TREATMENT MODALITIES IN POOR RESPONDER PATIENTS UNDERGOING ASSISTED REPRODUCTIVE TECHNIQUES

SUMMARY
Successful IVF/ICSI treatment depends on obtaining a sufficient oocyte sample and selecting patients carefully. COH (Controlled Ovarian Hyperstimulation) treatment in patients with poor ovarian response is one of the most important issues in IVF programmes. The ovarian response that follows ovarian stimulation is the most important determinant in ART treatment. Although many other stimulation protocols have been applied on patients with poor ovarian response, low pregnancy rates are currently reported.

KEYWORDS: Poor responder; Assisted reproductive technology; Treatment modalities

INTRODUCTION
In this review, treatment and stimulation protocols in poor responder patients undergoing ART will be discussed. The Pub Med database was searched in August 2013 with various combinations of the following terms in English: ART, poor responder, stimulation protocols, improvement in pregnancy rates, ovarian response, ovarian reserve, and IVF/ICSI treatment.

DEFINITION
Poor ovarian response was first reported by Garcia et al. [1] in 1983. Poor responder cases constitute 9-24% of ART cycles. The rate is reported as 50% in women over 40 years old [2]. FSH begins increasing 13 years before menopause. With increasing FSH, the number of follicles, oocytes, embryos and implantation rates decrease, and cycle cancellation rates increase. Despite improvements in ART, there is no consensus on the management of patients with poor responses. Parameters such as increased FSH levels, low E2 levels at hCG day (300-660 pg/ml), low oocyte samples (below 4-6), antral follicules below 3-5 on the day of hCG administration, patients with an advanced age, increased FSH dosage used and an extended stimulation period are used to describe poor ovarian response. The ESHRE meeting in Bologna at 2011 concluded with a new consensus to find a common language to describe poor ovarian response. This included, i) Advanced maternal age (>40) or other risk factors for POR, ii) previously obtaining < 4 oocytes with conventional stimulation, iii) Abnormal ovarian reserve tests (AFC <5-7, or AMH <0.5-1.1 ng/ml). Again, according to the Bologna criteria, regardless of age, a patient produces <4 oocytes at 2 cycles even with maximal stimulation and patients aged over 40 years who have poor ovarian reserve tests without using stimulation are also accepted as having POR [3]. Many factors, such as diminished ovarian reserve, advanced maternal age, low levels of FSH receptor numbers, pleomorphism of FSH receptors, Turner syndrome, flagyl X syndrome, previous history of radiotherapy and chemotherapy, mutations of the FMR1 gene, existence of FSH binding inhibitor at follicular liquid, degraded signal transduction as a result of FSH binding, existence of autoantibody against granulosa cells, deficiency of the vessel web that spread of gonadotropins, low levels of GnSAF (gonadotropin surge attenuating factor) were reported for POR etiology [4]. Previous surgical endometrioma, previous PID, obesity, environmental factors, smoking and functional ovarian cysts may also be counted as situations related to POR.

CLINICAL SITUATION
The number of embryos has a substantial importance for IVF/ICSI success rate in POR patients. Therefore high levels of gonadotropin dosages may provide more follicle stimulation. However, how this contributes to pregnancy rates is unclear. High rates of mitochondrial DNA mutations were reported at advanced maternal age [5]. Mitochondrial DNA deletions were also observed in the oocytes of women at advanced ages. As a result, it is thought that decreased energy production effects mitotic activity badly. A more decreased ovarian stromal blood flow is also determined with a 3D power doppler in patients who have POR [6]. Increased abortus rates were also reported in patients with poor ovarian response, as well as decreased pregnancy rates [7]. In patients with POR, the incidence of poor response at the second cycle was reported as 62% [8].

