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 mini-
mizing complications and the risk of treatment failure, while maximiz-
ing the chance of pregnancy and live birth. The question explored here is
whether an adaptive strategic approach using different GnRH ana-
logue control protocols shows any advantage over simple modification of
FSH dose, in women whose response to standard COS was pre-
dicted by anti-Müllerian hormone (AMH).

Individualization of COS regimens for patients undergoing in vitro ferti-
zation (IVF) has proven difficult primarily due to the variability in the
chronological decline of the total follicular cohort between indi-
viduals (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 individual-
ization 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 folli-
cular 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 preg-
nancy and live birth (Nelson et al., 2007).

The accurate prediction of oocyte yield in COS by AMH, independ-
ent of age, and the ability of AMH to detect women at risk of
extremes of ovarian response including, at one extreme, cycle cancel-
lation, 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 con-
straint 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 prospec-
tive cohort study performed in two independent centres of an AMH
ddictated 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 cat-
ergories. The criteria relating to safety were excessive oocyte yields and
incidence of OHSS. Criteria defining treatment burden were dur-
atIon 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 strat-
egies. 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 clini-
cal 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 evalu-
atIon was taken at any stage of the menstrual cycle, no parallel data on
antral follicle count were available for comparative analyses. Table 1
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 his-
torical 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
performed on stimulation day 8, and subsequent scans were performed
ultrasound assessment of follicular growth. The first response scan was
intra-assay coefficients of variation were 5.3 and 5.4%, respectively.

Follicular development occurred after 14 days of stimulation.
retrieval and continued for 12 days. Cycles were discontinued if negligible
gesterone, 400 mg/day, intravaginally (Cyclogest, Actavis UK or Crinone
in subsequent unstimulated cycles. Luteal phase supplementation with pro-
were transferred. Good quality embryos were cryopreserved for transfer

Definitions

‘Cycle cancellation’ occurred in women receiving maximal gonado-
trophin doses at both centres when <2 mature sized follicles were
observed after 2 weeks of stimulation. For women on lower doses, the
cycle was cancelled if <3 follicles were observed.

‘Low oocyte yield’ was defined as ≤2 oocytes obtained at retrieval, as
this represented −2 SDs from the mean number of oocytes collected and
allowed for a 66% yield per follicle at oocyte retrieval using the hCG cri-
tera of three follicles ≥17 mm.

Statistical analyses

Data were analysed using standard software (Minitab 14, PA, USA and
Stata 7, TX, USA). Normally distributed data is presented as mean ± stan-
dard deviation and for non-normally distributed variables as unadjusted
median (inter-quartile range). Variables were logarithmically transformed
to obtain normal distributions. Inter-group differences were assessed by
analysis of variance or, where further predictor variables were included,
by general linear models. Multivariate analysis was performed using logistic
regression. Cases with missing data on covariates were dropped from the
multivariate analysis. Interaction terms were assessed using the likelihood
ratio test.

Results

Female partner and outcome characteristics for the cohorts are
described in Table II. The centres differed in their patient character-
istics with Centre 2 having older patients with a lower circulating
AMH concentration and undergoing a lower proportion of intracyto-
plasmic sperm injection cycles. The duration of stimulation, starting
dose and total dose of gonadotrophins was reduced in Centre 2, reflect-
ing 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.

### 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).

### Clinical procedures

Similar criteria applied in both centres for cancellation, hCG adminis-
tration, oocyte retrieval, fertilization, embryo transfer, cryopreservation
and luteal phase support procedures as in the standard IVF group.

### 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 col-
lected at oocyte retrieval and all normally fertilized (2PN) embryos were cryo-
preserved in order to minimize both the incidence and degree of OHSS.

