Different ovarian stimulation protocols for women with diminished ovarian reserve

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Abstract

Purpose To review the available treatments for women with significantly diminished ovarian reserve and assess the efficacy of different ovarian stimulation protocols.

Methods Literature research performed among studies that have been published in the Pubmed, in the Scopus Search Machine and in Cochrane database of systematic reviews.

Results A lack of clear, uniform definition of the poor responders and a lack of large-scale randomized studies make data interpretation very difficult for precise conclusions. Optimistic data have been presented by the use of high doses of gonadotropins, flare up Gn RH-a protocol (standard or microdose), stop protocols, luteal onset of Gn RH-a and the short protocol. Natural cycle or a modified natural cycle seems to be an appropriate strategy. Low dose hCG in the first days of ovarian stimulation has promising results. Molecular biology tools (mutations, single nucleotide polymorphisms (SNPs)) have been also considered to assist the management of this group of patients.

Conclusions The ideal stimulation for these patients with diminished ovarian reserve remains a great challenge for the clinician, within the limits of our pharmaceutical quiver.

Keywords IVF · Stimulation protocols · Poor responders · Ovarian reserve · Mutations · Single nucleotide polymorphisms

Introduction

The standard goal of all fertility treatments is the improvement in pregnancy rates in patients with infertility problems. Within the past years, ovulation induction has contributed to the success of assisted reproduction techniques, in vitro fertilization (IVF) and embryo-transfer (ET). The efficacy of these techniques depends on a personalized protocol of controlled ovarian hyperstimulation (COH) and an adequate oocyte recruitment. The response of many patients to ovarian stimulation protocols used every time is not always as expected. Failure to respond adequately to standard protocols and to recruit adequate follicles is called ‘poor response’ [1] and result in lowering oocyte production, cycle cancellation and overall, decreased pregnancy rates. Poor response to ovarian stimulation induction is such a case of a poor expected outcome. It is very important to be able to identify the patients who will have a poor response and advise her properly, so that the couple and the doctor make the appropriate decisions and individualize the stimulation protocol.

The incidence of poor responders varies in the literature between 9 and 24% [2] and depends on the definition of a poor responder that individual IVF centers employ [3].

Reduced ovarian reserve represents the most frequent etiological factor [4, 5]. Although it is highly correlated with maternal age, it is also common in younger women and may be associated with advanced endometriosis or prior ovarian surgery [6]. Recent studies have shown, that poor ovarian response is a first sign of ovarian ageing (early ovarian failure or early menopause) [7–9]. The oocytes obtained at egg retrieval are often sub-optimal [10]. Two other situations that can lead to poor response are the elevated basal serum follicle-stimulating hormone (FSH) levels and the reduced inhibin production by granulosa cells [11]. Alternatively, a decreased number of FSH receptors in

Stimulation protocols commonly used for women with significantly diminished ovarian reserve.

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granulosa cells [12], a defective signal transduction after FSH receptor binding [13], an inappropriate local vascular network for the distribution of gonadotropins [14], the presence of autoantibodies against granulosa cells [5], an excess of vascular growth factor receptor (VEGFR-1) [15] and a lowered circulating gonadotropin surge-attenuating factor (GnSAF) bioactivity [16] have also been reported as possible etiologies.

In order to investigate the genetic background of ‘poor responders’, we examined a group of 35 poor responders and a group of 20 normal fertile controls to determine if three of the known inactivating mutations, T479C (Ile160Thr), C566T (Ala19Val), C1717T (Arg573Cys) are present in the FSH receptor gene. The first two are extracellular domain mutations, whereas the third is a transmembrane one. So far our results have shown that FSHR gene mutations are not frequent in Greek ‘poor responders’ [194].

For the patients, poor response causes a lot of stress, increases cost because of the increase of the attempts and some IVF programs will refuse to accept such patients, encouraging them into oocyte donation or adoption.

Difficulties in evaluating poor responders

Definition- predictive tests

Unfortunately, the definition for women with significantly diminished ovarian reserve varies greatly in the literature [3]. There is no universal definition for the “poor responders”, although numerous criteria have been proposed for the researchers to describe this situation. The number of developed follicles and the number of oocytes retrieved after a standard stimulation protocol, are the most important criteria. The proposed number varies among authors and ranges from less than three to less than five dominant follicles on the day of hCG administration [17–19] and/or less than three to less than five retrieved oocytes [20, 21]. Another correlative criterion that has been proposed is the peak E₂ level. A peak E₂ level of <300 to <500 pg/ml [19] or a level <100 pg/ml have been reported as crucial for defining poor response [22]. A basal (day 3) elevated serum FSH (≥ 7 to ≥ 15 m IU/ml) is an additional criterion [23–26]. An advanced patient’s age (≥ 40 years) [26, 27], at least one cancelled IVF cycle [28], an increased number of hMG or FSH ampoules used (>44) [29] or increased (>300 IU/day) gonadotropin used [30] and prolonged duration of gonadotropin stimulation [31] have been suggested as more common criteria either alone or in combination. Some researchers classified poor responders in two subgroups; the first, includes young (aged ≤ 37 years) and slim patients (weight ≤ 70 kg) who developed less than five follicles following 9 days of ovarian stimulation with 225 IU/day and did not reach oocyte retrieval, or required

>600 IU of gonadotropin per retrieved oocyte if they reached that stage. The second subgroup includes patients aged >37 years and weight >70 kg whose cycles were cancelled due to less than five follicles following 9 days of ovarian stimulation with 300 IU/day of gonadotropins [32].

