Review

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 aetiological 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.

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; Lashen *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 with serum oestradiol <100 pg/ml on day 5 of stimulation (Schoolcraft *et al.*, 1997) or with maximal oestradiol ranging from <300 to <600 pg/ml (Brzysky *et al.*, 1988; 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; Hugues *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 pre-ovulatory 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 (Treloar *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 near-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 (Tarin, 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 (Martinez *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 absolutely predictive and the best test is of course ovarian response to ovarian stimulation.

Measurement of serum FSH concentrations on day 3 of the cycle represents one of the most widely used prognostic tests in assessing the ovarian reserve. Basal serum FSH concentrations begin to rise on average a decade or more before the menopause (Ebbiary *et al*., 1994; Klein *et al*., 1996), and are inversely correlated with ovarian follicular responsiveness to maximal exogenous gonadotrophin stimulation (Cahill *et al*., 1994). This is caused by the negative feedback of the FSH-modulating proteins from the ovary, mainly inhibin-A and inhibin-B (Groome *et al*., 1996). Since inhibin-B is predominantly secreted by the early antral follicles, a decreased serum concentration of inhibin-B reflects a reduction of the antral follicle pool (Burger *et al*., 1998; 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 (Creus *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 (Licciardi *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 (Martinez *et al*., 2002).

A new promising biochemical marker that could be used as a predictor of ovarian reserve 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 lupron 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 premestrual 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 pregancy 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.

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

Modified from Tarlatzis *et al*. (2003).

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

Reference	*Study design*	*Regimen*	*Retrieved eggs (mean)*	*Cancellation rate (%)*	*Pregnancy rate/embryo transfer (%)*
Howles *et al*. (1987)	Prospective with historical control	Buserelin nasally from day 1 to day 3; HMG from day 3	0	–	42 (increased)
Scott *et al*. (1994)	Prospective with historical control	Leuprolide s.c from day 3; HMG high dose from day 5	1.8 versus 5.1 ($P = 0.05$)	0	0 versus 11
Padilla *et al*. (1996)	Prospective (no control)	Leuprolide flare-up	Significantly lower	11 (increased)	29 (increased)
Schoolcraft *et al*. (1997)	Prospective with historical control	Leuprolide s.c. from day 3; HMG high dose from day 5	10.9 (increased)	12.5 (decreased)	50 (increased)
Surrey *et al*. (1998)	Prospective with historical control	Leuprolide s.c from day 3; HMG high dose from day 5	7.3 versus 6.4 (NS)	7 versus 53 ($P = 0.05$)	41 versus 0 (NS)
Toth *et al*. (1996)	Retrospective	Flare-up Gn RH agonist	Significantly lower	20 versus 11 ($P = 0.01$)	–
Leondires *et al*. (1999)	Retrospective	Leuprolide s.c from day 3; HMG high dose from day 51	3.3 versus 16.5 (NS)	22 versus 8 ($P = 0.03$)	47 versus 60 (NS)

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; Leondires *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 of 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 (Smitz *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 (Dirnfeld *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; Baricca *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 ultrasonograpy (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.

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

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.

Reference	*Study design*	*Regimen*	*Retrieved eggs (mean)*	*Cancellation rate (%)*	*Pregnancy rate/embryo transfer (%)*
Bassil *et al*. (1999)	Prospective with historical control	Natural cycles	0.9 versus 1.5 (NS)	19 versus 48 (NS)	19 versus 0 (NS)
Morgia *et al*. (2004)	Prospective randomized	Natural cycles versus GnRH flare protocol	–	–	14 versus 10 (NS)
Kolibianakis *et al*. (2004)	Prospective	Natural cycles with late GnRH antagonist administration	0.9	32	0 versus 0
Ubaldi *et al*. (unpublished)	Open observational	Natural cycle with late GnRH antagonist administration	0.8	16	26

high doses gonadotrophins had failed to produce multiple pre-ovulatory follicles.

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.

References

Adashi E, Resnick C, D'Erole J *et al*. 1985 Insulin-like growth factors as intraovarian regulators of granulosa cell growth and function. *Endocrine Reviews* **6**, 400–420.

Akande VA, Fleming CF, Hunt LP *et al*. 2002 Biological versus chronological ageing of oocytes, distinguishable by raised FSH levels in relation to the success of IVF treatment *Human Reproduction* **17**, 2003–2008.

