

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 (Veenen *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 (Kolibanakis *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 \geq 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 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 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).

Table 1 Deployment of GnRH analogues and doses of follicle stimulating hormone in the groups categorized by anti-Müllerian hormone in the two centres

AMH group (pmol/l)	Centre 1		Centre 2	
	FSH daily dose	GnRH analogue	FSH daily dose	GnRH analogue
< 1.0	375	Antagonist	(Modified natural cycle)	(Antagonist)
1.0 to <5	375	Agonist	300	Antagonist
5.0 to <15	225	Agonist	225	Agonist
≥ 15.0	150	Agonist	150	Antagonist

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

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 (E_2) 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 E_2 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 E_2 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 E_2 exceeded 200 pg/ml (700 pmol/l).

Clinical procedures

Similar criteria applied in both centres for cancellation, hCG administration, 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 collected at oocyte retrieval and all normally fertilized (2PN) embryos were cryopreserved in order to minimize both the incidence and degree of OHSS.

'Cycle cancellation' occurred in women receiving maximal gonadotrophin 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 criteria 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 ± standard 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 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 gonadotrophins 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.

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

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

Values are presented as median (inter-quartile range) or mean ± SD. ^aOutcome percentages calculated per cycle started ($n = 370$ and 168), rates per oocyte retrieval (OR) and embryo transfer also provided. ^bNo 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 $r = -0.50$, $P < 0.001$).

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 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 III Patient characteristics and controlled ovarian stimulation details relative to anti-Müllerian hormone category for Centre 1

AMH category	1 to <5 pmol/l	5 to <15 pmol/l	≥ 15 pmol/l
Protocol	Agonist + 375 IU	Agonist + 225 IU	Agonist + 150 IU
Patients (n) % of cohort	74 (20%)	128 (34.6%)	148 (40%)
Age (years)	37.3 (34.6–39.3)	35.1 (32.7–37.3)	32.8 (28.8–36.2)
BMI (kg/m ²)	23.9 \pm 7.5	23.8 \pm 5.6	24.1 \pm 5.6
AMH (median (IQR))	2.6 (1.8–3.7)	9.2 (6.8–11.9)	22.4 (18.3–29.9)
Duration of stimulation (days (IQR))	14 (13–15)	14 (13–15)	13 (12–14)
Number of oocytes collected	5 (3–7)	10 (7–15)	14 (10–19)
Number of oocytes fertilized	3 (2–4)	6 (3–9)	7 (5–11)
Low oocyte yield n (%)	7/55 (12.7%)	3 (2.3%)	4 / 144 (2.8%)
Freeze all n (%)	1 (1.4%)	13 (10.1%)	27 (18.2%)
Hospitalized for OHSS	0 (0%)	3 (2%)	20 (13.9%)
Cancelled cycle n (%)	19 (25.7%)	3 (2.3%)	4 (2.7%)
Clinical pregnancy per cycle n (%)	6 (8.1%)	29/125 (23.2%)	47 (31.8%)
Clinical pregnancy per OR n (%)	6/55 (10.9%)	29/112 (25.9%)	47/144 (32.6%)
Clinical pregnancy per embryo transfer n (%)	6/54 (11.1%)	128 (34.6%)	47/117 (40.1%)

BMI, body mass index, OHSS, ovarian hyperstimulation syndrome. Values are either absolute numbers, median (inter-quartile range) or mean \pm 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

AMH category: Protocol:	1 to <5 pmol/l		5 to <15 pmol/l		≥ 15 pmol/l	
	Antagonist + 300 IU	P*	Agonist + 225/300 IU	P*	Antagonist + 150 IU	P*
Patients (n) % of cohort	61 (36.3%)		73 (43.4%)		34 (20.2%)	
Age (years)	39.0 (32.0–41.0)	0.005	37 (34–39.5)	<0.001	32.0 (30.0–35.2)	0.94
BMI (kg/m ²)	24.6 ± 4.9	0.57	24.2 ± 3.7	0.63	23.6 ± 3.3	0.59
AMH (median (IQR))	3.0 (2.0–3.8)	0.40	8.7 (7.2–11.4)	0.93	25.8 (23.6–34.9)	0.018
Duration of stimulation (days (IQR))	10 (8–11)	<0.001	11 (10–12)	<0.001	9 (8–11)	<0.001
Number of oocytes collected	3 (1–4)	<0.001	6 (4–10)	<0.001	10 (8.5–13.5)	<0.001
Number of oocytes fertilized	2 (1–4)	0.10	4 (3–6)	0.027	6 (4–8)	0.009
Low oocyte yield n (%)	20/56 (35.7%)	<0.001	1 (1.4%)	0.61	1/33 (3.0%)	1.0
Freeze all n (%)	0 (0%)	1.0	0 (0%)	0.04	0 (0%)	0.003
Hospitalized for OHSS	0 (0%)	1.0	1 (0%)	1.0	0 (0%)	0.021
Cancelled cycle n (%)	5 (8.2%)	0.005	0 (0%)	1.0	1 (2.9%)	1.0
Clinical pregnancy per cycle n (%)	9 (14.7%)	0.27	24 (32.9%)	0.13	21 (61.7%)	0.002
Clinical pregnancy per OR n (%)	9/56 (16.1%)	0.58	24/73 (32.9%)	0.18	21/33 (63.6%)	0.001
Clinical pregnancy per embryo transfer n (%)	9/48 (18.7%)	0.40	24/71 (33.8%)	0.31	21/33 (63.6%)	0.019

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.

(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.

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

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 (Pelinck *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 when only a single follicle is maturing (Pelinck *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 targeted 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 conventional IVF approach, and despite limited numbers in the current study we draw similar conclusions to previous studies in poor responder patients—that the use of antagonists in conjunction with high dose gonadotrophins (Mahutte and Arici, 2007) or modified natural protocols (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 disappointing (Tarlantzis *et al.*, 2003), and consequently the ethics of subjecting women to ovarian stimulation with a negligible prospect of pregnancy, irrespective of cost-effectiveness arguments, are questionable. Instead, counselling regarding oocyte donation, where available, and limiting the treatment to the context of clinical trials of novel concepts are more appropriate.

Patients with 'reduced' circulating concentrations of AMH (1–4.9 pmol/l) also demonstrate sub-optimal ovarian responses and clinical 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 populations; 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 (Huime *et al.*, 2007),

their role as an inferior strategy has recently been questioned (Kolibianakis *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. Furthermore, given the continued high incidence of failure to achieve pregnancy 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 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 (Vyjayanthi *et al.*, 2006), the necessity for multiple frozen embryo transfer is costly, time-consuming and challenging for patients (Fiddlers *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.

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