Revisiting gonadotrophin-releasing hormone agonist protocols and management of poor ovarian responses to gonadotrophins

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Within the past decade, gonadotrophin-releasing hormone (GnRH) agonists have contributed greatly to the success of cycles programmed for in-vitro fertilization and embryo transfer. However, apart from a preventive effect on the luteinizing hormone (LH) surge, most of the beneficial effects of these molecules are still only partly known. A precise analysis of regimens using GnRH agonists for ovarian stimulation shows that many parameters may interfere with the outcome of long-term and short-term protocols. The great variability between these protocols hampers our comprehension of the mechanisms involved in the overall clinical improvement seen with this therapy. The hypophyseal desensitization induced by GnRH agonists is greatly dependent on the dose and duration of their administration, but the residual gonadotrophin secretion is imperfectly estimated by hormonal measurements using radio-immunometric assays. Moreover, the specific role of GnRH agonist-induced ovarian quiescence on subsequent ovarian responsiveness to gonadotrophins and on endometrial receptivity deserves further investigation. Finally, a direct ovarian action of GnRH agonists on steroidogenesis, folliculogenesis and embryo quality is still controversial in humans. These putative deleterious effects of GnRH agonists have led some authors to recommend a reduction of both dose and duration of GnRH agonist administration for women identified by a poor response to gonadotrophins. Using this approach, a few reports have recently shown some clinical advantages for ovarian responsiveness but no convincing evidence for any improvement in pregnancy rate. It thus appears that the overall impact of GnRH agonists on reproductive function is still partly misunderstood.

Key words: GnRH agonist/gonadotrophins/hypophysis/ovary

Introduction

From their first introduction in the management of ovulation induction (Fleming et al., 1982), gonadotrophin-releasing agonists (GnRHa) have greatly contributed to the success of cycles for in-vitro fertilization (IVF) and embryo transfer. By inducing a hypophyseal desensitization, they allow a complete suppression of endogenous luteinizing hormone (LH) surges, fewer occurrences of premature oocyte luteinization and a significant reduction in the subsequent cancellation of cycles previously treated by gonadotrophins (Fleming and Coutts, 1985; Wildt et al., 1986; de Ziegler et al., 1987; MacLachlan et al., 1989; Abdalla et al., 1990). Moreover, they facilitate scheduling and they improve follicular recruitment, numbers of harvested oocytes and total number of embryos obtained (Neveu et al., 1987; Meldrum et al., 1989; Hughes et al., 1992; Liu et al., 1992; Oehninger et al., 1992; Filicori et al., 1996). Finally, the simultaneous increase in implantation rate testifies that benefits of GnRHa are only partly dependent on the improvement of the quantitative ovarian response to gonadotrophins. It thus appears that mechanisms involved in the overall improvement of IVF cycles treated with GnRHa are not limited to a hypophyseal

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step and may possibly include additional action at the ovarian and endometrial levels. Besides these positive effects, it has been simultaneously reported that GnRHα may be responsible for some adverse actions on ovarian function, especially steroidogenesis, leading to higher administration of exogenous gonadotrophins in order to achieve follicular maturation. In addition, the quality of the extra oocytes recruited by protocols including GnRHα may not be optimum. Therefore, some authors have questioned their current use in normo-ovulatory patients (Maroulis et al., 1991; Polson et al., 1991; Kingsland et al., 1992; Harrison et al., 1994), while others have recently suggested that both the dose and duration of GnRHα administration should be reduced, or that certain formulations should be selected, particularly for women whose ovarian response was previously known to be poor.

This recent progress in programming IVF cycles gives us the opportunity to revisit GnRHα protocols: our common reference to so-called short-term and long-term GnRHα protocols cannot really reflect the extreme heterogeneity in the mode of their prescription. Rather than contrasting these two approaches, it seems more relevant to analyse more closely their use, in order to recognize differences between them and to identify their specific benefit in terms of IVF outcome. By this means, we hope to present a new insight into the management of patients with poor ovarian response to gonadotrophins.

**GnRH agonists: structure and hypophyseal action**

**Structure**

From the natural GnRH, numerous superactive agonists have been synthesized with a D-amino acid substitution at position 6 (proteolysis site) and often with an ethylamide group instead of the C-terminal glycaminamide residue (high-affinity binding site), leading to an increased biological activity of such peptides. Among the agonists most commonly used in Europe, triptorelin (Decapeptyl) and nafarelin (Synarel) are the result of only one substitution at position 6 and have a relative potency of 100–200, whereas buserelin (Suprefact) and leuprorelin (Enantone), obtained from two substitutions at positions 6 and 10, have a relative potency of ~50 (Table I). These data require further consideration regarding differences of efficacy reported between analogues in some clinical studies. Several formulations are now available for clinical purposes: short-acting, requiring daily subcutaneous or intranasal administration; long-acting (sustained release formulations) with a 30–40 day effect from the initial injection.

**Hypophyseal action**

Repeated administration of GnRHα in vivo is characterized at the hypophyseal level by a biphasic pattern of gonadotrophin secretion composed of an initial stimulatory phase during the first 48 h (called the ‘flare-up effect’) followed by an inhibitory phase (Sandow et al., 1978). This functional state of hypophyseal desensitization is related to both GnRH receptor reduction (down-regulation) and intracellular uncoupling. It leads to a progressive reduction in gonadotrophin synthesis (decreased pituitary content) which is maintained during GnRHα administration and persists some days after cessation (‘refractory period’ to endogenous GnRH). These different components of GnRHα action are reflected in plasma LH and follicle stimulating hormone (FSH) variations described as follows.

### Table I. Main characteristics of gonadotrophin-releasing hormone (GnRH) agonists

<table>
<thead>
<tr>
<th>Generic name (native)</th>
<th>relative potency</th>
<th>half-life (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂</td>
<td>100</td>
<td>450</td>
</tr>
<tr>
<td>D-Trp</td>
<td>100</td>
<td>450</td>
</tr>
<tr>
<td>D-Nal</td>
<td>200</td>
<td>180</td>
</tr>
<tr>
<td>S-Ser</td>
<td>Ethylamide</td>
<td>50</td>
</tr>
<tr>
<td>(I-Bu)</td>
<td>Ethylamide</td>
<td>50</td>
</tr>
<tr>
<td>D-Leu</td>
<td>Ethylamide</td>
<td>50</td>
</tr>
<tr>
<td>Lucrin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**The initial stimulation phase (‘flare-up period’)**

Within 48 h following the first administration of GnRHa, a sharp increase is observed in plasma FSH and LH concentrations in relation to their release from the two gonadotrophin pools (Hoff et al., 1977). At doses currently used, this gonadotroph flare-up is more pronounced for LH than FSH (Lemay et al., 1983). However, several animal experiments (Navot et al., 1991; Scott et al., 1993a) and clinical reports (Shroick et al., 1985; Deaton et al., 1996) have recently shown that smaller doses of GnRHa may induce equivalent, even more prolonged, gonadotroph flare-up with a larger increase in plasma FSH (Scott et al., 1993a). As a consequence of gonadotroph secretion, gonadal steroid production (i.e. oestradiol and, to a lesser extent, progesterone) is initially stimulated, and some authors have demonstrated the prognostic value of the steroid pattern for subsequent ovarian response to exogenous gonadotrophins (Padilla et al., 1990; Winslow et al., 1991; Hugues et al., 1992).