MANAGEMENT OF POOR RESPONDERS
Although different COH protocols are applied for increasing the IVF success rate in these patients, there is no consensus about the optimal procedure. Increasing gonadotropin dosages during the COH procedure, using different types of gonadotropins, changing the start time of gonadotropins or GnRH analogs, utilization of OCS,
addition of GH, DHEA-S, CC, aromatase inhibitors, testosterone, E2, nitric oxide (L-arginine), aspirin, colony-stimulating factor, dexamethasone, pyridostigmine or another adjuvants or the usage of natural cycles and Assisted Reproductive Technology are recommended [9,10]. Despite being illegal in some countries, oocyte donation is an alternative method. However, this may not always be feasible for couples because of different religious and cultural concerns. A limited amount of oocyte maturation is observed as a result of decreased ovarian reserve in patients with POR. Therefore acceptable pregnancy rates have also been reported if IVF/ICSI is applied during a patient’s own cycles even when high LH surge possibilities exist. Increasing mature follicles and embryos is the main objective for patients with poor response. Yet there are two questions. Can we make a non-existent follicle grow? And: Can we improve the quality of a damaged oocyte? A successful ART is related to especially good COH. An inadequate response to gonadotropin results with the cancellation of the cycle, a decreased quality and number of embryos that can be frozen or transferred, a low pregnancy rate and psychological trauma.

Cycle Cancellation Criteria in Poor Responders

Similar cycle cancellation parameters are used in all IVF cycles. These are i) 3 or fewer follicles in USG, ii) highest E2 < 500 pg/ml.

Clinical and Laboratory Administration

Increasing the number of trials and the usage of ICSI instead of IVF in patients with poor ovarian response and aged over 40 seems to provide an increase in pregnancy rates [11]. Depending on the decreased reserve in patients with POR the development of a small number of oocytes can be monitored. Therefore IVF-ICSI applications during natural cycles (with the advantage of lower drug costs) were reported to give acceptable pregnancy rates in several series [12].

A decreased ovarian reserve is related to decreased oocyte quality. The end of the thirties and the early forties are related to increased aneuploidy rates, decreased natural fecundity, decreased numbers of follicle and worsening oocyte quality. Therefore, the transfer of the healthy oocyte cytoplasm to an oocyte with a poor prognosis using the microinjection procedure, and the transfer of the germinal vesicle of a defective oocyte to a denucleated healthy oocyte have become a current issue. In the In Vitro Maturation procedure, immature oocytes are gathered and grown in vitro and the ICSI procedure is applied. At the same time, better follicle development is provided with this procedure. In preliminary studies of in vitro maturation (IVM), decreased cancellation of cycle and acceptable pregnancy/implantation rates were also reported [13]. Assisted hatching is a newer lab technique that was developed when fertility experts observed that embryos with a thin zona pellucida had a higher rate of implantation during IVF. Higher clinical pregnancy and implantation rates have been observed after assisted hatching [14]. Increased pregnancy results were also reported with early embryo transfer in patients with POR [15].

Genetic studies on embryos and the transfer of elected healthy embryos are named ‘PIG’ (Preimplantation Genetic). This technique is used for single gene disease. And this procedure also seems to increase IVF success in patients with POR. Endometrial co-culture environments, also known as ‘imitation uteruses’, are systems that allow the development of embryos in a nearly natural environment before the transfer period. Higher implantation and pregnancy rates are obtained with co-culture techniques [16].

In recent years another technique initiated has been electroacupuncture treatment. Electro acupuncture is the usage of electric impulse stimulation with acupuncture needles. Increased oocyte quality and better pregnancy outcomes for electroacupuncture treatment undergoing patients with POR have been also reported [17]. Stem cell technology will also be used in the treatment of POR patients in the future. Studies on the use of embryonic stem cells in reproductive medicine offers hopes for patients who have no oocytes or sperm.

