Can we improve pregnancy rate in GnRH antagonist protocols for IVF?

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The recent introduction of GnRH antagonists in assisted reproductive technologies (ART) has simplified ovarian stimulation (1). This is mainly due to the immediate mode of antagonist action, which allows their administration during the follicular phase, when the inhibition of premature LH surge is required. In this way, the prolonged period of downregulation, necessary in the long agonist protocol, can be avoided, while FSH requirement is decreased in terms of total dose and duration (2).

Despite the above mentioned advantages, acceptance of GnRH antagonists in IVF, has been hampered by a lower clinical pregnancy rate as compared to the long agonist protocol (2). The difference observed was marginal in terms of clinical importance (5%), however, it has been a source of concern (3) and has stimulated research towards optimizing the existing GnRH antagonist protocols.

The standard GnRH antagonist protocol

As the standard GnRH antagonist protocol is considered the one used in the large multicentre comparative trials, with which GnRH agonists were introduced in ART (4-8).

In the daily dose antagonist protocol (4), antagonist is administered on day 6 of stimulation at a dose of 0.25mg, while in the single dose protocol, 3 mg of GnRH antagonist are administered on day 7 of stimulation, provided that E2 is >400 pg/ml (6).

The dose with which GnRH antagonists were used in ART was defined through dose-finding studies (9-11). However, every other parameter of the antagonist protocol was not evidence based. For instance, the optimal day on which GnRH antagonist should be started (day 6 or day 7 of stimulation) was an arbitrary choice. The same was true for the criteria used for hCG administration, which were not identical between the comparative analogue trials. On the other hand, no information was available regarding the optimal starting dose of FSH, the need to modify gonadotrophin dose at antagonist initiation, the need to add LH or the optimal mode of luteal support after antagonist stimulation.

It therefore appears that the introduction of GnRH antagonists in clinical practice was rather premature, since it occurred before important knowledge on antagonist use become available.

Research on the optimal GnRH antagonist protocol

Areas of interest regarding the use of GnRH antagonist have been the need for LH addition during ovarian stimulation with FSH, the use of a
fixed vs. a flexible scheme of antagonist administration, the use of a single vs. multiple dose antagonist protocol, the benefit from increasing the starting dose of gonadotropins, the benefit from increasing the dose of gonadotrophins at antagonist initiation, the importance of follicular phase prolongation, the replacement of hCG with GnRH agonist for triggering final oocyte maturation as well as the use of oral contraceptive pill (OCP) pretreatment.

**Modifications of the GnRH antagonist protocol in order to improve pregnancy rates**

**Criteria used for hCG administration**

In IVF cycles, the criteria for triggering final oocyte maturation have been established on an arbitrary basis (12). In a large randomized controlled trial (RCT) evaluating the effect of the prolongation of the follicular phase in antagonist cycles (administration of hCG as soon as ≥ 3 follicles of ≥ 17mm were present at ultrasound or two days later), it was shown that delaying hCG administration is associated with a decreased probability of ongoing pregnancy (difference in ongoing pregnancy rate: 10.6%, 95% CI: 1.4% - 19.5%) (12).

Although further studies are necessary to confirm these results and to explore the optimal timing of hCG administration, it appears that the prolongation of the follicular phase in antagonists cycles should be avoided. This however, is a frequent practice in order to avoid weekend oocyte retrievals, to reach follicles of large size or to increase the number of oocytes retrieved. In GnRH antagonist cycles, a potential adverse effect of the above manipulations on the probability of pregnancy cannot be excluded. On the other hand, the adoption of strict criteria for triggering final oocyte maturation, which ensures that the follicular phase ends in the same way for all patients, is necessary.

**Single vs. multiple dose GnRH antagonist protocol**

Until today the daily GnRH antagonist protocol has been used much more frequently than the single dose antagonist protocol. One RCT (13) has been published to compare the single dose (Cetrorelix 3mg, 87 patients) with the daily dose antagonist protocol (ganirelix 0.25mg, 89 patients). Patients were stimulated with recombinant FSH, while antagonist was started in a flexible manner by a follicle of 14 mm. A small difference (3.3%, 95% CI -11.2 to 17.6%) in clinical pregnancy rates per randomized patient was observed between the two antagonist protocols (45.9% and 42.6%, respectively). Although the single dose protocol warrants further evaluation, its potential to enhance pregnancy rates compared to the daily dose protocol appears to be small.

