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REVIEW

The journey from the old to the new AMH assay: how to avoid getting lost in the values

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Abstract Anti-Müllerian hormone (AMH) is set to dominate reproductive endocrinology because of its unique relationship with the ovarian reserve. To date half of the published articles have used the Diagnostic Systems Lab (DSL) assay and the other half the Immunotech (IOT) assay. Unfortunately, these assays utilize two different primary antibodies against AMH and different standards, and consequently the crude values reported can differ substantially, with the IOT assay giving values for AMH that are higher than those obtained with the DSL assay. With the recent consolidation of these two companies by Beckman Coulter, and their sole ownership of the patent to measure mammalian AMH, there is finally a single commercially available assay – the AMH Gen II assay, which will fully replace the DSL and IOT assays. The aim of this article is to briefly focus on the different assays for AMH evaluation in order to give readers hopefully helpful suggestions for a correct interpretation of the AMH measurement. A brief overview on the development and performance characteristics of the new assay, how it relates to previous values and previously developed nomograms and where the future lies for AMH is also provided.

KEYWORDS: AMH, anti-Müllerian hormone, IVF, ovarian reserve, pregnancy

Introduction

Anti-Müllerian hormone (AMH) is the molecule of the moment in reproductive medicine with increasing recognition that it could potentially revolutionize reproductive endocrinology. Presently, the principal field of application for AMH measurement remains the IVF setting, where the hormone is used to predict both poor and hyper-response (La Marca et al., 2010b), in the individualization of treatment strategies (Nelson et al., 2009) and to counsel couples regarding the likelihood of a live birth after assisted conception (Gleicher et al., 2010; La Marca et al., 2011; Li et al., 2010a; Nelson et al., 2007). However, its scope is potentially much larger. Consistent evidence indicates that AMH evaluation could also be useful as a diagnostic criterion for secondary amenorrhoea permitting to distinguish

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between WHO class II and III amenorrhoea (Fleming et al., 2006; La Marca et al., 2006a,b; Laven et al., 2004; Mulders et al., 2004; Pigny et al., 2003, 2006). AMH can also assess the severity of ovarian damage following ovarian surgery or chemotherapy (Anderson et al., 2006; Bath et al., 2003; Lie Fong et al., 2008; Lutchman Singh et al., 2007; van Beek et al., 2007), and AMH can be used in the specific follow up of granulosa cell tumours (La Marca et al., 2007). Lastly, because of AMH's unique relationship with primordial follicle number (Hansen et al., 2011), it has the potential to predict the onset of the menopause (Burger et al., 2007; La Marca et al., 2005; Sowers et al., 2008; Tehrani et al., 2009; van Disseldorp et al., 2008; van Rooij et al., 2004).

The remarkable gains in popularity in AMH has principally been due to recognition of its unique characteristics in the field of gynaecological endocrinology. Firstly, circulating AMH seems to be solely derived from the ovary, since bilateral oophorectomy in premenopausal women and menopause are associated with undetectable AMH concentrations (La Marca et al., 2005). Secondly, in the ovary it is only produced by a single-type cell, namely granulosa cells (Baarends et al., 1995; Bezard et al., 1987; Weenen et al., 2004), permitting its use as a biochemical marker of granulosa cell function. Moreover, it is synthesized and secreted only by granulosa cells from primary to small antral follicles ($\leq$4–6 mm) (Visser and Themmen, 2005; Weenen et al., 2004), and this confers to AMH the peculiar characteristic to be the only hormonal marker available to date for gonadotrophin-independent folliculogenesis (McGee and Hsueh, 2000). Other biological characteristics, such as being independent of circulating FSH (Fanchin et al., 2003a,b; La Marca et al., 2004) and being relatively stable across the menstrual cycle (La Marca et al., 2006a,b; Sowers et al., 2010; Wunder et al., 2008) and between cycles (Fanchin et al., 2005; van Disseldorp et al., 2009), combined with its ability to be measured by a commercially available enzyme-linked immunosorbent assay, have all contributed to its overall appeal and exponential increase in the number of published papers on AMH and in the number of clinics using AMH on a routine basis.

