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REVIEW

Can anti-Müllerian hormone concentrations be used to determine gonadotrophin dose and treatment protocol for ovarian stimulation?

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Abstract The ability to predict the response potential of women to ovarian stimulation may allow the development of individualized ovarian stimulation protocols. This tailored approach to ovarian stimulation could reduce the incidence of ovarian hyperstimulation syndrome in women predicted to have an excessive response to stimulation or could improve pregnancy outcomes in women classed as poor responders. Namely, variation of the type of gonadotrophin-releasing hormone (GnRH) analogue or the form and dosage of gonadotrophin used for stimulation could be adjusted according to an individual's response potential. The serum concentration of anti-Müllerian hormone (AMH) is established as a reliable marker of ovarian reserve, with decreasing concentrations correlated with reduced response potential. This review examines the current evidence evaluating individualized ovarian stimulation protocols using AMH concentration as a predictive marker of ovarian response. The rationale behind why specific treatment protocols based on individual response potential may be more suitable is also discussed. Based on current evidence, it appears that the

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use of AMH serum concentrations to predict ovarian response and optimize treatment strategies is a promising approach for improving pregnancy outcomes in women undergoing ovarian stimulation. However, prospective randomized controlled trials evaluating this approach are needed before any firm conclusions can be drawn.

KEYWORDS: anti-Müllerian hormone (AMH), gonadotrophin-releasing hormone, GnRH agonist, GnRH antagonist, ovarian response, ovarian stimulation

Introduction

It is now established that the serum concentration of anti-Müllerian hormone (AMH) can be used as a predictor of ovarian response to gonadotrophins during ovarian stimulation and, therefore, has the potential to determine the optimal treatment protocol for an individual undergoing assisted reproduction. This knowledge of a woman's response potential could be used to address safety and efficacy issues associated with ovarian stimulation by varying the type of gonadotrophin-releasing hormone (GnRH) analogue used or the type and daily dose of gonadotrophin. In May 2010, a panel of international experts gathered for a consensus meeting to discuss how prior knowledge of a woman's response to exogenous FSH injections could be used to determine the most suitable mode of ovarian stimulation. The aims of the meeting were to discuss the findings of work carried out so far and to address how future studies could provide a firm scientific basis for the use of individualized ovarian stimulation protocols. The meeting enjoyed extensive debate and explored numerous areas for future explorations and the summary is reported here.

Background

There has been much debate regarding the relative merits of the two basic methods of LH suppression in ovarian stimulation, as well as the optimal dose and origin of FSH to use. When applied in comprehensive treatment programmes, the consensus is that the use of the original long GnRH agonist protocol (Fleming et al., 1982) is associated with a greater oocyte yield and a higher risk of ovarian hyperstimulation syndrome (OHSS) compared with use of GnRH antagonists to control the LH surge (Al-Inany and Aboulghar, 2001). Despite the improved safety advantage with the GnRH antagonist protocol, the majority of cycles are performed using GnRH agonist down-regulation. For example, data from the audited German IVF Registry database shows that 54.8% of IVF cycles were performed with a GnRH agonist compared with 31.5% with a GnRH antagonist, for the period of 1998–2008 (Bals-Pratsch et al., 2010). This is probably due to the technical simplicity of the agonist protocol and also the increased incidence of suboptimal egg yields and reduced pregnancy rate using the antagonist protocol (Al-Inany et al., 2006, 2007). However, there is now increasing interest in the possibility of identifying and stratifying patients according to the most appropriate method of ovarian stimulation for the individual patient, with attention being paid to three principal critical criteria — efficacy, safety and treatment burden. The logical design of the individualized strategic approach should reflect the importance of safety in women likely to have an excessive response to exogenous FSH, while aiming to maximize egg yields in women in whom the response is limited. It is now accepted that the two best markers of ovarian reserve, antral follicle count (AFC) and circulating AMH concentrations, also prove to be strong markers of ovarian response to stimulation.

