F, V & V in ObGyn, 2011, 3 (2): 101-108

Poor responders in *in vitro fertilization* (IVF) therapy: the challenge continues

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Abstract

Poor responders represent more than a third of women undergoing assisted reproduction. Typically they are patients with advanced maternal age and low ovarian reserve. However, there is a younger group that unexpectedly demonstrates impaired response to controlled ovarian hyperstimulation. The etiologies in many of these cases are still unclear. In our program, the determination of basal cycle day 3 serum FSH, LH and E_2 levels, measurement of AMH, and the estimation of the basal antral follicular count by transvaginal ultrasonography, are the preferred screening tests for ovarian reserve in all IVF patients, and together with the woman's age, determine the ovarian stimulation regimen to be chosen for the cycle treatment. In spite of a variety of protocols and adjuvant therapies of unproven benefit, these patients have compromised outcomes and continue to represent a challenge to reproductive endocrinologists.

Key words: **Age, genetic factors, IVF, ovarian reserve, poor responder.**

Introduction

Infertile couples make up approximately 10% of the worldwide population of reproductive age, and assisted reproductive technologies (ART) currently account for 1.2% of total US live births, and up to 4% in some European countries (Nygren and Andersen, 2002; Schieve *et al.*, 2009). Current estimates indicate the cumulative birth of over 4 million babies from ART services worldwide, with usage reaching 5% or more in developed countries. Controlled ovarian hyperstimulation (COH) is a principal step of *in vitro* fertilization (IVF) therapy, which leads to the harvest of fertilizable oocytes. In the last two decades, the ovarian stimulation regimens have undergone significant modifications and improvements as a consequence of increased clinical experience and the availability of new hormonal preparations and adjuvant therapies. Notwithstanding overall increased pregnancy rates in IVF, a large group of patients referred to as "poor responders", consistently show from suboptimal to much compromised outcomes, both in terms of oocyte recovery and pregnancy rates. In our program the incidence of such cases surpasses a third of the total IVF population (see below). These patients are typically women of advanced maternal age and with a diminished ovarian reserve. But in addition, there are younger "poor responders", some with identifiable and others with non-identifiable causes, and all of them constitute a formidable challenge for the reproductive endocrinologist.

Assessment of the ovarian reserve

Georgeanna Jones and collaborators (Jones *et al.*, 1984, 1985) pioneered the use of gonadotropins for COH in IVF therapy. It was early identified that normally cycling, ovulatory women subjected to gonadotropin stimulation fell into one of three response categories, i.e., high, intermediate or low responders, and, furthermore, that the individual's response was similar on a subsequent stimulation cycle. The response category was based on the

assessment of the resulting serum estradiol (E_2) curve (E_2 pattern) and the consequent accompanying follicular response as monitored by ultrasonography. Moreover, the patient's response category and E_2 pattern were correlated with the capacity to achieve a pregnancy following IVF and embryo transfer (Muasher *et al.*, 1985). Almost three decades later, and following the introduction of improved gonadotropin preparations (from urinary, to highly purified, and later recombinant) and the use of adjuvant therapies (GnRH agonists and antagonists), these concepts have remained almost intact and continue to guide clinical management (Arslan *et al*., 2005).

Muasher and collaborators (Muasher *et al*., 1988) first reported that the measurement of serum levels of FSH, LH and E_2 on day 3 of the basal menstrual cycle was a predictor of COH response and IVF outcome. Subsequent studies established the clinical significance of defined thresholds for such hormones in addition to their relationship to the woman's age, thus further defining the concept of ovarian reserve (Scott *et al*., 1989; Toner *et al*., 1991a). It was earlier determined that basal FSH levels are better predictor of IVF performance than age (Toner *et al*., 1991a). Regression analyses indicated independent contributions of both basal FSH and age in predicting cancellation rate, peak E_2, number of oocytes retrieved, fertilized, and transferred, and ongoing pregnancy rates. The combined use of age and basal FSH in counseling patients improves the accuracy of prognosis, and provide an index of functional ovarian reserve.

Since then, many other tests have been introduced as candidates for the examination of the ovarian reserve (Broekman, 2009). Such screening tests include: the clomiphene citrate challenge test (CCCT), GnRH test, GnRH agonist test, measurement of serum inhibin B and anti-Mullerian hormone (AMH), and ultrasound examination of basal cycle ovarian volume, antral follicular count (AFC) and ovarian stromal blood flow (Arslan *et al.*, 2005). Recently, much attention has been given to the measurement of AMH. AMH is produced solely by the granulosa cells of growing pre-antral and small antral ovarian follicles, and shows little inter- and intra-cycle variability. AMH is an accurate predictor of excessive response to ovarian hyperstimulation (Broer *et al*., 2011).