**The secondary inhibition phase (‘desensitization period’)**

With a progressive decrease in plasma LH concentrations, a second inhibition phase is observed. During this period and as long as GnRHa administration is maintained, the pituitary seems completely refractory to GnRH action, as attested by the disappearance of LH pulsatile secretion and a lack of significant hypophyseal response to exogenous GnRH or oestradiol benzoate administration (Fraser, 1981; Bergquist et al., 1982; Insler et al., 1988; Bider et al., 1989; Caraty et al., 1990; Broekmans et al., 1993). The intensity and duration of hypophyseal desensitization are dose dependent, at least for LH (Bergquist et al., 1979; Oppenheimer et al., 1992; Broekmans et al., 1996). As far as FSH secretion is concerned, the desensitizing effect of GnRHa is less evident, suggesting its relative independence from GnRH control. However, it is well recognized that, during this period of desensitization, regular radioimmunoassays do not really reflect hormonal bioactivity: indeed, after a 3-week desensitization, LH bioactivity is completely suppressed but LH concentrations remain measurable by radioimmunoassay in relation to persistent secretion of non-biologically active hormones (α subunits and/or molecules with modified glycosylation). Moreover, FSH secretion is also modified: some studies have shown that the FSH bioactivity/immunoreactivity ratio tends to increase after GnRHa administration (Huhtaniemi et al., 1988; Matikainen et al., 1992). Conversely, other studies have reported the release of a FSH-deglycosylated form that could even act as an anti-hormone in conjunction with its ability to bind to FSH receptors and to decrease action of exogenous FSH (Dahl et al., 1988).

**The final refractory phase (‘recovery period’)**

After cessation of GnRHa administration, the hypophysis remains in a non-functional state for a period of time necessary for a complete recovery. This refractory period is usually determined by measurements of plasma gonadotrophins concentrations in basal and stimulated conditions. Its duration is dependent on both the dose and the formulation of GnRHa, being several weeks following administration of long-acting triptorelin but only 5–8 days after daily injection of its short-acting form (Barrière et al., 1991; Winslow et al., 1992; Porcu et al., 1994). This period seems to be even shorter when the dose of triptorelin is lower (Broekmans et al., 1996). Recovery of normal plasma concentrations is always slower for LH than for FSH.

It must be stressed that most of these data have been obtained after a long period of desensitization (3–4 weeks as realized in long-term protocols). Is it equivalent when GnRHa administration is shorter, as in short-term protocols? As discussed below, some data suggest that both the duration and mode of GnRHa administration may influence the quality of residual FSH and LH secretion.

Through their hypophyseal action, GnRHa prevent any premature endogenous LH surge and subsequent granulosa cell luteinization during ovarian stimulation for IVF. The question arises as to whether this is enough to explain the overall improvement of IVF prognosis? Is the enhancement of follicular recruitment and numbers of retrieved oocytes or total embryos related to a common mechanism in long- and short-term protocols? Furthermore, GnRHa administration seems to improve the pregnancy rate regardless of the number of transferred embryos. Thus, it seems likely that the preventive effect of GnRHa on the LH surge cannot totally account for their overall beneficial effect on reproductive function, and which mechanisms are involved is still a matter of discussion. In order to address this issue, several approaches designed for the clinical use of GnRHa during ovarian stimulation are examined.

**GnRH agonist protocols**

**Long-term GnRH agonist protocol**

The main aim of this protocol is to achieve a complete suppression of ovarian activity (via GnRHa-induced hypophyseal desensitization) before starting follicular stimulation with exogenous gonadotrophins. It must be stressed that gonadotroph desensitization may be qualitatively checked for its rapidity, its intensity (usually monitored by residual plasma LH and oestradiol concentrations) and its duration. In fact, all these parameters are critically dependent on numerous factors in
the GnRHa protocol: type of molecule, time of its first administration in the cycle, dose and duration of administration and formulation. Moreover, both quantitative and qualitative ovarian responses to exogenous gonadotrophins seem to be closely linked to the quality of this previous gonadotroph desensitization. Therefore, it is essential to analyse carefully the different ways of using these long-term protocols, even if involvement of multiple parameters may preclude any firm conclusion on the specific role of each factor.

**GnRHa molecules**

To determine if any differences between agonists with regard to structure and in-vitro activities on granulosa cells are clinically relevant, comparisons between several GnRHa molecules have been performed in long-term protocols. It may be presumed that both the rapidity and completeness of ovarian suppression are directly related to the biopotency of the compound. In fact, this is not always true: for instance, nafarelin, which has a high intrinsic biological potency, induces a dose-dependent LH and oestriadiol suppression (Monroe et al., 1986) that is lower than that achieved by leuprolide, a weaker in-vitro agent (Dantas et al., 1994). Several authors have observed a negative correlation between the intensity of ovarian suppression and the subsequent efficacy of exogenous gonadotrophins in stimulating follicular development. Following administration of nafarelin, which induces a modest hypothalamic LH suppression, the amount of gonadotrophins required for ovarian stimulation is lower than after leuprolide (Penzias et al., 1992), buserelin (Goldman et al., 1994) or triptorelin (Tanos et al., 1994). Moreover, the suppressive effect of the agonist on FSH secretion must be considered: the more effective FSH inhibitory action of buserelin could account for the lower oestrogenic response to gonadotrophins and the smaller number of mature oocytes collected as compared with other agonists (Balasch et al., 1992; Parinaud et al., 1992; Gianoroli et al., 1994). As far as pregnancy rate is concerned, no significant difference could be demonstrated between the efficiency of leuprolide and buserelin, irrespective of the protocol (Tarlatzis et al., 1994).

Collectively, these data show that each GnRHa results in a specific pattern of pituitary desensitization and follicular growth. However, no clear superiority of any GnRHa molecule has been established so far. As discussed below, a direct GnRHa effect on ovarian function must be also taken into account.