The dimeric glycoprotein AMH is a member of the transforming growth factor-β superfamily of peptide growth and differentiation factors (Cate et al., 1986), and it is likely that it plays a pivotal role in the regulation of folliculogenesis (Baarends et al., 1995; Grootegoed et al., 1994). In women, ovarian follicular granulosa cells produce AMH, with maximal expression occurring in late pre-antral and small antral follicles (<4 mm in diameter). Expression declines as pre-ovulatory follicles mature (Laven et al., 2004; Weenen et al., 2004) or undergo atresia. Anti-Müllerian hormone has also been proposed to inhibit the sensitivity of antral follicles to FSH in cyclic recruitment (Pellatt et al., 2010) and also to inhibit the aromatase enzyme, reducing oestrogen biosynthesis (Eilso Nielsen et al., 2010).

It has recently been shown, with powerful databases (a population study of over 9000 infertile women), that the age-related decline in circulating AMH serum concentrations parallels that of the non-growing follicles, and can, therefore, claim to be a marker of ovarian reserve (Nelson et al., 2011b). This model has recently been externally verified in over 15,000 patients (Nelson et al., 2011a). Even before this demonstration of principle, numerous studies showed that AMH concentrations were able to predict the number of oocytes collected after ovarian stimulation (a comprehensive review of this topic was published by La Marca et al., 2010). Although serum concentrations of AMH are indicative of both the ovarian reserve and the number of oocytes obtained after ovarian stimulation, studies to determine potential roles as markers of oocyte quality and implantation potential, independent of female age, require more extensive and systematic analyses than are so far available.

Prior to the recent developments of AMH and AFC as predictors of ovarian response to stimulation, the only indicators available were female age and serum FSH concentration, the latter of which is profoundly influenced by phase of cycle. In contrast, circulating concentrations of AMH are consistent across the menstrual cycle (Cook et al., 2000; La Marca et al., 2004, 2006). Although it is likely that women with a higher basal AMH concentration will experience greater variation in AMH values over time, a consistent pattern of variation has not been observed, apart from a possible modest dip around the mid-point of the menstrual cycle (Hehenkamp et al., 2006; Sowers et al., 2010). With the introduction of three-dimensional technology, measurement of the AFC has recently been shown to have similar discriminatory power to AMH (Broer et al., 2011; van Disseldorp et al., 2010). However, without the use of this technique, the AFC tends to have higher cycle-to-cycle variation, intra-cycle variation and inter-operator errors

compared with measurement of AMH concentrations (Broer et al., 2011; van Disseldorp et al., 2010).

AMH serum concentrations and prediction of response to ovarian stimulation

The first indication that AMH could predict responses to ovarian stimulation came from a small study by Seifer et al. (2002). Potential quantitative aspects of follicular recruitment were explored further by Fleming et al. (2006) and the first large prospective assessment of response categorization was reported by Nelson et al. (2007). This latter study demonstrated that AMH is a reliable marker, able to distinguish between responses to ovarian stimulation, with pragmatic values reported as follows: <1.1 pmol/l, non-response (failure to achieve criteria for human chorionic gonadotrophin, HCG); 1–5 pmol/l, poor response (≤2 eggs or fewer at oocyte retrieval); 5–15 pmol/l, normal response (3–20 eggs at oocyte retrieval, median = seven eggs); >15 pmol/l, high/excessive response (over 20 eggs at oocyte retrieval). These results are supported by meta-analyses that have demonstrated that the AMH serum concentration can predict both poor (Broer et al., 2009) and excessive (Broer et al., 2011) responses to stimulation with equivalent levels of accuracy and clinical value as the AFC.

AMH as a predictor of follicular recruitment

A key feature of the use of GnRH agonists prior to ovarian stimulation is maximal follicular recruitment. The number of follicles recruited per day during GnRH agonist ovarian stimulation differs by 1.9 between younger (<30 years old) and older (>35 years old) women (Fleming et al., 2006). In comparison, when categorized by different AMH concentrations, this differential is 2.9 follicles per day, indicating that the AMH concentration is a better predictor of follicular recruitment than age (Fleming et al., 2006). Furthermore, after 10 days of FSH stimulation, higher circulating concentrations of AMH are correlated with increased numbers of follicles recruited and, thus, an increased risk of excessive response (≥20 oocytes) and OHSS (Fleming et al., 2006).

