Introduction
Need for down-regulation
During the early years of IVF, it became apparent that premature LH surge could complicate ovarian stimulation and decrease pregnancy rates (Loumaye, 1990). Gonadotrophin-releasing hormone (GnRH) antagonists could solve this problem by immediately suppressing endogenous LH (Karten and Rivier, 1986). The history of ovarian stimulation might have been written differently if antagonists did not also cause histaminic reactions (Kiesel and Runenbaum, 1992). This impeded their use in assisted reproductive technologies for almost a decade.

GnRH agonists in ovarian stimulation for IVF
It was GnRH agonists (Porter et al., 1984) that, in the mid-1980s, formed what is perceived today as the gold standard of performing ovarian stimulation: administering medication to avoid premature LH surge approximately 3 weeks before such an event is possible.

Pituitary down-regulation using GnRH agonists decreased the proportion of cycle cancellation due to premature LH surge from approximately 20% to 2% (Loumaye, 1990) and led to a significant improvement in IVF outcome (Hughes et al., 1992).

Agonist usage was accompanied by occurrence of ovarian cysts, oestrogen deprivation symptoms, increased consumption of gonadotrophins and lack of immediate pituitary responsiveness following agonist discontinuation (Smitz et al., 1992). In order to simplify ovarian stimulation, several modifications of the long agonist protocol were proposed; however, they were proven inferior in terms of pregnancy rates (Daya, 2000).

GnRH antagonists: reappearance
The third generation of GnRH antagonists devoid of histaminic problems affecting earlier forms was introduced into clinical practice in the form of a daily (Diedrich et al., 1994) or a single-dose protocol (Olivennes et al., 1994). This allowed suppression of the premature LH surge in the mid-follicular phase, when it was really necessary, thereby offering a rational way to perform ovarian stimulation.

GnRH antagonists versus GnRH agonists
Five large randomized controlled trials (RCT) were performed to...
compare GnRH analogues (Albano et al., 2000; Borm and Mannaerts 2000; Olivennes et al., 2000; European and Middle East Orgalutran Study Group, 2001; Fluker et al., 2001). It was confirmed that antagonists could effectively suppress endogenous LH and result in a decreased amount of gonadotrophins, a shorter period of stimulation, a similar incidence of severe ovarian hyperstimulation syndrome and similar multiple pregnancy rates compared to agonists. Although significantly fewer oocytes were retrieved in the antagonist group, similar numbers of embryos of similar quality were transferred between the two groups. However, the expectation that pregnancy rates with antagonists would be at least equal to those achieved by GnRH agonists was not realized (Al Inany and Aboulghar, 2001). This might be due to several differences present between the two analogue stimulation schemes, the impact of which on pregnancy rates was not known prior to phase III trials.

The antagonist cycle is not preceded by a period of gonadotrophin suppression such as that in the agonist long protocol, but instead by the luteal phase of a natural cycle. As a consequence, in a small proportion of patients, abnormal steroid concentrations can be observed on the day gonadotrophin stimulation is scheduled to start. The importance of these abnormal steroids concentrations at initiation of stimulation was unknown, while no guidelines were available as to what should be the appropriate management in these cases.

Moreover, in the early follicular phase of an antagonist cycle, unsuppressed endogenous LH concentrations and significantly higher oestradiol concentrations are present as compared with an agonist cycle, while no data were available as to their potential effect on the probability of pregnancy. Similarly, the significance of the abrupt decrease in LH after antagonist initiation at a critical stage of follicular development was unknown, while the decision for the optimal moment at which final oocyte maturation should be triggered was arbitrary.

Meta-analysis considerations

The meta-analysis by Al-Inany and Aboulghar (2001) showed that the probability of clinical pregnancy was 5% lower with GnRH antagonists as compared with GnRH agonists. This was not expected and in order to optimize GnRH antagonist stimulation, the source of this difference had to be identified. On the other hand, its implications for clinical practice deserve further consideration.

According to the meta-analysis by Al-Inany and Aboulghar (2001), the number needed to treat with GnRH agonists to achieve one additional pregnancy as compared with the GnRH antagonists is 20 (the inverse of the 5% difference in clinical pregnancy rate observed in favour of the agonists). It was also shown by the same authors that for each patient treated with GnRH agonist, 21 days of additional treatment as compared with a patient treated with GnRH antagonist were required. Thus, 420 days of additional treatment are necessary to achieve an extra clinical pregnancy with agonists as compared with GnRH antagonists (number needed to treat times the additional duration of treatment, 20 times 21 days). This needs to be considered carefully in clinical practice before deciding what should be the preferred protocol for pituitary down-regulation.