**Time of administration**

The clinical objective of prompt and consistent suppression of ovarian activity has prompted investigation of the best time to start agonist administration: either the early follicular or the mid-luteal phase of the cycle. Among four prospective randomized studies, only two enrolled >100 patients: Ron-El et al. (1990) and Urbancsek and Witthaus (1996) showed that down-regulation is achieved more rapidly when the GnRHa is started in the mid-luteal phase. Contrasting findings were reported by Pellicer et al. (1989a) on a smaller number of patients and by Serafini et al. (1988), who treated ‘poor responders’. This suppressive effect was thought to be even more pronounced with the long-acting formulation of the agonist (Vauthier et al., 1989; Ron-El et al., 1990; Gonen et al., 1991) or with a combination of norethindrone acetate and GnRHa, with a lower incidence of ovarian cyst formation (Ditkoff and Sauer, 1996). However, none of these studies showed any clinical advantage in achieving a prompt and profound desensitization. It is evident that an aberrant oestriadiol flare-up, despite GnRHa induced suppression, is associated with impaired implantation (Penzias et al., 1994). Conversely, a prompt desensitization seems to induce a relative refractory state of the ovary to exogenous gonadotrophins (Goswami et al., 1996) and is associated with either an improved (Seifer et al., 1991) or a reduced fertilization rate (Chang et al., 1993). There is still no clear consequence in terms of pregnancy rate (Ron-El et al., 1991; Ferraretti et al., 1996; Urbancsek and Witthaus, 1996).

**Dose and duration of GnRHa administration**

**During the desensitization phase**

Consequences of a reduction in the GnRHa dose during the desensitization phase have been investigated in few studies using the long-acting form. After injection of 3.75 or 1.88 mg of triptorelin, no difference in cycle outcome was demonstrated (Balasch et al., 1992a; Simon et al., 1994). It may be more informative to check whether the duration of the desensitization phase influences the subsequent ovarian response to gonadotrophins and the IVF outcome. Using leuprolide for 14–115 days prior to ovulation induction, Scott et al. (1993b) did not observe any impact of the duration of hypo-oestrogenic state on ovarian responsiveness to gonadotrophins, nor on IVF success. Apart from the increased cost, Scott et al. (1993b) found no reason to believe that a patient must be stimulated as soon as suppression is achieved. This observation allows great flexibility in scheduling ovulation induction cycles but seems contradictory to other evidence in the literature indicating that implantation rate is higher in amenorrhoeic patients (Edwards et al., 1990), as discussed below. This study warrants confirmation in patients with poor ovarian reserve, especially in older women.
It is usual to administer the same dose of short-acting GnRHa during both the pituitary desensitization and ovarian stimulation phases. By contrast, it has been shown that the dose needed to maintain desensitization gradually decreases with the length of treatment (Sandow and Donnez, 1990). Thus, some authors tried to reduce GnRHa doses or even to stop its administration during exogenous gonadotrophin stimulation in normo-responder patients in order to improve ovarian responsiveness and/or implantation rate; however, the results are contradictory: Smitz et al. (1992a), by stopping buserelin (600 µg/day intranasally) once desensitization was achieved (usually 21 days), did not expose patients to a premature LH surge, but this may have resulted in lower quality supernumerary embryos. Conversely, for Pantos et al. (1994), discontinuation of buserelin administration 10 days after its initiation in the luteal phase (500 µg/day s.c.) increased the pregnancy rate without any risk of premature luteinization. Finally, Simon et al. (1994) reported that it is cost effective to reduce the triptorelin dose from 0.5 to 0.1 mg/day after pituitary desensitization without affecting either ovarian response or clinical outcome of an IVF attempt.

Altogether, these data challenge the concept of a standard protocol. Is there any advantage to calibration of a prescription according to an individual hormonal profile? As discussed below, the potential adverse ovarian effects of GnRHa have recently led some authors to reduce agonist doses in patients classified as poor responders.

**GnRHa formulations**

Although a single injection of a long-acting GnRHa formulation is more convenient for patients than daily administration of a short-acting one, it is essential to check whether long-acting formulations may have some adverse effects during the luteal phase and early pregnancy. Comparing short-acting and depot forms of leuprolide, Tsai et al. (1995) did not observe any difference in IVF outcome. Other studies compared different molecules, particularly short-acting buserelin and long-acting triptorelin: Gianaroli et al. (1994) found that the long-acting form is more convenient and reduces cost and side-effects. Nevertheless, Devreker et al. (1996) recently reported that short-acting formulations should be preferred because both implantation and pregnancy rates are impaired by the long-acting depot forms. They maintain that the GnRHa molecule could interfere with embryo quality, function of the corpus luteum or endometrium receptivity. It should be noted that, in this study, plasma LH values during the late follicular phase were significantly higher in patients receiving daily buserelin administration, without any adverse consequences on embryo quality and implantation rate.

In conclusion, analysis of long-term protocols is hampered by interference of several factors which individually interact with the rapidity, intensity and duration of the desensitization phase (Table II). At present, it is difficult to define the state of ‘ideal’ desensitization that can prevent premature luteinization without interfering with ovarian responsiveness, embryo quality, luteal function and endometrial receptivity. In addition, as measurements of plasma immunoreactive LH cannot reflect LH bioactivity, there are no specific criteria to measure hypophyseal desensitization adequately.

### Short-term GnRH agonist protocol

Specificity of short-term GnRHa protocols depends on almost simultaneous administration of short-acting agonist and exogenous gonadotrophins. Advantage is taken of effect of the initial rise (flare-up) of serum gonadotrophins on follicular recruitment, with a subsequent pituitary desensitization induced by daily agonist administration. Their clinical efficacy has been previously established (Fleming and Coutts, 1986; Barrière et al., 1987; Garcia et al., 1990; Acharya et al., 1992a,b). In clinical practice, short-term protocols are not easily comparable because they may differ on several points: previous progestogen (or contraceptive) administration for cycle programming, GnRHa molecules, respective timing of agonist and

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**Table II. Factors influencing main parameters of desensitization**

<table>
<thead>
<tr>
<th>Parameters of desensitization</th>
<th>Factors</th>
<th>Time of administration</th>
<th>Dose/duration of administration</th>
<th>Formulations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapidity</td>
<td>?</td>
<td>+</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Intensity</td>
<td>+</td>
<td>?</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Duration</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>+</td>
</tr>
<tr>
<td>Subsequent ovarian response</td>
<td>+</td>
<td>?</td>
<td>+/-</td>
<td>?</td>
</tr>
</tbody>
</table>
gonadotrophin administration, and dose and duration of agonist injection. The overall consequences of these apparently minor differences between short-term agonist protocols are considered below.

**Effect of progestogen pre-treatment on gonadotrophin flare-up**

Padilla et al. (1990) demonstrated the prognostic value of FSH, LH and oestradiol responses to the GnRHa action of leuprolide during the early follicular phase when used as an adjuvant to ovarian stimulation in IVF. This ‘Lupron screening test’ allows evaluation of both pituitary sensitivity to GnRH and ovarian responsiveness to endogenous gonadotrophins. These authors described four early oestradiol patterns and showed that a brief but significant elevation followed by a transient fall of plasma oestradiol concentrations may identify most patients who will succeed in IVF. By contrast, a prompt but persistent rise or the absence of significant variation in plasma oestradiol was associated with a poor prognosis in IVF outcome. In this latter case, early exogenous FSH supplementation improved the pregnancy rate (Padilla et al., 1991). Winslow et al. (1991) considered absolute differences between basal and post flare-up oestradiol values (ΔE2) as the best prognostic parameters of the IVF cycle (final hormonal values, number of oocytes and embryos, pregnancy rate). In these studies, leuprolide was used as agonist.