Akman MA, Erden HF, Tosun SB *et al*. 2000 Addition of GnRH antagonist in cycles of poor responders undergoing IVF. *Human Reproduction* **15**, 2145–2147.

Akman MA, Erden HF, Tosun SB *et al*. 2001 Comparison of agonistic flare-up-protocol and antagonistic multiple dose protocol in ovarian stimulation of poor responders: results of a prospective randomized trial. *Human Reproduction* **16**, 868–870.

Albano C, Smitz J, Camus M *et al*. 1997 Comparison of different doses of gonadotropin-releasing hormone antagonist cetrorelix during controlled ovarian hyperstimulation. *Fertility and Sterility* **67**, 917–922.

Bancsi LJMM, Broekmans FJM, Eijkemans MJC *et al*. 2002 Predictors of poor ovarian response in in-vitro fertilization: a prospective study comparing basal markers of ovarian reserve. *Fertiity and Sterility* **77**, 328–336.

Baricca A, Artini P, Del Monte P *et al*. 1993 In vivo and in vitro effect of growth hormone on estradiol secretion by granulosa cells. *Journal of Clinical Endocrinology and Metabolism* **77**, 61–67.

Barnhart K, Dunsmoor R, Coutifaris C 2002 Effect of endometriosis on in vitro fertilization. *Fertility and. Sterility* **77**, 1148–1155.

Battaglia DE, Goodwin P, Klein NA, Soules MR 1996 Influence of maternal age on meiotic spindle assembly in oocytes from naturally cycling women. *Human Reproduction* **11**, 2217–2222.

Battaglia C, Salvatori M, Maxia N *et al*. 1999 Adjuvant L-arginine treatment for in-vitro fertilization in poor responder patients. *Human Reproduction* **14**, 1690–1697.

Bassil S, Godin P, Donnez J 1999 Outcome of in-vitro fertilization through natural cycles in poor responders. *Human Reproduction* **14**, 1262–1265.

Benadiva CA, Kligman I, Munne S 1996 Aneuploidy 16 in human embryos increases significantly with maternal age. *Fertility and Sterility* **66**, 248–255.

Blumenfeld Z, Dirnfeld M, Gonen Y, Abramovici H 1994 Growth hormone co-treatment for ovulation induction may enhance conception in the co-treatment and succeeding cycles in clonidine negative but not clonidine positive patients. *Human Reproduction* **9**, 209–213.

Brook JD, Gosden RG, Chandley AC 1984 Maternal ageing and aneuploidy embryos: evidence from the mouse that biological and not chronological age is the important influence. *Human Genetics* **66**, 41–45.

Brown JR, Liu HC, Sewitch KF *et al*. 1995 Variability of day 3 follicle-stimulating hormone levels in eumenorrheic women. *Journal of Reproductive Medicine* **40**, 620–624.

Brzyski R, Muasher S, Droesch K *et al*. 1988 Follicular atresia associated with concurrent initiation of gonadotropin-releasing hormone agonist and follicle-stimulating hormone for oocyte recruitment. *Fertility and Sterility* **50**, 917–921.

Burger HG, Cahir N, Robertson DM *et al*. 1998 Serum inhibins A and B fall differentially as FSH rises in perimenopausal women. *Clinical Endocrinology (Oxford)* **48**, 809–813.

Burns WN, Oktay KH, Tekmal RR *et al*. 1996 Diminished alpha-inhibin messenger ribonucleic acid in in vitro fertilization–embryo transfer poor responders reflects declining follicle reserve. *Fertility and Sterility* **65**, 394–399.

Cahill DJ, Prosser CJ, Wardle PG *et al*. 1994 Relative influence of serum follicle stimulating hormone, age and other factors on ovarian response to gonadotrophin stimulation. *British Journal of Obstetrics and Gynaecology* **101**, 999–1002.

Cameron IT, O'Shea FC, Rolland JM *et al*. 1988 Occult ovarian failure: a syndrome of infertility, regular menses, and elevated follicle-stimulating hormone concentrations. *Journal of Clinical Endocrinology and Metabolism* **67**, 1190.

Chuang CC, Chen CD, Chao KH *et al*. 2003 Age is a better predictor of pregnancy potential than basal FSH levels in women undergoing in vitro fertilization. *Fertility and Sterility* **79**, 63–68.

Copperman AB 2003 Antagonists in poor responder patients. *Fertility and Sterility* **80**, 16–24.

