CHAPTER 25

IVM as an alternative for poor responders

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INTRODUCTION

In conventional in-vitro fertilization (IVF) treatment, infertile women are treated with gonadotropin-releasing hormone (GnRH)-agonist or -antagonist in combination with gonadotropins for approximately 2 or 3 weeks to induce the development of ovarian follicles, because the number of oocytes retrieved determines the number of embryos available for transfer, which in turn directly affects the pregnancy rate.

However, many patients respond poorly to ovarian stimulation. It has been estimated that up to 15% of all patients treated for IVF are poor responders to stimulation with exogenous gonadotropins[1]. Reports have indicated that, amongst patients undergoing IVF treatment, the prevalence of poor response to gonadotropin stimulation is between 9 and 24%[2]. Therefore, this has become a frequently encountered problem in all IVF treatment centers.

Poor response to gonadotropin stimulation occurs more often in older women, but may also present in young women with both an abnormal and a normal endocrinologic profile[3]. These patients are characterized typically by low estradiol concentrations combined with markedly reduced numbers of follicles in spite of stimulation with massive doses of gonadotropins[4]. Other patients appear to respond to gonadotropin stimulation but have a low estrogen level or few or slow-growing follicles. Finally, in some patients, the number of follicles in the ovaries seems normal following ovarian stimulation, but their size remains less than 12 mm in diameter on day 15 of the treatment cycle[5,6]. Oocyte quality is often compromised in these groups of patients and results in diminished clinical pregnancy rates, increased spontaneous abortion rates, and lower implantation rates when compared with age-matched controls.

A modified stimulation regimen may help to overcome poor ovarian response and oocyte growth retardation, but most patients still require longer stimulation time and higher gonadotropin doses. These patients seem resistant to gonadotropin stimulation. However, a higher dose of gonadotropin may negatively affect fertilization and pregnancy outcome[7]. Furthermore, many women also experience a higher cycle cancellation rate because of the smaller number and size of follicles.

Since the first successful live birth from in-vitro maturation (IVM) of immature oocytes was reported from a woman with polycystic ovary syndrome (PCOS)[8], immature oocyte retrieval followed by IVM has been applied as a clinical treatment, especially for infertile women with

PCOS[9]. Liu et al.[10] reported a 37.5% pregnancy rate following immature oocyte retrieval and IVM, suggesting that IVM may be a viable alternative to cancellation in this group of patients[11].

DEFINITION OF POOR RESPONDERS

There is no universal standard definition for 'poor responder' in the field of assisted reproductive technology (ART). However, it is common sense that such patients are characterized by lower-than-expected numbers of follicles and oocytes recruited in the face of exogenous gonadotropin stimulation. Several criteria have been used frequently to characterize poor responders: (1) the number of developed follicles in the ovaries; (2) the concentration of estradiol during the gonadotropin stimulation; (3) the increased basal FSH level; (4) other factors.

Number of follicles

The proposed number of follicles varies among the different reports. However, most reports indicate that less than three to five dominant follicles on the day of human chorionic gonadotropin (hCG) administration should be considered 'poor responders' in gonadotropin-stimulated IVF treatment cycles[12–16].

Estradiol level

Estradiol level is correlated with the number and size of follicles. A peak estradiol level of 300–660 pg/ml has been proposed as an important criterion for defining poor response to ovarian stimulation[15,17–19]. It also has been suggested that an estradiol level of less than 100 pg/ml on day 5 of stimulation should be defined as 'poor response'[16]. Poor response to ovarian stimulation for IVF treatment has been defined as a plasma estradiol level of less than 1000 pg/ml on the day of hCG administration and no more than four oocytes retrieved[20].