Our group reported on the value of various screening tests in a general infertility population undergoing IVF (Riggs *et al*., 2008). AMH correlated better than age, FSH, LH, E_2, and inhibin B, with the number of retrieved oocytes. Receiver operating characteristic curves estimated that AMH can accurately predict ovarian responsiveness to COH with high sensitivity and specificity, both in the low and high ranges of response. In further work, Riggs and colleagues (Riggs *et al*., 2011) showed that AMH was superior to other biomarkers of ovarian reserve in predicting low and high response in young women selected as oocyte donors, but that it was not predictive of embryo morphology or pregnancy outcome in the recipient population. In our program, the determination of basal cycle day 3 serum FSH, LH and E_2 levels, measurement of AMH, and the estimation of the basal AFC, are the preferred screening tests for ovarian reserve in all IVF patients, and together with the woman's age, determine the COH regimen to be chosen for the cycle treatment.

The etiologies of poor ovarian response

The definition of poor responder has differed widely in the literature and has included the woman's age, basal hormonal status (high FSH), previous cycle cancellation, and/or a poor response in a previous cycle with < 3-5 oocytes retrieved and/or a peak serum E_2 level < 500-900 pg/mL (Muasher, 1993). Notwithstanding definition inconsistencies, this group of women has the poorest prognosis for COH results and IVF pregnancy outcome. It has been reported that treatment cancelation owing to poor ovarian response is a significant problem seen in 12-30% of all stimulated cycles (Al-Azemi *et al*., 2011).

Poor response associated with advanced maternal age

Although neuroendocrine and uterine factors may reduce fertility with age, progressive depletion of the size of the pool of ovarian follicles is thought to be the major cause of this problem. Decline in primordial follicle number with ageing has been linked to an equivalent decline in oocyte quality with adverse factors affecting both nucleus (aneuploidy, abnormal spindle formation) and cytoplasm (reduction in mitochondrial number and ATP, abnormalities of the cytoskeleton) (Broekmans *et al*., 2009). Advanced maternal age is clearly associated with oocyte aneuploidy. We have also described anomalies of the zona pellucida in oocytes recovered from poor responders (Oehninger *et al*., 2006). Such anomalies were characterized by an abnormal protein backbone as measured with specific anti-ZP3 antibodies.

Poor response in younger women

As mentioned above, poor ovarian response to stimulation may be a consequence of advancing chronological age although it may also occur unexpectedly in relatively young patients. It appears that this latter group is heterogeneous and includes

women with either intrinsic and/or stimulation-derived defects, leading to a defined poor responder phenotype. The true pathogenesis of the poor ovarian response is unknown in a large proportion of these cases, although "ovarian failure" may be due to an immunological origin in some. Occasionally, a low ovarian reserve is secondary to previous ovarian surgery, severe endometriosis, and/or pelvic adhesive disease, iatrogenic (post-chemo- or radiotherapy), and/or associated with high body mass index or heavy smoking (Keay *et al*., 1997; Buyuk *et al*., 2011).

Greenhouse and colleagues (Greenhouse *et al*., 1998) described the presence of mutations affecting female fertility based on a mouse model with targeted mutagenesis. The authors elegantly demonstrated that the fate of the follicular pool can be altered by germ cell proliferation/apoptosis imbalances during fetal life; in addition multiple anomalies of the process of folliculogenesis at various developmental stages (from primordial to pre-ovulatory) could result in a "poor responder" phenotype, including mutations of Gdf-9, Connexin37, FSH and LH receptors, in addition to alterations of the pituitary secretion of FSH-β.

A few endocrine-related abnormalities have been observed in the clinical scenario. These include: a decreased number of FSH receptors (FSHR) in granulosa cells (Zeleznik *et al*., 1981), defective signal transduction after FSHR binding (Hernandez *et al*., 1992), and FSHR polymorphisms. Although inactivating FSHR mutations result in a severe reproductive phenotype, it is plausible that more subtle genetic variations of the receptor can contribute to functional perturbations, subfertility, and/or infertility. Two common FSHR polymorphisms have been associated with altered response to FSH during IVF and different basal FSH levels (Perez Mayorga *et al*., 2000; Sudo *et al*., 2002). These polymorphisms are two single nucleotide changes in exon 10 of the receptor, resulting in two amino acid substitutions (p.307Thr/Ala and p.680Asn/Ser).