Craft I, Gorgy A, Hill J *et al*. 1999 Will GnRH antagonist provide a new hope for patients considered 'difficult responders' to GnRH agonist protocols? *Human Reproduction* **14**, 2959–2962.

Creus M, Peñarrubia J, Fábregues F *et al*. 2000 Day 3 serum inhibin B and FSH and age as predictors of assisted reproduction treatment outcome. *Human Reproduction* **15**, 2341–2346.

D'Amato G, Caroppo E, Pasquadibisceglie A *et al*. 2004 A novel protocol of ovulation induction with delayed gonadotropin-releasing hormone antagonist administration combined with high-dose recombinant follicle-stimulating hormone and clomiphene citrate for poor responders and women over 35 years. *Fertility and Sterility* **81**, 1572–1577.

Davoren J, Hsueh A 1986 Growth hormone increases ovarian levels of immunoreactive somatomedin-C/insulin-like growth factor I in vivo. *Endocrinology* **118**, 888–890.

De Placido G, Alviggi C, Mollo A *et al*. 2000 Recombinant FSH is effective in poor responder patients to highly purified FSH. *Human Reproduction* **15,** 17–20.

De Vet A, Laven JSE, De Jong FH *et al*. 2002 Antimullerian hormone serum levels: a putative marker for ovarian ageing. *Fertility and Sterility* **77**, 357–362.

Deaton J, Baugness P, Huffman C, Miller K 1996 Pituitary response to early follicular phase minidose gonadotropin releasing hormone agonist (GnRH-a) therapy: evidence for a second flare. *Journal of Assisted Reproduction and Genetics* **13**, 390–394.

Dechaud H, Ferron G, Anahory T *et al*. 1998 Obesity and assisted reproduction techniques. *Contraception, Fertility and Sex* **26**, 564–567.

Dirnfeld M, Fruchter O, Yshai D *et al*. 1999 Cessation of gonadotropin-releasing hormone analogue (GnRH-a) upon down-regulation versus conventional long GnRH-a protocol in poor-responders undergoing in vitro fertilization. *Fertility Sterility* **72**, 406–411.

Dor J, Seidman DS, Ben-Shlomo I *et al*. 1992 The prognostic importance of the number of oocytes retrieved and estradiol levels in poor and normal responders in in vitro fertilization (IVF) treatment. *Journal of Assisted Reproduction and Genetics* **9**, 228–232.

Ebbiary AA, Lenton EA, Cooke ID 1994 Hypothalamic-pituitary ageing: progressive increase in FSH and LH concentrations throughout the reproductive life in regularly menstruating women. *Clinical Endocrinology (Oxford)* **41**, 199–206.

Edelstein M, Brzyski R, Jones G *et al*. 1999 Equivalency of human menopausal gonadotropin and follicle-stimulating hormone stimulation after gonadotropin-releasing hormone agonist suppression. *Fertility and Sterility* **53**, 103–106.

El-Nemr, A, Al-Shawaf T, Sabatini L *et al*. 1998 Effect of smoking on ovarian reserve and ovarian stimulation in in-vitro fertilization and embryo transfer. *Human Reproduction* **13**, 2192–2198.

El-Toukhy T, Khalaf Y, Hart R *et al*. 2002 Young age does not protect against the adverse effects of reduced ovarian reserve – an eight year study. *Human Reproduction* **17**, 1519–1524.

Engel JB, Felberbaum R, Reissmann T *et al*. 2001 Inhibin A/B in HMG or recombinant FSH ovarian stimulation with cetrorelix medication *Reproductive BioMedicine Online* **3**, 104–108.

Faber B, Mayer J, Cox B *et al*. 1998 Cessation of gonadotropin-releasing hormone agonist therapy combined with high-dose gonadotropin stimulation yields favorable pregnancy results in low responders. *Fertility and Sterility* **69**, 826–830.

Faddy MJ, Gosden RG, Gougeon A *et al*. 1992 Accelerated disappearance of ovarian follicles in mid-life: implications for forecasting menopause. *Human Reproduction* **7**, 1342–1346.

Fanchin R, de Ziegler D, Oliveness F *et al*. 1994 Exogenous follicle stimulating hormone ovarian reserve test (EFORT): a simple and reliable screening test for detecting 'poor responders' in in-vitro fertilization. *Human Reproduction* **9**, 1607–1611.