In the last 20 years there has been a constant evolution of the assay from single laboratory versions (Al-Qahtani et al., 2005; Groome et al., 2006; Hudson et al., 1990; Lee et al., 1996; Long et al., 2000), through to the more recent commercially available Diagnostic Systems Lab (DSL) and Immunotech (IOT) (also branded as the Immunotech Beckman Coulter (IBC)) assays. Consequently, at present half of the published articles have used the DSL assay and the other half the IOT assay. However, these current assays utilize two different primary antibodies against AMH and different standards and consequently the crude values reported by authors and between papers can differ substantially, with the IOT assay giving values for AMH that are higher than those obtained with the DSL assay. But with the recent consolidation of these two companies by Beckman Coulter there is finally a single commercially available assay – the AMH Generation II assay (AMH Gen II assay). This new assay is going to fully replace the DSL and the IOT assays, and combined with Beckman Coulter holding the international patent on measuring AMH in mammalian samples means that for the foreseeable future they will be the sole providers of the assay. Furthermore, the timeline to move the assay on to Beckman Coulter's automated Access Immunoassay platform will be over several years, so in the immediate future the manual Gen II assay will dominate. So, for clinicians and researchers trying to get to grips with the existing literature, there will be substantive confounding in the interpretation of results, namely as about which AMH assay was used: DSL, IOT or Gen II.

The aim of this article is to briefly focus on the different assays for the AMH evaluation in order to give readers hopefully helpful suggestions for a correct interpretation of the AMH measurement. A brief overview on the development and performance characteristics of the new assay, how it relates to previous values and previously developed nomograms, and where the future lies for AMH is also provided.

AMH assay design

AMH is synthesized as a large precursor with a short signal sequence followed by a pre-hormone that forms homodimers. Prior to secretion, the mature hormone undergoes glycosylation and dimerization to produce a 140 kDa dimer composed of identical disulphide-linked 70 kDa monomer subunits; each monomer contains an N-terminal domain (also called the 'pro' region) and a C-terminal domain (also called the 'mature' region). AMH is cleaved at a specific site between the N-terminal domain and the C-terminal domain of the 70-kDA monomer during cytoplasmic transit, to form two polypeptides of 58 kDa (N-terminal fragment; pro-region) and 12.5 kDa (C-terminal fragment, mature region). These two parts of the molecule remain in non-covalent attachment (Lee and Donahoe, 1993; MacLaughlin et al., 1992). Previous incarnations of the AMH assay have targeted different aspects of the AMH molecule. The first targeted epitopes in the pro-region (Lee et al., 1996), the second used a pair of antibodies which targeted both the pro-region and the mature region (Long et al., 2000) and a third assay directed its pair against the epitopes in the pro-region (Al-Qahtani et al., 2005). However, for all of these, careful attention to sample collection and storage was required because of instability with storage and freeze–thaw cycles. Further assay development was achieved by immunizing female null-AMH mice with recombinant human AMH and then screening the antibodies against recombinant human and rat AMH and other mammalian species (Al-Qahtani et al., 2005; Kevenaar et al., 2008; Weenen et al., 2004). The optimal combination of detector and capture antibodies F2B/7A and F2B/12H, respectively, were the foundation for the original DSL assay, with continued use of these antibodies in the Gen II assay (Kevenaar et al., 2006). Notably for this pair of monoclonal antibodies, the first antibody binds to the pro-region under reduced and non-reduced conditions but with longer exposure it will also bind to the mature region under non-reduced conditions. The second antibody binds to the mature region under non-reduced conditions (Groome et al., 2006). This ability to bind the mature region means that this assay is not affected by proteolysis of AMH in the sample. As noted in their original development the F2B/7A and F2B/12H antibodies will also detect AMH in human, monkey, bovine and other mammalian species. It is these antibodies which have been incorporated into the AMH Gen II assay.

