Among this group of patients, no patients suffered from severe symptoms of OHSS during the treatment cycle and pregnancy.

Table 26.1 Detailed information of the patients who underwent ovarian stimulation

	Value (range)
No of patients	123
Age (mean ± SD)	28.1 ± 3.4 (27–36)
Ampoules of gonadotropins used (mean ± SD)	16.4 ± 8.8 (8–52)
Dose (IU) of gonadotropins used (mean ± SD)	1228.6 ± 655.6 (600–3900)
Duration (days) of ovaries stimulated (mean ± SD)	7.0 ± 2.8 (4–14)
Endometrial thickness (mm) on day of hCG priming (mean ± SD)	10.5 ± 1.8 (8–15)

Table 26.2 Pregnancy outcome of the patients who underwent oocyte retrieval followed by in-vitro maturation

	Embryo transfer at		
	Day 3	*Day 5*	*Total*
No of patients (cycles)	106 (106)	17 (17)	123 (123)
Age (mean ± SD)	27.9 ± 3.4	29.2 ± 3.0	28.1 ± 3.4
No of oocytes retrieved (mean ± SD)	1236 (11.7 ± 6.5)	318 (18.7 ± 6.9)	1554 (12.6 ± 6.9)
No of mature oocytes retrieved at collection (%)	201 (14.2)	92 (28.9)	293 (18.9)
No of immature oocytes retrieved (mean ± SD)	1035 (9.8 ± 5.9)	226 (13.3 ± 7.3)	1261 (10.3 ± 6.2)
No of oocytes matured in-vitro 24 h postculture (%)	617 (59.6)	147 (65.0)	764 (60.6)
No of oocytes matured in-vitro 48 h postculture (%)	159 (15.4)	29 (12.8)	188 (14.9)
Total number of oocytes matured (%)	977 (79.0)	268 (84.3)	1245 (80.1)
No of oocytes fertilized (%)	760 (77.8)	207 (77.2)	967 (77.7)
No of embryos cleaved (%)	718 (94.5)	186 (89.9)	904 (93.5)
No of embryos transferred (mean ± SD)	459 (4.3 ± 1.6)	55 (3.2 ± 0.8)	514 (4.2 ± 1.5)
No of clinical pregnancies (%)	37 (34.9)	8 (47.1)	45 (36.6)
No of embryos implanted (%)	45 (9.8)	13 (23.6)	58 (11.3)

As shown in Figure 26.1, fertilization rates were not different between in-vivo (81.6% = 239/293) and in-vitro (24 h: 77.1% = 589/764; 48 h: 73.9% = 139/188) matured oocytes. However, the embryo cleavage rate was significantly lower ($p < 0.05$) in 48 h in-vitro matured oocytes (78.4% = 109/139) compared with in-vivo matured (96.2% = 230/239) and 24 h in-vitro matured (95.9% = 565/589) oocytes (Figure 26.2).

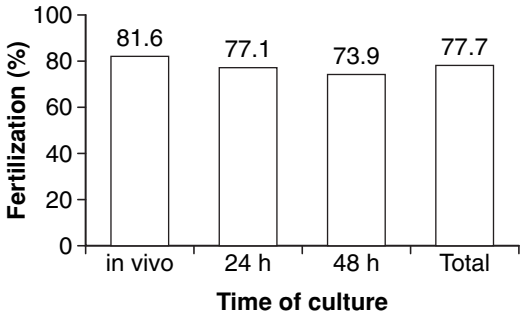

Figure 26.1 Comparison of fertilization rates in the oocytes matured in vivo (0 h), 24 h and 48 h following in-vitro maturation. There are no differences among groups

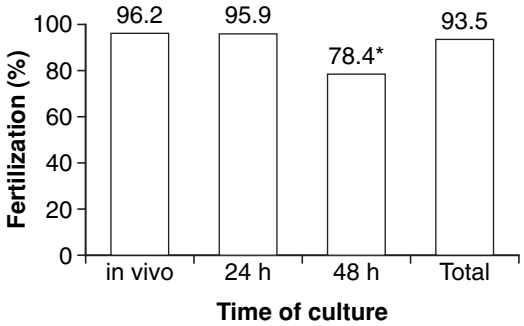

Figure 26.2 Comparison of cleavage rates in the oocytes matured in vivo (0 h), 24 h and 48 h following in-vitro maturation. Cleavage rate is significantly lower ($*p < 0.05$) in the oocytes matured 48 h following in-vitro maturation compared with other groups

CAN OHSS BE PREVENTED BY IVM TREATMENT?