We performed a similar study except that the IVF protocol involved a pretreatment with norethisterone (10 mg/day for 10–20 days) and administration of short-acting triptorelin (0.1 mg/day). We confirmed that serum oestradiol variations (ΔE2) during the flare-up period are closely correlated to most IVF parameters (final oestradiol value, number of retrieved oocytes, pregnancy rate) (Hugues et al., 1992). However, cycles pretreated with progestogen were characterized by: (i) a reduced gonadotrophin flare-up. Consequently, the threshold predictive of IVF success was lower than the previously reported 2-fold increase over the baseline oestradiol concentration (Cédrin-Durnerin et al., 1995): (ii) no increase in plasma progesterone values, considered by others as deleterious for oocyte maturity when observed in early follicular phase (Cédrin-Durnerin et al., 1996b). Thus corpus luteum rescue induced by GnRHa (Castracane et al., 1996) is totally prevented by a progestogen pretreatment; (iii) an identical pregnancy rate compared with cycles without any pretreatment.

Besides their interest for early identification of poor prognosis patterns, these studies have given rise to some major but still controversial issues, e.g. why does an initial biphasic oestradiol pattern result in a higher ovarian response by the time of human chorionic gonadotrophin (HCG) injection? It may be speculated that this early oestradiol drop before initiation of ovulation induction allows for granulosa cell FSH receptors to become more available and functional (Padilla et al., 1990). Is there any definitive proof that an increase in plasma progesterone during the early follicular phase adversely affects follicular development, oocyte maturity or the success rate of IVF as previously reported (Antoine et al., 1988; Brzyski et al., 1988; Loumaye et al., 1989a)? As suggested by Sims et al. (1994), it may rather indicate a progesterone threshold beyond which an impairment in follicular development may be observed. From a clinical point of view, such a risk may be easily prevented by a progestogen pre-treatment.

**Effect of duration in GnRH analogue administration**

In addition to the protocol first described, in which GnRHa administration was maintained up to the time of HCG injection, two simplified regimens were proposed: (i) a 3-day administration (‘ultra-short protocol’). Initially reported by Howles et al. (1987) and Macnamee et al. (1989), this abbreviated schedule still allows reduction of endogenous tonic LH secretion during the latter stages of follicular development. It must be stressed that mean plasma LH concentrations measured in the late follicular phase of an ultra-short protocol are even lower than those observed in the same period with a long-term protocol (Ron-El et al., 1992). However, this potentially cost-reducing protocol does not totally prevent the occurrence of an endogenous LH surge (Smitz et al., 1990; Acharya et al., 1992a,b). This observation may be explained by the relative shortness of the refractory period when the GnRHa is administered for only 3 days; (ii) a 7-day administration (‘mini-short protocol’). A 7-day course of GnRHa seems sufficient to provide a reliable protection against the risk of premature plasma LH elevation and yet yield similar overall IVF results as the long-term GnRHa protocol with a reduced amount of exogenous gonadotrophins (Hazout et al., 1993).

In a recent prospective study comparing the effectiveness of a 7 and a 14 day administration of GnRHa, we observed that stopping injection of triptorelin on day 7 leads to a sudden and dramatic decrease of plasma LH combined with a plasma oestradiol stagnation and an increased requirement for gonadotrophins (Cédrin-Durnerin et al., 1996a). These data, in agreement with those of Sungurtekin and Jansen (1995), would suggest a state of incomplete hypothalamic desensitization after a 7 day period of GnRHa administration, with a relative LH release following daily agonist administration. By using highly specific immunometric assays for LH α and β subunits and for dimeric LH, we observed a progressive decrease in LH β subunits within the 7 days of the agonist administration which persists...
during the whole follicular phase, regardless of the duration of GnRHa administration. By contrast, LH α subunit secretion seems to be dependent on duration of treatment, with a dramatic decrease concomitant with cessation of agonist administration that is in contrast with a constant synthesis when GnRHa administration is maintained (Hugues et al., 1997). Such persistence of LH α subunit secretion under GnRHa treatment was previously reported during long-term administration of triptorelin for precocious puberty (Lahlou et al., 1987). Moreover, as shown by Oppenheimer et al. (1992), each daily GnRHa administration can lead to a partial release of LH α subunit. Thus, in this early phase of hypothalamic desensitization, the limiting step of dimeric LH secretion could be the synthesis of LH α subunit. However, such an observation cannot account for the lower amount of exogenous gonadotrophins needed to sustain follicular development when GnRHa is maintained, because LH α subunits are usually considered as biologically inactive, with no effect on progesterone secretion (Lincoln et al., 1995). Is this effect related to modifications in hypothalamic FSH secretion? Immunoassay plasma FSH concentrations were not influenced by duration of treatment. Nevertheless, unlike LH (Meldrum et al., 1984), FSH bioactivity does not decrease during GnRHa administration (Matikainen et al., 1992) and a daily administration of 500 µg of buserelin induces a significant release of bio-FSH without any change in immunometric FSH activity (Huhtaniemi et al., 1988). It may therefore be speculated that the apparent beneficial effect of maintaining GnRHa administration for 14 days is related to the persistence of bio-FSH secretion. An additional study is in progress to test this hypothesis and to improve our knowledge of the structure and bioactivity of gonadotrophins secreted during the refractory period. From a practical point of view, these data lead us to maintain GnRHa administration up to the time of HCG administration in short-term protocols, especially for patients known to be poor responders to exogenous gonadotrophins.

Timing of administration of GnRH analogue and gonadotrophins

In short-term protocols, the starting day of GnRHa administration is usually the first or third day of the cycle. Smitz et al. (1990) and Benadiva et al. (1990) obtained better results when the GnRHa supply was started on day 3 of the cycle and attributed any rise of progesterone plasma concentration to an earlier administration of the agonist with a subsequent rescue of the corpus luteum. Similarly, Ron-El et al. (1992) found that high doses of exogenous gonadotrophins administered concomitantly with GnRHa may be responsible for progesterone secretion, which has been related to a potential deleterious effect on oocyte quality (Antoine et al., 1988; Bryszki et al., 1988; Loumaye et al., 1989a,b; Sims et al., 1994). Nevertheless, as far as is known, no correlation has been demonstrated between increasing plasma progesterone concentrations during the early follicular phase and subsequent reduction in pregnancy rate (Smitz et al., 1990; Cédric-Durnerin et al., 1996b).

Collectively, these data show the extreme and largely underestimated heterogeneity of protocols using GnRHa for ovarian stimulation. Recognizing these differences is the first step towards a better understanding of the success and failure of these regimens, which have been generally analysed under the general heading of long- and short-term protocols. Many aspects of the overall improvement in IVF outcome achieved by using GnRHa are still a matter of debate: short- and long-term effects of hypothalamic desensitization, consequences of gonadotroph flare-up or hypothalamic quiescence on ovarian function etc.