Another classification was also proposed [33]. This included patients with a low response to previous IVF but normal basal FSH levels, young patients with steady high FSH levels, and older patients with an abnormal endocrinological profile. None of these classifications represent any clinical advantage, because no significant differences in ovarian response have been observed [34].

From our experience, in our IVF unit, we have decided to consider a poor responder as a patient who fulfills the following criteria: three or fewer recruited follicles or collected oocytes and serum estradiol concentrations lower than 300 pg/mL (of one follicle) or 500 pg/mL (if 2 or 3 follicles) at the time of hCG administration [35].

There exist some screening test to detect ovarian reserve, but no poor ovarian response. Several tests have been suggested but none of them has significant predictive value. More common dynamic tests applied for the last two decades include the clomiphene challenge test [36], the “Lupron” screening test [37], the exogenous FSH ovarian reserve test [38], the elevated ratio of FSH/luteinizing hormone (LH) in day 3 blood tests [39] and the GnRH stimulation test [40]. High levels of serum FSH (12 or >15 m IU/ml) on cycle day 2 or 3 [41–43], elevated levels of serum estradiol (>30 or 75 pg/ml) on cycle day 2 or 3 [44] and decreased levels of serum inhibin B (45 pg/ml) on cycle day 2 or 3 [45] are considered to be more predictive. Recently, the accuracy of the reduced GnSAF production and bioactivity [16] and low insulin-like growth factor (IGF-I) in follicular fluid have been evaluated as predictive tests in poor responders [46]. Another novel marker for ovarian aging has been proposed by de Vet et al. [47]. Decreased serum concentrations of antimüllerian hormone is correlated with the number of antral follicles and age. Different sonographic tests have also been proposed as predictive for ovarian response. Decreased ovarian volume [48], decreased antral follicle count [49, 50], significantly decreased ovarian stromal blood flow [51] are some of them that provide better information for the ovarian status. But no powerful tests exist for predicting a successful outcome.

The lack of a uniform definition for poor responders is one of the difficulties to evaluate stimulation protocols and compare outcomes. Another problem in evaluating the literature is the study design. Only the powered prospective randomized trials would be helpful for evaluation of poor responders’ protocols, but is very difficult to design them, as poor responders are not a homogeneous group and each patient may have a different cause of diminished ovarian reserve.

A variety of protocols have been used to improve response in the poor responders. Although, the optimal protocol has not yet been defined, reproductive specialists have many options at their disposal [6, 52]. In this study, we present all the strategies that have been used as treatment for poor responders and the efficacy of various controlled hyperstimulation (COH) regimens, focusing in the most promising.

Materials and methods

This review aimed to identify and evaluate all the protocols that have been used for poor responders over the last few years. The literature research was performed among studies that have been published in the Pubmed, in the Scopus Search Machine and in Cochrane database of systematic reviews. The basic key words were “poor responders”, “IVF”, “stimulation protocols”, “ovarian reserve”. The relative studies identified were about 222, other prospective randomized or non randomized, some retrospective, with historical controls and some reviews. Available treatments for women with significantly diminished ovarian reserve were registered and all proposed protocols are presented in Table 1.

Results

High doses of gonadotropins

Obviously, a very logical approach to treat these patients who failed to respond to standard gonadotropin stimulation

protocols is to increase the daily and total dose of gonadotropins. The important role of gonadotropins in stimulation protocols for IVF-ET is already known [53]. In a recent study of the role of the FSH receptor gene polymorphisms [54], it was concluded that ovarian dysfunction infertile women tend to carry the Ser/Ser allelic variant, whereas good responders carry more often the Asn/Ser allelic variant, that has a higher FSH sensitivity and is related with increased number of follicles and oocytes in ovarian dysfunction patients. The Ser/Ser genotype variant requires a significantly higher gonadotropin dose. This variation may indicate a prompt response to FSH administration.

Data in the literature, though, are in conflict concerning the successful outcome of these protocols. Some researchers have reported improved outcome [55, 56]. According to most authors, the common initial dose for poor responders is at least 300 IU/day. But, although all poor responders protocols use high gonadotropin dosages (typically 300–450 IU/day), subsequent studies did not support any benefit from this method [17, 19, 57, 58]. In fact, Ben-Rafael and colleagues [59], reported poorer oocyte quality with the use of highly dosage of gonadotropins in normally responding patients. On the other hand, Garcia-Velasco and colleagues [60], concluded that poor responders with normal basal FSH levels, may benefit from increasing the doses of gonadotropins. Overall, in a review from the center for Clinical Effectiveness [61], searching for evidence for the effectiveness of increasing the total dose of FSH >3,000 IU for ovulation protocols in poor responders, no advantage of the higher dose protocol was found. At present, from currently available data, no recommendation for clinical practice can be offered [62].