Gerasimova and collaborators (Gerasimova *et al*., 2010) investigated whether genetic alterations of the FSHR contribute to COH response variability. The authors hypothesized that additional mutations/polymorphisms in the FSHR gene resulting in altered structure and function of the receptor may influence the number of oocytes produced. They studied women undergoing treatment with IVF falling into the edges of the normal distribution of ovarian response to FSH, with respect to age, extracted RNA from cumulus cells surrounding the oocytes, and analyzed the FSHR mRNA by RT-PCR and sequencing. Four abnormal FSHR splicing products were identified (three exon deletions and one intron insertion) in the FSHR mRNA in 37% (13 of 35) of women tested. All alterations affected the extracellular ligand-binding portion of the receptor without causing a frame shift. When transfected in HEK293T cells, all four splicing variants showed markedly decreased cAMP activation compared to controls. The authors concluded that FSHR variants can constitute an intrinsic genetic cause of some forms of infertility. Clearly more work is needed to corroborate and expand these interesting findings.

Clinical management

Low responders can be prospectively identified as patients with one or more of the following characteristics: advanced age (≥ 37 years), high basal cycle day 3 FSH (≥ 10 mIU/mL) or high basal E_2 levels (≥ 90 pg/mL), high FSH:LH ratio and low LH levels in basal cycle day 3 (Scott *et al.*, 1989; Evers *et al.*, 1998; Barroso *et al*., 2001), and/or low ovarian volume and/or a reduced AFC. If the patient had a previous IVF attempt, allocation to this response group in our program requires a previous cycle with a peak serum E_2 of < 900 pg/mL, and/or retrieval of ≤ 5 mature oocytes, and/or previous cancellation due to inadequate folliculogenesis (< 4 dominant follicles after 6 days of gonadotropin stimulation with 300 IU of FSH). Using such definition, low responders constitute a very large proportion of our patient population (Figure 1). In fact, 47% of cycles performed during the last decade were low responders. It is important to consider that there are poor responders < 37 years of age and even with a normal basal FSH and E_2 levels ("hidden" poor ovarian reserve); some of these patients can be identified upon basal ultrasound assessment of AFC and AMH, although as mentioned before AMH levels, although predictive of response, do not correlate with pregnancy outcome.

Review of the literature reveals that multiple COH strategies have been implemented in this group of challenging patients. They have included: high FSH doses, clomiphene citrate and hMG , micro-flare with a GnRH agonist , flare GnRH agonist protocol, stop-GnRH agonist protocol , growth hormone and other adjuvants, and use of GnRH antagonists, typically in combination with FSH + LH preparations (Muasher, 1993; Arslan *et al*., 2005; Pandian *et al*., 2010). Even re-assessment of natural cycle-IVF has been suggested as an alternative approach in this group of patients. This variety of protocols reflects high within-group variability, a probably multifactorial origin but more importantly, an overall compromised outcome.

In our program, poor responders are properly counseled about their compromised outcomes.