How do GnRH agonist protocols improve the outcome of IVF cycles?

Hypothalamic effects

It is commonly recognized that the main beneficial effect of GnRHa is to prevent any pre-ovulatory LH surge during ovarian stimulation. Nevertheless, despite pituitary desensitization, a small elevation in serum progesterone can occur at the end of the follicular phase in up to 20% of stimulated cycles. Considerable attention has been given to progesterone concentrations at the time of HCG administration after a report that pregnancy rate may be adversely affected (Schoolcraft et al., 1991). Some (Edelstein et al., 1990) have described a critical threshold progesterone concentration of 0.9 ng/ml on the day of HCG, above which a negative impact on pregnancy rate is seen (Silverberg et al., 1991; Fanchin et al., 1993). This subtle premature rise in serum progesterone was thought to impair endometrial receptivity rather than oocyte quality (Legro et al., 1993; Silverberg et al., 1994; Fanchin et al., 1996; Shulman et al., 1996). In support of this possibility is the recent report that premature progesterone elevation does not affect blastulation (Fanchin et al., 1997b), and recommendation has been made to cryopreserve embryos for a subsequent transfer. However, several other studies have not found a relationship between late follicular progesterone concentrations and IVF outcome (Edelstein et al., 1990; Givens et al., 1994; Abuzeid and Sasy, 1996; Hofmann et al., 1996). The mechanisms that account for the premature elevation of progesterone by GnRHa, despite suppressed endogenous gonadotrophins, are still
subsequent ovarian response to gonadotrophins is unlikely because LH concentrations are invariably low and the incidence of premature progesterone rise has been found to be identical in women receiving long and ultra-short GnRHa protocols (Fanchin et al., 1993; Hazout et al., 1993). Another theory proposes that progesterone elevation could result from exposure to large amounts of exogenous gonadotrophins (Fanchin et al., 1995). An increased FSH-induced LH receptivity would be more to be involved in this process than the presence of HCG in exogenous gonadotrophin preparations (Ubaldi et al., 1996). Another possibility is to examine the contribution of the adrenal gland to both progesterone and androgen production. These hormonal secretions may be reduced by simultaneous dexamethasone administration, but improvement of the subsequent IVF outcome by this treatment needs further confirmation (Eldar-Geva et al., 1997; Fanchin et al., 1997a). At present, using serum progesterone concentration thresholds on the day of HCG administration as a means of making a clinical decision regarding cancellation of fresh transfer and the cryopreservation of all embryos for future transfer must be questioned (Moffitt et al., 1997).

Role of ovarian quiescence

The role of ovarian quiescence may be considered when ovarian stimulation is achieved with a long-term GnRHa protocol or when a short-term one is programmed by oestro-progestogen or progestogen administration. The subsequent hypo-oestrogenic state may have some influence on both ovarian sensitivity to gonadotrophins and endometrium receptivity.

Effects of an induced period of agonadal state and subsequent ovarian response to gonadotrophins

Long before the first introduction of GnRHa, Jones et al. (1969) reported that ovarian quiescence induced by oestro-progestogens reduces the variability of the ovarian response to gonadotrophins by a synchronizing effect on the follicular cohort. However, in this situation, a larger amount of gonadotrophin is needed (Lewinthal et al., 1988). These reports fully agree with observations on the consequences of long-term GnRHa protocols which usually lead to the recruitment of a more homogeneous cohort of follicles with a lower sensitivity to exogenous gonadotrophins (Fleming et al., 1985). In another study, Schwartz et al. (1980) also underlined the high effectiveness of ovarian stimulation using exogenous gonadotrophins in patients with hypogonadotrophic hypogonadism, whose pregnancy rate was significantly higher than in normogonadotrophic women. For these authors, such a difference could be related to the natural agonadal state of these women prior to ovarian stimulation. Collectively, these data suggest that a spontaneous or induced state of hypogonadism contributes simultaneously to an increase in homogeneity of follicular recruitment and to a reduction in ovarian sensitivity to gonadotrophins (Filicori et al., 1994). It is easily understood that gonadotrophin suppression, which maintains the ovary in a quiescent state by controlling gonadotrophic-dependent follicular growth, could lead to a greater homogeneity of the cohort that will subsequently be recruited at the time of stimulation. In contrast, it is more difficult to explain the lower sensitivity to gonadotrophins. Indeed, Scheele et al. (1993) have shown, in an elegant study, that the follicular FSH threshold is unaffected by GnRHa administration. It cannot be ruled out that the low residual LH bioactivity induced by a long-term GnRHa administration may deprive granulosa cells of their androgenic substrate, in accordance with the ‘two cell, two gonadotrophin’ model (Hilgier et al., 1994; Hilgier, 1996). Finally, the involvement of paracrine ovarian factors or modifications of ovarian receptors to gonadotrophins may also contribute to the reduction of ovarian responsiveness.

Endometrial effect

As discussed by Edwards (1995), women in a state of amenorrhoea seem to display an enhanced fertility; this group includes acyclic and agonadal women <49 years of age who were given oocyte donation and hormone replacement therapy, cyclic women with endometriosis aged <40 years who become highly fertile after 4 months of pituitary down-regulation and women in their late teenage years who have just begun to have complete menstrual cycles after their adolescent period. In these situations, a significantly higher rate of implantation has been reported, but the reasons for this enhanced fertility in amenorrhoeic women are still partially unknown. One hypothesis is that steroid-sensitive systems in the uterus, such as progesterone-dependent pinopodes, have been impaired during many years of regular cycles and require some time to recover from this constant stimulus (Edwards, 1995). Conversely, it has been shown in some reports (Forman et al., 1988; Pellicer et al., 1989b; 1996; Simon et al., 1995a) that high serum oestradiol concentrations following HCG administration are detrimental to uterine receptivity. Nevertheless, this assumption has been challenged by others (Chenette et al., 1990; Toner et al., 1991) and the steroid threshold concentrations required to achieve implantation are still a matter of debate (Younis et al., 1994; de Ziegler, 1995).
Collectively, these data support the concept that a transient state of hypophysial desensitization induced by GnRHa improves IVF cycle success through a beneficial effect on both ovarian folliculogenesis and endometrial receptivity.

**Ovarian direct effects of GnRH agonists**

There is still controversial information regarding a possible extra-pituitary action of GnRH and its agonists. In hypophysectomized animals, direct negative effects on ovarian function have been reported. In humans, there is evidence for GnRH binding sites within the follicle (Latouche et al., 1989) and the corpus luteum (Bramley et al., 1986). Their specific effects on steroidogenesis, folliculogenesis and oocyte quality are considered below.

