Anti-Müllerian hormone-based approach to controlled ovarian stimulation for assisted conception

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BACKGROUND: Individualization of controlled ovarian stimulation (COS) for assisted conception is complicated by variable ovarian response to follicle stimulating hormone. We hypothesized that anti-Müllerian hormone (AMH), a predictor of oocyte yield, may facilitate treatment strategies for women undergoing COS, to optimize safety and clinical pregnancy rates.

METHODS: Prospective cohort study of 538 patients in two centres with differential COS strategies based on a centralized AMH measurement.

RESULTS: AMH was associated with oocyte yield after ovarian stimulation in both centres, and a ‘reduced’ AMH (1 to <5 pmol/l) was associated with a reduced clinical pregnancy rate. Women with a ‘normal’ AMH (5 to <15 pmol/l) treated with a long GnRH-agonist protocol (both centres) showed a low incidence of excess response (0%) and poor response (0%). In women with ‘high’ AMH (>15 pmol/l), the antagonist protocol eliminated the need for complete cryopreservation of embryos due to excess response (P < 0.001) and showed a higher fresh cycle clinical pregnancy rate than agonist cycles [OR 4.40 (95% CI 1.95–9.93), P < 0.001].

CONCLUSIONS: The use of circulating AMH to individualize treatment strategies for COS may result in reduced clinical risk, optimized treatment burden and maintained pregnancy rates, and is worthy of prospective randomized examination.

Key words: anti-Müllerian hormone / GNRH AG/ANTAG / ovarian stimulation

Introduction

The optimal strategy for controlled ovarian stimulation (COS) in programmes of assisted reproduction is the subject of much current debate. The principle elements address the mode and degree of ovarian stimulation, including the means of luteinizing hormone (LH) surge blockade, and also the debate of single versus multiple embryo transfer. There is not necessarily a direct connection between these two issues, but the arguments can become confused. There is published criticism to the ‘one size fits all’ approach, using the standard long course GnRH-agonist down-regulation with exogenous follicle stimulating hormone (FSH) in variable doses, due to dangers of excess response at one extreme and demanding treatment burden at the other (Heijnen et al., 2007). Despite these concerns, and the potential for GnRH-antagonist control to reduce the incidence of ovarian stimulation syndrome (OHSS), the long course GnRH agonist, first published in 1982 (Fleming et al., 1982) probably remains the most popular mode of treatment amongst practitioners—because it is simple, clinically convenient and effective.

It is clear that the two issues referred to, excessive responses and demanding treatment burden, predominate in patients with high ovarian responses and reduced ovarian responses, respectively. Correspondingly, a programme designed to treat women based upon their capacity of ovarian response will be the most likely to show the optimized combination of maintained pregnancy potential and maximized clinical safety. This requires two major components: an accurate means of predicting ovarian responses and appropriate strategic approaches to COS adapted to that response. This concept...
should facilitate an initial optimal treatment strategy, potentially minimizing complications and the risk of treatment failure, while maximizing the chance of pregnancy and live birth. The question explored here is whether an adaptive strategic approach using different GnRH analogue control protocols shows any advantage over simple modification of FSH dose, in women whose response to standard COS was predicted by anti-Müllerian hormone (AMH).

Individualization of COS regimens for patients undergoing in vitro fertilization (IVF) has proven difficult primarily due to the variability in the chronological decline of the total follicular cohort between individuals (Faddy, 2000) and the limited ability of tests of ovarian reserve to detect extremes of response to COS (Broekmans et al., 2006; Fauser et al., 2008). A wide variety of indices has been proposed to define the extremes of ovarian response including cycle cancellation and hyperstimulation (Fauser et al., 2008), but translation to individualization of treatment for first treatment cycles has been limited. Two studies have examined and tested nomograms incorporating multiple phenotypic, ultrasound derived and biochemical indices to dictate starting doses of exogenous gonadotrophins (Popovic-Todorovic et al., 2003; Howles et al., 2006). The clinical application of these nomograms required distinct combination of factors influencing responses. In one study, these included total number of antral follicles, total Power Doppler score and ovarian volume on days 2–5, age and smoking status (Popovic-Todorovic et al., 2003), whereas in the second study basal FSH, body mass index (BMI), age and the number of follicles with a diameter <11 mm were used (Howles et al., 2006). The greatest weight in both studies was given to antral follicular counts (AFCs). However, AFC has a limited clinical value for pregnancy prediction (Broekmans et al., 2006). In contrast, AMH (Müllerian-inhibiting substance), a member of the transforming growth factor-β family and predominantly a product of pre-antral and small antral follicles (Weenen et al., 2004) and thereby a close correlate of AFC, is not only predictive of the ovarian responses to COS (van Rooij et al., 2002; Penarrubia et al., 2005; Fleming et al., 2006; Nelson et al., 2007) but it is also able to predict clinical pregnancy and live birth (Nelson et al., 2007).