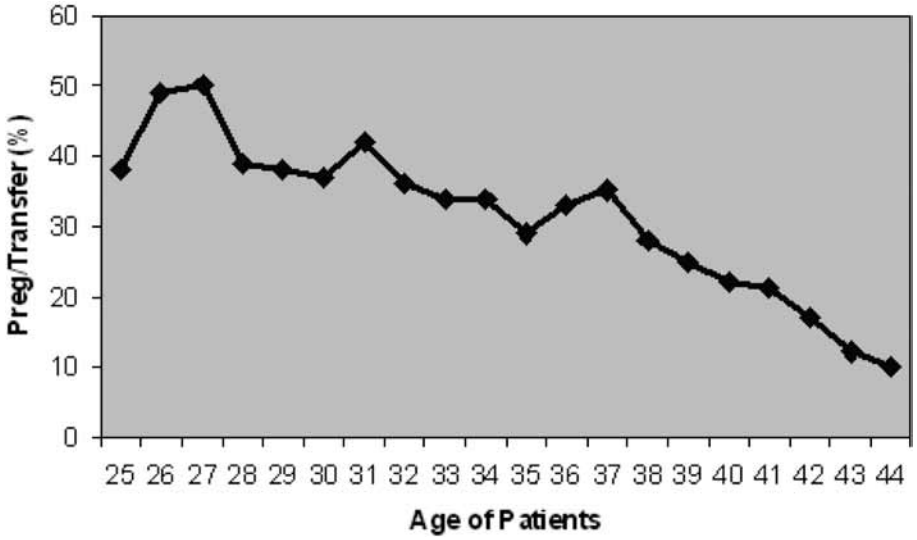

Fig. 1. — IVF results for all patients treated at the Jones Institute from 1995 through 2010 (total of 5,289 transferred cycles): relationship of age and clinical pregnancy rates (%). Pregnancy diagnosis followed confirmation of intrauterine gestational sac and heart beat at 7 weeks gestation. Note that the decline of pregnancy is more marked after 37-38 years.

Couples with advanced maternal age and/or high basal serum FSH (or very low AMH levels), with otherwise idiopathic or male subfertility, are counseled that intrauterine insemination therapy may offer the same likelihood of successful pregnancy as IVF (cumulative pregnancy rates), and may be a more cost-effective approach (Goverde *et al.*, 2000). If IVF is performed, poor responders are subjected to stimulation with one of two protocols. In both regimens recombinant FSH is used at doses higher than in intermediate responders, either in a micro flare GnRH agonist protocol or with a GnRH antagonist protocol. For both regimens, patients can be pretreated or not with oral contraceptives (OCP) for 3 weeks and gonadotropins are initiated after 4-5 days of the last active pill. In the micro flare regimen, the GnRH agonist is started on day 2 at the dose of 50 micrograms twice daily and continued until hCG administration. Recombinant FSH is initiated on day 5 at the dose of 250-350 IU daily (Scott and Navot, 1994). For the GnRH antagonist regimen, rFSH (at the same dose) is started on day 3, and the antagonist used in either a flexible or fixed regimen. In many of these poor responder patients, particularly if OCP are used, LH is added to the stimulation, typically as hMG or rLH (75 IU); other programs use hCG 20 IU instead (Meldrum and Schoolcraft, 2010).

Other approaches have been suggested. Dragisic and colleagues (Dragisic *et al.*, 2004) reported lower cancellation rates and improved IVF outcome by a combination of estrogen therapy and GnRH antagonist started in the mid luteal phase of the preceding menstrual cycle. This is an interesting maneuver in order to suppress early follicular recruitment that typically occurs in the late luteal phase in patients with a pre-menopausal status, and aiming to improve synchronization of follicular growth. Others found comparable results using the luteal E_2 patch and GnRH antagonist suppression protocol before gonadotropin stimulation versus a microdose GnRH agonist protocol for patients with a history of poor IVF outcomes (Weitzman et *al.*, 2009). Nilson and collaborators (Nilson *et al.*, 2010) used a GnRH antagonist for luteolysis in poor responder patients undergoing IVF treatment. The authors hypothesized that daily low dose GnRH antagonist administration given during the late luteal phase to induce luteolysis could secure a more synchronous cohort of recruitable follicles. The authors concluded that despite GnRH antagonist administration in the late luteal phase and menstrual bleeding, FSH was not sufficiently reduced to secure a more synchronic cohort of recruitable follicles. Finally, Elassar and colleagues (Elassar *et al.*, 2011) compared luteal phase E_2 versus luteal phase E_2 and antagonist protocol for COH before IVF in poor responders and did not find differences in any outcomes.

Bromer *et al.*, (2007) summarized the use of pretreatment modalities used prior to COH proposed to increase the success rate. No clear evidence from well-designed clinical trials has shown a benefit of any of these treatments, including low-dose aspirin, metformin, growth hormone, OCP, or corticosteroid supplementation, versus placebo or no supplementation.