Although studies on gaining differentiated gamete cells from embryonic stem cells are currently experimental, successful results have been reported. With the beginning of the formation of embryonic bodies in mouse stem cell cultures, the expression of the marker of germ cells were shown. When germ cell markers expressing cells was cultured with retinoic acid solution to obtain a male germ cell, these cells transformed into preseperum cells. Again, culturing the embryonic bodies with a solution containing neonatal testis tissue was successful for gaining ovarian tissue containing oocyte-like structures. These oocyte-like structures have been shown to express markers specific for oocytes and the indicator for meiosis SPC3 expression [18]. Again, differentiating the in vitro male gamete cell from the mouse embryonic stem cell and the injection of the cell obtained into the oocyte has been successful in obtaining blastocyst formation [19]. Non-tail sperm gain in mouse and fertilization was shown when injected into the oocytes and was reported by other studies [20]. It was shown that 0.1% of human embryonic stem cells were differentiated to primordial germ cells by the surface and gene expression markers [21]. In another study in the mouse, oocyte gain from embryonic stem cells was successful [22]. Trofoblastes differentiation from embryonic bodies gained from an embryonic stem cell and human chorionic gonadotrophine secretion from these trofoblastes were also shown [23]. There are some limitations in the use of stem cells in treatment. It is known the potential of stem cells to differentiate various cells, although the mechanisms of this differentiation have not been ascertained. Before the use in the treatment all of the mechanisms in differentiation, the potential side effects and in vivo situation of these cells must be illuminated. The use of stem cells also has some ethical problems. In the use of stem cells in clinical practice in the future more studies will have to be made on animal experiments.

Treatment of Poor Responders

Assuming that ovarian reserve tests predict an acceptable success rate, the next question is what stimulation protocol would optimize patients’ chances for success. Despite technological progress, IVF is still currently an expensive treatment. More gonadotropin doses are used in patients with POR and this increases costs. Prolonged treatments and high rates of cycle cancellation are other important problems in POR. The best ovarian hyperstimulation procedure in these patients should
provide low cycle cancellation rates, a sufficient number of mature oocytes, lower cost, optimal pregnancies, more live births. However, the best treatment for PORs should be discussed.

### Administration of Gonadotropin

The first and basic approach to poor responder patients is to increase the dose of gonadotropins when inadequate response is obtained with standard dose ovarian stimulation in COH cycles. Decreased cycle cancellation rates and increased pregnancy rates were reported in a study in which gonadotropin doses were increased from 350 UI to 400 UI [24]. Manzi et al. [25] reported that they got more oocytes with 150 UI increment of the daily FSH dose. Yet an increment for pregnancy rates was not reported. In another study, the FSH dose was increased to 450 UI because there was no adequate response to 250 UI and more follicles were gathered. Yet pregnancy rates were observed to be low [26]. Again, in another study in patients with POR, it was observed that increasing the gonadotropin dose had no effect on the level of E2, the number of embryos and pregnancy rates [27]. In spite of increased FSH dose in patients with POR, poor oocyte retrieval may be related to poor ovarian reserve. Although increasing the FSH dose seems to fail for oocyte retrieval, recombinant FSH is shown to be more potent than urinary products for numbers of oocyte retrieved, more embryos obtained and higher pregnancy rates.

FSH and LH play the same role in folliculogenesis and ovulation. Barrenetxea et al. [28] have reported that the addition of rLH to stimulation protocol after the seventh day of the cycle has no effect on clinical pregnancy, implantation rates and cycle dynamics. In addition, a recent prospective randomized study demonstrated that the additional exogenous LH activity in the form of either recombinant LH or low-dose recombinant hCG did not improve the cycle outcomes and the pregnancy rates in poor responders [29].

### Administration of GnRH Analogues

In recent years, the advantages of the microdose GnRH agonist flare-up technique have been reported in some studies [30]. In several studies, GnRH antagonists have been found to have an effect [31]. In a previous study that compares two stimulation protocols, we have observed no difference between them [32].

