Management of poor responders in IVF

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Abstract
Correct controlled ovarian stimulation is of paramount importance in assisted reproductive technologies. Therefore, analysis of the ovarian reserve of the patient is mandatory to tailor the best ovarian stimulation regimen. When the ovarian reserve is reduced, the induction of a multifollicular growth remains a challenge. Several factors could be associated with reduced ovarian response. However, reduced ovarian reserve either in older patients or in young patients represents the most frequent aetiopathological factor. Whatever is the aetiology, one of the main problems is how to predict a reduced ovarian response, and although several tests have been suggested, no very accurate predictive test is available. A variety of different stimulation protocols have been suggested but the lack of any large-scale, prospective, randomized, controlled trials of the different management strategies and the lack of a uniform definition of the population may result in comparisons of heterogeneous groups of patients, making it difficult to draw any definitive conclusions. Natural cycle IVF may represent an easy and cheap approach in the management of this group of patients. Although no controlled large prospective randomized studies are available to compare the natural IVF procedure with ovarian stimulation IVF in poor responder patients, the efficacy of natural cycle IVF is hampered by high cancellation rates mainly due to untimely LH surge. The use of gonadotrophin-releasing hormone antagonists in the late follicular phase, which reduces the premature LH rise rate, and the improvements in laboratory conditions and fertilization techniques, increase the embryo transfer rates, making this procedure more cost-effective.

Keywords: ICSI, IVF, natural cycle, ovarian stimulation, poor responder

Introduction
Before starting ovarian stimulation, a prospective analysis of the ovarian reserve of the patient, definition of the goals of the ovarian stimulation and selection of the correct stimulation protocol are mandatory (Penzias, 2004). In patients with reduced ovarian response to stimulation regimens, even more attention is required to achieve acceptable reproductive outcome and very often the induction of a multifollicular response remains a challenge. Although it is difficult to standardize the characteristics that categorize a patient as a ‘poor responder’, it has been estimated that among patients undergoing IVF treatment, the prevalence of poor ovarian response is 9–24% (Keay et al., 1997).

Several factors could be associated with reduced ovarian response to ovarian stimulation: reduced ovarian reserve either in older patients (Akande et al., 2002) or in young patients with early ovarian ageing (Nikolau and Templeton 2003), previous ovarian surgery (Nargund et al., 1995), and pelvic adhesions (Keay et al., 1998). Whatever is the aetiology, it is of paramount importance to try to predict poor ovarian response to ovarian stimulation in order to tailor the correct stimulation regimen. Unfortunately, although several tests have been suggested, there is no very accurate predictive test available to assess ovarian response, and apparently the ideal test is the response of the ovaries to ovarian stimulation. Finally, a variety of different stimulation protocols have been suggested, but the lack of any large-scale, prospective, randomized, controlled trials of the different management strategies does not allow any definitive conclusion to be drawn. Most studies in fact, compare patients with their previous failed cycles, where patients who had a poor ovarian response but conceived would automatically have been excluded.
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Definition

The reported prevalence of ‘poor responders’ amongst patients undergoing ovarian stimulation for IVF is 9–24% (Keay et al., 1997). However, it is very difficult to make a more accurate estimation because of the lack of uniformity in the definition of this group of patients.

The variable numbers of mature follicles on the day of human chorionic gonadotrophin (HCG) administration noted on ultrasound (less than two to less than five) (Serafini et al., 1988; Feldberg et al., 1994; Land et al., 1996; Lindheim et al., 1996; Fridstrom et al., 1997; Schoolcraft et al., 1997; Surrey et al., 1998; Laschen et al., 1999) and the number of oocytes retrieved (less than four to less than six) (Faber et al., 1998; Rombauts et al., 1998) have been used by most authors as criteria to define poor ovarian response. Another parameter widely used is peak serum oestradiol concentrations. Patients criteria to define poor ovarian response. Another parameter used by several authors is the total gonadotrophin dose used and/or the daily stimulation dose (Hofmann et al., 1993; Feldberg et al., 1994; Lindheim et al., 1996; Schoolcraft et al., 1997) and similarly a poor ovarian response is considered when it is not possible to recruit more than two dominant follicles with a peak serum oestradiol concentration <500 pg/ml. Another parameter used by several authors is the total gonadotrophin dose used and/or the daily stimulation dose (Hofmann et al., 1989; Hughes et al., 1991; Ibrahim et al., 1991; Salat-Baroux et al., 1993; Feldberg et al., 1994; Manzi et al., 1994; Lindheim et al., 1996; Surrey et al., 1998) are considered ‘poor responders’. Other authors use a combination of these parameters (Dor et al., 1992; Feldberg et al., 1994; Lindheim et al., 1996; Schoolcraft et al., 1997) and similarly a poor ovarian response is considered when it is not possible to recruit more than two dominant follicles with a peak serum oestradiol concentration <500 pg/ml. Another parameter used by several authors is the total gonadotrophin dose used and/or the daily stimulation dose (Hofmann et al., 1989; Hughes et al., 1991; Ibrahim et al., 1991; Salat-Baroux et al., 1993; Hughes et al., 1994; Karande et al., 1997) and/or prolonged duration of gonadotrophin stimulation (Toth et al., 1996).