The accurate prediction of oocyte yield in COS by AMH, independent of age, and the ability of AMH to detect women at risk of extremes of ovarian response including, at one extreme, cycle cancellation, poor response and at the other extreme, ovarian stimulation and excess response, would suggest that it is an ideal candidate for individualization of stimulation strategies. Furthermore, AMH levels in most studies are stable across the menstrual cycle (Cook et al., 2000; La Marca et al., 2006; Streuli et al., 2008), removing the constraint of early follicular blood samples or ultrasound scans.

We previously suggested that clinical categories of AMH would allow optimization of treatment strategies prior to the first cycle of ovarian stimulation (Nelson et al., 2007). Adaptive strategies can be effected either through simple differential dose of FSH within cycles controlled by a GnRH agonist or alternatively, deploying different GnRH analogue control with or without additional variable FSH doses. Numerous studies have shown that GnRH antagonists yield a lower degree of response in normal women (Kolibianakis et al., 2006; Heijnen et al., 2007) and it would therefore be logical to deploy these elements while treating women with predicted high response (high circulating AMH). We now describe the first prospective cohort study performed in two independent centres of an AMH dictated approach to individualization of COS in women undergoing their first IVF cycle, using the predetermined values to dictate either FSH dose in a GnRH-agonist-controlled programme or a programme of modified GnRH analogue strategy. The end-points addressed were safety in high responding women, treatment burden in women with predicted reduced responses and clinical pregnancy rates in all categories. The criteria relating to safety were excessive oocyte yields and incidence of OHSS. Criteria defining treatment burden were duration of FSH injections and cycle cancellation.

Materials and Methods

Subjects and protocol stratification

Successive patients undergoing their first assisted reproduction cycles at the Glasgow Royal Infirmary, Glasgow, UK (Centre 1, n = 370) and Glasgow Centre for Reproductive Medicine, Glasgow, UK (Centre 2, n = 168) between October 2006 and October 2007 were allocated to the distinct programme designs. Centre 1 is state funded and Centre 2 a standalone private centre, both centres operate completely independently and autonomously developed their respective AMH-based stimulation strategies. Treatment was limited to women aged <45 years in Centre 1 and <44 year in Centre 2, with an upper BMI limit of <35 kg/m² in both centres.

Stratification of the stimulation protocol in both centres was based on plasma AMH determined 1 month before starting the treatment (sample taken at any point in the menstrual cycle), and the AMH assay for both centres was performed centrally in combined batches. Four clinical categories of patients determined exclusively by AMH and defined as previously described (Nelson et al., 2007) were used in both centres: (i) AMH <1 pmol/l, (ii) AMH 1 to <5 pmol/l, (iii) AMH 5 to <15 pmol/l and (iv) AMH ≥ 15 pmol/l. As the sample for AMH evaluation was taken at any stage of the menstrual cycle, no parallel data on antral follicle count were available for comparative analyses. Table I shows the different strategies deployed for the groups in the two centres, revealing that in Groups 3 and 4 the same starting dose of FSH was used in each centre, but different approaches to control LH by the GnRH analogues in Groups 2 and 4. Centre 1 used long course GnRH-agonist control for most cases, whereas Centre 2 used the GnRH-antagonist control in the high and lower responding categories. FSH stimulant Centre 1 used Gonal F (MerkSerono, Feltham, UK) for all categories, whereas Centre 2 used Gonal F for the two groups with lower AMH levels and Menopur (Ferring UK, Slough, UK) for the two categories with AMH >4.9 pmol/l. When the patient’s weight was >75 kg, the FSH starting dose was increased by 75 IU in Centre 2 (the numbers qualifying were nine cases in Group 2, nine cases in Group 3, and nine cases in Group 4). In general, the FSH dosing strategies were based on historical experience, serving the local population at Centre 1, whereby a starting dose of 150 IU had been shown to yield lower responses than a standard starting dose of 225 IU. The deployment of higher starting doses in reduced and poor responder patients was a common established practice. Although not evidence based, it was considered inappropriate to change too many standard operating procedures at this time.

The modified natural cycle used by Centre 2 for the predicted negligible responders was similar to that described by Pelinck et al. (2007), in which a follicle of 14 mm was identified around 16 days prior to the expected following menses, at which point FSH (150 IU per day) combined with GnRH-antagonist treatment was administered for 2 or 3 days, prior to ovulation induction with human chorionic gonadotrophin (hCG; Ovitrelle, MerkSerono, Feltham, UK).
hCG (Ovitrelle, MerkSerono, Feltham, UK), provided two follicles were according to the follicular response. Ovulation was induced with 6500 IU performed on stimulation day 8, and subsequent scans were performed ultrasound assessment of follicular growth. The first response scan was in intra-assay coefficients of variation were 5.3 and 5.4%, respectively.