It is also important to realize that the meta-analysis published by the Cochrane group (Al-Inany and Aboulghar, 2001) suggested that GnRH antagonists were associated with a lower pregnancy rate than GnRH agonists, in the way the two analogues were used and compared in phase III trials. GnRH agonists were employed in an optimal way, following approximately 15 years of experience. The same claim, however, cannot be made for GnRH antagonists.

The current review examines existing knowledge on the use of GnRH antagonists, the protocol modifications applied so far and their effectiveness in improving pregnancy rates, and delineates further research that needs to be carried out. Potential extra-pituitary effects of GnRH antagonists, as well as their safety, have been reviewed elsewhere (Ortmann et al., 2000; Tarlatzis and Kolbianakis 2003; Tarlatzis and Billi 2004).

Elevated progesterone at initiation of stimulation

In all phase III trials, it is explicitly stated that ovarian stimulation was started only after down-regulation was confirmed in the agonist group. In what proportion of patients randomized to antagonist treatment abnormal steroid concentrations were present on the day stimulation should start, or how these patients were managed, is not mentioned in any of the phase III trials. It has to be assumed either that all patients had normal hormonal concentrations, or that stimulation was started in all patients regardless of their hormonal status. The second scenario does not ensure equality of these patients with those in the agonist group, while the first is probably not true.

Approximately 5% of patients planned to start an antagonist cycle will present with elevated progesterone concentrations on the day stimulation is scheduled to start (Kolibianakis et al., 2004a). In a prospective study including 420 patients, initiation of stimulation was postponed for 1 or 2 days in the presence of elevated progesterone concentrations (20 patients) and was started only if repeat progesterone concentrations returned to normal range. Progesterone concentrations, despite normalization before stimulation was started, were significantly higher during the follicular phase compared with those observed in 390 patients with normal progesterone concentrations at initiation of stimulation (Kolibianakis et al., 2004a). More importantly, the probability of pregnancy was significantly decreased in patients with elevated progesterone at initiation of stimulation (5%) as compared with those having normal levels (31.87%).

It appears that patients treated with GnRH antagonists should not start stimulation in the presence of abnormal progesterone concentrations, even if these concentrations return to normal within 1–2 days.

Increasing the starting dose of gonadotrophins

Significantly lower numbers of cumulus–oocyte complexes (COC) were retrieved in the antagonist as compared to the agonist group in phase III trials (Al-Inany and Aboulghar, 2001). This might have been a source of the difference in pregnancy rates observed between the two analogues. It was postulated that by increasing the starting dose of gonadotrophins, this difference might be eliminated leading to an improvement in pregnancy rates.
Two RCT have been performed so far that have examined the concept of an increase in the starting dose of gonadotrophins. The study by Wilkland et al. (2001) analysed 117 patients, while the study by Out et al. (2004) included 257 patients. Both studies were successful inasmuch as they increased the number of COC retrieved, enough to compensate for the difference observed between agonists and antagonists in phase III trials (Wilkland et al., 2001: +1.9 COC; Out et al., 2004: +1.6 COC). The studies were not adequately powered to address clinically important differences in pregnancy rates. However, the odds ratio for pregnancy rate is currently not in favour of increasing the starting dose of recombinant FSH (odds ratio: 0.81, 95% CI 0.51–1.28; Table 1).

Interestingly, Fanchin et al. (2003) showed that luteal oestradiol administration reduces the pace of growth, improves size homogeneity of antral follicles on day 8 of r-FSH treatment and increases the number of follicles reaching maturation at once. The effect of this manipulation on pregnancy rates remains to be assessed.

**Fixed versus flexible antagonist administration**

Three randomized controlled trials including 373 patients have so far been performed comparing a fixed (on day 6) versus a flexible protocol (by a follicle of 14–15 mm) of GnRH antagonist administration (Ludwig et al., 2002; Escudero et al., 2004; Mochtar, 2004). Randomization was performed prior to initiation of stimulation. A trend towards a lower probability of pregnancy is present with the use of flexible protocol (odds ratio 0.70, 95% CI 0.45–1.10; Table 2). However, the power of the stratified analysis is currently too low to allow for solid conclusions to be drawn.