**Effects on steroidogenesis.**

In rodents, a direct inhibitory effect of GnRH on FSH-induced steroidogenesis was demonstrated in cultured granulosa cells (Hsueh and Erickson, 1979) and specific high affinity GnRH receptors in ovarian tissue were also reported (Clayton et al., 1979). More recently it has been shown that GnRH receptor expression is developmentally regulated, with an inverse relationship between gene expression and granulosa cell differentiation measured by LH receptor mRNA levels (Whitelaw et al., 1995), suggesting that GnRH is involved in the process of apoptosis and follicular atresia (Billig et al., 1994).

In humans, evidence for a direct ovarian effect of GnRH and its agonists is less clear because the methodological approach is often restricted to in-vitro cultures of luteinized granulosa cells obtained from IVF programmes. However, this question requires some consideration; indeed, both characterization of specific binding sites and purification of a GnRH-like protein in human ovaries attest to the existence of a complete ligand–receptor system for GnRH. Moreover, detectable concentrations of bioactive peptide may be measured within follicular fluid after nasal buserelin administration (Latouche et al., 1989b), although it was not observed 48 h after leuprolide injection (Dodson et al., 1988).

In in-vitro studies, contradictory effects of GnRHa on steroid production have been shown. As for progesterone synthesis in vitro, some investigators have found an inhibitory effect (Tureck et al., 1982), while others have reported no effect (Casper et al., 1984; Dodson et al., 1988) or a stimulatory effect only at low concentrations of the agonist (Parinaud et al., 1988; Guerrero et al., 1993). Discrepancies between these studies may be related to differences in the maturity of collected granulosa cells and in GnRHa concentrations used in vitro. Regarding oestradiol synthesis, similar divergent effects of GnRHa have been published and this could be related to the nature of GnRHa molecules. Indeed, Bussenot et al. (1993) reported that oestradiol production would be increased only in the presence of the two Gly6 and Gly10-substituted agonists (buserelin and leuprolide, whereas molecules substituted only for Gly6 (triptorelin) had no effect. This observation suggests that modification in position 10 of the native molecule may be involved in its ovarian bioactivity and/or its affinity for granulosa GnRH receptors. However, any stimulating effect of leuprolide was not confirmed in other reports (Guerrero et al., 1993; Fabbri et al., 1996).

Clinical studies have dealt with measurements of follicular fluid and steroid concentrations within the follicular fluid and steroid production by granulosa cells exposed in vivo to GnRHa during ovarian stimulation. Results are also contradictory. Follicular fluid oestradiol concentrations seem to decrease in leuprolide-treated patients (Brysztaki et al., 1990) whereas, using the same GnRHa, Pellicer and Miro (1990) observed an increase in in-vitro oestradiol production. The only clinical study comparing three agonists has shown a weaker oestradiol production following buserelin administration (Parinaud et al., 1992), attesting to its potential antigonadotrophic effect on small follicles as previously shown in rats (Reddy et al., 1980) and monkeys (Gougeon et al., 1992). As far as progesterone production is concerned, results were diverse: normal (Stone et al., 1988), increased (Hartshorne, 1989) or decreased in relation to a conversion to the inactive 20-OH derivative (Pellicer and Miro, 1990) or to a reduced stimulating effect of FSH on LH receptor synthesis (Maruo et al., 1985). From these data, it is not possible to demonstrate clearly any conclusive effects of GnRHa on human granulosa cell steroidogenesis.

Effects of GnRHa on luteal cell steroid production are inhibitory: their luteolytic action (Shriock et al., 1985; Smitz et al., 1992b) seems to be secondary to abolition of gonadotrophin pulsatile secretion and/or to a direct negative effect upon steroid synthesis (Pellicer and Miro, 1990). These conclusions justify systematically supplementing the luteal phase in protocols with GnRHa (Bourgain et al., 1994).

**Effects on folliculogenesis**

In monkeys, follicle quality appears to be altered by the introduction of GnRHa in ovarian stimulation protocols: fewer follicles with normally dissociated granulosa walls and a larger number of follicles in late atresia have been reported when using short-term protocols. However, this could be either a consequence of the gonadotrophin flare-up or related to a direct effect of the GnRHa (Lefèvre et al.,
1991). To our knowledge, such effects have not been proved in humans. For Brzyski et al. (1990), significant lower oestradiol concentrations in follicles from leuprolide-treated patients, in the presence of metaphase II oocytes, would reflect an adverse effect of the agonist on the maturity of the cumulus–oocyte complex. However, a similar asynchrony was also observed in protocols without GnRHα (Hammit et al., 1993). It is thus unclear whether the follicular environment is modified by GnRHα exposure.

Effects on oocyte and embryo quality

Most studies have used animal species (rat, rabbit, monkey), for which it has been demonstrated that GnRH and GnRHα induce complete oocyte maturation and meiosis resumption in follicle-enclosed oocytes (Hillensjo and Le Maire, 1980; Erikson et al., 1983; Yoshimura et al., 1992) or in isolated oocytes (Lefevre et al., 1988). Although receptors for GnRH were found in oocytes (Dekel et al., 1988), mechanisms by which GnRHα induces oocyte maturation have yet to be elucidated because of an apparently associated increasing degeneration (Yoshimura et al., 1991). In humans, some clinical studies have reported a higher incidence of immature (Ron-El et al., 1991; Wojcik et al., 1995) or fractured zona oocytes (Testart et al., 1989a; Cordeiro et al., 1993). More recently, a higher incidence of diploid oocytes and prematurely condensed sperm chromosomes was observed in GnRHα treated patients, indicating impairment of the nuclear and cytoplasmic maturation processes in some oocytes (Racowsky et al., 1997). However, these reports were unable to separate a specific deleterious effect of GnRHα from the negative consequences of the larger cohort of retrieved oocytes, the high doses of gonadotrophins administered in these protocols, or from the aetiology of infertility (Selva et al., 1991). Nevertheless, one of the major advances with the use of GnRHα is undoubtedly the ability to postpone HCG administration with less concern for development of a premature LH surge, permitting the majority of oocytes to achieve greater maturity before aspiration (Hammit et al., 1993).

Finally, Plachot et al. (1988) reported in a multicentric study that the rate of triploid human embryos observed after IVF was significantly higher when GnRHα were included in the treatment protocol, with a trend for more chromosomally abnormal oocytes. Furthermore, accelerated development of embryos produced in GnRHα treated cycles was also reported (Keenan et al., 1991). These data could account for the lower implantation rate of embryos obtained from IVF programmes incorporating GnRHα (Testart et al., 1989b; 1993).

Collectively, these data are still contradictory, at least in humans. Differences between in-vitro experiments preclude any significant comparison, and the absence of an in-vivo model to separate any specific effect of GnRHα administration from the role of gonadotrophins does not allow any firm conclusions to be made. In any case, these putative effects of GnRHα on ovarian function cannot account for their beneficial action on IVF outcome.