The AMH assay

The AMH assay used was the commercial ELISA kit provided by DSL (Webster, TX, USA), with values presented in concentration of picomoles per litre (conversion factor to pmol/l = ng/ml × 7.143). Inter and intra-assay coefficients of variation were 5.3 and 5.4%, respectively.

Definitions

‘Freeze all’ (excess response) was diagnosed when ≥21 oocytes were collected at oocyte retrieval and all normally fertilized (2PN) embryos were cryopreserved in order to minimize both the incidence and degree of OHSS.

Agonist-controlled cycles (both centres)

Down-regulation with the depot GnRH agonist (Prostap SR 3.75 mg, Wyeth, Maidenhead, UK) was initiated on cycle day 21. Ovarian stimulation with exogenous gonadotrophins commenced 2 weeks later, when the circulating estradiol (E2) was <100 pg/ml combined with a thin endometrium and no ovarian cysts >40 mm on transvaginal ultrasound scan. Follicular responses were monitored with serum E2 concentrations and transvaginal ultrasound assessment of follicular growth. The first response scan was performed on stimulation day 8, and subsequent scans were performed according to the follicular response. Ovulation was induced with 6500 IU hCG (Ovitrelle, MerkSerono, Feltham, UK), provided two follicles were ≥17 mm in diameter and serum E2 was ≥200 pg/ml. Oocyte retrieval and fertilization in vitro was performed according to standard procedures as described previously (Nelson et al., 2007). A maximum of two embryos were usually transferred, although in one case three embryos were transferred. Good quality embryos were cryopreserved for transfer in subsequent unstimulated cycles. Luteal phase supplementation with progesterone, 400 mg/day, intravaginally (Cyclogest, Actavis UK or Crinone gel, MerkSerono, Feltham, UK) was started on the evening of the oocyte retrieval and continued for 12 days. Cycles were discontinued if negligible follicular development occurred after 14 days of stimulation.

Antagonist-controlled cycles

Ovarian stimulation was performed with exogenous gonadotrophins initiated on the third or fourth cycle day. The GnRH antagonist Cetrotide (0.25 mg/day s.c.; merkSerono, Feltham, UK) or Orgalutran (0.25 mg/day s.c.; Organon, Cambridge, UK) treatment was commenced on stimulation days 4–7 when serum E2 exceeded 200 pg/ml (700 pmol/l). A maximum of two embryos were usually transferred, although in one case three embryos were transferred. Good quality embryos were cryopreserved for transfer in subsequent unstimulated cycles. Luteal phase supplementation with progesterone, 400 mg/day, intravaginally (Cyclogest, Actavis UK or Crinone gel, MerkSerono, Feltham, UK) was started on the evening of the oocyte retrieval and continued for 12 days. Cycles were discontinued if negligible follicular development occurred after 14 days of stimulation.

Table I Deployment of GnRH analogues and doses of follicle stimulating hormone in the groups categorized by anti-Müllerian hormone in the two centres

<table>
<thead>
<tr>
<th>AMH group (pmol/l)</th>
<th>Centre 1</th>
<th>GnRH analogue</th>
<th>FSH daily dose</th>
<th>Centre 2</th>
<th>GnRH analogue</th>
<th>FSH daily dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1.0</td>
<td></td>
<td>Antagonist</td>
<td>375</td>
<td>(Modified natural cycle)</td>
<td>(Antagonist)</td>
<td></td>
</tr>
<tr>
<td>1.0 to &lt;5</td>
<td></td>
<td>Agonist</td>
<td>375</td>
<td>300</td>
<td>Agonist</td>
<td>225</td>
</tr>
<tr>
<td>5.0 to &lt;15</td>
<td></td>
<td>Agonist</td>
<td>225</td>
<td>150</td>
<td>Agonist</td>
<td>150</td>
</tr>
<tr>
<td>≥15.0</td>
<td></td>
<td>Agonist</td>
<td>150</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AMH, anti-Müllerian hormone; FSH, follicle stimulating hormone.

Results

Female partner and outcome characteristics for the cohorts are described in Table II. The centres differed in their patient characteristics with Centre 2 having older patients with a lower circulating AMH concentration and undergoing a lower proportion of intracytoplasmic sperm injection cycles. The duration of stimulation, starting dose and total dose of gonadotrophin was reduced in Centre 2, reflecting the higher deployment of antagonist protocols. Significantly fewer oocytes were retrieved at Centre 2 (P < 0.001, adjusted for age and AMH), and a higher fertilization rate was observed.