The use of recombinant FSH (r FSH)

During the 1990s, the introduction of recombinant FSH (r FSH) became widely available for ovulation induction protocols. Many studies from then favored the use of rFSH with regard to oocyte maturation, number of mature oocytes retrieved, number of high quality embryos and pregnancy rates. Indeed, minimizing LH, oocyte/embryo quality improved and several prospective studies in poor responders demonstrated significant benefits with r FSH, especially young [19, 63, 64]. But these initial promising results were not confirmed by others [65–69]. A study of Drakakis and colleagues showed that, a gonadotropin with a lower cost, such as hMG, can be employed in intracytoplasmic sperm injection (ICSI) without affecting the outcome adversely [70]. Also, in a recent meta-analysis there was no clinical significant difference confirmed between r FSH and h MG in IVF/intracytoplasmic sperm injection cycles (ICSI) [71]. Therefore, there is no conclusive evidence that rFSH has

Table 1 Synopsis of different ovarian stimulation protocols for poor responders (see text for references)

Protocol
High doses of gonadotropins
r FSH
Luteal initiation of FSH
LH
Adjunctive GH
Gn RH agonists- Luteal onset of Gn RH agonists
“Stop” Gn RH agonist protocols
Gn RH-a: ‘flare’ regimens
Microdose Gn RH agonist flare regimen
Short protocol
Gn RH antagonists
Natural cycle or modified natural cycle
Androgen supplementation
Administration of letrozole
Low level hCG in early stimulation phase

better results in poor responders, but larger randomized studies are needed in order to have definite conclusions.

Luteal initiation of FSH

Another alternative approach is the initiation of FSH administration during the late luteal phase for enhancing follicular recruitment in prior poor responders. It is well known that healthy, small antral follicles are present in the late follicular phase and their further development occurs under action of the premenstrual FSH rise [72]. Earlier administration of FSH might increase the number of recruited follicles by opening the recruitment window in the late luteal phase of the preceding cycle. However, in a prospective randomized trial that Rombauts and colleagues designed [20], the results were not encouraging, with high cancellation cycles and lower pregnancy rates, although follicular maturation was achieved sooner after the onset of menses.

The use of LH

The necessary and optimal dose of LH in order to achieve pregnancy in assisted reproduction has not been determined yet. Levels of <1.5 IU/L have been insufficient to maintain aromatase activity and E2 production [73]. Similarly, low periovulatory levels (<3 IU/L) in patients undergoing IVF are associated with impaired fertilization and increased early pregnancy loss [74, 75]. Small doses of LH when used early in ovarian stimulation in IVF-ET cycles have beneficial effect in quality of oocytes, a fact very important, especially in cases in which few embryos are available for transfer [76]. In poor responders, LH administration in the early phase of folliculogenesis, early during the stimulation protocol, may have beneficial effect on the maturity and fertilizability of oocytes, as well as the number of embryos transferred. However, the data do not show that increased pregnancy rates are achieved in patients for whom LH was added to r FSH for ovarian stimulation [70, 77]. In a recent meta-analysis [78] where randomized controlled trials comparing controlled ovarian hyperstimulation (COH) with r FSH alone or in combination with r LH in IVF/ICSI, were analyzed, there was no evidence of statistical difference in pregnancy outcomes when rLH was used, probably due to the small number of patients. Nevertheless, further large randomized trials should be undertaken because all these data suggest a point towards a beneficial effect of co-treatment with rLH, in particular with respect to pregnancy-loss and poor responders.

Adjunctive use of growth hormone (GH)

An alternative concept to the use of gonadotropins is the supplementation with growth hormone (GH) or growth

hormone-releasing hormone (GH-RH). GH seems to stimulate ovarian steroidogenesis, follicular development and enhances the ovarian response to FSH [79]. GH modulates the action of FSH on granulosa cells by up-regulating the local synthesis of insulin-like growth factor (IGF-I) [80–82]. The IGF-I acts in synergy with FSH, amplifying its effect on granulosa and theca cells [79, 83]. The usual GH dose used in different studies was 4 to 12 IU sc, commencing on the day of ovarian stimulation with gonadotropins. Initial results in small groups of poor responders were encouraging, reporting higher number of oocytes collected and improved pregnancy rates [84–86]. Another randomized double-blind, placebo-controlled study by Chang-Hoon and colleagues [87] used pyridostigmine (120 mg/day orally from the day of down-regulation until the day of h CG), an acetylcholinesterase inhibitor which by enhancing the action of acetylcholine, can increase GH secretion. These results compared with placebo showed significant number of ampoules used, higher number of oocytes collected and improved, but no significantly pregnancy rates. However, other randomized prospective trials failed to prove any beneficial effect [88–92].