**Evaluation of steroid levels at initiation of stimulation**

Whether ovarian stimulation should be started in patients with elevated E2 and/or progesterone levels treated with GnRH antagonists was not known in the initial comparative trials between the two analogues. In a prospective study (14) the probability of ongoing pregnancy was compared between patients with normal steroid levels and those with elevated progesterone levels on day 2 of an antagonist cycle (~5% of the total patients). In the latter category, stimulation was started only if progesterone levels returned to normal within 1-2 days. Ongoing pregnancy rate was significantly decreased in these patients compared to those with normal steroid levels at initiation of stimulation (5% vs. 31.8%, respectively). It therefore appears that, similarly to GnRH agonists, the baseline hormonal status of patients treated with GnRH antagonists should be evaluated prior to initiation of stimulation.

**Fixed vs. flexible antagonist administration**

In the large comparative studies between GnRH analogues, antagonist initiation was performed on day 6 of stimulation in the daily antagonist protocol (4) and on day 7 of stimulation or later in the single dose protocol (6). In theory, however, antagonists should be administered when there is follicular development and/or production of estradiol (E2) by the developing follicles that might give rise to a premature elevation in pituitary LH. This might occur as early as day three of
Table 1. RCTs comparing the fixed vs. the flexible antagonist protocol.

<table>
<thead>
<tr>
<th>Study</th>
<th>Gonadotrophin</th>
<th>Antagonist</th>
<th>Criteria for antagonist initiation</th>
<th>Earliest evaluation day</th>
<th>Mean stimulation day in which antagonist was started</th>
<th>Ongoing pregnancy rate at 12 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fixed</td>
</tr>
<tr>
<td>Ludwig 2003</td>
<td>Follitropin a</td>
<td>Cetrotide 0.25mg</td>
<td>Follicle of 14-15mm</td>
<td>Not mentioned</td>
<td>7.8 ±2.1 days</td>
<td>15%</td>
</tr>
<tr>
<td>60 patients</td>
<td></td>
<td>Cetrotide 3mg</td>
<td></td>
<td></td>
<td>7.8 ±1.9 days</td>
<td></td>
</tr>
<tr>
<td>Kolibianakis 2003a</td>
<td>Follitropin b</td>
<td>Ganirelix 0.25mg</td>
<td>Follicle of ≥ 15mm</td>
<td>Day 6 of stimulation</td>
<td>6.1±1.3 days</td>
<td>29.2%</td>
</tr>
<tr>
<td>111 patients</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Escudero 2004</td>
<td>Follitropin a</td>
<td>Cetrotide 0.25mg</td>
<td>Follicle of ≥ 14mm</td>
<td>Not mentioned</td>
<td>7.2±0.2 days</td>
<td>44.1%b</td>
</tr>
<tr>
<td>109 patients</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Mochtar 2004</td>
<td>Follitropin b</td>
<td>Ganirelix 0.25mg</td>
<td>Follicle of ≥ 15mm</td>
<td>Day 5 of stimulation</td>
<td>6.6±1.3 days</td>
<td>31.1%</td>
</tr>
<tr>
<td>205 patients</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

a dose of FSH was increased by 100 IU at antagonist initiation in the flexible group.
b clinical pregnancy rate at 7 weeks

stimulation (15), or might not have occurred yet on day 6 of stimulation. Therefore, flexible administration of GnRH antagonists is worth evaluating.

Four randomised controlled trials have so far been performed comparing a fixed (on day 6 of stimulation) vs. a flexible (by a follicle diameter of 14-15 mm) protocol of GnRH antagonist administration (16). Although currently the difference between the two antagonist schemes is not significant, it appears that the use of flexible antagonist administration by a follicle of 14-15mm does not enhance the probability of pregnancy. However, the population analyzed is not large enough to allow solid conclusions to be drawn.

Interestingly, the criteria used to time antagonist initiation as well as the earliest day the on which patients should start evaluation, in order to examine if these criteria are satisfied, have not been assessed so far. In the four RCTs that compared a fixed vs. a flexible protocol of antagonist administration, flexible antagonist administration led to a later and not earlier than day 6 of stimulation antagonist initiation (Table 1). Earlier administration of GnRH antagonist needs to be further explored (1).

The effect of the signal used to trigger final oocyte maturation on pregnancy rates

Due to immediate reversibility of GnRH antagonist action, hCG can be replaced by GnRH agonist for triggering of final oocyte maturation (17). This might also lead to a decreased risk of developing ovarian hyperstimulation syndrome (OHSS) in high risk patients (18). However, such a modification of GnRH antagonist protocol was shown to decrease the probability of pregnancy (19-21) and thus cannot be recommended for application in routine clinical practice.

Oral contraceptive pill (OCP) pretreatment and probability of pregnancy

OCP pretreatment has been used to assist in cycle programming in antagonist cycles, while it might exert a positive effect on synchronization of follicular development (22). Two RCTs have been performed so far to compare antagonist protocols with or without OCP pretreatment (23,24).
Pregnancy rates in these studies appear to be similar (39.7% vs. 42.1%; 20.4% vs. 23.6% respectively, ns). However, the effect of the time interval from OCP discontinuation to initiation of stimulation, on IVF outcome (25), remains to be assessed.