A more meaningful comparison is probably that between fixed (on day 6) and delayed antagonist administration (by a follicle of 14–15 mm), in which patients are randomized on day 6 of stimulation. Randomization in this case is performed in patients who do not meet the pre-specified

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**Table 1.** Stratified analysis of randomized controlled trials assessing the value of an increased starting dose of recombinant FSH. Calculation of odds ratios on pregnancy rates per started cycle was performed by Revman 4.1 software using a random effects model. OR = odds ratio, CI = confidence interval.

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>Higher dose (nmol/l)</th>
<th>Standard dose (nmol/l)</th>
<th>OR (fixed) 95% CI</th>
<th>Weight</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wikland, 2001</td>
<td>15/59</td>
<td>15/58</td>
<td></td>
<td>27.33</td>
<td>0.98 (0.43, 2.24)</td>
</tr>
<tr>
<td>Out et al., 2004</td>
<td>32/126</td>
<td>41/131</td>
<td></td>
<td>72.67</td>
<td>0.75 (0.43, 1.29)</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>185</td>
<td>189</td>
<td></td>
<td>100.00</td>
<td>0.81 (0.51, 1.28)</td>
</tr>
</tbody>
</table>

Test for heterogeneity: chi-squared = 0.28, df = 1 (P = 0.60), F = 0%.
Test for overall effect: Z = 0.91 (P = 0.36).

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**Table 2.** Stratified analysis of a fixed versus flexible antagonist administration. Calculation of odds ratios on pregnancy rates per started cycle was performed by Revman 4.1 software using a random effects model. CI = confidence interval.

<table>
<thead>
<tr>
<th>Study or sub-category</th>
<th>Higher dose (nmol/l)</th>
<th>Standard dose (nmol/l)</th>
<th>OR (fixed) 95% CI</th>
<th>Weight</th>
<th>OR (fixed) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ludwig et al., 2002</td>
<td>7/40</td>
<td>4/20</td>
<td></td>
<td>9.84</td>
<td>0.85 (0.22, 3.33)</td>
</tr>
<tr>
<td>Escudero et al., 2004</td>
<td>20/50</td>
<td>26/59</td>
<td></td>
<td>32.01</td>
<td>0.85 (0.39, 1.82)</td>
</tr>
<tr>
<td>Mochtar et al., 2004</td>
<td>23/101</td>
<td>34/103</td>
<td></td>
<td>58.15</td>
<td>0.60 (0.32, 1.11)</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>191</td>
<td>182</td>
<td></td>
<td>100.00</td>
<td>0.70 (0.45, 1.10)</td>
</tr>
</tbody>
</table>

Test for heterogeneity: chi-squared = 0.56, df = 2 (P = 0.76), F = 0%.
Test for overall effect: Z = 1.53 (P = 0.13).
criteria of follicular development for antagonist initiation and in whom antagonist is started either on day 6 or it is delayed until the criteria of follicular development are reached.

It can easily be conceived that even if delayed antagonist administration results in a decreased probability of pregnancy, a comparison between a fixed and a flexible antagonist protocol, when randomization is performed prior to initiation of stimulation, is difficult to detect a significant difference between the two protocols. It has been shown that approximately 50% of patients will have a follicle of 15 mm on day 6 of stimulation (Kolibianakis et al., 2003a). Consequently, a comparison of fixed versus flexible protocols, in this case, means that a substantial proportion patients randomized in either group will in fact start the antagonist on the same day (day 6), thus decreasing the probability of detecting a potentially existing difference between the two protocols.

Delayed antagonist administration beyond day 6 of stimulation has been associated with increased endometrial advancement at oocyte retrieval, which in extreme cases may result in a decreased probability of pregnancy (Kolibianakis et al., 2002). Moreover, delayed antagonist administration and increase in FSH at antagonist initiation was shown to result in a significantly decreased implantation rate compared to fixed antagonist administration on day 6 and no increase of FSH (8.8 versus 23.9% respectively; Kolibianakis et al., 2003a).

Administration of antagonist from day 1 of stimulation

Administration of antagonist from day 1 of stimulation resulted in significantly lower LH and oestradiol concentrations as compared with administration from day 6 of stimulation in a small RCT which included 60 patients (Kolibianakis et al., 2003b). This was not accompanied by differences in the number and proportion of follicles or metaphase II oocytes between the two groups. The resultant induction of deficient aromatization and disproportionate production of oestradiol per follicle appeared to have no impact on ongoing implantation rate (day 1, 33.9%; day 6, 30.5%) (Kolibianakis et al., 2003b).

Administration of antagonist from day 1 of stimulation did not appear to improve pregnancy rates; however, as shown in a prospective series including 63 patients (Kolibianakis et al., 2004b), it is an efficient protocol (ongoing pregnancy rate: 39.7%, 95% CI 30.1–50.8%) that might be worth investigating in patients with polycystic ovarian syndrome, characterized by high LH concentrations during the follicular phase.