Recently, Kailasam et al. (2004) considered as poor responders those patients who failed to develop more than three preovulatory follicles after using more than 300 IU FSH daily or when it required more than 3000 IU FSH to recruit less than four follicles. However, a poor ovarian response can be confirmed only after having had a failed standard ovarian stimulation, and at least one cancelled IVF cycle is another logical criterion suggested to define a poor responder patient (Schachter et al., 2001).

These criteria have been used alone or in combination as required for inclusion in the various protocols, and this is the main reason why the various clinical trials published may not be comparable because the various approaches studied may apply to heterogeneous groups of patients.

Aetiology of poor ovarian response

Although several possible aetiologies have been suggested, it seems that a diminished ovarian reserve is the principal factor in poor ovarian response (Pellicer et al., 1998).

Women have a finite number of germ cells whose number peaks at 6–7 million by gestation week 20. From mid-gestation onward and throughout reproductive life, an irreversible attrition progressively diminishes the germ cell pool of the gonad (Peters, 1976), mainly though follicular atresia. The rate of follicular attrition is not constant but rather follows a bi-exponential pattern, with the change in exponential rate being determined by the number of remaining oocytes rather than age (Faddy et al., 1992). Normally, there is a marked increase in the rate of follicular disappearance from age 37–38 years onwards when the total number of follicles reaches around 25,000 (Faddy et al., 1992). From this critical number of follicles left to the time of the menopause (1000 remaining follicles), takes about 13 years, regardless of age at menopause (Faddy et al., 1992).

The rate of follicle disappearance and its relation to the important reproductive events in a woman’s life has been very extensively studied by various reproductive biologists, suggesting that after a period of optimal fertility from age 18–31 years, oocyte quality decreases in parallel to the progressive loss of follicle numbers, becoming severely impaired after age 37–38 years (25,000 follicles) (Faddy et al., 1992; Gougeon et al., 1996). Several theories have been formulated to explain declining oocyte quality with age. In the so-called ‘production line’ hypothesis, oocyte quality is established during fetal life, and oocytes that are less susceptible to non-disjunction are ovulated first, leaving poor quality oocytes to be ovulated later in life (Henderson and Edwards, 1968; Polani and Crolla, 1991). For this reason, the associated reproductive impairments may be more closely related to decline in ovarian follicle number than to age, or to put it another way, to the interval before the menopause rather than the age at which that occurs. Therefore, in young women, premature reduction of ovarian follicle numbers, whether by excessive atresia or accidental or iatrogenic damage, could be expected to lead to an advancement of all reproductive problems. This aspect could be very important, as those women who will become menopausal earlier (40–45 years) will have an accelerated decline in fertility (25,000 remaining follicles) at an unexpectedly young age. It has been estimated that 10% of women in the general population become menopausal by the age of 45 (Tarloar et al., 1981; van Noord et al., 1997) and because of the long latent phase (about 13 years), there could be 10% of women in their early 30s with reduced fertility which is otherwise unexplained (Scott et al., 1993; Hofmann et al., 1996).

However, besides the ‘production line’ hypothesis supported by some experimental data in mice (Brook et al., 1984; Meredith and Butcher, 1985) and in women (Hardy and Kuh, 1999; Freeman et al., 2000), other data on female ovarian ageing point to an increased frequency of meiotic non-disjunction as time goes by, which is the most important mechanism responsible of the majority of aneuploidies in early embryos (Lamb et al., 1997). In fact, oocyte quality seems to be less impaired in younger patients with elevated FSH concentrations and reduced ovarian reserve and the implantation rates between young patients (<35 years) with high or low basal serum FSH appear to be comparable (Chuang et al. 2003). Similarly, van Rooij et al. (2003), comparing women >40 years old with normal serum FSH with younger women who had higher basal serum FSH concentrations, reported higher cycle cancellation rates and lower numbers of oocytes retrieved in this latter group of patients; however, once the oocytes were retrieved, the younger women had normal implantation rates. These results suggest that ovarian reserve is a better predictor of oocyte production capacity than of oocyte quality, whereas age affects oocyte quality. This phenomenon can be explained by
an age-dependent accumulation of damage due to either compromised granulosa cell function (Warburton, 1989) or progressively defective micro-circulation around the leading follicle with reduced oxygen concentrations in the follicular fluid (van Blerkom et al., 1997) or a gradual increase in intracellular oxidative stress (Tarín, 1995).

This hypothesis is, however, disputed and other authors reported a reduction of the implantation rates in young women with high basal serum FSH concentrations after IVF (Toner et al., 1991; Scott et al., 1995; Akande et al., 2002; El-Toukhy et al., 2002), suggesting that aneuploidy before (Roberts and O’Neill, 1995) or after (Benadiva et al., 1996) fertilization due to disruption of meiotic spindle assembly (Battaglia et al., 1996) or mitochondrial DNA deletions (Keefe et al., 1995) are not due simply to time related exposure to risk of damage, but were probably present from fetal life. More universally agreed is the very poor reproductive outcome in women approaching 40 years old with poor ovarian response. In this group of women, in fact, it is necessary to sum the effect of the reduced ovarian reserve to the diminished oocyte quality. The result is that there is a higher proportion of ‘poor quality’ oocyte due to either the ‘production line’ hypothesis (Henderson and Edwards, 1968; Polani and Crolla, 1991) or to time-related exposure to risk of damage (te Velde and Pearsons, 2002).