However, at extremely low concentrations of AMH, this test may lose some of its sensitivity. For example, AMH concentrations in women nearing menopause, although universally low, have been observed to vary considerably during longitudinal observations (Robertson et al., 2011). The authors of this study proposed that these distinct AMH patterns that emerge as ovarian follicle reserve decreases with age may be reflective of the intermittent pattern of the emergence of follicles close to menopause. Therefore, it may be more accurate to say that AMH is a marker of potentially functional follicles rather than an indicator of total follicle number.

AMH as a predictor of pregnancy in IVF

It is generally considered that age is the primary driver of treatment success in IVF programmes. However, it is now clear that this phenomenon cannot be considered in isolation. Recent explorations of large databases have determined that oocyte yield plays a critical role in predicting IVF success (Sunkara et al., 2011). As AMH predicts oocyte yield, it is likely that AMH (or any similar marker of ovarian reserve) will predict the likelihood of success prior to treatment. In this regard, La Marca et al. (2011) explored this dynamic and produced a model showing that older woman with a lower AMH concentration showed the lowest chance of success (in the region of 5% per cycle) while younger woman with a higher AMH concentration showed the highest chance of a successful outcome (La Marca et al., 2011). The critically important aspect of this model is that, in the middle range, it was AMH (possibly through egg yield) rather than young age that was the better predictor of success.

Ovarian reserve testing and the implications for a strategic approach to ovarian stimulation

When the concentration of FSH in the circulation increases to a concentration that recruits all FSH-sensitive follicles to grow, the result is multiple follicular development (Baird, 1983). Correspondingly, the response to FSH injections is dictated by ovarian FSH exposure and the functional ovarian reserve, and this varies widely between individuals (te Velde and Pearson, 2002). Ovarian reserve tests provide knowledge of a patient's response potential, allowing for the management of expectations and alteration of treatment strategies with appropriate manipulation of gonadotrophin treatments. For patients predicted to have a poor ovarian response, clinicians may decide to counsel patients not to proceed with treatment or alter their treatment protocol or even to suggest egg donation at an early stage in their management. For patients anticipated to have an excessive ovarian response, clinicians can provide guidance on the potential risks associated with treatment in addition to increased monitoring during treatment, and can recommend alterations in treatment schedules accordingly.

This logical approach to a stratified treatment programme was first explored by Nelson et al. (2009) using AMH as the only indicator of response with which to decide on the most appropriate treatment strategy. Of course, there will be many alternative strategies to be explored, but the basis underlying this programme was to use a protocol involving moderate follicular recruitment in high-responding women and a simple maximizing approach in normal-responding women and to lower the treatment burden of ovarian stimulation in women predicted to have a reduced response. Underlying this proposal is a desire for the conventional IVF laboratory to work with between 5 and 15 eggs whilst maintaining a low risk of excessive responses and OHSS.

Much of the data discussed below was derived from an extended database of results from the same programme. A number of valuable observations can be drawn from these analyses, providing guidance for potentially fruitful prospective examinations.

The predicted poor response and an individualized approach

An important distinction of the poor responder in ovarian stimulation is a previous treatment cycle with a low egg

yield, a failure to achieve a viable pregnancy and a desire to repeat the treatment procedure. This combination is not a good basis to conduct prospective scientific analyses; nevertheless, useful data can still be derived from such studies. There are conflicting results regarding the merit of increasing the FSH dose for patients categorized as poor responders or those predicted to have a poor response. In a study by Popovic-Todorovic et al. (2003), patients ($n = 262$) received an individualized recombinant FSH dose (100–250 IU/day), based on a model incorporating the AFC, ovarian volume, ovarian flow, female age and smoking habits, or received a standard dose of 150 IU/day (Popovic-Todorovic et al., 2003). The individualized dose reduced the need for dose adjustments during stimulation and resulted in higher ongoing pregnancy rates. Moreover, a significantly lower proportion of patients receiving an individualized dose of recombinant FSH experienced a poor response to stimulation compared with those treated with a standard dose (1.5% versus 10.7%, respectively; $P < 0.05$).