OHSS is a serious and potentially life-threatening complication in patients who undergo ovarian stimulation for IVF treatment. Even in the mild cases of OHSS, ovarian enlargement, abdominal distension, and weight gain may occur. In severe cases of OHSS, ascites, pleural effusion, hypovolemia with oliguria, pericardial effusion, the adult respiratory distress syndrome (ARDS), hypercoagulability with thromboembolic sequelae, and multi-organ failure may occur[4]. There are several risk factors associated with the development of OHSS during ovarian stimulation for women undergoing IVF treatment. These include young age, low body weight, a high level of estradiol, and large numbers of follicles during gonadotropin stimulation. Anovulatory women with PCOS are especially at an increased risk for OHSS.

The mechanism of OHSS induced by gonadotropin stimulation is not fully understood. However, it is known that OHSS is triggered by hCG administration and is associated with very high levels of estradiol, subsequently increased capillary permeability, and extravasations of fluid in the abdominal cavity. Interestingly, hCG administration did not lead to OHSS in these patients when the size of the leading follicles reached 12–14 mm in diameter. The range of gonadotropins used in these patients was from 600 IU to 3900 IU (average: 1228.6 ± 655.6 IU). The mean stimulation period with gonadotropin for the group of patients was 7.0 ± 2.8 days (ranging from 4 days to 14 days) (Table 26.1).

Various strategies for preventing or diminishing OHSS and its severity have been suggested. The first approach is to abandon the treatment cycle[21]. The second, in view of the increased risk of OHSS associated with pregnancy, is to cryopreserve all resulting embryos[22]. In order to reduce the incidence of severe OHSS without compromising pregnancy rates in the treatment cycles, some other approaches, such as minimizing the dose of gonadotropin[23,24], reducing the dose of hCG for triggering ovulation[25], giving intravenous administration of albumin[26] or other plasma-expanding agents before or during oocyte retrieval[5,6], and withholding gonadotropin (coasting) during COH, have been proposed. Among these, it is suggested that coasting is an effective measure in the prevention of OHSS without jeopardizing the pregnancy outcome[27]. Recently, it has been reported that the use of GnRH antagonists/agonists instead of hCG to

trigger ovulation may prevent OHSS[28]. However, OHSS will never be completely eliminated by these proposed methodologies, suggesting that the only way to avoid iatrogenic OHSS is to avoid ovarian stimulation using gonadotropins[29].

El-Sheikh et al.[15] reported that OHSS might be efficiently prevented by limited ovarian stimulation (LOS) in women with PCOS. Interestingly, they retrieved mature oocytes when the leading follicles reached a mean diameter of 12 mm following hCG administration, resulting in eight clinical pregnancies out of 20 patients. At the same time, the incidence and severity of OHSS were efficiently reduced[16]. In contrast, the results of the present study indicate that hCG administration triggers maturation of oocytes in some small follicles. Our results show that 18.9% of oocytes were mature at the time of oocyte retrieval when hCG was given once the follicles had reached a diameter of 12–14 mm (Table 26.2). This implies that these medium size follicles possess luteinizing hormone (LH) receptors that respond to the LH surge for oocyte maturation in vivo in women with PCOS. Yang et al.[30] reported that the pattern of cumulus cells at the time of oocyte collection plays a predictive role in the maturation of oocytes recovered from patients with PCOS in hCG-primed IVM cycles. They indicated that there were LH receptors in cumulus cells as detected by semiquantitative reverse transcription polymerase chain reaction (RT-PCR)[30].

It was reported that the cleavage and blastocyst development rates are different among oocytes matured in vivo, 24 h and 48 h following IVM[31], suggesting that the oocytes reaching MII faster following IVM have better embryonic developmental competence. In this study, we confirm that although fertilization rates were not different between groups (Figure 26.1), the cleavage rates were significantly different between in-vivo matured and in-vitro matured oocytes and between 24 h and 48 h in-vitro matured oocytes (Figure 26.2). Also, we found that the quality of embryos differed among oocytes matured in vivo and in vitro and among the oocytes matured at different times following IVM. More interestingly, among these 123 patients, 17 of them underwent blastocyst transfer. Eight of them became pregnant following blastocyst transfer.

Although it is hard to tell whether the implanted embryos were produced from in-vivo or in-vitro matured oocytes, the results from the present study indicate an improvement of clinical pregnancy rate (36.6%) when using embryos generated from both in-vivo and in-vitro matured oocytes. Our previous experiences with IVF alone (using mature oocytes only) in women who were over-responders and who had a high risk of OHSS during ovarian stimulation cycles resulted in only a 10–15% chance of clinical pregnancy per embryo transfer.

SUMMARY

Mature and immature oocyte retrieval followed by IVM is an efficient method for the prevention of OHSS during ovarian stimulation without compromising the pregnancy outcome for IVF treatment cycles in women with PCOS. The important criteria for this alternative is to stop gonadotropin stimulation when there are ultrasonographic signs of OHSS risk, where there are more than 20 growing follicles with a mean diameter >10 mm, and to administer hCG when the leading follicle reaches 12–14 mm in diameter.

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