### GnRH Agonists

GnRH agonists are one of the main medicines in COH cycles. GnRH agonists suppress the production of pituitary gonadotropin and become effective in preventing premature LH surge and increasing the gonadotropin required. Decreasing the GnRH dose in patients with POR decreases the gonadotropin required and increases the number of oocytes [33]. The existence of GnRH receptors in human ovarian tissue is that shown by studies indicates that agonists may have direct and negative effects besides pituitary effects. This situation has caused debate about the classical usage of GnRH agonist in patients who have a limited ovarian reserve. Therefore, modified agonist protocols have been identified in patients with POR. There are many different modified GnRH-a protocol for patients who have previously shown a poor response to long luteal GnRH-a protocol – mostly by altering the dose and timing of administration. Among the various types of modified GnRH-a protocol, microdose flare-up is one of the most popular regimens.

Some studies supporting short and flare protocols against long protocols have been reported in the literature [34,35]. Decreased cancellation of cycles, increased pregnancy rates without premature LH surge for microdose flare-up protocol and a thoroughly decreased dose (40-80µg) have been reported [36]. In microdose GnRH agonist (GnRH-a) flare-up protocol; the ovarian suppression is not excessive and the initial stimulation of GnRH receptors and consequent secretion of endogenous gonadotropins reinforce the effects of exogenously administered gonadotropins. These are main advantages of this protocol.

In a recent review comparing two agonist protocols, there was no statistically significant difference between two protocols. These protocols are stop and non-stop long GnRH agonist protocols. In the stop agonist protocol, GnRH agonist was initiated in the midluteal phase and was stopped upon adequate down regulation. In the non-stop protocol, a standard long GnRH agonist was applied and GnRH agonist administration continued until the day of hCG administration [37].

In a study that considers co-flare 450 cycles in POR patients retrospectively, a 24 % cycle cancellation, a 20 % pregnancy rate per cycle and a 14 % live birth rate were reported. Lower cycle cancellation rate is detected in patients with ‘Estradiol doubling’. Flare effect at the beginning seems a better stimulation indicator but has no significant effect on pregnancy outcomes [38].

### GnRH Antagonists

Defining the extra-pituitary effects of GnRH and the possible importance of these effects on poor ovarian responders caused the seeking of different cycles to come to a head, rather than of classical long luteal agonist cycles. At first, the attempt was made to overcome these negative effects with modified agonist procedures. With the invention of GnRH antagonists, the usage of these medicines in POR patients has become a current issue. Most recently, the use of GnRH antagonists has been suggested as the preferred ovarian stimulation protocol in poor responders. In recent times, the use of GnRH antagonists in patients with POR has given a new perspective to clinicians. And the number studies on this issue has gradually increased. Lower cycle cancellation rates, a greater number of oocytes, a greater number of transferable embryos and higher clinical pregnancy rates with antagonists have been reported in studies comparing GnRH antagonists with the usage of long luteal GnRH analogs in particular in poor responders [39]. As the reason, a greater excessive suppression of GnRH analogs on the ovaries in patients who already have a poor ovarian reserve has been claimed. Nevertheless, FSH and LH blood serum levels are suppressed excessively at the third day of cycles using GnRHa, when ovarian stimulation starts, and blood serum FSH and LH levels are frequently 5-8 IU at cycles using GnRH antagonists. Early LH peak is prevented with the addition of GnRH antagonists at late follicular period of the stimulation protocol. Thus GnRH antagonist protocols, in proportion to GnRH agonist long protocols, do not suppress endogenous FSH and LH at the early follicular period and allow natural follicle election [40].
In a study performed on 48 POR patients, equal pregnancy rates have been reported between microdose flare-up and antagonist protocols [41]. The cycle cancellation rate was found to be higher in the antagonist group in comparison to the long protocol group in a study that performed the evaluation when the leading follicle reached 16 mm [42]. In the study series of Humaidan et al. [43] with 72 patients, comparing flexible GnRH antagonist protocols with the long protocol, significant differences were detected for number of follicles, number of oocytes, implantation and pregnancy rates.