In apparent contrast, in a smaller study by Klinkert et al. (2005), increasing the starting dose of recombinant FSH from 150 to 300 IU/day ($n = 52$) in predicted poor responders (AFC <5 follicles between 2–5 mm) did not improve pregnancy rates (Klinkert et al., 2005). This may appear to be a divergent finding, but one explanation could be that the Popovic-Todorovic study included well-defined normal ovulatory women, with normal cycles and basal FSH concentrations, whereas the Klinkert study examined an older group of women with elevated FSH values, who were predicted to be low-responders. The clinical benefits of increasing the FSH dose, however, remain unproven. In a pseudo-randomized trial ($n = 122$; Lekamge et al., 2008), women predicted to have a poor response based on their AMH concentration did not experience improvements in oocyte yield or pregnancy rates with increased recombinant FSH stimulation (150 versus 200–300 IU/day). A recent meta-analysis (Pandian et al., 2010) concluded that there is currently insufficient evidence to recommend a particular treatment for women defined as poor responders. However, a recent Cochrane review (Duffy et al., 2010) of a small number of studies addressing the possible use of growth hormone in these patients suggested that it may improve the clinical outcome of women undergoing IVF. A clonidine test may identify which patients are most likely to benefit from treatment with growth hormone in combination with gonadotrophin stimulation (Blumenfeld et al., 1991), as this combination appears to have a synergistic effect on pregnancy outcomes in clonidine-negative patients. These results would need to be confirmed in adequately powered randomized controlled trials, which would also need to provide reassurance on the safety profile of this treatment strategy.

Despite the limitations of these studies, it can be concluded that increased FSH doses will achieve, at best, modest benefits in these patients and that prospective evaluations of the relative benefits of different methods of LH control (GnRH agonist or antagonist) should also be explored. Overall, more randomized controlled trials are needed to establish the effectiveness of individualizing the FSH dose or whether the AMH serum concentration, in association with other factors such as body mass index, could be used to optimize the dosage of gonadotrophins in order to improve pregnancy outcomes in poor responders.

Indications from the AMH-based programme: poor responders (AMH <5 pmol/l)

The pragmatic determinant of a poor response to ovarian stimulation determined by Nelson et al. (2007) was an upper AMH value of 5 pmol/l. For women <40 years of age, AMH serum concentrations <5 pmol/l were associated with reduced egg yields and clinical pregnancy rates compared with women with higher AMH values. However, in women >40 years of age, AMH serum concentrations <5 pmol/l are within the normal range and are more reflective of the overall decline in reproductive capacity with age. These observations suggest that a reduced ovarian reserve may have implications for adverse embryo quality in younger women.

In the study by Nelson et al. (2009; **Table 1**), treatment with a GnRH antagonist protocol reduced the burden of treatment in poor responders compared with a GnRH agonist protocol, but did not influence either the proportion of cases achieving egg collection or pregnancy rates (Nelson et al., 2009). The GnRH antagonist protocol required fewer days of FSH stimulation in reduced responders; nevertheless, the prognosis for these patients remained poor, with clinical pregnancy rates reaching a maximum of 16% (Nelson et al., 2009). Moreover, a LH surge occurred in 9% of poor responders treated with GnRH antagonists, which was discomforting and gives cause for concern.

The ability to reliably identify poor responders prior to treatment could allow prospective studies comparing different approaches to ovarian stimulation to be conducted without patients needing to have had a prior unsuccessful cycle (a requirement that undermines the scientific integrity of such studies).