LH supplementation in antagonist cycles

The addition of 75 IU of recombinant LH (recLH) to recombinant FSH (recFSH) at antagonist initiation was tested in a RCT that included 218 patients, and did not appear to enhance delivery rates (recFSH only: 23%, recFSH + recLH: 25.2%; Cedrin-Durnerin et al., 2004). Similarly, in a further RCT that recruited 151 patients (AbouHgheir et al., 2004), no improvement in clinical pregnancy rates could be shown by increasing the dose of human menopausal gonadotrophins by 75 IU when GnRH antagonist was started (increase in HMG: 36.2%, no increase in HMG: 32.1%). Furthermore, no difference could be observed in pregnancy rates between patients randomized to receive recLH and recFSH from initiation of stimulation (n = 62) or recFSH only (n = 65) (12.9 versus 18.4% respectively; Griesinger et al., 2005). Thus, LH supplementation in GnRH antagonist cycles does not appear to be beneficial.

The above studies did not have the power to address clinically important differences in pregnancy rates. However, the basis for LH supplementation in normo-ovulatory/WHO II women treated with GnRH antagonists is questionable. In a prospective study of patients stimulated with recFSH only and GnRH antagonists, it was shown that the lower the LH concentrations on day 8 of stimulation, 2 days after antagonist initiation, the higher the pregnancy rates achieved (Kolibianakis et al., 2004c) (Table 3).

In a dose-finding study (Ganirelix Dose-Finding Study Group, 1998), very low LH and oestradiol concentrations, caused by high antagonist doses, were associated with a decreased probability of pregnancy. However, there is no indication that this was due to an effect on embryo/oocyte quality (Kol et al., 1999) or endometrium quality (Simon et al., 2004).

Kol et al. (1999) showed that the implantation potential of frozen embryos from cycles stimulated with GnRH antagonists/gonadotrophins is not dependent on the dose of the antagonist used. Moreover, Simon et al. (2004) showed that there is no difference in endometrial development after daily treatment with low (0.25 mg) or high (2 mg) dose ganirelix, while endometrial development after GnRH antagonist treatment mimics the non-stimulated endometrium better than after GnRH agonist treatment.

Interestingly, the decreased probability of pregnancy with very low LH concentrations has occurred in the presence of detectable antagonist concentrations on the day of transfer (Ganirelix Dose-Finding Study Group, 1998), which might have reduced the implantation potential of the transferred embryos (Casan et al., 1999; Raga et al., 1999).

Criteria for triggering final oocyte maturation

Almost each antagonist study performed so far has used its own criteria for triggering final oocyte maturation (Kolibianakis et al., 2004d). Obviously, this has occurred on an empirical basis.

In a large RCT, which included 413 patients, it was shown that prolongation of follicular phase significantly decreases the probability of ongoing pregnancy in antagonist cycles [early human chorionic gonadotrophin (HCG) administration: 35.6% versus late HCG administration: 25.0%, P < 0.027; Kolibianakis et al., 2004d] (Table 4). Although the optimal moment for triggering final oocyte maturation has not yet been defined, it is probably no later than the first day that three or
more follicles ≥17 mm are present at ultrasound (Kolibianakis et al., 2004d). However, earlier administration of HCG is worth investigating.

**Replacement of HCG by GnRH agonist**

At present, no conclusive data have been published regarding the triggering of final oocyte maturation by GnRH agonists instead of HCG (Fauser et al., 2002). However, the feasibility of such an intervention has been put in doubt by two recent RCTs (Westergaard et al., 2004: 96 patients, Griesinger et al., 2004: 106 patients), presented in meetings, which showed that the use of agonist is associated with a significantly lower pregnancy rate as compared to HCG (Westergaard et al., 2004 clinical pregnancy rate: agonist 7.5 versus HCG 39%; Griesinger et al., 2004 ongoing pregnancy rate: agonist 3.8 versus HCG 27.8%). Currently, the use of GnRH agonist to trigger final oocyte maturation should be performed with caution and only within the context of a randomized controlled trial.

**GnRH antagonists in poor responders**

The value of GnRH antagonists in poor responders is not clear at present, as the majority of studies performed are either retrospective (Nikolettos et al., 2001; Fasouliotis et al., 2003; Kolibianakis et al. 2004e) or prospective but not randomized trials (D’Amato et al., 2004). There is only one RCT in which GnRH antagonists were compared with GnRH agonists in poor responders (Akman et al., 2001). That study, however, although showing promising results for GnRH antagonists, was underpowered to allow solid conclusions to be drawn. There is a need for further well-designed studies in this category of patients with poor prognosis in order to evaluate the use of GnRH antagonists.