Although reduced ovarian reserve is the most important and frequent aetiological factor in reduced ovarian response to ovarian stimulation, in some patients the presence of a polymorphic FSH receptor in which the asparagine of the receptor protein is replaced by serine at position 680 requires higher FSH concentrations for normal function, and it is probably not related to reproductive ageing (Perez Mayorga et al., 2000). Moreover, low responses have also been associated with the presence of ovarian antibodies (Meyer et al., 1990), reduced aromatase activity (Hurst et al., 1992), decreased blood flow measured by Doppler ultrasonography (Pellicer et al., 1994) and reduced circulating surge-attenuating gonadotrophin factor (GnSAF) bioactivity (Martínez et al., 2002). Finally, possible acquired factors such as obesity (Dechaud et al., 1998) chemotherapy, radiotherapy, pelvic surgery (Tulandi et al., 2002), pelvic infections or tubal disease (Keay et al., 1998), severe endometriosis (Barnhart et al., 2002), and heavy smoking (El-Nemr et al., 1998) can be also associated with a poor ovarian response.

**Prediction**

Correct identification of those who are at risk for poor response prior to stimulation is useful in counselling patients, and may be helpful in tailoring the best stimulation protocol and dosage of gonadotrophin to individual patients. Before analysing the tests that have been described as predictors of ovarian reserve, it is important to emphasize that no test is a good predictor of clinical outcome (Burns et al., 1996; Welt et al., 1999), and it is clearly associated with the FSH rise in the early follicular phase. Subtle serum increase of FSH represents an early signal of the decline of the ovarian response despite regular menses, and it is associated with otherwise unexplained infertility (Cameron et al., 1988; Muasher et al., 1988). There is a wide variation in what can be considered ‘high’ FSH values, and basal high FSH concentrations >10 or >12 or >15 mIU/ml have been reported as predictive of poor ovarian response and poor clinical outcome (Cameron et al., 1988; Scott et al., 1989; Toner et al., 1991; Faber et al., 1998). Moreover, even a single elevated FSH value might denote a reduced ovarian reserve (Scott et al., 1990). However, when basal serum FSH concentrations are used to try to predict the ovarian reserve, it is necessary to consider the possible inter-cycle variability of day 3 FSH reported by some (Brown et al., 1995) but not all authors (Peñarrubia et al., 2004), which suggests that basal hormone values should be sampled within 3 months of the assisted reproduction cycle (Cres et al., 2000). Another marker of reduced ovarian reserve is the increased basal oestradiol concentrations in the presence of ‘normal’ FSH concentrations due to early follicular recruitment that occurs consequently to a premature luteal elevation of FSH. This early luteal recruitment is probably due to a diminished follicular cohort that produces less inhibin. Elevated oestradiol concentrations may be able to suppress FSH into the normal range in women who have substantially diminished ovarian reserve and thus may cause false-negative FSH test results. Basal oestradiol concentrations >30 or >45 or >70 pg/ml have been associated with poor IVF outcome (Liccari et al., 1995; Smotrich et al., 1995). Higher inhibin B concentrations throughout the stimulation were related to a higher oocyte yield (Engel et al., 2001), whereas lower serum concentrations of inhibin-B on day 3 very likely reflect lower follicle numbers and may serve as a good predictor of clinical outcome (Burns et al., 1996; Seifer et al., 1997). In patients with day 3 serum inhibin-B concentrations <45 pg/ml, the number of oocytes retrieved is lower, the cycle cancellation rate is higher and the clinical outcome is poorer compared with patients with day 3 inhibin-B concentrations ≥45pg/ml (Seifer et al., 1997).

Recently Martinez et al. (2002) suggested a role of GnSAF, an ovarian factor that has a specific biological effect of reducing pituitary responsiveness to GnRH, in the prediction of ovarian reserve. The authors demonstrated that on the day of HCG administration, circulating GnSAF concentrations are lower in women with reduced ovarian response to ovarian stimulation compared with normally responding patients. Moreover, they observed the lack of GnSAF bioactivity during the follicular phase of spontaneous cycles and a much more reduced and slower increase in circulating GnSAF following FSH stimulation in women with previous poor ovarian response to ovarian stimulation compared with patients with previously normal ovarian response (Martínez et al., 2002).
A new promising biochemical marker that could be used as a predictor of ovarian potential is the anti-Mullerian hormone. Because anti-Mullerian hormone is produced exclusively by the small growing follicles and secreted into the circulation, serum concentrations of anti-Mullerian hormone were significantly decreased over time in young normo-ovulatory women, whereas other markers associated with ovarian ageing, such as serum concentrations of FSH and inhibin-B, did not change during the same interval (De Vet et al., 2002). Ultimately, the high heritability found for age at menopause suggests genetic control by an unknown number of genes. With developments in molecular genetics, it might become possible to construct 'DNA fingerprints' that will identify women with a genetic predisposition to 'early ovarian ageing' (Te Velde and Pearson, 2002). Some authors have suggested dynamic tests of ovarian reserve such as the clomiphene challenge test (Navot et al., 1987), the lupon screening test (Padilla et al., 1991), change in oestradiol concentrations after exogenous FSH stimulation (Fanchin et al., 1994) and the GnRH stimulation test (Karande and Gleicher, 1999) as useful tools to predict ovarian response.