Primate studies demonstrated interactions between FSH and androgens during follicular development

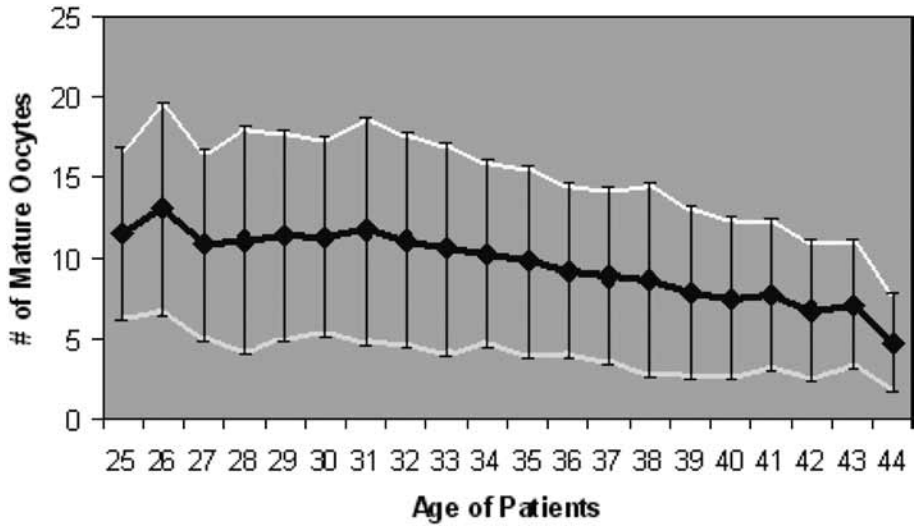

Fig. 2. — IVF results for all patients treated at the Jones Institute from 1995 through 2010 (total of 5,289 transferred cycles): relationship between age and total number of mature oocytes harvested (mean ± standard deviation). Note that the decline of the number of recovered mature oocytes is more marked after 37-38 years, and the standard deviations are larger in the younger women, pointing to the "hidden or occult" poor ovarian reserve cases, and independent of age.

and a highly significant positive correlation between the FSHR and androgen receptor (AR) mRNA levels. Furthermore, testosterone administration that resulted in supra-physiological levels augmented granulosa cell FSHR expression and it was suggested that androgens promote follicular growth by sensitizing granulosa cells to FSH action (Weil *et al*., 1999). In human granulosa cells from small antral follicles, androgen receptor mRNA and androgen levels in follicular fluid correlate with FSH receptor mRNA (Nielsen *et al*., 2010). Recently, the current status of the use of androgens in the context of poor ovarian response was analyzed (Feigenberg et *al.*, 2009). It was concluded that the variations in patient selection, type of androgens employed and the different duration of exposure preclude drawing any definite conclusions. Aromatase inhibitors block the conversion of androgens to estrogens, thereby promoting an androgen-rich intrafollicular environment. The evidence presented suggested a potential beneficial role for the use of aromatase inhibitors in treating women who have previously experienced failure of standard IVF protocols. The optimal dose and duration of this treatment is yet to be determined.

Although the results of studies concerning LH supplementation in poor responders are conflicting, the latest Cochrane review on the use of recombinant LH for ovarian stimulation supports its use in poor responders, based on pooled pregnancy estimates (Mochtar *et al*., 2007). Kolibianikis *et al*. (2009) concluded in a systematic review and meta-analysis that the addition of growth hormone to gonadotropins in ovarian stimulation of poor responders treated by IVF was beneficial. Nevertheless, the total number of patients analyzed was small and thus further prospective and randomized clinical trials are warranted to prove or disprove this finding.

Pandian *et al*. (2010) recently reported on a meta-analysis of interventions for 'poor responders' to COH in IVF. Only randomized controlled trials comparing one type of intervention versus a standard long protocol were included. The number of oocytes retrieved was significantly lower in the conventional GnRH agonist long protocol compared to stop protocol and GnRH antagonist protocol. Total dose of gonadotropins used was significantly higher in the GnRH agonist long protocol group compared to the stop protocol and GnRH antagonist groups. Cancellation rates were significantly higher in the GnRH agonist flare up group compared to the GnRH agonist long protocol group. It was concluded that there is insufficient evidence to support the routine use of any particular intervention either for pituitary desensitization, ovarian stimulation or adjuvant therapy in the management of poor responders to controlled ovarian stimulation in IVF.

Conclusions and perspectives

It is generally agreed that an ideal COH protocol for IVF should have the following features: (i) mimic physiological conditions as possible; (ii) be of lowest possible complexity in terms of number of patient' visits, injections, and comfort, depicting highest patient satisfaction; (iii) have minimal risks and side effects; (iv) lowest possible cost; and (iv) result in the maximally achievable pregnancy rate, both in the fresh and subsequent cycles with cryopreserved/

thawed embryos, thereby optimizing the total reproductive potential (Toner *et al*., 1991b).