In a recent Cochrane Review [93], a meta-analysis of all the trials that used GH adjunctive therapy in women undergoing ovulation induction, no significant difference was found either in the number of follicles and oocytes or in the number of ampoules of gonadotropins used. At the present, published data do not support a beneficial effect with the supplementation of GH or GH-RH in poor responders' protocols.

GnRH agonists

The use of GnRH agonists (GnRH-a) in IVF protocols has been beneficial for ovarian stimulation. ART improved in terms of reduction in cycle cancellation by the almost abolishment of spontaneous LH surges and reduction of inadequate follicular development, improving clinical pregnancy rates [94]. Additionally, patients who respond poorly to hMG stimulation with an insufficient number of growing follicles and a peak estradiol level less than 300 pg/ml are probably the appropriate population to use GnRH-a [95]. Superovulation strategies combining the use of GnRH-a and HMG or rFSH fall into three categories, the long, the short and ultra-short protocols. The superiority of long protocol is already defined in IVF/ICSI cycles [96, 97]. The prolongation of Gn RH-a in patients with a high level of E₂ on day 15 until hMG initiation has been investigated and there was no adverse effects on cycle treatment. In the contrary, this long period of 22–38 days of “frozen ovary” has a clear improvement of embryo cleavage speed, pregnancy rate as well as a significant higher rate of multiple pregnancies [98].

On the other hand, poor responders present a poor response to a protocol employing GnRH analogs. An alternative way of administering GnRH-a that would improve pregnancy rates for these patients has, therefore, been investigated.

Luteal onset of GnRH-a

This protocol is characterized by the use of low doses of GnRH agonists (0.1–1 µg) commencing in the midluteal phase of the cycle and ending at the time of menses or shortly thereafter, in combination with high doses of gonadotropins. In this way, the reduced effect of the GnRH agonists on their ovarian receptors [99, 100] results in reduced ovarian suppression and in increased ovarian response. The administration of GnRH-a during the midluteal phase to achieve initial pituitary gonadotropin down-regulation has been used in poor responder patients. Improved oocyte yield and pregnancy rates was found in large nonrandomized series [101–104]. However, McKenna and colleagues [105] reported modest gains from this approach and Horvath and colleagues [106] significantly increased gonadotropin requirements before ovarian stimulation. Although, these data tend to suggest a benefit for the addition of luteal phase GnRH-a in patients who fail to respond to hMG alone, Ashrafi and colleagues [107] concluded that there is no advantage in the use of GnRH-a compared to the older regimens of clomiphene plus HMG and hMG alone. The cancellation rate was similar for the three protocols and hMG requirement was higher with the use of GnRH-a.

Another alternative is to diminish the dose of GnRH-a initiated during luteal phase to allow down-regulation without complete inhibition of ovarian response to exogenous gonadotropins. Kowalik and colleagues [100] used this protocol in patients who were not necessarily poor responders and reported higher E₂ levels, though implantation rates were not affected.

In a prospective study by Olivennes and colleagues [25] GnRH-a was initiated in the midluteal phase and decreased in half as soon as gonadotropin stimulation started. The use of total gonadotropin doses was lower, the stimulation was more rapid and increased number of oocytes, better quality of embryos obtained and low cancellation rates was noted. Feldberg and colleagues [24] also reported a beneficial effect in diminishing the starting dose of GnRH-a (0.1 µg/day) until menstruation and then lowering the dose in half during COH.

“Stop” GnRH-a protocols

Some IVF programs have explored stopping GnRH-a once pituitary suppression has occurred and then begin the

ovarian stimulation with hMG or rFSH [60, 108]. In these two prospective randomized controlled trials no statistical improvement in pregnancy rates or in cancellation rates was found.

But others, in prospective trials with historical controls [25, 40, 104, 109–111] and a retrospective study [24], who administered a low-dose GnRH-a in midluteal phase, but then discontinued with the onset of menses or shortly after, demonstrated improved outcomes, increased number of oocytes collected, high pregnancy rates and lower cancellation rates. Various GnRH-a do not seem to effect the benefit of this protocol.

GnRH-a: ‘flare’ regimens

A widely employed protocol is the so called “flare” regimen. The administration of GnRH-a is initiated in the follicular phase and the gonadotropin stimulation commences few days later. The concept in this protocol is to add the agonistic effect of GnRH-a to the action of gonadotropins, eliminating excessive ovarian suppression. The results with this protocol are also conflicting in the literature. Katayama and colleagues [112] reported higher pregnancy rates, decreased miscarriage rates and decreased gonadotropin doses required. In similar results, concluded the study by Garcia and colleagues [113]. Other sequential studies [31, 114] noted also, higher pregnancy and implantation rates, with lower cancellation cycles. However, these encouraging results have not been confirmed by others. Many study groups investigating the “flare” regimen protocol versus luteal phase GnRH initiation protocols, have reported poorer or similar outcome in normal responding patients [115–121] and similar results in poor responders. The poorer outcomes may be explained by the significant increase that was noted in LH serum levels, progesterone and testosterone levels at the first follicular phase GnRH-a dose and remained high until the day of hCG administration [122]. Probably a direct effect of the GnRH-a on enhancing LH secretion to rescue the corpus luteum from the prior cycle or/and the local hyperandrogenic environment during folliculogenesis, which has a degenerative effect on the oocyte are the causes of the poor results.