Fasouliotis SJ, Laufer N, Sabbagh-Ehrlich S *et al*. 2003 GnRH-antagonist versus GnRH-agonist in ovarian stimulation of poor responders undergoing IVF. *Journal Assisted Reproduction and Genetics* **20**, 455–460.

Feldberg D, Farhi J, Ashkenazi J *et al*. 1994 Minidose gonadotropin releasing hormone agonist is the treatment of choice in poor responders with high follicle-stimulating hormone levels. *Fertility and Sterility* **62**, 343–346.

Freeman SB, Yang Q, Allran K *et al*. 2000 Women with reduced ovarian complement may have an increased risk for a child with Down syndrome. *American Journal Human Genetics* **66**, 1680–1683.

Fridstrom M, Akerlof E, Sjoblom P, Hillensjo T 1997 Serum levels of luteinizing and follicle-stimulating hormones in normal and poor-responding patients undergoing ovarian stimulation with urofollitropin after pituitary down regulation. *Gynecological Endocrinology* **11**, 25–28.

Garcia-Velasco JA, Isaza V, Requena A *et al*. 2000 High doses of gonadotrophins combined with stop versus non-stop protocol of GnRH analogue administration in low responder IVF patients: a prospective, randomized controlled trial. *Human Reproduction* **15**, 2292–2296.

Gelety T, Pearlstone A, Surrey E 1995 Short-term endocrine response to gonadotropin-releasing hormone agonist initiated in the early follicular, mid luteal or late luteal phase in normally cycling women. *Fertility and Sterility* **64**, 1074–1080.

Gougeon A 1996 Regulation of ovarian follicular development in primates: facts and hypotheses. *Endocrine Reviews* **17**, 121–155.

Groome NP, Illingworth PJ, O'Brien M *et al*. 1996 Measurement of dimeric inhibin B throughout the human menstrual cycle. *Journal of Clinical Endocrinology and Metabolism* **81**, 1401–1405.

Hardy R, Kuh D 1999 Reproductive characteristics and the age at the inception of the perimenopause in a British National Cohort. *American Journal of Epidemiology* **149**, 612–620.

Henderson SA, Edwards RG 1968 Chiasma frequency and maternal age in mammals. *Nature* **218**, 22–28.

Hofmann G, Toner J, Muasher S, Jones G 1989 High-dose follicle-stimulating hormone (FSH) ovarian stimulation in low-responder patients for in vitro fertilization. *Journal of In Vitro Fertilization and Embryo Transfer* **6**, 285–289.

Hofmann GE, Sosnowski J, Scott RT, Thie J 1996 Efficacy of selection criteria of ovarian reserve screening using the clomiphene citrate challenge test in a tertiary fertility centre population. *Fertility and Sterility*, **66**, 49–53.

Howles CM, Macnamee MC, Edwards RG 1987 Short term use of an LHRH agonist to treat poor responders entering an in-vitro fertilization programme. *Human Reproduction* **2**, 655–656.

Hsu C, Hammond J 1988 Concomitant effects of growth hormone on secretion of insulin-like growth factor I and progesterone by cultured porcine granulosa cells in vitro. *Endocrinology* **120**, 198–207.

Hughes EG, Fedorkow DM, Daya S *et al*. 1992 The routine use of gonadotropin-releasing hormone agonists prior to in vitro fertilization and gamete intrafallopian transfer: a meta-analysis of randomized controlled trials. *Fertility and Sterility* **58**, 888–896.

Hughes S, Huang Z, Morris I *et al*. 1994 A double-blind crossover controlled study to evaluate the effect of biosynthetic growth hormone on ovarian stimulation in previous poor responders to in-vitro fertilization. *Human Reproduction* **9**, 13–18.

Hugues J, Torresani T, Herve F *et al*. 1991 Interest of growth hormone-releasing hormone administration for improvement of ovarian responsiveness to gonadotropins in poor responder women. *Fertility and Sterility* **55**, 945–951.

Hurst B, Zacur H, Schlaff W, Berkovitz G 1992 Use of granulosa–luteal cell culture to evaluate low and high clinical responses to menotropin stimulation. *Journal of Endocrinological Investigation* **15**, 567–572.

Ibrahim Z, Matson P, Buck P, Lieberman B 1991 The use of biosynthetic human growth hormone to augment ovulation induction in buserelin acetate/human menopausal gonadotropin in women with a poor ovarian response. *Fertility and Sterility* **55**, 202–204.