As expected, patients with ‘freeze all/excess response’ were younger (32.0 years (27.6–40.8) versus 35.0 years (32.2–44.2), P < 0.001) and had higher AMH (23.1 pmol/l (12.5–38.7) versus 10.4 (5.0–19.6), P < 0.001) than those receiving a fresh embryo transfer. Conversely, patients with cancelled cycles were older (cancelled 37.7 years (33.7–39.9); non-cancelled 34.7 years (31.6–37.4); P = 0.003) and had lower AMH (cancelled 1.8 pmol/l (0.9–3.2); non-cancelled 12.4 pmol/l (6.2–21.2); P < 0.001). The combination of complete cryopreservation and cycle cancellation was responsible for 95% of the cases where an embryo transfer did not take place in the treatment cycle. Miscarriage rates did not differ between centres.
AMH was strongly associated with oocyte yield after ovarian stimulation in both centres (Centre 1 \( r = 0.53, P < 0.001 \); Centre 2 \( r = 0.64, P < 0.001 \)), despite the use of different strategies and FSH doses. Maternal age was negatively associated with AMH (Centre 1 \( r = -0.39, P < 0.001 \); Centre 2 \( r = -0.45, P < 0.001 \)) and oocyte yield (Centre 1 \( r = -0.27, P < 0.001 \); Centre 2 \( r = -0.50, P < 0.001 \)).

### Table II Baseline and outcome characteristics of the patient cohorts treated at the two centres

<table>
<thead>
<tr>
<th></th>
<th>Centre 1</th>
<th>Centre 2</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of patients</strong></td>
<td>370 (31.9–37.7)</td>
<td>168</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Age at stimulation (years)</strong></td>
<td>34.8 (21.9–37.7)</td>
<td>37 (34.0–39.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>24.0 ± 5.9</td>
<td>24.2 ± 4.1</td>
<td>0.64</td>
</tr>
<tr>
<td><strong>Procedure</strong></td>
<td>IVF 186 (50.2%)</td>
<td>103 (61.3%)</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>ICSI 184 (49.8%)</td>
<td>65 (38.7%)</td>
<td></td>
</tr>
<tr>
<td><strong>Type of stimulation</strong></td>
<td>Long course 352</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Antagonist cycle 20</td>
<td>95</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>AMH (pmol/l)</strong></td>
<td>11.4 (4.9–20.4)</td>
<td>7.6 (3.4–13.2)</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>AMH category</strong></td>
<td>&lt;1 pmol/l 20</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 to &lt;5 pmol/l 74</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 to &lt;15 pmol/l 128</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥15 pmol/l 148</td>
<td>34</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Starting dose of drug</strong></td>
<td>150 IU 148</td>
<td>34</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>225 IU 128</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td></td>
<td>300 IU 0</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td></td>
<td>375 IU 74</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Duration of stimulation (days)</strong></td>
<td>14 (12.2–15.0)</td>
<td>10 (9–12)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Total dose (IU)</strong></td>
<td>2925 (2250–3900)</td>
<td>2737 (1856–3300)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Stimulation outcomes</strong></td>
<td>Freeze all 41 (11.1%)</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Cancelled cycle 36 (10.8%)</td>
<td>6 (3.6%)</td>
<td>0.004</td>
</tr>
<tr>
<td><strong>Number of oocytes</strong></td>
<td>11 (9–16)</td>
<td>5 (3–9.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Number with &gt;21 oocytes</strong></td>
<td>10 (9–14)</td>
<td>4 (2–6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Number of oocytes inseminated</strong></td>
<td>6 (3–10)</td>
<td>4 (2–6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Number of oocytes normally fertilized</strong></td>
<td>71 (58–83)</td>
<td>83.3 (59.6–100)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>62 (44–75)</td>
<td>67 (50–92)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Number of oocytes normally fertilized</strong></td>
<td>7 (4–11)</td>
<td>4 (2–5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>5 (3–8)</td>
<td>4 (2–7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Number of embryos transferred</strong></td>
<td>30</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 258 (117)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Patients with frozen embryos</strong></td>
<td>116 (31.3%)</td>
<td>32 (20.2%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Number of embryos frozen</strong></td>
<td>6 (4–13)</td>
<td>3 (2–3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Cohort outcomes</strong></td>
<td>No transfer 81 (21.9%)</td>
<td>16 (9.5%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Not pregnant 166 (44.8%)</td>
<td>84 (50%)</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>Ectopic 2 (0.5%)</td>
<td>1 (0.5%)</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>Miscarriage–FH seen 2 (0.5%)</td>
<td>1 (0.5%)</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>Miscarriage–no sac seen 25 (6.8%)</td>
<td>11 (6.5%)</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>Miscarriage–sac seen 6 (1.6%)</td>
<td>1 (0.5%)</td>
<td>0.33</td>
</tr>
<tr>
<td><strong>Clinical pregnancy</strong></td>
<td>88 (23.8%)</td>
<td>54 (32.1%)</td>
<td>0.089</td>
</tr>
<tr>
<td><strong>Clinical pregnancy per OR</strong></td>
<td>88/289 (26.9%)</td>
<td>54/162 (33.3%)</td>
<td>0.52</td>
</tr>
<tr>
<td><strong>Clinical pregnancy per embryo transfer</strong></td>
<td>88/330 (30.4%)</td>
<td>54/152 (35.5%)</td>
<td>0.034</td>
</tr>
</tbody>
</table>