Microdose flare regimen

In 1994 the microdose flare protocol for poor responders was introduced [123]. This protocol is an alternative scheme to the flare protocol, where the dosage of GnRH-a administered is decreased (till 25–50 µg as single dose) [124] and pretreatment with oral contraceptives or progesterone is employed [125]. In the study of Scott and colleagues, significant improvement was reported in peak estradiol, follicle recruitment and mature oocytes at

retrieval. Other investigations also [22, 126] have reported significantly fewer cycle cancellations, improved implantation rates and extremely ongoing pregnancy rates. Additionally, low dose Gn RH-a administered in the early follicular phase either with or without supplementation of GH has been reported to enhance ovarian response and pregnancy rates in poor responders.

Oral contraceptive (OC) or progestin pretreatment could potentially intermit the function of a persistent corpus luteum derived from a prior cycle. Several studies in normal responders have reported that the administration of OC or progestins before ovarian stimulation, improved ovarian response, eliminating premature LH surges, decreased required gonadotropin doses, abolished cyst formation and shortened the duration of gonadotrope suppression with no adverse effect on pregnancy rates [127–129]. Pretreatment with a progestin for 12–20 days before initiation of a flare regimen in poor responders, diminished LH and progesterone levels during the early stages of gonadotropin stimulation [130]. In another study [131], the comparison between three protocols in a small group of poor responders (flare regimen- luteal phase GnRH-a down regulation- gonadotropin stimulation alone) and OC-pretreated gonadotropin stimulated protocol, showed higher pregnancy rates and lower cancellation cycles in the group pretreated with OC. The effect of OC on the hypophyseal and ovarian hormonal response after GnRH-a injection in a flare protocol is not clear. Although, OC may result in hypothalamic and gonadotropin suppression for variable duration after their cessation, this suppression may blunt any flare response to GnRH-a in the early follicular phase following OC [132, 133]. Al-Mizyen and colleagues studied the effect of pre-IVF treatment with either a gestogen or OC in poor responders undergoing flare protocol and concluded that the pretreatment did not improve the outcome of IVF-ET [134]. Similarly, in a retrospective analysis [135] comparing a microdose flare protocol with a long luteal with decreasing dose of Gn RH-a, high cancellation rates were observed and lower clinical pregnancies, with the microdose protocol. A recent study [136] that compared the FSH flare with and without pretreatment with OC in poor responders, reported that the administration of OC suppressed the FSH before the injection of the Gn RH-a, but did not blunt the FSH flare. On the other hand, it blunted the LH flare, which may be beneficial. Larger studies is required to asses any outcome advantages or disadvantages of pretreatment with OC in flare protocols, although there is a trend towards improvement.

Short protocol

The results of ovarian stimulation with the short protocol using rFSH with or without the use of LH in IVF/ICSI was

recently evaluated in patients with many failed attempts and maternity age ≥ 37 years [137]. FSH levels of >9 mIU/ml are associated with poor results even with the use of HMG. The quality and the number of embryos transferred were better in the r FSH + HMG group. This study concluded that intricate cases have good chances for achieving a pregnancy using the short protocol and the outcome is further improved when LH is added from the beginning of ovarian stimulation. It seems the development rate of embryos is faster when the follicles are exposed to LH from the beginning of the stimulation protocol, rather than only during the later phase of the follicular stimulation [138].

However, the matter for which GnRH-a protocol is preferable in poor responders remains controversial. Some investigators [114–139] insist that the GnRH-a short protocol appears to improve the ovarian outcome in patients at risk for reduced ovarian responsiveness. Another randomized, prospective study comparing the short and the long protocol in poor responders [140], both specifically modified (modified flare protocol versus GnRH agonist mini-dose) [24], concluded that despite the superiority of mini-dose protocol, that still appears to yield the best results, some poor responders have better performance on the short protocol.

In the study of Detti and colleagues [141], a comparison of three downregulation approaches for poor responders (stop protocol- microdose flare- short protocol) supported that the microdose flare protocol for poor responders demonstrates a trend towards higher delivery rates.