Jia X, Kalmijn J, Hsueh A 1986 Growth hormone enhances follicle-stimulating hormone-induced differentiation of cultured rat granulosa cells. *Endocrinology* **118**, 1401–1406.

Kailasam C, Keay SD, Wilson P *et al*. 2004 Defining poor ovarian response during IVF cycles, in women aged <40 years, and its relationship with treatment outcome. *Human Reproduction* **19**, 1544–1547.

Karande V, Gleicher N 1999 A rational approach to the management of low responders in IVF. *Human Reproduction* **14**, 1744–1749.

Karande VC, Jones GS, Veeck LL, Muasher SJ 1990 High-dose follicle-stimulating hormone stimulation at the onset of the menstrual cycle does not improve the in vitro fertilization outcome in low-responder patients. *Fertility and Sterility* **53**, 486–489.

Karande V, Morris R, Rinehart J *et al*. 1997 Limited success using the 'flare' protocol in poor responders in cycles with low basal follicle-stimulating hormone levels during in vitro fertilization. *Fertility and Sterility* **67**, 900–903.

Keay SD, Liversedge NH, Mathur RS, Jenkins JM 1997 Assisted conception following poor ovarian response to gonadotrophin stimulation. *British Journal of Obstetrics and Gynecology* **104,** 521–527.

Keay SD, Liversedge NH, Jenkins JM 1998 Could ovarian infection impair ovarian response to gonadotrophin stimulation? *British Journal of Obstetrics and Gynaecology* **105**, 252–254.

Keefe DL, Niven-Fairchild T, Powell S, Buradagunta S 1995 Mitochondrial deoxyribonucleic acid deletions in oocytes and reproductive aging in women. *Fertility and Sterility* **64**, 577–583.

Kenisberg D, Littman BA, Williams RF *et al*. 1984 Medical hypophysectomy II: Variability of ovarian response to gonadotrophin therapy. *Fertility Sterility* **42**, 116–126.

Klein NA, Illingworth PJ, Groome NP *et al*. 1996 Decreased inhibin B secretion is associated with the monotropic FSH rise in older, ovulatory women: a study of serum and follicular fluid levels of dimeric inhibin A and B in spontaneous menstrual cycles. *Journal of Clinical Endocrinology and Metabolism* **81**, 2742–2745.

Kolibianakis E, Zikopoulos K, Camus M *et al*. 2004 Modified natural cycles for IVF does not offer a realistic chance of parenthood in poor responders with high day 3 FSH levels, as a last resort prior to oocyte donation. *Human Reproduction* **19**, 2545–2549.

Kowalik A, Barmat L, Damario M *et al*. 1998 Ovarian estradiol production in vivo: inhibitory effect of leuprolide acetate. *Journal of Reproductive Medicine* **43**, 413–417.

Lamb N, Feingold E, Savage A *et al*. 1997 Characterization of susceptible chiasma configurations that increase the risk of maternal non-disjunction of chromosome 21. *Human Molecular Genetics* **6**, 1391–1399.

Land J, Yarmolinskaya M, Dumoulin J, Evers J 1996 High-dose human menopausal gonadotropin stimulation in poor responders does not improve in vitro fertilization outcome. *Fertility and Sterility* **65**, 961–965.

Lashen H, Ledger W, Lopez-Bernal A, Barlow D 1999 Poor responders to ovulation induction: is proceeding to in-vitro fertilization worthwhile? *Human Reproduction* **14**, 964–969.

Leondires MP, Escalpes M, Segars JH *et al*. 1999 Microdose follicular phase gonadotropin-releasing hormone agonist (GnRH-a) compared with luteal phase GnRH-a for ovarian stimulation at in vitro fertilization. *Fertility and Sterility* **72**, 1018–1023.

Leroy I, d'Acremont MF, Brailly-Tabard S *et al*. 1995 A single injection of GnRH antagonist (Cetrorelix) postpones the LH surge: further evidence for the role of GnRH during the LH surge. *Fertility and Sterility* **62**, 461–467.

Levy T, Limor R, Villa Y *et al*. 1993. Another look at cotreatment with growth hormone and human menopausal gonadotropins in poor responders. *Human Reproduction* **8**, 834–839.

Licciardi FL, Liu H-C, Rosenwaks Z 1995 Day 3 estradiol serum concentrations as prognosticators of stimulation response and pregnancy outcome in patients undergoing in vitro fertilization. *Fertility and Sterility* **64**, 991–994.