Values are presented as median (inter-quartile range) or mean ± SD. *Outcome percentages calculated per cycle started (\( n = 370 \) and 168), rates per oocyte retrieval (OR) and embryo transfer also provided. aNo transfer includes women who either had cycle cancelled due to failure of response to gonadotrophins (Centre 1 \( n = 36 \); Centre 2 \( n = 6 \), all embryos frozen (Centre 1 \( n = 41 \); Centre 2 \( n = 0 \)) no oocytes at OR (Centre 1 \( n = 0 \); Centre 2 \( n = 3 \)) or no fertilization (Centre 1 \( n = 5 \); Centre 2 \( n = 7 \)). IVF, in vitro fertilization; ICSI, intracytoplasmic; AMH, anti-Müllerian hormone; BMI, body mass index.
AMH and ovarian stimulation strategy

0.001). AMH and age were unrelated to BMI. AMH, age and centre were all independent predictors of oocyte yield with the greatest contribution from AMH (AMH contribution to variance (CTV) 32.3%, \( P < 0.001 \); age CTV 2.4% \( P = 0.002 \); centre CTV 6.4% \( P < 0.001 \)).

**Analyses by AMH indicated response category**

The predicted negligible response category (AMH < 1.0 pmol/l)

Centres 1 and 2 treated 20 and 6 patients in this category, using strategies of antagonist or modified natural IVF, respectively. These women were older, median age 39.3 (37.4–42.0), and 12 (60%) were cancelled due to poor response to ovarian stimulation. A median of three oocytes (2–6) was obtained at oocyte retrieval after COS. Oocyte retrieval was successful in four of the six cases of the modified natural cycles. No pregnancy was achieved in this group using either strategy.

The predicted ‘reduced’ response category (AMH ≥ 1.0, < 5.0 pmol/l)

Women in this category exhibited a sub-optimal response to COS in both centres (Tables III and IV), and low clinical pregnancy rates compared with women with an AMH of 5–15 or > 15 pmol/l, irrespective of treatment strategy. Table IV shows that the antagonist protocol was associated with fewer days of stimulation (10 days (IQR 8–11) versus 14 days (13–15); \( P < 0.001 \)) and a significant reduction in risk of cancellation (\( P = 0.005 \)). After adjustment for maternal age and AMH, antagonist protocols were associated with a substantial drop in cycle cancellation [OR 0.20 (95% CI 0.06–0.65); \( P = 0.008 \)] and a trend towards higher pregnancy rates [OR 2.89 (95% CI 0.88–9.50); \( P = 0.09 \)].

The predicted ‘normal’ response category (AMH ≥ 5.0, < 15.0 pmol/l)

Both centres deployed the same protocol in this category, but women attending Centre 2 were significantly older (\( P < 0.001 \)) and yielded fewer oocytes (\( P < 0.001 \)) than their equivalent group in Centre 1 (Table IV). Centre 2 showed a negligible over-response in this category, whereas Centre 1 showed an incidence of 10%, and consequently the number of women not receiving a fresh embryo transfer was also increased (\( P = 0.04 \)) compared with Centre 2. Pregnancy rates of women in this category did not differ between treatment centres (Table IV).

The ‘high’ response category (AMH ≥ 15.0 pmol/l)

Women with an AMH of ≥ 15 pmol/l were younger, produced high oocyte numbers and higher clinical pregnancy rates than other AMH categories after COS (Tables III and IV, both centres). Table IV shows that the antagonist protocol required fewer days of stimulation (9 days (8–11) versus 13 days (12–14); \( P < 0.001 \)) and was associated with elimination of the need for complete cryopreservation of embryos due to excess response, and reduced hospitalization for OHSS. All cycle cancellations (\( n = 5 \)) within this latter group were due to social reasons. The antagonist protocol yielded fewer (\( P < 0.001 \)) oocytes than the agonist protocol, with a mean of 10 compared with 14 in the agonist protocol (Table III). The difference in the yields of normally fertilized embryos (six in the antagonist and seven in the agonist protocol) was less pronounced (Tables III and IV). Correspondingly, the antagonist protocol was not associated with a significant reduction in the number of good quality embryos available for cryopreservation in those women who also had a fresh embryo transfer (Centre 1 0 (0–4.5); Centre 2 0 (0–2) \( P = 0.09 \)). Fresh cycle clinical pregnancy rates were higher in Centre 2.
Discussion