Basal FSH level

An age-related decline in fecundity is observed as women progress through reproductive life and ovarian reserves decline. It has been suggested that the age-related decline in oocyte quantity and quality is the result of defects in the follicle originating from development in the fetal ovary. There is a premature reduction in follicle numbers in 'poor responders'[21] and it is identifiable by a rise in FSH level in the early follicular phase[22], that reflects an effort by the pituitary gland to maintain the normal follicle response[23]. Therefore, it has been suggested that the basal FSH level is increased from 6.5 mIU/ml to 15.0 mIU/ml (average 10.0 mIU/ml) in 'poor responders'[24–26].

Other factors

Some other indicators have been also implicated in poor responders. These are a failed response to the 'clomiphene challenge test'[27] and the 'lupron screening test'[28], an advanced patient age of more than 40 years[25], at least one previous cancelled IVF treatment cycle[29], increased numbers of gonadotropin ampoules used (more than 300 IU/day)[26], and a prolonged duration of gonadotropin stimulation[30]. Recent studies have shown convincingly that poor ovarian response is a first sign of ovarian aging (early ovarian failure or early menopause)[31–33].

In a variety of studies, these criteria have been used either alone or in combination, thereby highlighting the complexity, the lack of uniformity in definitions, and also the major difficulties encountered when comparing the different strategies proposed to overcome this problem.

PREDICTION OF POOR RESPONDERS

There is no accurate prediction of low ovarian response. Despite the multitude of predictive tests for low ovarian response, the 'poor

responder' is revealed definitively only during ovarian stimulation. However, multivariate analyses involving basal FSH and inhibin levels combined with antral follicle count may significantly improve the prediction of poor ovarian response in IVF treatment[34].

An increased serum FSH level on day 3 of the menstrual cycle is a biomarker for ovarian reserve decline and is believed to indicate a reduced reproductive potential[35]. Women with elevated FSH levels may require consistently more gonadotropin stimulation than women with a low range of FSH levels[36]. It has been known that women with higher basal FSH levels have a poor outcome compared with those with a normal range of FSH levels[37,38]. As FSH levels rise, there is a progressive decline in the pregnancy rate, suggesting that basal FSH is a better predictor than age with regard to pregnancy and cancellation rates[39]. Therefore, basal serum FSH level is the most widely used test of ovarian reserve and is strongly associated with poor ovarian response to gonadotropin stimulation[40]. However, there remains a group of young women with an apparently normal basal FSH level and ovarian reserve who do not respond well. In this case, the small antral follicle counting may be a better prognostic indicator of poor response before controlled ovarian stimulation for IVF treatment[41]. Another ultrasound marker of ovarian response may be ovarian volume[42].

An additional biomarker of ovarian reserve and response is inhibin. Inhibin-A and inhibin-B are secreted by granulosa cells of the developing follicles and exert a suppressive effect on FSH. It has been reported that lower inhibin-B levels are associated with fewer oocytes, higher cancellation rates, and lower pregnancy rates compared with patients with normal inhibin-B levels[34,43]. Although other biochemical markers of ovarian reserve and response have been proposed[44,45], it seems that no biomarker is absolute for the prediction of poor response to ovarian stimulation. Therefore, an accurate prediction of poor response should be based on multivariate analyses.

ETIOLOGIES OF POOR RESPONDERS

The mechanism of a poor response to gonadotropin stimulation is still unclear. Although several possible etiologies have been suggested, a diminished ovarian reserve is still thought to be the principal reason for poor ovarian response[6]. Alternatively some other factors, such as a decreased number of FSH receptors available in the granulosa cells[46], defective signal transduction after FSH receptor binding[47], an inappropriate local vascular network for the distribution of gonadotropins[5], premature luteinization due to an abnormal negative ovarian feedback at the level of the anterior pituitary, and the presence of autoantibodies against granulosa cells, as well as lowered circulating gonadotropin surge-attenuating factor (GnSAF) bioactivity[48], have all been suspected. In addition, vascularization around ovaries appears to play a very important role in the recruitment, growth, and maturation of follicles in both natural and stimulated IVF cycles[49], suggesting that the severe adhesions caused by previous pelvic infection or inflammation may result in an encumbrance for this process.