GnRH antagonists regimens (GnRH- ant)

The use of GnRH antagonists gave new hope to the management of poor responders. The administration of Gn RH antagonists aims to avoid the premature LH surge and to utilize the maximum of the ovarian oocyte cohort by minimizing the suppressing effects of the GnRH-a on the ovarian receptors, eliminating ovarian suppression at the stage of follicle recruitment [142]. Premature luteinization is markedly reduced during the period of treatment with the antagonist as compared to the period preceding the onset of its administration [143]. The clinical use of Gn RHant provides several advantages for the patient, including a shorter duration of stimulation, fewer doses of gonadotropins, no increased risk of cyst formation and no hormonal withdrawal symptoms [144–146]. However, the GnRH-ant cycles have been found to result in lower E_2 levels than agonist cycles [147]. This may be the cause of lower pregnancy and implantation rates in an antagonist protocol. But even with a modified antagonist protocol (longer duration and increased r FSH dosage), no difference in E_2 levels was noted [148].

Many IVF programs currently use GnRH antagonists for poor responders. The first publication to describe the use of GnRH antagonists in poor responders appeared in 1999 [149]. Poor responders were stimulated with a combination of gonadotropins and clomiphene citrate, and were started on daily GnRH-ant once the leading follicle reached at least 14 mm in diameter. Compared to their response in a previous GnRH agonist cycle, modest improvements in cycle cancellation rate, oocyte yield, and gonadotropin requirements were realized with the GnRH antagonists. Another retrospective analysis of poor responders treated with GnRH antagonists reported lower gonadotropin consumption and shorter durations of stimulation in antagonist cycles, compared to previous cycles using a GnRH agonist [150].

There have been few prospective, randomized trials comparing GnRH antagonists to alternate protocols [151–155]. None of these studies had sufficient power to evaluate a difference in pregnancy rates, and in most cases, IVF outcomes were comparable. In a study of Marci and colleagues [156], a GnRH-ant protocol was compared to standard long protocol in poor responders. Fewer cycles were canceled with the use of the antagonist protocol and a difference in pregnancy rates was noted in favor of the antagonist protocol, but not statistically significant.

In a recent meta-analysis for GnRH-ant in ovarian stimulation in patients with poor response to gonadotropins, no differences in clinical outcomes were found, except a significantly higher number of cumulus-oocyte complexes in GnRH-ant multiple dose protocol as compared to Gn RH-a long protocol [157].

Although all poor-responder protocols utilize high gonadotropin doses (typically 300–450 IU per day), other tactics are usually also employed. A novel protocol of ovulation induction, based on the combined administration of OC, high dose rFSH, and delayed GnRH-ant administration, for poor responders compared with the outcome of a standard long protocol [158] resulted in decreased cancellation rates, oocyte retrieval, pregnancy and implantation rates increased, whereas these parameters remained unchanged in patients receiving the standard long protocol.

A multicentre prospective longitudinal study including a group of poor responders, serving as their own controls was recently conducted [159]. This protocol, termed CRASH, included the administration of Gn RHant in the late luteal phase, on cycle day 23, stimulation with r FSH started on cycle day 2, followed by a flexible GnRH-ant protocol. The results showed significantly more follicles, more oocytes and transferable embryos. The implantation and pregnancy rates were similar with the clinical outcome of normal responders.

Improved fertilization and implantation rates were reported with LH supplementation in donor cycles utilizing

GnRH antagonists [160]. De Placido and colleagues [161], reported in their study, where a flexible protocol providing a gradual increase in the dose of Gn RHant in association with recombinant LH administration was compared with the standard Gn RH-a flare-up protocol, that a significant increase in the mean number of mature oocytes has been observed in the Gn RHant group. A number of prospective, randomized trials in normal responders failed to demonstrate an advantage for the addition of LH when using GnRH antagonists. In two recent randomized studies [162, 163], patients undergoing ovarian stimulation with GnRH antagonists to either r FSH, or a combination of r FSH and LH, LH supplementation significantly increased serum E₂ levels, but had no significant effect on fertilization or implantation rates. However, a recent retrospective analysis on poor responders treated with a GnRH antagonist found comparable outcomes, whether stimulation was achieved with or without supplemental LH [164]. In women ≥ 40 years of age, exogenous LH was associated with fewer oocytes at retrieval and fewer embryos available for transfer. Thus, at present, the data are not in favor of LH supplementation in women treated with a GnRH antagonist [165].

The benefit of pretreatment with or without OC in a GnRH-ant protocol in potential poor prognosis patients has recently been investigated [166]. In this retrospective study the comparison of the Gn RHant protocol with or without OC, the pregnancy outcomes were similar in the two groups. Although pretreatment with OC may help with scheduling flexibility, higher doses of gonadotropines for ovarian stimulation are required.

A recent meta-analysis [167] that compared the efficacy of Gn RHant versus Gn RH-a, as co adjuvant therapy for ovarian stimulation in poor ovarian responders in IVF/ICSI, has not proved any difference between the two regimens with respect to cycle cancellation rate, number of mature oocytes and clinical pregnancy rate, per oocyte retrieval or per embryo transfer.