Patients with extremely low AMH concentrations (<1.0 pmol/l) and pregnancy potential

In the study by Nelson et al. (2009), patients with AMH concentrations <1.0 pmol/l were predicted to have a negligible chance of response. Indeed, no pregnancies were achieved in this group, regardless of the treatment protocol used (**Table 1**), although it should be noted that only 26 patients were included in this category. Furthermore, in a recent retrospective study by Weghofer et al. (2011), patients with extremely low AMH concentrations (defined as 0.7–2.9 pmol/l) were shown to have a moderate but reasonable chance of pregnancy (7.9% per cycle started) when treated with a microdose agonist protocol, a daily gonadotrophin dose of 600 IU and dehydroepiandrosterone supplementation (Weghofer et al., 2011). Age was also a key factor affecting the chance of pregnancy in patients with AMH concentrations 0.7–2.9 pmol/l, as clinical pregnancy rates per cycle started were significantly lower in women aged >42 years compared with those aged ≤42 years (11.0% versus 3.7%, $P = 0.031$). Therefore, as patients with AMH concentrations <1.0 pmol/l may still have a reasonable chance of achieving a clinical

Table 1 Treatment strategy based on predicted response due to serum anti-Müllerian hormone concentration used in the two-centre trial of Nelson et al. (2009).

Predicted response	*AMH concentration (pmol/l)*	*Treatment protocol*				
		Centre 1		*Centre 2*		*Optimal*
		GnRH analogue	*FSH dose (IU)*	*GnRH control*	*FSH dose (IU)*	
Negligible	<1	Antagonist	375	Modified natural cycle		No treatment
Reduced	1—<5	Agonist	300	Antagonist	300	Antagonist
Normal	5—15	Agonist	225	Agonist	225	Agonist
High	>15	Agonist	150	Antagonist	150	Antagonist

Table adapted from Nelson et al. (2009). AMH measured using the DSL assay. AMH = Anti-Müllerian hormone; GnRH = gonadotrophin-releasing hormone.

pregnancy, this should not be used as a factor driving the decision to withhold fertility treatment.

Improving the outcome for women classed as high/excessive responders

A meta-analysis by Broer et al. (2011), including nine studies and 1500 patients, established that AMH is a good predictor of excessive ovarian response (Broer et al., 2011). Thus, in women with a high AMH concentration, an individualized (reduced) dose of FSH could potentially improve both safety and pregnancy outcomes. However, well-designed randomized controlled trials are needed to confirm whether this is an effective strategic approach and also to further explore sources of complications.

In a preliminary prospective study of women aged <35 years old undergoing assisted reproductive technology, the CONSORT dosing algorithm (incorporating basal FSH, body mass index, age and AFC) was used to predict the optimal FSH starting dose for women at risk of developing OHSS (Olivennes et al., 2009). Individualizing the FSH dose, based on the CONSORT algorithm, resulted in adequate oocyte yields and good pregnancy rates (Olivennes et al., 2009). However, there were high rates of cancellations in the low-dose group (75 IU FSH) owing to inadequate response, and OHSS still occurred in a significant proportion of the patients. Moreover, as there was no control group, the study lacked comparative evidence of improved clinical outcomes. It has been suggested that incorporating a powerful marker such as AMH into the CONSORT algorithm could potentially improve its clinical efficacy (La Marca et al., 2010).

Indications from the AMH-based programme: high responders (AMH >15 pmol/l)

The pragmatic determinant of patients at risk of an excessive response determined by Nelson et al. (2007) was a AMH value >15 pmol/l. The ovarian response to moderate doses of FSH differed profoundly between antagonist and agonist cycles in this group of patients due to the biology of follicular recruitment. In GnRH agonist cycles, all follicles achieving constitutive sensitivity to FSH are recruited to grow and develop when the threshold circulating concentration of FSH is breached. This results in maximal follicular recruitment and, therefore, women with a high ovarian reserve will be at risk of excessive follicular recruitment. At the start of cycles using a GnRH antagonist protocol, there is significant follicular recruitment and selection undertaken by endogenous control mechanisms prior to starting the FSH injections. This leads to a smaller leading cohort of follicles — a potential advantage in women with a high ovarian reserve.