Endometrial direct effects of GnRH agonists

As no GnRH binding sites have been demonstrated at the endometrial level, direct effects are unlikely. Nevertheless, comparison of implantation rates between different protocols have led some authors to believe that the beneficial effects of GnRHα are related to an improved endometrial receptivity (Testart et al., 1989b; 1993; Rutherford et al., 1988). However, this positive effect of agonists is not constant (Hassiakos et al., 1990; Remohi et al., 1994), and a direct action of GnRHα on endometrium remains speculative. We must also consider that, as LH/HCG receptors have been recently recognized at the endometrial level (Reshef et al., 1990; Han et al., 1996; Toth et al., 1996), another way for GnRHα to influence uterine receptivity might be through the reduction of gonadotrophin synthesis, with subsequent consequences on endometrial LH receptor function. This hypothesis deserves further investigation.

It seems that the mechanisms involved in the overall improvement of IVF outcome mediated by GnRHα are largely unknown. Apart from the compelling evidence for prevention of any pre-ovulatory LH surge, most of the other consequences of agonist administration are still being discussed: the residual secretion of endogenous bioactive gonadotrophins following hypophyseal desensitization differs according to the schedule of GnRHα administration; the reduced ovarian sensitivity to exogenous gonadotrophins is poorly understood but is dependent on the degree of hypophyseal desensitization; the putative deleterious direct effects of the agonist on ovarian steroidogenesis and folliculogenesis have not been proven in humans; modifications of oocyte and embryo quality in IVF cycles with agonist treatment are not conclusive. Nevertheless, these potential negative effects of GnRHα have led some authors to reconsider their prescription in women whose ovarian response to exogenous gonadotrophins was poor. Is there any clinical evidence to recommend suppression or reduction of GnRHα doses in this particular group of patients?

How to manage poor ovarian response to gonadotrophins?

Before addressing this question, it must be noted that both the definition and pathophysiology of what is commonly
called ‘a poor response’ to gonadotrophins are still controversial. Identification of these patients is usually based on results of a previous IVF stimulation and the presence of one of the following characteristics: three or fewer recruited dominant follicles or collected oocytes; serum oestradiol concentrations lower than 300 or 500 pg/ml at the time of HCG administration; In fact, these criteria are not universally accepted, and some authors include patients whose cycle was cancelled because of a spontaneous LH surge or who require a large dose of gonadotrophins.

Is there any common feature in poor responders to gonadotrophins? In some of them, an incipient ovarian failure can be predicted by hormonal markers in basal and stimulated conditions; indeed, ovarian reserve and prediction of ovarian stimulation response have been, so far, evaluated by a single basal FSH determination during the early follicular phase (Muasher et al., 1988; Scott et al., 1989, 1990; Hansen et al., 1996; Magarelli et al., 1996; Kim et al., 1997) or by dynamic challenge tests: clomiphene citrate (Scott et al., 1995; Scott and Hofmann, 1995) or GnRH agonist stimulation test (Galtier-Dereure et al., 1996; Yamashita et al., 1996). Moreover, the prognostic value of day 3 plasma oestradiol determination has been reported (Licciardi et al., 1995; Smotrich et al., 1995). Finally, some authors have shown that ovarian volume, determined by transvaginal ultrasonography, may be clinically useful in the prediction of response to ovulation induction (Syrop et al., 1995; Lass et al., 1997; Tomas et al., 1997). In other women with apparently normal ovarian function, an alteration of the somatotrophic axis has been suggested (Giudice, 1992; Salobir et al., 1996). However, in poor responders, growth hormone-releasing hormone or growth hormone supplementation significantly improved ovarian stimulation in spite of a significant increase in plasma insulin-like growth factor-I concentrations, showing that the somatotrophic axis plays only a permissive role in ovarian function (Hugues et al., 1991; Salat-Baroux et al., 1993; Homburg and Ostergaard, 1995). It is thus likely that alterations in intra-ovarian factors or gonadotrophin receptor regulation are involved in some poor responder patients.

The large heterogeneity within the group of poor responders easily explains our current difficulties in managing ovarian stimulation in this group, and emphasizes that every new therapeutic approach must be analysed carefully, with special attention given to the criteria of patient inclusion. Keeping in mind these restrictions, let us examine the recent reports on adaptations of GnRHa protocols in this situation.

In the late 1980s, introduction of GnRHa in ovarian stimulation protocols clearly improved prognosis of human menopausal gonadotrophin-treated cycles whose cancellation rate was high in cases of an endogenous LH surge or a premature luteinization. In this particular group of patients, protocols with GnRHa were associated with an improved rate of follicular recruitment and a higher number of collected oocytes and pregnancies (Awadallah et al., 1987; Friedman et al., 1988; Palermo et al., 1988; Serafini et al., 1988; MacLachlan et al., 1989; Salat-Baroux et al., 1988; Antoine et al., 1990). However, it is still unclear whether this improvement is related only to the prevention of the endogenous LH surge or to other factors. Moreover, these conclusions must be tempered by other reports that FSH plasma concentrations are reduced by GnRHa administration (Ben-Rafael et al., 1991) and that the overall pregnancy rate may not be improved (Droesch et al., 1989; Sathanandan et al., 1989; Van Kasteren et al., 1995). Nowadays, ovarian stimulation with GnRHa in association with gonadotrophins is currently used for IVF; and a subgroup of patients has been identified as poor responders to these protocols. The two kinds of adaptation from current protocols that have been proposed are discussed below.

**Increment of gonadotrophin doses**

An increase of the doses of exogenous gonadotrophins is currently performed in this situation but is not supported by any clear pathophysiological approach. Furthermore, its beneficial effect may be reduced by inducing a receptor down-regulation, as previously observed with HCG administration. In fact, an incremental increase in gonadotrophin supply may be responsible for various effects, depending on ovarian sensitivity.

**In patients with a normal response to gonadotrophins alone**

A slight increase in dose (150–225 IU/day) to improve ovarian recruitment exposes the patient to the risk of lower oocyte quality (Ben Rafael et al., 1987) in relation to the hormonal modifications (Ben Rafael et al., 1986), and to an inadequate luteal phase or altered endometrial receptivity (Edwards et al., 1980). In this group of patients, the introduction of GnRHa has produced different effects: a slight reduction in the number of ampoules needed to achieve follicular maturation, which is secondary to the hormonal flare-up induced by a short-term protocol (Luxman et al., 1995). Conversely, a higher consumption of exogenous gonadotrophins after pituitary desensitization has been induced by a long-term protocol (Lewinthal et al., 1988), but the consequences for IVF outcome are still unclear. Stadtmayer et al. (1994) found that the pregnancy rate was inversely correlated with the number of ampoules
administered, while others did not find any detrimental effect (Simon et al., 1995b). Thus, at present, there is no proven advantage to increasing the usually recommended supply of gonadotrophins for normo-responder patients. In poor-responder women

It has been established that a greater amount of gonadotrophins may only allow a slight improvement in serum oestradiol concentrations (Jenkins et al., 1991; Manzi et al., 1994) or number of oocytes (Land et al., 1996), but does not increase the pregnancy rate because the fertilization rate is unchanged or even reduced (Benadiva et al., 1988; Karande et al., 1990; Hershlag et al., 1990; Van Hoof et al., 1993). It is thus clear that the problem of poor response cannot simply be overcome by increasing the dose of gonadotrophins. This is not surprising if we consider that only 1% of receptor occupancy by a substrate is usually enough to achieve a significant biological cell response. It remains to be shown whether alterations in gonadotrophin receptivity and/or in intra-cellular machinery can account for ovarian hypo-responsiveness to exogenous stimulation.