Nevertheless, interest in the use of GnRH antagonists in poor responders has continued. GnRH antagonists may be associated with simpler stimulation protocols, lower gonadotropin requirements, reduced patient costs, and shorter downtimes between consecutive cycles. However, the greatest advantage of GnRH antagonists may lie in the ability to assess ovarian reserves immediately prior to deciding whether or not to initiate gonadotropin stimulation. The ability to respond to cycle-to-cycle variation in antral follicle counts may allow the optimization of oocyte yield and reduce cycle cancellation rates [168]. It remains to be seen if this approach (initiating gonadotropins only in cycles where an adequate antral follicle count is present) also translates into higher clinical pregnancy rates for poor responders.

Natural cycle

IVF following a natural cycle has recently received renewed attention

The first successful IVF treatment was performed in a natural cycle [169]. This practice was abandoned due to premature LH surges and subsequent high cancellation rates. In a review by Pelenck and colleagues [170], the cancellation rate in natural cycles was reported to range from 14.3 to 62.5%. Natural cycles in IVF have been used for patients who have shown a poor response in at least two previous attempts with gonadotropin stimulation [171]. It has been suggested in recent years that IVF following a natural cycle may be a promising alternative for poor responders [172]. The biological advantages of the natural cycle may provide a single oocyte of better quality and thus allow the transfer of a healthier embryo into a more receptive endometrial environment [173, 174]. It is also a less expensive and more time efficient, avoids most of the risks and complications of ovarian stimulation [175], spares the endometrium from the adverse effects of ovarian stimulation [176] and is more psychologically friendly to the patient [177].

The results of two prospective studies [171, 178] support the hypothesis that the naturally developed follicles of poor responders may produce fewer oocytes but oocytes of better quality than their hyperstimulated ovaries. It is clear that, in poor responders, natural cycle is superior once an oocyte has been retrieved and fertilization and implantation rates are significantly better. It is more likely that poor responders produce the same number of embryos suitable for transfer, with or without exogenous gonadotropins. Overall, these data suggest that poor responders may benefit from natural cycle IVF treatment, but should not be offered in these patient with high early follicular phase levels of serum FSH.

Furthermore, GnRH antagonists are likely to reduce the rate of cancellation of cycles due to premature luteinization. A recent study [179] investigated the efficacy of GnRH-ant supplementation during natural cycles in poor responders. A modified natural protocol was compared to a Gn RHant protocol and to a long protocol. The authors concluded that the use of GnRH-ant during natural IVF cycles can be an optional treatment in poor responders. Another study [180] suggested that the use of Gn RH antagonists in a modified natural cycle decreases the occurrence of a premature LH rise and showed a similar pregnancy rate to a standard protocol.

Large scale prospective studies should be performed in order to evaluate these interesting results.

Androgen supplementation

In order to raise the insulin-like growth factor-1 (IGF-1) concentration and thus to enhance the gonadotropin effect,

dehydroepiandrosterone (DHEA) has been used 2 months prior to ovarian stimulation in patients who previously had a poor response. Preliminary results were encouraging [181, 182] In a recent study [183] was investigated the usefulness of testosterone pretreatment in poor responders.(transdermal application) Authors concluded that this may be a useful approach for patients known to be poor responders but having normal basal FSH concentrations. Another study reported a significant increase in plasma testosterone levels but did not significantly improvement of the antral follicle count [184]. The authors concluded that there is no significant benefit from androgen administration on the ovarian response to FSH. However, subsequent clinical trials are needed to determine whether an optimal dose and/or a longer duration of testosterone administration may be helpful.

Low hCG dose in early stimulation phase

In our IVF Unit, we have performed a randomized, pilot clinical study in a period of 12 months. A total of 120 patients were enrolled and randomized while 114 completed the study. Of these, 58 belonged to Group A (received hCG along with rFSH for ovarian stimulation) and 56 to Group B (received rLH along with rFSH). hCG was added only for the first 4 days of the cycle and in a dose of 200 IU. The short protocol was employed.

We had statistically significantly higher implantation and pregnancy rates in the group with hCG administration (unpublished data, submitted for publication). Our study has shown, for the first time to our best knowledge, that the administration of 200 iu of hCG daily, in addition to rFSH, is a safe and possibly better alternative of human recombinant LH supplementation for patients undergoing IVF/ICSI-ET. In our study we used hCG supplementation during the first 5 days of ovarian stimulation in the short protocol. No negative impact of low-dose hCG administration was detected in patients receiving this treatment.

In most of the currently used ovarian stimulation protocols, serum LH is clearly suppressed through pituitary down regulation by GnRH agonists or antagonists. In the short protocol, LH is suppressed during the final days of the follicular growth (days 7–11). It seems, from this study, that the administration of 200 IU of hCG daily from days 3–7 during the follicular growth permits a sufficient LH level in this period of ovulation induction. Our results have shown that with hCG we had lower number of gonadotropin ampoules used, higher fertilization rate, higher implantation rate and better pregnancy rate. In addition, the percentage of mature oocytes and the number and quality of embryos was comparable between rLH and hCG, thus showing that hCG, in the specific dose and way of administration, had no harmful effect on ovarian stimulation.