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

AMH, anti-Müllerian hormone; FSH, follicle stimulating hormone.
### 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>Number of patients</td>
<td>370</td>
<td>168</td>
<td></td>
</tr>
<tr>
<td>Age at stimulation (years)</td>
<td>34.8 (31.9–37.7)</td>
<td>37 (34.0–39.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.0 ± 5.9</td>
<td>24.2 ± 4.1</td>
<td>0.64</td>
</tr>
<tr>
<td>Procedure</td>
<td>IVF</td>
<td>186 (50.2%)</td>
<td>103 (61.3%)</td>
</tr>
<tr>
<td></td>
<td>ICSI</td>
<td>184 (49.8%)</td>
<td>65 (38.7%)</td>
</tr>
<tr>
<td>Type of stimulation</td>
<td>Long course</td>
<td>352</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>Antagonist cycle</td>
<td>20</td>
<td>95</td>
</tr>
<tr>
<td>AMH (pmol/l)</td>
<td>11.4 (4.9–20.4)</td>
<td>7.6 (3.4–13.2)</td>
<td>0.003</td>
</tr>
<tr>
<td>AMH category</td>
<td>&lt;1 pmol/l</td>
<td>20</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>1 to &lt;5 pmol/l</td>
<td>74</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>5 to &lt;15 pmol/l</td>
<td>128</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>≥ 15 pmol/l</td>
<td>148</td>
<td>34</td>
</tr>
<tr>
<td>Starting dose of drug</td>
<td>150 IU</td>
<td>148</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>225 IU</td>
<td>128</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>300 IU</td>
<td>0</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>375 IU</td>
<td>74</td>
<td>0</td>
</tr>
<tr>
<td>Duration of stimulation (days)</td>
<td>14 (12.2–15.0)</td>
<td>10 (9–12)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total dose (IU)</td>
<td>2925 (2250–3900)</td>
<td>2737 (1856–3300)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stimulation outcomes¹</td>
<td>Freeze all</td>
<td>41</td>
<td>1 (11.1%)</td>
</tr>
<tr>
<td></td>
<td>Cancelled cycle</td>
<td>36 (10.8%)</td>
<td>6 (3.6%)</td>
</tr>
<tr>
<td>Number of oocytes</td>
<td>11 (9–16)</td>
<td>5 (3–9.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Number with &gt;21 oocytes</td>
<td>41</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Number of oocytes inseminated</td>
<td>10 (9–14)</td>
<td>4 (2–6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Number of oocytes normally fertilized</td>
<td>6 (3–10)</td>
<td>4 (2–6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Normal fertilization rate (%)</td>
<td>IVF</td>
<td>71 (58–83)</td>
<td>83.3 (59.6–100)</td>
</tr>
<tr>
<td></td>
<td>ICSI</td>
<td>62 (44–75)</td>
<td>67 (50–92)</td>
</tr>
<tr>
<td>Number of oocytes normally fertilized</td>
<td>IVF</td>
<td>7 (4–11)</td>
<td>4 (2–5)</td>
</tr>
<tr>
<td></td>
<td>ICSI</td>
<td>5 (3–8)</td>
<td>4 (2–7)</td>
</tr>
<tr>
<td>Number of embryos transferred</td>
<td>1</td>
<td>30</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>258</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Patients with frozen embryos</td>
<td>116 (31.3%)</td>
<td>32 (20.2%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Number of embryos frozen</td>
<td>6 (4–13)</td>
<td>3 (2–3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cohort outcomes¹</td>
<td>No transfer¹</td>
<td>81 (21.9%)</td>
<td>16 (9.5%)</td>
</tr>
<tr>
<td></td>
<td>Not pregnant</td>
<td>166 (44.8%)</td>
<td>84 (50%)</td>
</tr>
<tr>
<td></td>
<td>Ectopic</td>
<td>2 (0.5%)</td>
<td>1 (0.5%)</td>
</tr>
<tr>
<td></td>
<td>Miscarriage–FH seen</td>
<td>2 (0.5%)</td>
<td>1 (0.5%)</td>
</tr>
<tr>
<td></td>
<td>Miscarriage–no sac seen</td>
<td>25 (6.8%)</td>
<td>11 (6.5%)</td>
</tr>
<tr>
<td></td>
<td>Miscarriage–sac seen</td>
<td>6 (1.6%)</td>
<td>1 (0.5%)</td>
</tr>
<tr>
<td></td>
<td>Clinical pregnancy</td>
<td>88 (23.8%)</td>
<td>54 (32.1%)</td>
</tr>
<tr>
<td></td>
<td>Clinical pregnancy per OR</td>
<td>88/289 (26.9%)</td>
<td>54/162 (33.3%)</td>
</tr>
<tr>
<td></td>
<td>Clinical pregnancy per embryo transfer</td>
<td>88/330 (30.4%)</td>
<td>54/152 (35.5%)</td>
</tr>
</tbody>
</table>

Values are presented as median (inter-quartile range) or mean ± SD. ¹Outcome percentages calculated per cycle started (n = 370 and 168), rates per oocyte retrieval (OR) and embryo transfer also provided. ²No 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 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 <
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 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 also 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 = 0.08].

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.