In general, it is accepted that the elevated FSH levels represent quantitative and qualitative limitations in follicle development, but do not always occur simultaneously[50]. It has been reported that the ovarian response to FSH stimulation is dependent upon the FSH receptor genotype, in which is expressed a less active FSH receptor requiring higher levels of FSH for stimulation[51]. Different isoforms of FSH have been described with differing receptor binding immunoactivity[52]. FSH binding inhibitors or FSH antibodies may affect the binding and result in a low ovarian response to FSH.

A frequently occurring variant of the FSH receptor has been reported in which the enzyme asparagine of the receptor protein is replaced by serine at position 680[51]. This change leads to a slightly less active FSH receptor that requires higher FSH levels for normal function and is probably not related to reproductive aging[53].

Therefore, it has been proposed that in cases of elevated FSH further investigations should be made, such as FSH receptor genotyping, dynamic ovarian testing, measurement of antral follicle count, and another potential biochemical marker – anti-Müllerian hormone[54,55].

MANAGEMENT OF POOR RESPONDERS

High dose of gonadotropins

Most authorities recommend a high dose of gonadotropins for poor responders. However, the results remain controversial. Some reports indicate that the increased dose of gonadotropins would improve oocyte yield[56–58]. Although some reports indicate that there is no benefit from the increase in FSH dose[12,16,24–26], large prospective randomized trials are needed to elucidate this issue further.

The type of gonadotropin used has been suggested to have the different potencies of ovarian response as a result of its increased purity and isoform properties[15,59]. The combination of FSH and LH may also benefit poor responders as compared with FSH alone[60]. Clomiphene citrate, when administered in conjunction with exogenous gonadotropins, may be a more potent stimulator of FSH than mid luteal GnRH-agonist among poor responders who failed responding to other ovarian stimulation protocols[61,62], indicating that the number of oocytes is not increased but the follicular growth and oocyte quality seem to be improved.

Downregulation with GnRH-agonist

Initially a GnRH-agonist was used to avoid a premature LH surge during ovarian stimulation. It was initially thought that using GnRH for downregulation might improve the response of poor responders[63]. However, using a GnRH-agonist for downregulation depletes endogenous FSH and LH, making it more difficult to achieve an adequate follicular response[64]. Therefore, modified GnRH-agonist downregulation protocols have been proposed.

The GnRH-agonist flare, or the short protocol, has been applied to poor responders to avoid the suppressive effects of GnRH-agonist downregulation on endogenous gonadotropins. This may benefit the initial pituitary release of FSH and LH in response to GnRH-agonist initiation. Although there is an improvement in oocyte quality seen by this modification of GnRH-agonist downregulation, there is little or no improvement in clinical outcome[65–67].

A microdose of GnRH-agonist flare protocol has the advantages of the standard flare. At least in theory, the regimens of microdose of GnRH-agonist flare would be suited to patients with a low ovarian response. Because approximately 1% of the normal GnRH-agonist dose could initiate pituitary release of gonadotropins, it results in delayed desensitization of the pituitary and allows for significant follicular recruitment and response[68]. Several microdoses of GnRH-agonist flare protocols have been tested, and most studies conducted to assess the standard dose flare protocols demonstrate a degree of improvement[16,69]. A significant improvement was demonstrated with the use of the microdose of GnRH-agonist regimens[70]. However, further clinical investigations are needed to confirm its outcome.

GnRH-antagonist protocols

The relatively new GnRH-antagonist regimens were brought into clinical use for eliminating the premature LH surge. A GnRH-antagonist offers potential advantages for the treatment of poor responders. Use of a GnRH-antagonist avoids the suppression of the early follicular phase endogenous gonadotropins by a GnRH-agonist. The synergic effect of endogenous FSH with high-dose exogenous gonadotropins may maximize the delivery of gonadotropin to the cohort of recruitable follicles in the early follicular phase.