Recently, the ultrasonography characteristics of the ovary have been suggested to be predictive of ovarian potential during ovarian stimulation. The antral follicle count as well as ovarian volume appeared to be indicative of poor response in assisted reproduction. Pellicer et al. (1998) reported a high correlation between the number of selectable follicles (2–5 mm) measured by transvaginal three-dimensional ultrasonography and the number of selectable follicles in histological slices. According to this observation, it is possible that the number of antral follicles originating from the cohort of growing follicles reflects the size of the pool of resting follicles, and thus ovarian reserve. Bancsi et al. (2002), analysing the odds ratios, statistical significance and receiver operating curve (ROC) area under the curve (AUC) for six basal ovarian reserve markers, observed that the number of antral follicles as a single predictor was the most powerful prognosticator of reduced ovarian response expressed by the largest ROC AUC of 0.87. This discriminative potential for poor response could increase if the day 3 inhibin-B and FSH are also considered. In a multivariate analysis with these three variables, the antral follicle count was selected in the first step, followed by inhibin-B in step two, and finally by FSH in step three. The ROC AUC increased in a stepwise manner from 0.87 to 0.92 (Bancsi et al., 2002). Similar results were recently reported in a prospective study where various markers of ovarian reserve (FSH, LH, oestradiol, inhibin-B, antral follicle count) were evaluated in the natural cycle preceding assisted reproductive therapy in 60 women as prognosticators of their ovarian response to ovarian stimulation (Loverro et al., 2003).

Management of poor ovarian response

In poor responder patients, the induction of a controlled multifollicular growth is a big challenge. Several stimulation protocols with different doses of gonadotrophins have been suggested but unfortunately no protocol is really effective and the ideal approach to this group of patients has not been well established. Moreover, whatever protocol is used, the clinical outcome is poorer than that observed in normoresponder patients and it seems to be related to female age, to the number of oocytes retrieved and to the number of embryos transferred (Ulug et al., 2003).

Gonadotrophins

When the standard dose of gonadotrophins (225–300 IU) fails to induce a proper multifollicular growth, the obvious clinical approach is to increase the dose of gonadotrophins, and high doses of gonadotrophins have been used by the vast majority of the authors in poor responder patients. In a prospective study (Van Hooff et al., 1993) and a retrospective study (Karande et al., 1990; Land et al., 1996), increasing the starting dose up to 450 IU was ineffective in enhancing ovarian response and/or in increasing pregnancy rates (Table 1). These data are not in agreement with a prospective study with historical controls, where the authors increased the daily gonadotrophin dose from 300 IU to 450 IU and obtained increased pregnancy rates and reduced cancellation rates (Hofmann et al., 1989). However, in another retrospective study, the use of a daily dose higher than 450 IU failed to significantly improve ovarian response and clinical outcome (Karande et al., 1990). These patients are likely to have a reduced ovarian reserve with an outcome that will be poor independently of the dosage administered and their clinical pregnancy rates seem to be inversely correlated with the amount of gonadotrophins used for ovarian stimulation (Karande et al., 1990; Land et al., 1996). Some authors have suggested the use of urinary or recombinant FSH in order to improve ovarian response (Out et al., 1996). Increased numbers of oocytes retrieved, embryos obtained and pregnancies were reported in two very small prospective studies with the use of recombinant FSH (Raga et al., 1999; De Placido et al., 2000). However, these results could not be confirmed by other authors who reported comparable outcomes between gonadotrophin preparations (Edelstein et al., 1999).

Based on the physiological model of the late luteal phase recruitment of the antral follicles and on their initial development under the premestral FSH rise, it was suggested to start FSH therapy during the late luteal phase (Rombauts et al., 1998). Unfortunately, in a prospective randomized trial it was not possible to demonstrate any benefit of this technique with increased cancellation rate, decreased number of oocytes retrieved and increased doses of gonadotrophins administered (Rombauts et al., 1998).