In a study performed by Fsouliotis et al. [44], the stimulation was carried out with antagonist protocols at a new cycle on 53 patients who had not been able to become pregnant with the long protocol at a previous cycle. Higher implantation, pregnancy rates and pregnancy ongoing rate were detected in the antagonist group. Marci et al. [45], who compared ovarian response in the antagonist protocol with the standard long protocol, reported a greater number of follicles and lower cancellation rates in the antagonist group.

In a recent meta-analysis comparing GnRH agonists and antagonists, better outcomes were determined for antagonist protocols in proportion to analogs, a lower cycle cancellation rate, a greater number of oocytes and metaphase 2 oocytes and a higher clinical pregnancy rate. Nevertheless, there was no significant difference between antagonist protocols with flare-up protocols, and a greater number of oocytes were gathered in flare-up protocols than antagonist protocols [12].

Despite these theoretical advantages of GnRH antagonists, there is some concern that the use of GnRH antagonists in poor responders may have adverse effects on ovarian steroidogenesis, follicular growth, embryo development and the implantation process [46].

In the study of Di Luigi et al. [47] that compared the microdose leuprolid asetat protocol with protocols that were started with GnRH antagonists and E2 replacement at the luteal phase, no significant difference was detected for cycle cancellation, number of oocytes and clinical pregnancy rates between these two groups.

In a study including 300 consecutive cycles, similar pregnancy rates were found between the microdose flare-up group and antagonist [48].

### Aromatase Inhibitors

Aromatase inhibitors inhibit the last step of estradiol synthesis. Letrozole is a third generation selective aromatase inhibitor first used to decrease gonadotropin dose at ovulation induction. It was shown that aromatase inhibitors have positive effects on follicular response against FSH in poor responder patients [49]. In our earlier study including 70 patients undergoing the flexible antagonist protocol, it was shown that adjunctive letrozole administration seems to restore an IVF cycle by decreasing the rate of cycle cancellation and seems to reduce the cost by reducing the total gonadotropin dosage [50].

In a study including 12 POR patients, less requirement for gonadotropin was detected with 2.5 mg (7 days) letrozole [51]. Moreover, in another study, 71 of 147 patients with a cancelled cycle received a high dosage of FSH/hMG+antagonist and 2.5 mg Letrozole, and when this group’s outcomes were compared with the group that received the same protocol without the addition of letrozole, increased testosterone in follicular liquid, and rostenedion concentrations, the number of oocytes gathered and implantation rates were detected to be significantly higher in letrozole group [52].

### Additional Treatments

The passage of egg cells to the functional pool that is sensitive to reproductive hormones takes about four months. At present, modern medicine is able to intervene the last 15-20 days of this process. Some hormones have been detected that are believed to affect earlier periods of this process.

#### DHEA (dehydroepiandrosterone)

Dehydroepiandrostosterone is an endogenous steroid that originates from the zona reticularis (80 %) of the adrenal cortex and from ovarian theca cells (20 %). Dehydroepiandrosterone is an essential prohormone in ovarian follicular steroidogenesis. The level decreases with age. This hormone converts to estrogen and androgen in women. This conversion is in favor of androgens. In case reports and laboratory studies, it was shown that test-tube babies and fertilization administrations of DHEA increase pregnancy rates and live birth rates in patients with poor ovarian reserve and advanced ages (>38) [53]. At the same time it was shown that DHEA decreases abortion rates. In our earlier study, it was shown that the addition of DHEA provides enhancement of IVF outcomes [54]. It is thought that DHEA increases the number of oocytes passed in functional reserve and decreases aneuploidy by the optimum effect on oocyte cytoplasm [55]. Therefore 6-8 weeks administrations of DHEA (75 mg/day) might soon become routine in POR patients.