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**Table 3.** Ongoing pregnancy rates per oocyte retrieval and ongoing implantation rates across groups of patients defined according to percentile analysis of LH concentrations on day 8 of stimulation. Ovarian stimulation was performed with 200 IU fixed dose of recombinant FSH and GnRH antagonist was started on day 6 of stimulation (modified from Kolibianakis et al., 2004c).

<table>
<thead>
<tr>
<th>Percentile</th>
<th>LH level on day 8</th>
<th>Ongoing pregnancy rate per oocyte retrieval (%)</th>
<th>Ongoing implantation rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>0–25th</td>
<td>0.3</td>
<td>0.1</td>
<td>0.5</td>
</tr>
<tr>
<td>25–75th</td>
<td>1.0</td>
<td>0.6</td>
<td>1.9</td>
</tr>
<tr>
<td>75–100th</td>
<td>3.3</td>
<td>1.9</td>
<td>8.4</td>
</tr>
</tbody>
</table>

*aExact chi-square for trend.

**Table 4.** Pregnancy outcome in the early-human chorionic gonadotrophin (HCG) group, in which HCG was administered as soon as ≥3 follicles of 17 mm were present at ultrasound and in the late-HCG group, in which HCG was administered 2 days after this criterion was met. Ovarian stimulation was performed with recombinant FSH and GnRH antagonist was started on day 6 of stimulation. Modified from Kolibianakis et al. (2004d).

<table>
<thead>
<tr>
<th></th>
<th>Early-HCG group</th>
<th>Late-HCG group</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ongoing pregnancy rate per oocyte retrieval (%)</td>
<td>35.6 (69/194)</td>
<td>25 (49/196)</td>
<td>0.027</td>
</tr>
<tr>
<td>Ongoing implantation rate (%)</td>
<td>22.6 (87/385)</td>
<td>15.1 (58/383)</td>
<td>0.009</td>
</tr>
</tbody>
</table>
Luteal phase support in GnRH antagonist cycles

It was expected that the immediate reversibility of GnRH antagonist action would make the need for luteal support unnecessary. However, this was not confirmed in early antagonist studies (Albano et al., 2003c). Luteal support was universally accepted as mandatory, although the proof for that did not meet evidence-based medicine standards.

It was later suggested that endometrial development during an unsupported luteal phase was abnormal (Kolibianakis et al., 2003c). Moreover, it was shown that LH concentrations during the luteal phase after an antagonist cycle were very low and not different from those observed following HMG only stimulation (Tavaniotou et al., 2001). This indicated that reversibility of analogue action is only part of the problem of abnormal LH concentrations in the luteal phase after ovarian stimulation for IVF.

The most convincing evidence that luteal support is necessary was provided by an RCT in which patients received different signals for triggering final oocyte maturation without luteal support (Beckers et al., 2003). The study was discontinued due to the extremely low pregnancy rates observed after 40 patients had been included. Thus, luteal support remains mandatory in IVF when GnRH antagonists are used to inhibit the premature LH surge.

Conclusion

Knowledge has been gathered in post-phase III trials period regarding the use of GnRH antagonists. This makes it possible to gradually develop a much clearer view as to what an optimized antagonist protocol should be. This optimized GnRH antagonist protocol needs to be ultimately compared with the long agonist protocol to ensure at least equality in terms of pregnancy rates.

On the basis of the available studies, it can be supported that prolongation of follicular phase decreases the probability of pregnancy. It also appears that patients with elevated progesterone at initiation of stimulation have significantly fewer chances of achieving an ongoing pregnancy. Luteal support remains mandatory, while the replacement of HCG by GnRH agonist does not appear to be feasible. Although not conclusive, currently available data are not in favour of increasing the starting dose of gonadotrophins, of LH supplementation or of using a flexible antagonist protocol. The use of a single dose antagonist protocol appears to result in similar pregnancy rates compared with the single dose GnRH agonist protocol (Vlaivas-Glevic et al., 2003) although further studies are necessary.

The long-term future of antagonists will probably be based on their main advantage over agonists. Antagonists offer what patients should have been given in the mid-1980s, when inhibitions of premature LH surge become standard in ovarian stimulation: an effective, convenient and safe way to perform IVF.

References


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