Lindheim S, Barad D, Witt B *et al*. 1996 Short-term gonadotropin suppression with oral contraceptives benefits poor responders prior to controlled ovarian hyperstimulation. *Journal of Assisted Reproduction and Genetics* **16**, 745–747.

Loverro G, Nappi L, Mei L *et al*. 2003 Evaluation of functional ovarian reserve in 60 patients. Evaluation of functional ovarian reserve in 60 patients. *Reproductive BioMedicine Online* **7**, 200–204.

Manzi D, Thorton K, Scott L, Nulsen J 1994 The value of increasing the dose of human menopausal gonadotropins in women who initially demonstrate a poor response. *Fertility and Sterility* **62**, 251–256.

Martinez F, Barri PN, Coroleu B *et al*. 2002 Women with poor response to IVF have lowered circulating gonadotrophin surge-attenuating factor (GnSAF) bioactivity during spontaneous and stimulated cycles. *Human Reproduction* **17**, 634–640.

Meredith S, Butcher RL 1985 Role of decreased numbers of follicles on reproductive performance in young and aged rats. *Biology of Reproduction* **32**, 788–794.

Meyer W, Lavy G, DeCherney A *et al*. 1990 Evidence of gonadal and gonadotropin antibodies in women with a suboptimal ovarian response to exogenous gonadotropin. *Obstetrics and Gynecology* **75**, 795–799.

Morgia F, Sbracia M, Schimberni M *et al*. 2004 A controlled trial of natural cycles versus microdose gonadotropin-releasing hormone analog flare cycles in poor responders undergoing in vitro fertilization. *Fertility Sterility* **81**, 1542–1547.

Muasher SJ, Oehninger S, Simonetti S *et al*. 1988 The value of basal and/or stimulated serum gonadotropin levels in prediction of stimulation response and in vitro fertilization outcome. *Fertility and Sterility* **50**, 298–307.

Nargund G, Cheng WC, Parsons J 1995 The impact of ovarian cystectomy on ovarian response to stimulation during in-vitro fertilization cycles. *Human Reproduction* **17**, 1207–1211.

Navot D, Rosenwaks Z, Margalioth EJ 1987 Prognostic assessment of female fecundity. *Lancet* **1**, 645–647.

Nikolaou D, Templeton A 2003 Early ovarian ageing: a hypothesis. *Human Reproduction* **18**, 1137–1139.

Out H, Bernadette M, Driessen S, Bennink H 1996 Recombinant follicle stimulating hormone (rFSH; Puregon) in assisted