<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>Agonist + 375 IU</td>
<td>Agonist + 225 IU</td>
<td>Agonist + 150 IU</td>
</tr>
<tr>
<td>Patients (% of cohort)</td>
<td>74 (20%)</td>
<td>128 (34.6%)</td>
<td>148 (40%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>37.3 (34.6–39.3)</td>
<td>35.1 (32.7–37.3)</td>
<td>32.8 (28.8–36.2)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.9 ± 7.5</td>
<td>23.8 ± 5.6</td>
<td>24.1 ± 5.6</td>
</tr>
<tr>
<td>AMH (median (IQR))</td>
<td>2.6 (1.8–3.7)</td>
<td>9.2 (6.8–11.9)</td>
<td>22.4 (18.3–29.9)</td>
</tr>
<tr>
<td>Duration of stimulation (days (IQR))</td>
<td>14 (13–15)</td>
<td>14 (13–15)</td>
<td>13 (12–14)</td>
</tr>
<tr>
<td>Number of oocytes collected</td>
<td>5 (3–7)</td>
<td>10 (7–15)</td>
<td>14 (10–19)</td>
</tr>
<tr>
<td>Number of oocytes fertilized</td>
<td>3 (2–4)</td>
<td>6 (3–9)</td>
<td>7 (5–11)</td>
</tr>
<tr>
<td>Low oocyte yield n (%)</td>
<td>7/55 (12.7%)</td>
<td>3 (2.3%)</td>
<td>4 /144 (2.8%)</td>
</tr>
<tr>
<td>Freeze all n (%)</td>
<td>1 (1.4%)</td>
<td>13 (10.1%)</td>
<td>27 (18.2%)</td>
</tr>
<tr>
<td>Hospitalized for OHSS</td>
<td>0 (0%)</td>
<td>3 (2%)</td>
<td>20 (13.9%)</td>
</tr>
<tr>
<td>Cancelled cycle n (%)</td>
<td>19 (25.7%)</td>
<td>3 (2.3%)</td>
<td>4 (2.7%)</td>
</tr>
<tr>
<td>Clinical pregnancy per cycle n (%)</td>
<td>6 (8.1%)</td>
<td>29/125 (23.2%)</td>
<td>47 (31.8%)</td>
</tr>
<tr>
<td>Clinical pregnancy per OR n (%)</td>
<td>6/55 (10.9%)</td>
<td>29/112 (25.9%)</td>
<td>47/144 (32.6%)</td>
</tr>
<tr>
<td>Clinical pregnancy per embryo transfer n (%)</td>
<td>6/54 (11.1%)</td>
<td>128 (34.6%)</td>
<td>47/117 (40.1%)</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.
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</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.6 ± 4.9</td>
<td>24.2 ± 3.7</td>
<td>0.63</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</td>
</tr>
<tr>
<td>Duration of stimulation (days (IQR))</td>
<td>10 (8–11)</td>
<td>&lt;0.001</td>
<td>11 (10–12)</td>
</tr>
<tr>
<td>Number of oocytes collected</td>
<td>3 (1–4)</td>
<td>&lt;0.001</td>
<td>6 (4–10)</td>
</tr>
<tr>
<td>Number of oocytes fertilized</td>
<td>2 (1–4)</td>
<td>&lt;0.001</td>
<td>4 (3–6)</td>
</tr>
<tr>
<td>Low oocyte yield n (%)</td>
<td>0 (0%)</td>
<td>1.0</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Hospitalized for OHSS</td>
<td>0 (0%)</td>
<td>0.005</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Cancelled cycle n (%)</td>
<td>5 (8.2%)</td>
<td>&lt;0.001</td>
<td>1 (1.4%)</td>
</tr>
<tr>
<td>Clinical pregnancy per cycle n (%)</td>
<td>9 (14.7%)</td>
<td>0.27</td>
<td>24 (32.9%)</td>
</tr>
<tr>
<td>Clinical pregnancy per OR n (%)</td>
<td>9/56 (16.1%)</td>
<td>0.58</td>
<td>24/73 (32.9%)</td>
</tr>
<tr>
<td>Clinical pregnancy per embryo transfer n (%)</td>
<td>9/48 (18.7%)</td>
<td>0.40</td>
<td>24/71 (33.8%)</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. *: comparison with data from Centre 1.

(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 increased incidence of OHSS and ‘freeze all’ cases compared 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

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.

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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 (Pelincck 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 treatment 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 suppression from its reduced pregnancy rate, appear to be poor patient acceptance and a concomitant increase in fresh transfer and corresponding clinical pregnancy rates. The lower ovarian response in this category is probably 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 cryopreservation is not associated with an overall reduction in cumulative pregnancy 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 strategic 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 concentrations 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...
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 circulating 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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