#### GH (Growth Hormone)

It is known that growth hormone has a beneficial effect on ovarian function. In laboratory studies, it has been shown that GH provides for increased oocyte maturation and capacity for repair of DNA in human cells. In a study including women aged over 40, the addition of GH to treatment increased pregnancy and live birth rates significantly [56]. Again in another study, positive outcomes were reported [57]. The usage of GH has become a routine for treatment protocols in patients who have no response [58]. But the addition of GH-releasing factor in poor responders undergoing IVF treatment does not appear to beneficial.

#### Metformin

Today, the use of metformin is not offered to treat anovulatory infertile women. In patients with reduced ovarian reserve, metformin worsens the response to gonadotropins. It is thought that the use of metformin reduces response to stimulation by reducing androgen levels [59].

#### COC Pre-treatment

COC pre-treatment suppresses endogenous gonadotropins and sensitizes estrogen receptors depending on estrogen components inside. Coperman et al. [40] reported increased pregnancy rate,
and decreased cancellation rate in the antagonist cycles of poor responder patients who received OCP treatment, when compared with patients not receiving OC pills.

**Androgen**

Androgens play a critical role in follicular growth. Androgen receptors have been identified in the human ovary. The addition of androgen during the early follicular phase might have a beneficial effect on the number of small antral follicles and improve the ovarian sensitivity to FSH. In a meta-analysis, it was detected that the use of transdermal testosterone in POR patients has benefits for live birth rates, clinical pregnancies per cycle, and gonadotropin doses used in group increased testosterone significantly [60].

**Luteal Phase Supplementation**

Luteal supplementation with either hCG or progesterone significantly improves fertility outcomes compared with no treatment. Addition of oral estrogen to progesterone also improves implantation rates. Luteal support is therefore mostly offered in IVF/ICSI protocols. However, it has a potential risk of OHSS [61].

**Expert Commentary**

Poor response criteria are increased cycle cancellation rates, poor embryo development and decreased pregnancy rates in POR patients. Assessment of ovarian reserve before COH is important for the choice of an adequate protocol. Various solution strategies have been tried for increasing IVF success in POR patients. Variations have been made for type, dose and timing of gonadotropins, agonists and antagonists, however, none of these has, as yet, proven to be superior. The addition of adjuvants to treatment has contributed to IVF success. Individualizing COH protocols for each patient seems more appropriate.

**5-Year View**

The main factor that affects fertility, as a result of the effect of aging on the ovaries, are defects in oocyte quality. Many stimulation protocols have been offered to increase the quality and number of oocytes. The importance of adjuvant and hormone supports is likely to grow with developing technology and growing knowledge. Developments in IVF technology increase day by day. Electroacupuncture is one of these technologies. In recent years, an increasing number of stem cell studies are offering some hope. In the future, we may be able to retrieve oocytes from stem cells.

**Financial & Competing Interests Disclosure**

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.

**Key Issues**

- A short protocol has flare up effect on pituitary gonadotropin release, on the other hand, the use of a long protocol results in more coordinated follicular growth.
- Use of GnRH antagonists has better results regarding stimulation time, gonadotropin total dosage, and retrieval of oocytes. But more comparative studies are required.
- There are no differences between the long GnRH agonist protocols and CC+ rFSH in GnRH antagonist protocols.
- GnRH antagonist and short GnRH agonist protocols seem similar with regard to pregnancy rates.
- Natural cycle IVF can be an alternative to standard ovarian stimulation. Natural cycle IVF is less invasive and less costly. This can be offered for poor responders who do not produce more oocytes with ovarian stimulation.
- Short GnRH and long GnRH agonist protocols have no differences.
- Recombinant FSH is shown to be more potent than urinary products for the number of oocytes retrieved, more embryos obtained, and higher pregnancy rates in IVF protocols.
- Shortening the duration of embryo culture might be associated with an improvement in pregnancy rates. Early embryo transfer in POR might therefore be beneficial.
- Adjuvant treatment allows an increase in the success of IVF. In particular, the addition of GH appears to improve the probability of pregnancy.

**References**