reproduction: more oocytes, more pregnancies. Results from five comparative studies. *Human Reproduction Update* **2**, 162–167.
Padilla S, Smith R, Garcia J 1991 The lupron screening test: tailoring the use of leuprolide acetate in ovarian stimulation for in vitro fertilization. *Fertility and Sterility* **56**, 79–83.
Padilla S, Dugan K, Shalika S, Smith R 1996 Use of the flare-up protocol with high dose follicle stimulating hormone and human menopausal gonadotropins for in vitro fertilization in poor responders. *Fertility and Sterility* **65**, 796–799.
Pelinck MJ, Hoek A, Simons AHM *et al*. 2002 Efficacy of natural cycle IVF: a review of the literature. *Human Reproduction Update* **8** 129–139.
Pellicer A, Ballester MJ, Serrano MD *et al*. 1994 Aetiological factors involved in the low response to gonadotrophins in infertile women with normal basal serum follicle stimulating hormone levels. *Human Reproduction* **9**, 806–811.
Pellicer A, Ardiles G, Neuspiller F *et al*. 1998 Evaluation of the ovarian reserve in young low responders with normal basal levels of follicle-stimulating hormone using three-dimensional ultrasonography. *Fertility and Sterility* **70**, 671–675.
Peñarrubia J, Fábregues F, Manau D *et al*. 2004 Initial analysis of variability among basal hormone biomarkers of ovarian reserve. *Reproductive BioMedicine Online* **8**, 191–195.
Penzias AS 2004 Improving results with assisted reproductive technologies: individualized patient-tailored strategies for ovulation induction. *Reproductive BioMedicine Online* **9,** 43–46.
Perez Mayorga M, Gromoll J, Behere HM *et al*. 2000 Ovarian response to to FSH stimulation depends on the FSH receptor genotype. *Journal of Clinical Endocrinology and Metabolism* **85**, 3365–3369.
Peters H 1976 Intrauterine gonadal development. *Fertility and Sterility* **27**, 493–500.
Pinkas H, Orvieto R, Avrech OM *et al*. 2000 Gonadotropin stimulation following GnRH-a priming for poor responders in in vitro fertilization-embryo transfer program. *Gynecological Endocrinology* **14**, 11–14.
Polani PE, Crolla JA 1991 A test of the production line hypothesis of mammalian oogenesis. Human Genetics **88**, 64–70.
Raga F, Bonilla-Musoles F, Casan E *et al*. 1999 Recombinant FSH stimulation in poor responders with normal basal concentration of FSH: improved reproductive outcome. *Human Reproduction* **14**, 1431–1434.
Roberts CG, O'Neill C 1995 Increase in the rate of diploidy with maternal age in unfertilized in-vitro fertilization oocytes. *Human Reproduction* **10**, 2139–2141.
Rombauts L, Suikkari A, MacLachlan V *et al*. 1998 Recruitment of follicles by recombinant human follicle-stimulating hormone commencing in the luteal phase of the ovarian cycle. *Fertility and Sterility* **69**, 665–669.
Rongieres-Bertrand F, Olivennes F, Righini C *et al*. 1999. Revival of the natural cycles in in-vitro fertilization with the use of a new gonadotrophin-releasing hormone antagonist (Cetrorelix): a pilot study with minimal stimulation. *Human Reproduction* **14**, 683–688.
Salat-Baroux J, Rolten D, Alvarez S, Antoine J 1993 Comparison of growth hormone responses to growth hormone-releasing factor and clonidine in women with normal or poor ovarian response to gonadotropin stimulation. *Fertility and Sterility* **60**, 791–799.
Schachter, M, Friedler S, Raziel A *et al*. 2001 Improvement of IVF outcome in poor responders by discontinuation of GnRH analogue during the gonadotropin stimulation phase – a function of improved embryo quality *Journal of Assisted Reproduction and Genetics* **18**, 197–204.
Schoolcraft W, Schlenker T, Gee M *et al*. 1997 Improved controlled ovarian hyperstimulation in poor responder *in vitro* fertilization patients with a microdose follicle-stimulating hormone flare, growth hormone protocol. *Fertility and Sterility* **67**, 93–97.
Scott RT, Navot D 1994 Enhancement of ovarian responsiveness with microdoses of gonadotropin-releasing hormone agonists during ovulation induction for in vitro fertilization. *Fertility and Sterility* **61**, 880–885.
Scott RT, Toner JP, Muasher SJ *et al*. 1989 Follicle-stimulating hormone levels on cycle day 3, are predictive of in vitro fertilization outcome. *Fertility and Sterility* **51**, 651–654.
Scott RT, Hofmann GE, Oehninger S, Muasher SJ 1990 Intercycle variability of day 3 follicle stimulating hormone levels and its effect on stimulation quality in in vitro fertilization and embryo transfer. *Fertility and Sterility* **54**, 297–302.
Scott RT, Leonardi MR, Hofman GE *et al*. 1993 A prospective evaluation of clomiphene citrate challenge test screening of the general infertility population. *Obstetrics and. Gynecology* **82**, 539–544.
Scott RT, Opsahl MS, Leonardi MR *et al*. 1995 Life table analysis of pregnancy rates in a general infertility population relative to ovarian reserve and patient age. *Human Reproduction* **10**, 1706–1710.
Seifer DB, Lambert-Masserlian B, Hogan JW *et al*. 1997 Day 3 serum inhibin-B is predictive of assisted reproductive technologies outcome. *Fertility and Sterility* **67**, 110–114.
Serafini P, Stone B, Kerin J *et al*. 1988 An alternate approach to controlled ovarian hyperstimulation in 'poor responders': pretreatment with a gonadotropin-releasing hormone analog. *Fertility and Sterility* **49**, 90–95.
Shaller A, Fleming R, Jamieson M *et al*. 1992 Absence of effect of adjuvant growth hormone therapy on follicular responses to exogenous gonadotropins in women: normal and poor responders. *Fertility and Sterility* **58**, 919–923.
Shapiro D, Carter M, Mitchell-Leef D, Wininger D 2002 Plateau or drop in estradiol (oestradiol) on the day after initiation of the GnRH antagonist Antagon™ in in vitro fertilization (IVF) treatment cycles does not affect pregnancy outcome. *Fertility and Sterility* **78** (suppl. 1): S22.
Smitz J, Van Den Abbeel E, Bollen N *et al*. 1992 The effect of gonadotrophin-releasing hormone (GnRH) agonist in the follicular phase on in-vitro fertilization outcome in normo-ovulatory women. *Human Reproduction* **7**, 1098–1102.
Smotrich D, Widra E, Gindoff P *et al*. 1995. Prognostic value of day 3 estradiol on in vitro fertilization outcome. *Fertility and Sterility* **64**, 1136–1140.
Suikkari A, MacLachan V, Koistinen R *et al*. 1996 Double-blind placebo controlled study: human biosynthetic growth for assisted reproductive technology. *Fertility and Sterility* **65**, 800–805.
Surrey E, Bower J, Hill D *et al*. 1998 Clinical and endocrine effects of a microdose GnRH agonist flare regimen administered to poor responders who are undergoing in vitro fertilization. *Fertility and Sterility* **69**, 419–424.
Tarin JJ 1995 Aetiology of age-associated aneuploidy: a mechanism based on the 'free radical theory of ageing'. *Human Reproduction* **10**, 1563–1565.
Te Velde ER, Pearson PL 2002 The variability of female reproductive ageing. *Human Reproduction Update* **8**, 141–154.
Toner JP, Philput CB, Jones GS Muasher SJ 1991 Basal follicle-stimulating hormone is a better predictor of *in vitro* performance than age. *Fertility and Sterility* **55**, 784–791.
Toth T, Awwad J, Veeck L *et al*. 1996 Suppression and flare regimens of gonadotropin-releasing hormone agonist: use in women with different basal gonadotropin values in an in vitro fertilization program. *Journal of Reproductive Medicine* **41**, 321–326.
Treloar AE 1981 Menstrual cyclicity and the perimenopause. *Maturitas* **3**, 49–64.
Tulandi T, Sammour A, Valenti D *et al*. 2002 Ovarian reserve after uterine artery embolization for leiomyomata. *Fertility and Sterility* **78**, 197–198.
Ubaldi, F, Rienzi L, Baroni E *et al*. 2004 Natural cycles with minimal stimulation in poor responder patients: cost-effectiveness analysis. *Human Reproduction* **19** (suppl. 1), O-008.
Ulug U, Ben-Shalomo I, Turan E *et al*. 2003 Conception rates following assisted reproduction in poor responder patients: a retrospective study in 300 consecutive cycles. *Reproductive BioMedicine Online* **6**, 439–443.
van Blerkom J, Antczak M, Schrader R 1997 The developmental