In the study by Nelson et al. (2009; **Table 1**), the safety record of GnRH antagonist ovarian stimulation was superior to that of GnRH agonist cycles for the treatment of high responders. The antagonist protocol eliminated the need for complete cryopreservation of embryos due to excessive response ($P < 0.001$), coupled with significant reductions in the incidence of hospitalizations owing to the development of OHSS (13.9% in the agonist group versus 0.0% in the antagonist group; $P = 0.02$) (Nelson et al., 2009). These results are consistent with those of other studies that have demonstrated a reduced incidence of OHSS with GnRH antagonist protocols compared with agonist protocols (Kolibianakis et al., 2006; Lainas et al., 2007).

The antagonist protocol, in high responders, was also associated with higher fresh-cycle clinical pregnancy rates (odds ratio 4.40, 95% confidence interval 1.95—9.93; $P < 0.001$), required fewer days of FSH stimulation and was associated with lower egg yields compared with the agonist protocol (Nelson et al., 2009). Furthermore, patients with low egg yields using this protocol achieved pregnancy rates comparable with those with normal or high egg yields (Nelson et al., 2009). Analyses of the extended programme have identified a fourth response group — patients with AMH serum concentrations >40 pmol/l remain at risk of developing an excessive response and OHSS despite the use of a 'mild' antagonist protocol.

Further evidence of the value of tailored stimulation protocols based on patients' AMH values comes from a recent retrospective study conducted by Yates et al. (2011). This study evaluated data from 769 women undergoing IVF at one UK tertiary care unit, where 346 women underwent conventional stimulation protocols and 423 were treated under AMH-tailored protocols (Yates et al., 2011). Pregnancy rates per cycle started and live birth rates were significantly higher in women undergoing AMH-tailored protocols compared with those receiving conventional stimulation (27.7% versus 17.9%, $P = 0.002$, and 23.9% versus 15.9%, $P = 0.007$, respectively). Furthermore, the incidence of OHSS was

significantly lower in the AMH group (6.9 versus 2.3%, $P = 0.002$). The cost of fertility drug treatment was 29% lower per patient and the overall cost for the clinical management of OHSS was reduced by 43% in the AMH group. The authors of this study concluded that the use of GnRH antagonist protocols as part of the AMH-tailored treatment strategy may have contributed to some of these observed improvements. These results would have to be validated in prospective studies to determine whether AMH-based protocols improve the outcomes for predicted high responders.

A retrospective evaluation of patients in the randomized assessor-blinded controlled trials MERiT (GnRH agonist) and MEGASET (GnRH antagonist) assessed hyper-responder patients with AMH values >37.4 pmol/l (La Marca et al., 2012). This evaluation concluded that patients above this AMH threshold who were treated with recombinant FSH had a significantly lower live birth rate than those treated with highly purified human menopausal gonadotrophin (22% versus 34%, $P = 0.015$). As expected, patients in the recombinant FSH treatment group had higher numbers of oocytes retrieved and a higher proportion of excessive responders.

Current evidence suggests that a simple AMH test may identify patients whose treatment will be safer using a mild GnRH antagonist protocol. Furthermore, the AMH test may be able to identify those patients who are at risk of excessive response despite using this protocol. Correspondingly, prospective examinations can now be undertaken to examine the merits of different FSH dose regimes and also to identify whether the source of FSH and LH activity may confers safety and clinical advantages as hypothesised previously (Fleming and Jenkins, 2010). Such studies may also be able to establish whether the AMH-based tailored approach to stimulation will result in significant cost benefits for patients undergoing assisted reproduction treatment.

Improving the outcome for women classed as normal or safe responders

Indications from the AMH-based programme: normal responders (AMH 5—15 pmol/l)

Patients with a AMH concentration 5—15 pmol/l, when treated with a conventional long GnRH agonist protocol, were identified as unlikely to suffer from either a poor or excessive response to stimulation (Nelson et al., 2007). This was confirmed in the prospective comparative study (Nelson et al., 2009). The extended programme has confirmed these findings, as this treatment strategy resulted in a negligible incidence of cancelled oocyte retrievals or excessive responses and OHSS.