A recent study suggested that a mild stimulation approach may be beneficial for both oocyte/embryo qualities, with reduction of embryo aneuploidy; this was a randomized controlled trial comparing a gonadotropin/GnRH antagonist (no suppression) against a down regulation protocol with a GnRH agonist and a higher gonadotropin treatment (Baart *et al*., 2007). However, on the other side of the COH management spectrum, "high-performing" IVF programs in the US using full stimulation protocols with relatively high gonadotropin doses are reporting very high implantation and pregnancy rates (2-3 times higher than natural conception rates), so that potentially negative impacts of COH protocols on oocyte quality and on the endometrium clearly deserve further exploration (Van Voorhis *et al*., 2010). In addition, murine data revealed no effect of gonadotropins on chromosome aneuploidy or mosaicism in mouse preimplantation embryos (Fauzdar *et al*., 2009).

Pharmaceutical advances in recombinant technology and availability of short and long acting compounds, with development of oral regimens, are expected. Lately, advances in recombinant technology resulted in the introduction of corifollitropin alfa, a hybrid molecule with sustained FSH activity and reduced injection frequency. This molecule has a prolonged elimination half-life and enhanced *in vivo* bioactivity compared with wild-type FSH (Fauser *et al*., 2009). If proven effective, this new treatment option may be simpler and more convenient for patients compared with conventional long protocols of daily rFSH injections in combination with GnRH agonist co-treatment. Devroey *et al*., (2009) reported on a double-blind, non-inferiority trial comparing corifollitropin alfa and rFSH during the first seven days of ovarian stimulation using a GnRH antagonist protocol. In women < 35 years of age with normal body weight, results demonstrated a high ongoing pregnancy rate, equal to that achieved with daily rFSH. A prospective controlled trial is currently being performed in the United States in women 35-42 years of age, which should provide information in groups of low-poor responders.

Fine-tuning of COH protocols can be performed nowadays with the available battery of hormonal preparations and adjuvant therapies. In addition, new developments in the horizon may bring further novel alternatives including more bioactive gonadotropin agonists with effects of variable duration. However, management controversies still exist in the clinical setting, particularly as it applies to treatment of poor responders, whether of advanced age or not. The debates on the use of GnRH agonist (micro flare regimen) or antagonist adjuvant therapy in poor responders, and the addition of LH, continue. Consequently, large and prospectively controlled studies are needed to answer all these important questions. It is clear that in these groups of women with low response, the aim to develop a large cohort of oocytes for the purpose of freezing supernumerary embryos does not apply like it does in the intermediate and high responders. Conversely, here the aim should be to obtain a smaller cohort of fertilizable oocytes, probably not using extremely high gonadotropin doses, but if aneuploidy is intrinsic, results will be most likely compromised irrespective of the ovulation augmentation regimen (Jones *et al*., 2010). The use of "friendlier" stimulations is attractive, but clinical experience demonstrates that in many of these cases relatively high gonadotropin doses are needed in many of these cases to recruit a minimal number of follicles.

In light of the excellent results being reported with oocyte vitrification, banking of oocytes in women delaying conception can be a valid alternative (Nagy *et al*., 2009). The introduction of a small amount of ooplasm from a donor oocyte or zygote (ooplasmic or cytoplasmic transfer) may alter the function of oocytes, with probable deficiencies. Cytoplasmic transfer from fertile donor oocytes into compromised oocytes from patients with poor response and/or recurrent implantation failure after assisted reproduction has led to healthy births. Transfer of small amounts of cytoplasm probably involves mRNAs, proteins and mitochondria, as well as other factors and organelles (Barritt *et al*., 2001). However, potential developmental problems involving specific epigenetic and mitochondrial incompatibilities have seriously hampered progress of this research area.

An important question to be answered is whether high gonadotropin doses do result in increased oocyte aneuploidy, and if this is the case, are there genetic/DNA instability predisposing factors, and is this effect age-dependent? For other types of poor responders, whether genetic or other in origin (i.e., genetic phenotypes due to pituitary/follicular cells endocrinopathies), newly developed protocols and/or adjuvant alternatives should be sought. But only the unveiling of the underlying pathophysiology will permit this. Although COH has a fundamental role in ART, there are still lingering questions about potential detrimental effects on oogenesis, embryo quality, endometrial receptivity and perinatal outcomes (Santos *et al*., 2010). Consequently, further clinical knowledge is needed in order to ascertain the safety of all COH regimens, and importantly, to increase efficacy in poor responders.

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