potential of the human oocyte is related to the dissolved oxygen content of follicular fluid: association with vascular endothelial growth factor levels and perifollicular blood flow characteristics. *Human Reproduction* **12**, 1047–1055.

van Hooff MH, Alberda AT, Huisman GJ *et al*. 1993 Doubling the human menopausal gonadotrophin dose in the course of an in-vitro fertilization treatment cycle in low responders: a randomized study. *Human Reproduction* **8**, 369–373.

van Noord PA, Dubas JS, Dorland M *et al*. 1997 Age at natural menopause in a population-based screening cohort: the role of menarche, fecundity and lifestyle factors. *Fertility and Sterility* **68**, 95–102.

van Rooij IA, Bancsi LF, Broekmans FJ *et al*. 2003 Women older than 40 years of age and those with elevated follicle-stimulating hormone levels differ in poor response rate and embryo quality in in vitro fertilization. *Fertility and Sterility* **79**, 482–488.

Warburton D 1989 The effect of maternal age on the frequency of trisomy: change in meiosis or *in utero* selection? *Progress in Clinical and Biological Research* **311**, 165–181.

Welt CK, McNicholl DJ, Taylor AE, Hall JE 1999 Female reproductive aging is marked by decreased secretion of dimeric inhibin. *Journal of Clinical Endocrinology and Metabolism* **84**, 105–111.

Yoshimura Y, Nakamura Y, Ando M *et al*. 1992 Direct effect of gonadotropin-releasing hormone agonists on the rabbit ovarian follicle. *Fertility and Sterility* **57**, 1091–1097.

Paper based on contribution presented at the International Serono Symposium 'From the Oocyte to the Embryo: a Pathway to Life' in Stresa, Milan, Italy, September 24–25, 2004.

Received 1 September 2004; refereed 9 September 2004; accepted 5 November 2004.