This is the first prospective cohort study examining the clinical utility of AMH-determined strategy of COS for assisted conception, and it demonstrates the potential for maintained or improved clinical pregnancy rates and minimization of the risk of harm due to ovarian over-response. We have shown that strict application of a mixed treatment strategy, rather than simple modification of FSH dose, can influence clinical outcome in both the high and reduced response categories of patients. In the high responder category a profound reduction of excess responses to stimulation, and an increased proportion of cases having fresh embryo transfer resulted in a higher fresh clinical pregnancy rate. In the ‘reduced’ responder category, the antagonist protocol resulted in a reduced treatment burden, reduced cycle cancellation and a trend towards increased clinical efficacy. The net effect of this stratification upon the ‘normal’ response group, here treated with GnRH agonists in both centres, is profound reduction of the recognized complications of excess and sub-optimal responses. Differences between the centres in this group may relate to patient profile and/or the origin of the FSH used.

We have confirmed that women with an extremely low AMH (<1.0 pmol/l) have a severely diminished ovarian reserve and have a severely reduced prospect for clinical pregnancy using IVF, irrespective of age and whether COS or modified natural cycle is undertaken. We identify that women with a ‘normal’ AMH (5–15 pmol/l) exhibit an uncomplicated response to COS with agonist down-regulation and conventional clinical pregnancy rates are maintained. For women with an elevated AMH we establish the significant merit of antagonist cycles with a reduction in complete embryo cryopreservation (excess response) and a substantive increase in fresh clinical pregnancy rates, due mainly to the absence of excess response.

Extensive evidence supporting the use of the long GnRH-agonist protocol has led to its widespread adoption as the basic standard of care (Macklon et al., 2006). In addition to the initial reports of improved success rates (Al-Inany and Aboulghar, 2002), a major clinical advantage has been the contribution to the planning of the clinical procedures including response monitoring and oocyte retrieval because the initiation of exogenous gonadotrophins after pituitary desensitization can be manipulated to suite clinical procedures, without a detrimental effect on IVF outcome (Chang et al., 1993). The use of agonist protocols across the spectrum of ovarian response is, however, associated with, on the one hand, a substantial risk of cycle cancellation due to poor response or, on the other, need for complete embryo cryopreservation to minimize the risk of OHSS (Mathur et al., 2007). The results shown

(Table IV, P < 0.001). However, in Centre 1, 18% of these good prognosis patients underwent freezing of all embryos for subsequent transfer, denying them a contribution to the fresh pregnancy rate as shown.

Evaluation of principle end-points

The aims of the adaptive programmes were to address safety and treatment burden. Comparison of the two patient cohorts as demonstrated by the statistical evaluations shown in Table IV indicates that the deployment of the antagonist protocol in predicted high responders resulted in a reduction of indicators of excess response; lower oocyte yields, negligible incidence of OHSS and ‘freeze all’ cases compared with the agonist protocol, despite deployment of the same FSH dose. The use of the antagonist strategy was associated with higher fresh clinical pregnancy rate, probably related to the 18% ‘freeze all’ rate in the agonist-controlled group. At the other extreme, the clearest distinction between the ‘reduced responder’ groups (AMH: 1–5 pmol/l) was the shorter duration of FSH injections in the antagonist-controlled groups (Table IV), and a reduction in cycle cancellation, indicating a significantly reduced treatment burden.

Table IV Patient characteristics and controlled ovarian stimulation details relative to anti-Müllerian hormone category for Centre 2