An explanation of the better ovulation profile in hCG treatment cycles could be the different isoform of hCG as compared to rLH. Differences in the carbohydrate molecule may make the molecule more sensitive to the binding receptor. In addition, the longer plasma half-life of hCG (half life of hCG is 33 h while half life of rLH is 10–12 h) results to a better and prolonged effect in the ovarian stimulation process. It seems, therefore, that its longer plasma half-life and its greater potency (roughly six to eight times greater than that of LH) permit highly effective and more stable occupancy of the LH/hCG receptors. The fact that serum E₂ levels in patients who received rLH were statistically significantly lower than in patients treated with hCG, shows that indeed the occupation of the LH/hCG receptor in the rLH-administered patients is less compared to the hCG stimulated patients.

Due to the above, we believe that hCG addition may be a promising alternative for poor responder patients. Large studies from various centers have to verify these data before we consider hCG addition as a successful option for poor responder patients.

Other regimens

Recent data suggest a potential beneficial effect of aromatase inhibition by the administration of letrozole prior to gonadotropin stimulation [185, 186]. Letrozole is a highly selective, non-steroidal aromatase inhibitor, that has successfully be used for induction of ovulation in women with polycystic ovarian syndrome [187]. Recent data suggests that letrozole may improve ovarian response to FSH in poor responders and reduce gonadotropin dose required for stimulation in women with unexplained infertility [186, 188]. Another study [189], demonstrated that addition of letrozole to gonadotropins increased the number of pre-ovulatory follicles without having a negative effect on pregnancy rates.

The precise mechanism of the ovarian effects of letrozole is not yet known. It has been hypothesized that, because of the selective inhibition of aromatase, letrozole may significantly inhibits the overall production of estrogens. Consequent withdrawal of the negative feedback effects of estrogens may allow the pituitary to produce more endogenous FSH. Attenuated aromatization may also lead to accumulation of follicular androgens, which may increase the follicular sensitivity through amplification of FSH receptor gene expression [190] or stimulate IGF-I which may act in synergy with FSH [191]. A recent study [192] proposed the administration of letrozole, as an alternative to natural cycle IVF, as a low cost protocol in women with poor ovarian response.

Letrozole and other aromatase inhibitors have not been extensively used in women of reproductive age, so they

have to be evaluated more carefully with larger randomized studies.

Aspirin

The effect of adjuvant low-dose aspirin on utero-ovarian blood flow and ovarian responsiveness in poor responders undergoing IVF, was evaluated in a recent study [193]. Authors concluded that supplementation with low-dose aspirine failed to improve either ovarian and uterine blood flow or ovarian responsiveness in poor responders.

Conclusions

Despite the numerous predictive tests for diminished ovarian reserve, the poor responder is most times revealed during ovarian stimulation. The fact that there is no uniform definition of the poor response makes the clinical trials incomparable. Furthermore, an accurate prediction of low ovarian response would help the clinician to use the most suitable protocol for ovarian stimulation for each patient and the most appropriate alternative to IVF treatment, diminishing the cost and the psychological decay.

There have been no reports of large-scale, prospective, randomized, controlled trials of the various management strategies. Some of the few prospective or prospective randomized control studies include small number of patients and do not have the power to prove their results.

However, a trend for an overall improvement in ovarian response has been shown with some of these protocols. There are, also some investigations that give hope for ameliorating pregnancy rates.

The use of high doses of gonadotropins to stimulate the ovaries is inevitable due to the diminished ovarian reserve. Additionally, the use of recombinant LH might improve outcome.

Optimistic data have been presented by the use of flare up Gn RH-a protocol (standard or microdose). Significant improvement was demonstrated with the use of stop protocols, luteal onset of Gn RH-a and the short protocol. Pretreatment with OC may help the ovarian response, either on GnRH-a or GnRH-ant protocols.

Natural cycle or a modified natural cycle seems to be an appropriate strategy for those poor responders who are capable of producing one or two follicles. Finally, aromatase inhibitors, as letrozole may be a low-cost approach with similar to other protocols results.

The impact of polymorphisms Asn/Ser is also under consideration but, for the moment, it seems that it plays a small part. In order to ensure that a beneficial effect exists, an array of molecular tools will be needed and hundreds of thousands of polymorphisms must be examined in appro-

priate phenotypic groups such as ‘poor responders’. However, genotyping of patients scheduled for ovarian stimulation could be an attractive tool to individualize FSH dosage according to the genetic differences in ovarian sensitivity.

In conclusion, there is a great need of well-designed, large-scale, randomized controlled studies to assess the efficacy of all these protocols used for poor responders. The ideal stimulation for these patients with diminished ovarian reserve remains still of great challenge for the clinician, as the low number of oocytes and the poor quality cannot be reversed within the limits of our pharmaceutical quiver.

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