GnRH analogues

In normoresponder patients the combination of gonadotrophins and gonadotrophin-releasing hormone (GnRH) agonists lowers cancellation rate, raises the number of pre-ovulatory follicles, oocytes retrieved and good quality embryos for transfer, thus leading to better pregnancy rates (Hughes et al., 1992). Conversely, in poor responder patients it is not clear whether the use of GnRH agonists is advantageous or detrimental. The GnRH agonists may have a direct ovarian effect, acting to modulate ovarian steroidogenesis and oocyte maturation, and sometimes may induce excessive oversuppression with insufficient concentrations of serum oestradiol and a reduced or absent follicular response (Yashimura et al., 1992; Kowalik et al., 1998). For this reason, in all those patients who fail to obtain adequate multifollicular growth with the long late-luteal GnRH agonist protocols, the options are either to decrease the length of suppression by decreasing the duration of GnRH agonist use...
(short and ultrashort regimens) or to lower or to stop (after pituitary suppression) the dose of GnRH agonists initiated during the luteal phase to allow for down-regulation without complete inhibition of ovarian response.

With regard to the flare-up regimens (short and ultrashort protocols), two theoretical advantages can be considered: mild ovarian suppression and the flare-up effect, with the potent release of endogenous gonadotrophin (FSH and LH) in the 48 h after the initiation of the GnRH agonist, which enhances the effect of the exogenous gonadotrophin. However, this short potent release of endogenous gonadotrophins may induce enhanced ovarian androgen release, corpus luteum rescue, and a secondary decline in oocyte quality and ongoing pregnancy rates compared with those who receive a GnRH agonist in the mid-luteal phase to induce gonadotrophic down-regulation before initiation of gonadotrophin therapy (Gelety et al., 1995). With the aim of reducing these negative effects, some authors have suggested the so-called ‘mini’ or ‘micro’ dose flare-up GnRH agonist regimens. The lowest dose of GnRH agonist that can be successfully used to induce release of gonadotrophins in humans has not been elucidated. Deaton et al. (1996) noted increases in both FSH and LH concentrations in humans on cycle day 3 after administration of a single 25 or 50 µg dose of leuprolide acetate on cycle day 2. Whatever is the lowest dose of GnRH agonist with the use of ‘mini’ or ‘micro’ dose flare-up GnRH agonist regimens, very encouraging results have been reported by some authors (Schoolcraft et al., 1997; Surrey et al., 1998) (Table 2).

However, despite these findings, in another study it was not possible to confirm the results reported in the aforementioned

### Table 1. Alteration of the dose of gonadotrophins (high versus low) to induce an increased ovarian response.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study design</th>
<th>Regimen</th>
<th>Retrieved eggs (mean)</th>
<th>Cancellation rate (%)</th>
<th>Pregnancy rate/embryo transfer (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hofmann et al. (1989)</td>
<td>Prospective</td>
<td>6 versus 4 ampoules of HMG</td>
<td>2.6 versus 2 (NS)</td>
<td>9 versus 33 (P = 0.03)</td>
<td>33 versus 6 (P = 0.02)</td>
</tr>
<tr>
<td>Karande et al. (1990)</td>
<td>Retrospective</td>
<td>6 versus 4 ampoules of HMG</td>
<td>No change</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td>Van Hooff et al. (1993)</td>
<td>Prospective</td>
<td>Doubling HMG dose on day 5 versus unchanged HMG dose</td>
<td>No change</td>
<td>No change</td>
<td>No change</td>
</tr>
<tr>
<td>Land et al. (1996)</td>
<td>Retrospective</td>
<td>6 versus 3 ampoules of HMG</td>
<td>5.9 versus 7.5 (P = 0.05)</td>
<td>33 versus 70 (NS)</td>
<td>No change</td>
</tr>
</tbody>
</table>

Modified from Tarlatzis et al. (2003).

### Table 2. Evaluation of the efficacy of GnRH agonist flare-up versus long protocols in published studies.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study design</th>
<th>Regimen</th>
<th>Retrieved eggs (mean)</th>
<th>Cancellation rate (%)</th>
<th>Pregnancy rate/embryo transfer (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Howles et al. (1987)</td>
<td>Prospective with historical control</td>
<td>Buserelin nasally from day 1 to day 3; HMG from day 3</td>
<td>0</td>
<td>–</td>
<td>42 (increased)</td>
</tr>
<tr>
<td>Scott et al. (1994)</td>
<td>Prospective with historical control</td>
<td>Leuprolide s.c from day 3; HMG high dose from day 5</td>
<td>1.8 versus 5.1 (P = 0.05)</td>
<td>0</td>
<td>0 versus 11</td>
</tr>
<tr>
<td>Padilla et al. (1996)</td>
<td>Prospective (no control)</td>
<td>Leuprolide flare-up</td>
<td>Significantly lower</td>
<td>11 (increased)</td>
<td>29 (increased)</td>
</tr>
<tr>
<td>Schoolcraft et al. (1997)</td>
<td>Prospective with historical control</td>
<td>Leuprolide s.c from day 3; HMG high dose from day 5</td>
<td>10.9 (increased)</td>
<td>12.5 (decreased)</td>
<td>50 (increased)</td>
</tr>
<tr>
<td>Surrey et al. (1998)</td>
<td>Prospective with historical control</td>
<td>Leuprolide s.c from day 3; HMG high dose from day 5</td>
<td>7.3 versus 6.4 (NS)</td>
<td>7 versus 53 (P = 0.05)</td>
<td>41 versus 0 (NS)</td>
</tr>
<tr>
<td>Toth et al. (1996)</td>
<td>Retrospective</td>
<td>Flare-up Gn RH agonist</td>
<td>Significantly lower</td>
<td>20 versus 11 (P = 0.01)</td>
<td>–</td>
</tr>
<tr>
<td>Leonidires et al. (1999)</td>
<td>Retrospective</td>
<td>Leuprolide s.c from day 3; HMG high dose from day 5</td>
<td>3.3 versus 16.5 (NS)</td>
<td>22 versus 8 (P = 0.03)</td>
<td>47 versus 60 (NS)</td>
</tr>
</tbody>
</table>