A clear trend was observed between increasing AMH serum concentrations and egg yield, with clinical pregnancy and implantation rates decreasing with age. Overall, the use of a GnRH agonist protocol in this group was reliable, uncomplicated and safe, achieving maximum follicular recruitment. The first logical exploration in these patients would be to test whether the apparent clinical profile achieved using the GnRH agonist approach can be matched by using a GnRH antagonist. It is likely that the latter would achieve a shorter treatment cycle, but it remains to be established in these patients whether it would achieve a reduced egg yield and whether this would compromise the clinical outcome. The endocrine environment in cycles which are down-regulated is more controlled than that of a cycle controlled by GnRH antagonists, and all follicular growth is dictated by the exogenous gonadotrophins. It could be postulated that the particular characteristics of the different gonadotrophins, with their different origins, isoform profiles and LH activity, may be explored more precisely in these patients.

Is it advantageous to use AMH to determine when to use GNRH antagonists or agonists?

The basis of the stratified strategic approach is the hypothesis that patients with different AMH serum concentrations require different concentrations of follicular recruitment, which in turn influences the choice of GnRH analogue used. Each category of patients behaves in a manner that is intrinsically and distinctly different from the others. Therefore, for each category of patients different questions can be prospectively examined and suggestions for this are proposed in **Table 2**. For women predicted to have an excessive response, in whom a GnRH antagonist protocol may be most suitable, prospective studies could determine whether the source, potency or dosage of the form of FSH stimulation could impact safety outcomes (**Table 2**). In poor responders, determining the optimal protocol to improve clinical outcomes whilst minimizing treatment burden would be the ultimate goal of future prospective research (**Table 2**).

In addition to indicating the most appropriate GnRH analogue to use for a specific patient category, it is possible

Table 2 Suggested prospective examinations of individualized ovarian stimulation.

Category	*Comparisons*	*Derivation*
High/ excessive	Source, potency and doses of FSH in GnRH antagonist cycles	Safety
Normal/safe	GnRH agonist (long down-regulation) versus GnRH antagonist	Oocyte yields
	Source of FSH and effects of LH activity in GnRH agonist (long down-regulation) cycles	Oocyte yields
Poor responders	Comparison of down-regulation, GnRH antagonist, flare-agonist protocols	Clinical outcome, treatment burden

GnRH = gonadotrophin-releasing hormone.

Table 3 Optimizing ovarian stimulation strategies based on the anti-Müllerian hormone serum concentration.

AMH concentration	*Recruitable antral follicles reflected by the AFC*	*Stimulation requirement*	*Additional requirements*	*Gonadotrophin dose*	*GnRH analogue*
High	High	Mild	Reduced gonadotrophin potency	Low	Antagonist
Normal	Moderate	Maximizing	Maximum recruitment recommended	Standard/moderate	Agonist
Low	Reduced	Maximizing	Minimizing treatment burden	Standard/high	Long agonist

AMH = anti-Müllerian hormone; GnRH = gonadotrophin-releasing hormone.

that clinical advantages of particular formulations of gonadotrophin may be revealed in these more closely categorized circumstances. In patients predicted to have a poor response, maximal follicular recruitment is the aim, and the most potent form of gonadotrophin may represent the best form of ovarian stimulation. Additionally, in these patients, an agonist protocol may be more beneficial owing to the improved follicular recruitment associated with its use (**Table 3**).

In terms of the most appropriate GnRH analogue for excessive responders, as discussed earlier, a GnRH antagonist protocol may be more suitable (**Table 3**), owing to its association with the development of a smaller cohort of leading follicles and the potential for improved safety outcomes, such as a reduced incidence of OHSS. In predicted high responders, particularly those with a AMH serum concentration >40 pmol/l, an advantage of using the GnRH antagonist protocol is the possibility of triggering ovulation with a GnRH agonist instead of HCG. This approach can reduce or even eliminate the chances of OHSS compared with the use of HCG (Humaidan et al., 2009, 2011) and cannot be used in cycles down-regulated with an agonist.