<table>
<thead>
<tr>
<th>AMH category</th>
<th>1 to &lt;5 pmol/l</th>
<th>5 to &lt;15 pmol/l</th>
<th>≥15 pmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protocol:</td>
<td>Antagonist + 300 IU</td>
<td>Agonist + 225/300 IU</td>
<td>Antagonist + 150 IU</td>
</tr>
<tr>
<td>Patients (n) % of cohort</td>
<td>61 (36.3%)</td>
<td>73 (43.4%)</td>
<td>34 (20.2%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>39.0 (32.0–41.0)</td>
<td>37 (34–39.5)</td>
<td>&lt;0.001 32.0 (30.0–35.2)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.6 ± 4.9</td>
<td>24.2 ± 3.7</td>
<td>0.63 23.6 ± 3.3</td>
</tr>
<tr>
<td>AMH (median (IQR))</td>
<td>3.0 (2.0–3.8)</td>
<td>8.7 (7.2–11.4)</td>
<td>0.93 25.8 (23.6–34.9)</td>
</tr>
<tr>
<td>Duration of stimulation (days (IQR))</td>
<td>10 (8–11)</td>
<td>&lt;0.001 11 (10–12)</td>
<td>&lt;0.001 9 (8–11)</td>
</tr>
<tr>
<td>Number of oocytes collected</td>
<td>3 (1–4)</td>
<td>&lt;0.001 6 (4–10)</td>
<td>&lt;0.001 10 (8.5–13.5)</td>
</tr>
<tr>
<td>Number of oocytes fertilized</td>
<td>2 (1–4)</td>
<td>&lt;0.001 6 (4–8)</td>
<td>0.009</td>
</tr>
<tr>
<td>Low oocyte yield n (%)</td>
<td>20/56 (35.7%)</td>
<td>&lt;0.001 1 (1.4%)</td>
<td>0.61 1/33 (3.0%)</td>
</tr>
<tr>
<td>Freeze all n (%)</td>
<td>0 (0%)</td>
<td>1.0 0 (0%)</td>
<td>0.04 0 (0%)</td>
</tr>
<tr>
<td>Hospitalized for OHSS</td>
<td>0 (0%)</td>
<td>1.0 1 (0%)</td>
<td>1.0 0 (0%)</td>
</tr>
<tr>
<td>Canceled cycle n (%)</td>
<td>5 (8.2%)</td>
<td>0.005 0 (0%)</td>
<td>1.0 1 (2.9%)</td>
</tr>
<tr>
<td>Clinical pregnancy per cycle n (%)</td>
<td>9 (14.7%)</td>
<td>0.27 24 (32.9%)</td>
<td>0.13 21 (61.7%)</td>
</tr>
<tr>
<td>Clinical pregnancy per OR n (%)</td>
<td>9/56 (16.1%)</td>
<td>0.58 24/73 (32.9%)</td>
<td>0.18 21/33 (63.6%)</td>
</tr>
<tr>
<td>Clinical pregnancy per embryo transfer n (%)</td>
<td>9/48 (18.7%)</td>
<td>0.40 24/71 (33.8%)</td>
<td>0.31 21/33 (63.6%)</td>
</tr>
</tbody>
</table>

BMI, body mass index; OHSS, ovarian hyperstimulation syndrome. Values are either absolute numbers, median (inter-quartile range) or mean ± standard deviation. Outcome percentages calculated per cycle started, rates per oocyte retrieval (OR) and embryo transfer also provided. P*: comparison with data from Centre 1.
above identify that selection of individuals who have minimal risk of
either extreme complication is feasible using AMH, and that qualifying
patients can safely undergo conventional COS for IVF with maintained
standard oocyte yields and success rates (HFEA, 2007). Differences in
the results between the two centres in this patient category were
modest and may be attributable to demographic differences and also
the origin of the FSH drug used.

Previous attempts to address alternative ‘one size fits all’ blanket
strategies to overcome agonist-related risks apparent over the whole
range of ovarian response, reduce drug costs and improve patient
acceptability have included modified natural cycle (Pelinc et al.,
2007) and mild IVF (Heijnen et al., 2007). Both these strategies are
associated with a reduction in success rates per treatment cycle. Mild
IVF with single embryo transfer required, on average, one extra treat-
ment cycle to achieve equivalent cumulative live birth rates at 1 year.
The complications associated with the modified natural cycle, apart
from its reduced pregnancy rate, appear to be poor patient acceptance
and an inconsistent ability of the GnRH antagonist to maintain LH sup-
pression when only a single follicle is maturing (Pelinc et al., 2007).

The utilization of any single approach for all individuals undergoing
COS is limited by the variability in ovarian responses, in turn dictated
by ovarian reserve. Optimization of the strategy for the first cycle of
ovarian stimulation should have tangible benefits in terms of cost-
effectiveness, patient safety and treatment burden. Furthermore,
improvements in embryo cryopreservation techniques will allow tar-
geted single embryo transfer, with reduced demand for repeated
cycles of COS and oocyte pick-up operations.

Until recently, indices of ovarian response have performed poorly
(Broekmans et al., 2006). However, the strong association of AMH
with oocyte yield independent of age, underlies its ability to predict
high responders as well as those with a sub-optimal response
(Nelson et al., 2007). Indeed women with extremely low AMH
(<1.0 pmol/l), are at substantial risk of cycle cancellation using a con-
ventional IVF approach, and despite limited numbers in the current
study we draw similar conclusions to previous studies in poor respon-
der patients—that the use of antagonists in conjunction with high dose
gonadotrophins (Mauhette and Arici, 2007) or modified natural proto-
cols (Kolibianakis et al., 2004) are not associated with an improvement
in the clinical pregnancy rate. The management of such women with
an exceptionally low ovarian reserve is challenging and frequently dis-
appointing (Tarlatzis et al., 2003), and consequently the ethics of sub-
jecting women to ovarian stimulation with a negligible prospect of
pregnancy, irrespective of cost-effectiveness arguments, are question-
able. Instead, counselling regarding oocyte donation, where available,
and limiting the treatment to the context of clinical trials of novel con-
cepts are more appropriate.