Modified from Tarlatzis et al. (2003).
reports (Leonidres et al., 1999) (Table 2), and larger controlled and randomized studies are needed to support the real efficacy of the ‘mini’ or ‘micro’ dose flare-up GnRH agonist regimens in poor responder patients.

All the published studies that attempted to evaluate the efficacy of flare-up (short, ultrashort, mini-, micro-dose flare-up) regimens are prospective non-randomized trials with or without historical control (Scott and Navot, 1994; Padilla et al., 1996; Howles et al., 1997; Schoolcraft et al., 1997; Surrey et al., 1998) or retrospective (Toth et al., 1996; Leonidres et al., 1999) and although widely used there are no large prospective randomized controlled trials that can be used to assess their efficacy compared with standard protocols (Table 2).

The other option to the standard long luteal phase GnRH agonist protocols is the use of relatively low doses of GnRH agonist started in the mid-luteal phase of the cycle and suspended at the time of menses or on the day of the gonadotrophin administration. With this approach, it is possible to obtain a reduction in the inhibitory direct effect of the GnRH agonist on the gonads (Yashimura et al., 1992; Kowalik et al., 1998) and the pituitary suppression that avoids the premature release of LH during the follicular phase (Smits et al., 1992). Although most of the published trials (prospective studies with historical controls) report a reduction in the amount of gonadotrophin administered and improved results in terms of number of oocytes retrieved and clinical outcome (Faber et al., 1998; Karande et al., 1999; Pinkas et al., 2000; Schachter et al., 2001) with the use of ‘GnRH agonist stopped’ protocol, in two prospective randomized controlled trials improvements in reproductive outcome were not reported (Dininfeld et al., 1999; Garcia-Velasco et al., 2000).

**GnRH antagonists**

The use of GnRH antagonists in the mid-late follicular phase during ovarian stimulation prevents the premature LH surge while not causing suppression in the early follicular phase (Kenisberg et al., 1984). With this stimulation regimen, it is possible to obtain a more natural follicular recruitment without any inhibitory effect possibly induced by the GnRH agonist and therefore it has been suggested by several authors as a suitable protocol for poor responders. However, with this approach also, there are conflicting results in the literature. In two prospective studies (one with historical controls and one randomized), no significant improvements in the reproductive outcome were reported (Craft et al., 1999; Akman et al., 2000), whereas in another prospective randomized study where the antagonist regimen was compared with the flare-up regimen, better results have been reported with the latter protocol (Akman et al., 2001) (Table 3). Similarly in a prospective randomized study, D’Amato et al. (2004) retrieved a significantly higher number of oocytes and a significantly lower cancellation rate by using GnRH antagonists in combination with clomiphene citrate and gonadotrophins. Similar results were reported in one retrospective study (Fasulotis et al., 2003) (Table 3). Copperman (2003) observed better clinical results when the antagonist cycles were pretreated with the use of oral contraceptive (OC) pills. In this retrospective study, the authors reported a significantly increased number of oocytes and pregnancy rates and a significantly reduced cancellation rates. These results are in contrast to those of Shapiro et al. (2002), who reported significantly increased cancellation rates in the group of poor responder patients pretreated with OC compared with patients not receiving OC pretreatment. The limited data available show conflicting results on the use of GnRH antagonists in poor responder patients. Similarly to all other stimulation regimens analysed, larger controlled prospective randomized trials using GnRH antagonists are needed to assess the efficacy of GnRH antagonist protocols in poor responder patients.

**Alternative approaches in addition to ovarian stimulation regimens**

Alternative approaches have been proposed with the aim to strengthen the effect of exogenous gonadotrophins. It has been suggested that the use of growth hormone (GH) might modulate the action of FSH on granulosa cells by up-regulating the local synthesis of insulin-like growth factor-I (IGF-I) (Davoren et al., 1986; Hsu et al., 1988; Baricza et al., 1993) The IGF-I amplifies the effect of FSH at the level of both the granulosa and theca cell (Adashi et al., 1985; Jia et al., 1986). Unfortunately, several prospective randomized controlled studies failed to demonstrate any benefit from the use of GH as adjunctive therapy to ovarian stimulation in poor responder patients (Shaller et al., 1992; Levy et al., 1993; Suikkari et al., 1996). Probably only a selected group of patients may benefit by the use of GH together with standard stimulation protocols. Blumenfeld et al. (1994), in fact, noted enhanced pregnancy rates and a reduction of gonadotrophin dose with GH co-treatment for ovulation induction only in patients who failed to mount a normal GH response to the clonidine challenge test. For this reason, an uncontrolled use of GH may be useless.