Increase in the starting dose of FSH

Fewer oocytes were retrieved in the antagonist as compared to the agonist group in the comparative introductory trials between the two analogues (2). It was thus believed that an improvement in pregnancy rates might occur if an increase in the number of oocytes retrieved was achieved in the antagonist protocol. Two RCTs have so far been performed to evaluate the above concept (26-27). It was shown that a higher starting dose of FSH results indeed in a significantly increased number of COCs retrieved, however, it does not appear to be associated with higher pregnancy rates compared to the standard dose (Wikland et al, 2001 (26): 25.4% vs. 25.9%; Out et al 2004 (27): 25% vs. 31.%; respectively, ns).

Increase of gonadotrophin dose at antagonist initiation

Antagonist initiation in the mid-follicular phase results in a sharp decrease of endogenous LH and to a lesser extent to endogenous FSH at a critical point of follicular development. For that reason it has been suggested that an increase in the exogenous gonadotropin dose when antagonist is started might be beneficial for IVF outcome. Although the data currently present are not conclusive, the only RCT which tested this concept (28) suggested that an increase of HMG dose at GnRH antagonist initiation does not appear to enhance the probability of pregnancy (pregnancy rate: no HMG increase: 32.1% vs. HMG increase: 36.2%, ns).

LH supplementation in antagonist cycles

In order to inhibit a premature LH surge antagonists bind the GnRH pituitary receptor and result in a deep suppression of endogenous LH in a matter of hours. Existing evidence both in antagonist cycles (10) and in agonist cycles (29) suggested a decreased probability of pregnancy in the presence of low LH levels. For that reason, the concept of LH supplementation during an antagonist cycle was considered as worth evaluating in order to improve pregnancy rates.

However, the addition of 75 IU of recombinant LH to recombinant FSH at GnRH antagonist initiation in the mid-follicular phase (Cedrin-Durnerin 2004 (30); 218 patients) does not appear to enhance pregnancy rates (LH addition: 23.7% vs. FSH only 22.1%, ns) Similarly, LH addition from initiation of stimulation (Griesinger et al 2005 (21); 127 patients) does not appear to increase pregnancy rates (LH addition: 12.9% vs. FSH only: 18.5%, ns).

Luteal phase supplementation

Supplementation of the luteal phase is mandatory in GnRH antagonist cycles as it is in GnRH agonist cycles (9, 31). The optimal type of luteal phase support, however, has not yet been established. On the basis of published abstracts (Fatemi et al 2005 (32); 123 patients) it appears that the addition of E2 to progesterone does not enhance pregnancy rates in antagonist cycles (progesterone only: 28.3% vs. progesterone +E2: 22.2%, ns).

CONCLUSION

Several modifications of the GnRH antagonist protocol have been attempted so far with the aim to increase pregnancy rates. Solid conclusions cannot be drawn regarding the value of LH addition in antagonist cycles, the use of a single vs. multiple antagonist protocol, the optimal type of luteal phase supplementation, the value of increasing the starting gonadotrophin dose or the dose of gonadotrophin at antagonist initiation in the mid-follicular phase. This is mainly due to limitations in sample size of the studies performed. However, the best estimate on the basis of the available data is that the above modifications of the GnRH antagonist protocol do not enhance the probability of pregnancy.
On the other hand, there is little doubt that replacement of hCG with GnRH agonist results in a decreased probability of pregnancy in the general population, while elevated progesterone levels on day two of the cycle appear to be associated with a decreased probability of pregnancy. More importantly, although the optimal criteria for administering hCG still need to be established, it has been shown that administering hCG later than the day when ≥3 follicles ≥17 mm are present, is associated with decreased pregnancy rates. In addition, flexible antagonist administration needs to be further explored, allowing an earlier than day 6 of stimulation antagonist administration, if required.

Several of the modifications of the GnRH antagonist protocol evaluated may not appear to enhance pregnancy rates, however, they may still be useful in optimizing the antagonist protocol. These include pretreatment with OCP which can be used to assist in cycle scheduling, use of GnRH agonists to avoid occurrence of OHSS in high risk patients (with cryopreservation of the embryos produced) and the use of the single dose protocol, which avoids the need for daily injections.

It is expected that additional comparative trials between GnRH agonists and GnRH antagonists should take into advantage existing knowledge accumulated, in order to enhance the probability of pregnancy, avoiding unnecessary modifications of the antagonist protocol and by adopting those shown to be beneficial. In a different case the repetition of the results of the five large comparative trials is likely to occur, making more difficult the acceptance of GnRH antagonists in ovarian stimulation for IVF.

REFERENCES


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