Therefore, the use of a GnRH-antagonist regimen probably reduces the duration of ovarian stimulation in comparison with the conventional GnRH-agonist regimens[20]. However, asynchrony of the follicular cohort can result in the development of a single dominant follicle and is a potential problem of the GnRH-antagonist protocol. This risk may be avoided by taking the oral contraceptive pill[71,72]. Although a randomized control trial comparing microdoses of GnRH-agonist and GnRH-antagonist protocols demonstrated equivalent rates of treatment cancellation, pregnancy, and implantation[73], another report indicated that the GnRH-agonist flare protocol appears to be more effective than the GnRH-antagonist protocol in terms of mature oocytes retrieved, fertilization rate, and high-quality embryos transferred in poor responders[74].

Growth hormone

It was hypothesized that growth hormone (GH) can stimulate ovarian steroidogenesis and follicular development, and enhances the ovarian response to FSH[75]. The action of GH is believed to be mediated via insulin-like growth factor-1 (IGF-1) that acts in synergy with FSH, amplifying its effects on the granulosa cells[76]. It has been reported that GH-releasing hormone (GH-RH) causes an increase in endogenous GH secretion[77]. However, use of GH-RH seems not to improve the final cancellation and pregnancy rates compared to the controls. Pyridostigmine is an acetylcholinesterase inhibitor that can increase GH secretion by enhancing the action of acetylcholine[78]. Nevertheless, the published data so far do not support any benefit from using GH, GH-RH, or pyridostigmine as adjuvant therapy in poor responders.

Oral contraceptive pill

Oral contraceptive pill pretreatment (OCP) might benefit the ovarian response of poor responders. OCP administration is now widely used to suppress endogenous gonadotropins before controlled ovarian stimulation. OCP pretreatment seems to generate and to sensitize more estrogen receptors, and OCP administration prior to the GnRH-agonist protocol was associated with a higher pregnancy rate and lower cancellation rate[79]. However, a recent study indicated that pretreatment with OCP appears to be associated with no significant difference in ongoing pregnancy rate compared to controls and a significantly higher rate of early pregnancy loss[80]. Therefore, conclusive results are still awaited.

Low-dose aspirin treatment

Antiphospholipid antibodies (APAs) have clinical significance because of their association with thromboembolic events and adverse pregnancy outcome[81]. Among patients who have recurrent spontaneous abortions, prednisolone and low-dose aspirin therapy have been proven to be effective in maintaining and prolonging pregnancy in women with autoimmune conditions, including those with positive APA[82]. Many studies have indicated that the number of follicles, oocyte yield, and implantation and pregnancy rates are improved with a low dose of aspirin combined with either prednisolone or immunoglobulin G[83–85]. However, more recent studies indicate that low-dose aspirin does not improve ovarian stimulation, endometrial response, or pregnancy rates for IVF treatment[86–88]. Therefore, a well-designed clinical trial is needed to confirm the benefit of low-dose aspirin treatment for poor responders.

IVM FOR POOR RESPONDERS

No hCG priming prior to oocyte retrieval

Regardless of the modification of stimulation protocol, poor responders still experience a higher cancellation rate because of the small

number or size of follicles. It has been reported that an acceptable pregnancy rate was obtained following immature oocyte retrieval and IVM without hCG administration before oocyte collection, suggesting that IVM may be a viable alternative to cancellation in poor responders to conventional stimulated IVF cycles[10,11]. As mentioned above, poor response to gonadotropin stimulation occurs more often in older women, but may also be present in young women with an abnormal or normal endocrinologic profile. Some poor responders appear to respond to stimulation but have a low estrogen level or few or slow growing follicles. These groups of patients require a prolonged stimulation time and higher doses of gonadotropins. Following gonadotropin stimulation, the number of follicles may be normal, but their size may be smaller than in the usual treatment cycles[89]. In these cases, IVM treatment may be a novel option for the patients instead of longer gonadotropin stimulation or treatment cancellation. Our experience demonstrates that acceptable pregnancy rates are obtained when IVM treatment is applied to these poor responders before treatment cancellation (Table 25.1). Prior to immature oocyte retrieval, the patients can have priming either with or without hCG. Indeed, there were also two pregnancies following IVM when immature oocytes were retrieved after hCG administration from such poor responders before treatment cancellation[90].