One of the factors associated with poor ovarian response is the decreased blood flow measured by Doppler ultrasonography (Pellicer et al., 1994). Recently Battaglia et al. (1999) in a prospective randomized study used oral l-arginine with the aim to improve uterine and follicular Doppler flow and then improving ovarian response to gonadotrophin in poor responder women. In this study, l-arginine oral supplementation during ovarian stimulation induced an increase in plasma l-arginine, l-citrulline and nitrite/nitrate concentrations and was associated with increased follicular fluid concentrations of l-arginine and its derivatives. The above data were related to decreased blood flow resistance in the perifollicular arteries. Three pregnancies were observed in the l-arginine group. This result might be due to the increased number of transferred embryos, to better embryo quality, and/or to improved endometrial receptivity. These preliminary interesting results need further studies to be confirmed.

**Natural cycles**

In poor responder patients with a diminished ovarian reserve, only very few follicles can be recruited and very few oocytes, if any, can be retrieved after ovarian stimulation. For this reason, some authors have suggested the use of the patient’s own natural cycle oocyte(s), reporting good clinical pregnancy rates per embryo transfer despite high cancellation rates mainly due to untimely LH surge (Bassil et al., 1999).
Although many studies have been published on the efficacy of natural IVF cycles, only four prospective studies with historical controls involved solely poor responder patients (Bassil et al., 1999; Feldman et al., 2001; Kolibianakis et al., 2004; Morgia et al., 2004) (Table 4). The clinical pregnancy rates per embryo transfer were acceptable in the studies by Bassil et al. (1999), Feldman et al. (2001) and Morgia et al. (2004), but very disappointing results have been recently reported in the paper by Kolibianakis et al. (2004). In this latter study, the mean basal serum FSH concentration of the patients was 20 mIU/ml and the oocyte retrieval was performed 32 h after HCG administration. It is possible that selecting patients with lower serum basal FSH concentrations and postponing oocyte retrieval could improve oocyte quality and clinical outcome. The most important drawback of this approach is the high cancellation rates. In order to reduce the incidence of premature LH surge, GnRH antagonists have been already used in a single dose in the late follicular phase of natural IVF cycles in normoresponder patients, obtaining good clinical results (Rongieres-Bertrand et al., 1999). Encouraged by the acceptable results of the natural IVF cycles in poor responder patients (Bassil et al., 1999; Feldman et al., 2001) and by the use of GnRH antagonists to reduce the incidence of the untimely LH surge (Rongieres-Bertrand et al., 1999), from January 2002, it was decided to use GnRH antagonists in a multiple dose protocol (Albano et al., 1997) in natural intracytoplasmic sperm injection (ICSI) cycles of poor responder patients where ovarian stimulation strategies with

Table 3. Use of GnRH antagonist protocols during ovarian stimulation: results from prospective and retrospective studies.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study design</th>
<th>Regimen</th>
<th>Retrieved eggs (mean)</th>
<th>Cancellation rate (%)</th>
<th>Pregnancy rate/embryo transfer (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Craft et al. (1999)</td>
<td>Prospective with historical control</td>
<td>CC and HMG from day 2 and cetrorelix from day 6</td>
<td>6.4 versus 4.7 (NS)</td>
<td>29 versus 56 (P = 0.06)</td>
<td>23 versus 10 (NS)</td>
</tr>
<tr>
<td>Akman et al. (2000)</td>
<td>Prospective</td>
<td>600 IU FSH/HMG from day 2 and cetrorelix from follicle 14 mm</td>
<td>3.2 versus 3.4 (NS)</td>
<td>25 versus 20 (NS)</td>
<td>20 versus 6 (NS)</td>
</tr>
<tr>
<td>Akman et al. (2001)</td>
<td>Prospective</td>
<td>600 IU FSH/HMG from randomized day 2 and cetrorelix from follicle 14 mm</td>
<td>4.5 versus 5.5 (P = 0.03)</td>
<td>0 (NS)</td>
<td>22 versus 15 (NS)</td>
</tr>
<tr>
<td>D’Amato (2004)</td>
<td>Prospective randomized controlled administration</td>
<td>CC and rec-FSH from day 3 and late GnRH antagonist administration</td>
<td>5.5 versus 3.3 (P = 0.01)</td>
<td>5 versus 34 (P = 0.001)</td>
<td>22 versus 15 (NS)</td>
</tr>
<tr>
<td>Fasouliotis (2003)</td>
<td>Retrospective</td>
<td>HMG high dose and GnRH antagonist</td>
<td>No change</td>
<td>No change (NS)</td>
<td>26 versus 12 (NS)</td>
</tr>
</tbody>
</table>

Modified from Tarlatzis et al. (2004).