**Adaptation of GnRH agonist protocols**

As GnRHa itself may be involved in the poor response to gonadotrophins through its potential deleterious effects on reproductive function, it has been claimed by some authors (Davis and Rosenwaks, 1993) that the dose of agonist must be calibrated in order to obtain a significant flare-up effect (only in short-term protocol) and to prevent an endogenous LH surge. Although there are no studies that define the range of dosages that may be successfully used for flare-up stimulations, it was shown in baboons that doses which are equivalent to 1, 5 and 10 µg in humans induce a significant flare-up, with a rise in FSH which is greater in magnitude and lasts longer than that for LH (Scott et al., 1993a). Moreover, it was reported that the doses of GnRHa required to maintain pituitary suppression decrease with the length of treatment (Sandow and Donnez, 1990). It thus seemed logical to evaluate the effects of a reduction in doses and duration of GnRHa administration in poor-responder patients.

**Adaptation of GnRHa dosage in long-term protocol**

To address this issue, clinical investigations were recently performed in patients whose poor ovarian responsiveness to gonadotrophins was not similarly defined. A reduction in GnRHa dosage at the end of the desensitization period was proposed for women with a high basal FSH (Ben Rafael et al., 1993; Feldberg et al., 1994). A significant improvement in the follicular and hormonal ovarian response was observed following administration of low doses of short-acting triptorelin (starting with 0.1 mg and reduced to 0.05 mg/day) as compared with higher doses of short-acting or with long-acting formulations. A lower amount of gonadotrophins was needed in a shorter period to achieve follicular development but the pregnancy rate was not improved. A similar approach was investigated by Olivennes et al. (1996) with the use of low doses of leuprolide (0.5-0.25 mg/day) for patients whose basal FSH concentrations were above the normal range and who were not pregnant following a previous IVF attempt with a long-acting triptorelin protocol. A significant improvement in the hormonal response was only observed on day 8 of the stimulation, but oestradiol values at the time of HCG administration (>2000 pg/ml) in both groups testify that selected patients could not really be considered as poor responders. Finally, Muasher et al. (1996) recently evaluated the consequences of discontinuing leuprolide administration (0.5 mg/day started on day 21) with onset of menses when high dosage gonadotrophin stimulation was initiated. They selected patients with elevated FSH or oestradiol values at day 3 of a spontaneous cycle and/or a history of poor response (<5 oocytes) in a previous IVF cycle with a current long-term protocol. Despite a still high rate of cancellation, they observed a significant improvement in follicular recruitment and a good implantation rate. It must be noted that only one patient triggered a premature LH surge, despite cessation of leuprolide for a mean of 13 days prior to HCG injection.

Although this new approach of GnRHa protocols was not applied to patients selected on similar criteria, there was a trend for an overall improvement in ovarian response with the use of short-acting rather than depot forms and lower doses for a shorter period of time. The results require confirmation in poor responders whose day 3 FSH concentrations are normal.

**Adaptation of GnRHa dosage in short-term protocol**

A similar approach was proposed for women undergoing an IVF cycle with a short-term GnRHa protocol. To our knowledge, few studies have really evaluated the beneficial effects of a reduction in GnRHa dosage on IVF outcome. Scott and Navot (1994) studied women with a poor responsiveness defined as a weak follicular development in a previous cycle using a long-term protocol and with normal basal FSH values. Following administration of oral contraceptives, a flare-up was induced by leuprolide 20 µg twice daily and a similar dose of gonadotrophins was used. Their data demonstrated that micro-doses of GnRHa triggered the release of significant amounts of endogenous gonadotrophins and were still adequate to suppress prema-
ture LH surge. Furthermore, this protocol resulted in the development of more follicles and higher peak oestradiol concentrations, as well as the recovery of more mature oocytes and the transfer of a greater number of embryos. Similar conclusions were more recently reported by Schoolcraft et al. (1997). Although these results indicate that a flare-up protocol using micro-doses of GnRHa may be of value in poor responders, dose–range studies are still required to define the magnitude of the gonadotrophin release and the rate of desensitization and their impact on clinically oriented endpoints. Indeed, the nature of the experimental design in both studies precludes comparison of pregnancy rates.

Two other studies evaluated the benefits of high doses of gonadotrophins in poor responders with a lack of flare-up oestradiol response (Padilla et al., 1996) or with normal FSH concentrations at the onset of stimulation (Karande et al., 1997). In these reports, current doses of GnRHa were used and better pregnancy rates were obtained when gonadotrophins were administered in a step-down fashion (Padilla et al., 1996). These results emphasize that the regimen of gonadotrophin administration must be taken into account in the evaluation of new protocols and that, in these poor responders, milder forms of ovarian stimulation could be applied in ‘tailor-made’ modes to improve qualitatively follicular development (Edwards et al., 1996; Edwards et al., 1997).

Collectively, these data do not allow adequate prediction of how to optimize GnRHa protocols in patients with a previous poor ovarian responsiveness. However, they provide a new insight into the potential deleterious effects of large doses of GnRHa in some, but not all, women. The consequences of such a GnRHa dosage reduction on qualitative hypophysal secretion during the flare-up period, as well as during the desensitization and the recovery periods, remains to be determined.

Conclusions

The addition of GnRH agonists to ovarian stimulation regimens for IVF has several recognized advantages, including reduced cancellation rates, increased ease of scheduling and fewer cases of premature luteinization. Although the increased number of oocytes obtained per retrieval generally results in an increase in the number of embryos available for transfer, oocyte quality may not be optimum. Mechanisms involved in the several sites of action of GnRHa on reproductive function are still partly unknown. Consequences of the induced hypophysal desensitization are quite different from one protocol to another, and evaluation of the residual gonadotrophin secretion under this therapy remains critical. This may probably explain the emergence of poor responses to gonadotrophins with protocols including GnRHa. The recent trend for a reduction in both dose and duration of GnRHa administration also reflects the fear that these compounds may adversely affect oocyte quality and endometrial receptivity. Although some studies have reported a slight improvement in ovarian response, further investigations are needed to evaluate the effects of the GnRHa reduction on pregnancy rate. Introduction of GnRH antagonists in the near future will probably be helpful in the recognition of the specific effects of the agonists on reproductive function.

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