Table 25.1 Results of in-vitro maturation and fertilization of oocytes retrieved from poor responders during stimulation cycles without hCG priming*

No of cycle (couples)	19 (18)
Age	30.6 ± 3.7
No of oocytes retrieved	
Total	170
Mean	9.0 ± 8.1
No of oocytes matured (%)	135 (79.4)
No of oocytes fertilized (%)	96 (71.1)
No of embryos cleaved (%)	89 (92.7)
No of embryos transferred	
Total	45
Mean	2.4 ± 0.9
No of clinical pregnancy (%)	6 (31.6)
No of implantation (%)	7 (15.6)

* Data from IVF Center, Nanjing Medical University, China

hCG priming prior to oocyte retrieval

It has been reported that the IVM pregnancy rate is potentially improved by priming with hCG prior to immature oocyte retrieval[91–93]. It is possible that priming with 10 000 IU hCG 36 hours before oocyte retrieval followed by IVM would optimize the successful pregnancy rate in such poor responders because at least some in-vivo matured oocytes can be collected after hCG administration. Indeed, these mature oocytes pooled together with IVM of immature oocytes will maximize successful IVF treatment without cycle cancellation. Recently, Maria Infertility Hospital, Seoul, Korea, has tried this alternative IVM treatment for poor responders after hCG priming, and the results are promising (Table 25.2). As a criterion for this alternative, the size of follicles was still less than 10 mm in diameter after stimulation for more than 7 days. The patients were given 10 000 IU of hCG and oocyte collection was performed 36 h later. Interestingly, approximately 15% (1.7 ± 0.5) mature oocytes were retrieved at collection. These in-vivo matured oocytes pooled with in-vitro matured oocytes will give a higher chance for embryo transfer and potential pregnancy. Reasonable clinical pregnancy and implantation rates (40.4% and 15.8%) have been achieved by application of hCG priming in poor responders who are undergoing ovarian stimulation.

Table 25.2 Results of mature and immature oocytes retrieved followed by IVM from poor responders during stimulation cycles with hCG priming*

No of patients (cycles)	50 (55)
Age (mean ± SD)	32.3 ± 3.4
No of oocytes retrieved (mean ± SD)	641 (11.7 ± 8.3)
No of oocytes matured on collection day (%)	94 (14.7)
No of immature oocytes retrieved (%)	547 (85.3)
No of oocytes matured following culture (%)	406 (74.2)
Total no of oocytes matured (%)	500 (78.0)
No of oocytes fertilized (%)	359 (71.8)
No of cycles completed (%)	52 (94.6)
No of embryos transferred (mean ± SD)	203 (3.9 ± 1.1)
No of clinical pregnancies (%)	21 (40.4)
No of embryos implanted (%)	32 (15.8)

* Data from Maria Infertility Hospital, Seoul, Korea

SUMMARY

Although poor responders have been identified in conventional stimulation IVF cycles, the mechanism to this poor response to gonadotropin stimulation is still unclear. The chance of achieving pregnancy in this group of patients seems significantly reduced. Patients with a poor or retarded response to stimulation seem not to benefit from a stimulation protocol of higher dose gonadotropin, and a higher dose of gonadotropin may negatively influence oocyte quality, fertilization, and pregnancy outcome. Therefore, an increased cancellation rate and decreased pregnancy rates are also noted among these poor responders. The results from the data presented suggest that IVM treatment may be a viable alternative to cancellation of IVF treatment cycles in these poor responders from the conventional stimulation IVF cycles. In conclusion, mature and immature oocyte retrieval following hCG priming from poor responders during stimulation cycles following by IVM is a novel method for this group of patients.

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