The AMH test

Until recently, two commercial immunoassays were available for the measurement of AMH concentrations — the Diagnostic Systems Laboratory (DSL) assay and the Immunotech-Beckman assay — which led to some confusion regarding values for clinical interpretation. These have now been combined into a single assay, the AMH Gen II enzyme-linked immunosorbent assay kit, which can detect AMH concentrations >0.57 pmol/l with a minimum limit of quantitation of 1.1 pmol/l (Beckman Coulter, Webster, TX, USA; (Kumar et al., 2010); this assay should be used for all future evaluations of AMH. With the Gen II AMH assay, the pivotal cut-off values are <1.1 pmol/l for negligible response, 1.1–6.9 pmol/l for reduced response, 7–19.9 pmol/l for normal or safe response and >20 pmol/l for potential excessive response derived from the DSL cut-off values reported in the Nelson et al. (2009) study (Table 1).

Presently, the AMH assay is relatively expensive compared with basal FSH tests. In the USA, expenses associated with the use of the AMH assay have been estimated to be in the region of $150–400 while those associated with the basal FSH test are approximately $125–150 (Butts and Seifer, 2008). However, owing to cycle variability, FSH serum concentrations need to be tested on day 3 of at least two menstrual cycles and need to be repeated frequently in order to obtain prediction rates comparable to the AMH test. As a result, the total cost of these repeated tests might not differ greatly from that of a single AMH assay. However, the test is not currently established on an automated platform and requires individual assay performance, which leads to a reluctance for pathology laboratories to use it. Thus, the development of an automated platform could lead to the cost of AMH assays being lowered in the coming years.

Predictive tests of ovarian responsiveness are important for determining optimal protocols for reproductive assistance. Even suboptimal predictive tests may aid clinical management by helping clinicians decide how best to counsel patients regarding their potential response to ovarian stimulation. As AMH is stable across the menstrual cycle, its use may confer practical benefits over other ovarian reserve tests, as clinicians may use any AMH measurement taken prior to commencing an assisted reproduction cycle. Indeed, a recent study observed that AMH serum concentrations demonstrated less individual intra- and inter-cycle variations than AFC and therefore AMH may be considered a more reliable means of predicting ovarian reserve than AFC (van Disseldorp et al., 2010). The AMH serum concentration during ovarian stimulation is also proposed to be a good marker of ovarian response, albeit not as reliable as the baseline AMH serum concentration (Lee et al., 2010).

As demonstrated by the two-centre trial of Nelson et al. (2009), individualization of a ovarian stimulation regimen is possible based exclusively on the AMH serum concentration. The circulating basal AMH concentration can be used to guide the choice of GnRH analogue or gonadotrophin and to optimize the gonadotrophin dose. Furthermore, a study by Nakhuda et al. (2010) demonstrated that the AMH serum concentration could also successfully be used in oocyte donors, as a measure to determine the optimal gonadotrophin dose to avoid the risk of OHSS (Nakhuda et al., 2010). Application of these results could allow for the development of logical strategies for individualized ovarian stimulation. Alternatively, as a low AMH concentration in women <40 years of age has significant implications on their ovarian reserve, routine screening for this trait by gynaecologists could serve as a form of preventative medicine, potentially reducing the need for assisted reproduction treatment. If low AMH concentrations are detected on a routine visit, clinicians could advise patients that delaying

having children until an older age could significantly reduce their chances of achieving a successful pregnancy, even with the use of assisted reproduction treatment.

Conclusions

There is now sufficient evidence to allow the conclusion that estimates of ovarian reserve are effective predictors of ovarian responses to FSH. Anti-Müllerian hormone appears to be the most reliable of these indicators, and criteria diagnosing categories of response are now established. The characteristics of follicular recruitment in ovarian stimulation cycles differ when they are controlled with different types of GnRH analogues. When used in relatively unselected patient populations, there are modest differences in clinical outcomes including egg yields, OHSS and pregnancy rates. However, when patients are selected based upon their ovarian reserve, some of the differences are magnified to a remarkable degree. It is possible, therefore, that other differences may be revealed when tested in appropriate groups of patients determined by their AMH concentration. Further research is needed to establish whether individualized treatment protocols based on basal AMH serum concentrations will result in improved clinical outcomes by reducing poor response rates, lowering the incidence of OHSS and increasing live birth rates.

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