Table 4. Efficacy of natural IVF cycles in poor responder patients: results from four prospective studies with historical controls.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study design</th>
<th>Regimen</th>
<th>Retrieved eggs (mean)</th>
<th>Cancellation rate (%)</th>
<th>Pregnancy rate/embryo transfer (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bassil et al. (1999)</td>
<td>Prospective with historical control</td>
<td>Natural cycles</td>
<td>0.9 versus 1.5 (NS)</td>
<td>19 versus 48 (NS)</td>
<td>19 versus 0 (NS)</td>
</tr>
<tr>
<td>Morgia et al. (2004)</td>
<td>Prospective randomized</td>
<td>Natural cycles versus GnRH flare protocol</td>
<td>–</td>
<td>–</td>
<td>14 versus 10 (NS)</td>
</tr>
<tr>
<td>Kolibianakis et al. (2004)</td>
<td>Prospective</td>
<td>Natural cycles with late GnRH antagonist administration</td>
<td>0.9</td>
<td>32</td>
<td>0 versus 0</td>
</tr>
<tr>
<td>Ubaldi et al. (unpublished)</td>
<td>Open observational</td>
<td>Natural cycle with late GnRH antagonist administration</td>
<td>0.8</td>
<td>16</td>
<td>26</td>
</tr>
</tbody>
</table>
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This modified natural IVF cycle was used in 157 poor responder patients who underwent a total of 258 consecutive cycles. As soon as the dominant follicle was 14–15 mm, 0.25 mg of GnRH antagonist was administered in the evening and continued every 24 h until the day of HCG administration. Since oestradiol secretion could be impaired in natural cycles after the use of GnRH antagonist (Leroy et al., 1995), 75–100 IU of rec-FSH was used, starting on the same day as GnRH antagonist administration and continued until the evening before the day of HCG administration. When the mean diameter of the leading follicle reached 16–17 mm, ovulation was triggered by intramuscular administration of 10,000 IU of HCG and oocyte retrieval was performed 35 h later without anaesthesia. In this group of patients, a mean of 2.5 ± 1.6 ampoules of antagonist and 174.8 ± 131.2 IU of recombinant FSH per cycle were used. This amount of gonadotrophins is minimal compared with that used in poor responder patients after ovarian stimulation protocols. The most important drawback of this approach is the relatively low chance to perform an embryo transfer. Experience suggests that only in 51.5% (133/258) of the started cycles was it possible to obtain one embryo to transfer. The patients should be counselled and well informed about these figures. However, the cancellation rate is less dramatic because the physical (very few injections no side effects, no anaesthesia and hospital stay), emotional (less anxiety and stress as the patient does not have to worry about the ovarian response to the stimulation) and financial burdens of these couples are low and ICSI can theoretically be tried again in the next cycle. Overall, the clinical pregnancy rates observed in this group of patients were 13.5% per initiated cycle, 22.2% per patient and 26.3% per embryo transfer, with an overall implantation rate of 27.4% (Table 4).

A very important issue in favour of the natural cycle with minimal stimulation in poor responder patients is its cost-effectiveness compared with conventionally stimulated cycles. Recently, in a cost-effectiveness analysis it was calculated that a natural cycle, adjusted for reductions for incomplete cycles, at the present institution costs 1550 euros, whereas a conventionally stimulated cycle costs 6050 euros (Ubaldi et al., 2004). In this study, a clinical pregnancy rate per started cycle of 12.6% was observed in natural IVF cycles versus 8.4% in conventionally stimulated cycle. Thus the total cost of one clinical pregnancy using natural cycles with minimal stimulation is 12,300 euros, whereas the cost rises to 72,050 euros when conventionally stimulated cycles are used. Now, if it is considered that with the same amount of money spent for a conventionally stimulated cycle it is possible to undergo four natural IVF cycle with minimal stimulation, and assuming that pregnancy rate remains stable over four sequential attempts, the probability of conceiving with the same amount of money spent would be approximately 40% for the natural cycle (theoretical cumulative pregnancy rate for four attempts) as compared with 8% for conventional ovarian stimulation (one attempt). In conclusion, it can be said that natural IVF cycle is a low-risk and easy procedure with a pregnancy rate of about 7% per started cycle and 16% per embryo transfer (Pelinck et al., 2002). However, to assess the real efficacy of this approach in poor responder patients, large prospective randomized controlled studies are needed.

Conclusions

One of the most important problems in the management of poor responder patients is the difficulty in predicting poor ovarian response to ovarian stimulation in order to tailor the correct stimulation regimen. Unfortunately, although several tests have been suggested, no very accurate predictive test is available to assess ovarian response. A variety of different stimulation protocols have been suggested, but the lack of any large-scale, prospective, randomized, controlled trials of the different management strategies and the lack of a uniform definition of the population which may result in comparisons of heterogeneous groups of patients makes it difficult to draw any definitive conclusions. Natural IVF cycle with minimal stimulation can be considered as an easy and cheap approach in the management of poor responder patients. Although no controlled large prospective randomized studies are available to compare the natural IVF procedure with ovarian stimulation IVF in poor responder patients, the efficacy of natural cycle IVF is hampered by high cancellation rates because of the untimely LH surge. The use of GnRH antagonists in the late follicular phase, which reduces the premature LH rise rate, and the improvements in laboratory conditions and fertilization techniques, increase embryo transfer rates, making this procedure more effective.

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