Patients with ‘reduced’ circulating concentrations of AMH (1–
4.9 pmol/l) also demonstrate sub-optimal ovarian responses and cli-
cal pregnancy rates, independent of treatment strategy (or FSH dose)
and maternal age. The use of antagonists in this study was, however,
associated with a reduction in the duration of stimulation previously
recorded in a more general population (Kolibianakis et al., 2006)
and also in oocyte yield (again recorded previously in general popu-
lations; Al-Inany et al., 2006). However, there was a reduction in
cycle cancellation, with a trend towards increase in clinical pregnancy
rates. Although antagonists have been classically used in unfavourable
patients with generally lower pregnancy rates (Huurne et al., 2007),
their role as an inferior strategy has recently been questioned (Kolibia-
nakis et al., 2006; de Klerk et al., 2007). Notably our observed
decrease in cycle cancellation with the use of antagonists in these
patients, who would be classed as ‘poor responders’, based on
oocyte yield, may eventually translate to improved outcomes with
one extra clinical pregnancy achieved for 15 patients treated. Further-
more, given the continued high incidence of failure to achieve preg-
nancy within this group, the reduction in treatment burden, physical
discomfort and depressive symptoms during and after treatment
failure with antagonists is significant (de Klerk et al., 2007).

We demonstrate that the deployment of GnRH antagonists may
show clear advantages in women with a high ovarian response to
exogenous FSH, mitigating the need for complete cryopreservation
with a concomitant increase in fresh transfer and corresponding clinical
pregnancy rates. The lower ovarian response in this category is prob-
ably mainly attributable to the deployment of the antagonist protocol
(Centre 2) although other demographic factors, or FSH origin, may
have contributed to the major differences shown. It is clear that
these desired outcomes are not achieved by simply using modest
FSH doses in cycles of COS controlled by GnRH agonists in these
patients (as in Centre 1). The substantive decrease in the risk of
ovarian stimulation and consequent maternal morbidity achieved
with the GnRH antagonist use (Al-Inany et al., 2006) is also associated
with the additional benefits in unit workload due to reduced necessity
for frozen embryo transfer. Although elective complete cryopreserva-
tion is not associated with an overall reduction in cumulative preg-
nancy rate (Vijayanthi et al., 2006), the necessity for multiple frozen
embryo transfer is costly, time-consuming and challenging for patients
(Fiddelers et al., 2007). Equally important, in this exploration of stra-
tegic adaptability, is the determination that deployment of standard
GnRH-agonist control in women with ‘normal’ AMH levels is effective
and safe and operates within predictable limits.

Given the non-randomized study design and the differences between
centres and their patients, it is difficult to infer much from apparent
differences in pregnancy rates. Furthermore, apart from the deployment
of more GnRH antagonist use in Centre 2, gonadotrophins of different
origins were also used in the two centres. However, we contend that
the main end-points of treatment burden and excessive responses to
FSH are substantive and most likely to be related to the GnRH analogue
used. In this context, potential advantages and disadvantages of
the different drugs may be explored with greater precision in the
patients of different categories defined by AMH.

Of the other markers of responses to COS, it is unlikely that early
follicular phase FSH could be deployed in the manner described here
as it is unable to differentiate between normal and high or excess
responder (Nelson et al., 2007), which is a critical component of
this concept. It remains to be seen whether AFC could be deployed
in the same manner, although so far the comparisons of AMH and
AFC have shown potential equivalence in distinguishing poor and
normal responder patients (Broer et al., 2008) and AMH is better in
identifying high responders (Nardo et al., 2008).

In summary, this large prospective cohort study, indicates that the
novel concept of categorization of patients by circulating AMH con-
centrations alone has realistic potential to indicate treatment strategies
for COS for IVF. Furthermore, it suggests that the adoption of AMH
driven differential stimulation strategies may profoundly influence
both treatment burden and clinical outcome. Finally, this cohort

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study will also inform future formal assessment in randomized controlled trials of AMH as a determinant of differential stimulation strategies, both with respect to powering of the studies and appropriate clinical strategies to be examined. Additional studies should address whether the specific critical AMH concentrations used here are ideal or applicable universally, and whether other factors may further influence decisions. The use of AMH will also provide a framework in which to explore the impact of the known characteristics of the different gonadotrophins in reproducibly defined patients.

In conclusion, we demonstrate that a single measurement of circulatory AMH can be used to individualize treatment strategies for IVF, potentially resulting in reduced clinical risk, along with optimized treatment burden, and clinical pregnancy rates, with application of GnRH-antagonist protocols appearing to be advantageous for patients at the anticipated extremes of ovarian response.

References


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