A further issue with the assays to date has been the varying sources of the AMH standard used for the calibration. Although in the IOT and Gen II kit the AMH calibrators have been made in heat-inactivated bovine calf serum using native AMH (Kumar et al., 2010), the full details of the source and values assigned to the standards have not been released by Beckman Coulter because this is regarded as proprietary information. An international standard in accordance with the International Federation of Clinical Chemistry would be welcomed by the clinical and research community and would allow manufacturers including Beckman Coulter to collectively standardize future novel assays. At present there is no appetite by the National Institute for Biological Standards and Control to establish an international standard because of the limited use of the assay and a single manufacturer. Consequently, given that there is only the AMH Gen II assay with the intended movement onto the Beckman Coulter Access platform using the same AMH standard, the current single standard source will remain the international standard albeit its characteristics being confidential. The AMH Gen II assay therefore reflects a hybrid of the previous two assays combining the DSL antibodies and thereby cross-species reactivity, with the calibration of the IOT standards.

Performance characteristics and stability of analytes with the new assay

Although many laboratories have reported AMH values all the way down to zero, resulting in clinicians placing weight on small increments of AMH, this has been inappropriate as it does not recognize that all laboratory methods have limits when it comes to the reliability of a method measuring the amount of an analyte in a subject's sample. Two such critical performance characteristics are defined at the lower end of the measurement scale. The first is the smallest amount that a method can reliably detect to determine the presence or absence of an analyte. This is the limit of detection (LoD). The second characteristic is the smallest amount the method can reliably measure quantitatively. This is the limit of quantitation (LoQ). For the AMH Gen II assay the LoD is 0.08 ng/ml and the LoQ of the AMH Gen II assay is 0.16 ng/ml (Kumar et al., 2010). Consequently the AMH Gen II cannot reliably determine between 0 and 0.16 ng/ml and therefore values should not be reported below 0.16 ng/ml.

Importantly, given that samples are often transported between clinics and laboratories, the average variation between fresh samples and those stored for 7 days at room temperature is approximately 4%, and if the sample is frozen it is 1% (Kumar et al., 2010). Although plasma specimens do demonstrate more variation than serum samples under stress conditions, despite this the maximum variability in samples is approximately 6% between fresh and frozen serum/lithium–heparin plasma specimens (Kumar et al., 2010). At present, stability studies on whole blood have not been performed but are urgently required given the potential transportation of samples to central laboratories from phlebotomy sites.

Conversion from old assays to new values

Initial studies comparing the DSL and IOT assays have shown that AMH concentrations, although linearly related ($r = 0.88$), were almost 30% lower with the DSL assay compared with the IOT assay (Freour et al., 2007; Taieb et al., 2008). Differential conversion factors have been generated by various authors; however, this reflects that the assays themselves have not been static with the DSL assay being recalibrated and residual matrix effects being resolved during its commercial lifetime. Consequently more recent studies have shown very similar results between the two assays (Streuli et al., 2009; Bersinger et al., 2007; Lee et al., 2011). The most recent of these simply assessed correlation between the two assays, which is inevitably high given that they are both measuring AMH ($r = 0.967$; BC = 1.102 × DSL−0.042) (Lee et al., 2011). Notably, true assessment of the agreement of the assays would be by Bland Altman plots and Passing–Bablok regression.

Since the AMH Gen II assay has been calibrated to the IOT standards, the values derived from the AMH Gen II will be essentially identical to those previously obtained by the IOT assay (Kumar et al., 2010). However, the AMH Gen II assay values will differ substantively, relative to values previously obtained from the DSL assay. Specifically, both serum and lithium-heparin plasma samples measured by the AMH Gen II assay show significant associations as assessed by Passing-Bablok regression with values obtained from the IOT assay ($r = 0.99$; $P < 0.0001$; $y = 1.0x$; **Figure 1**). The relationship between the DSL assay and the AMH Gen II assay has recently been independently assessed in a

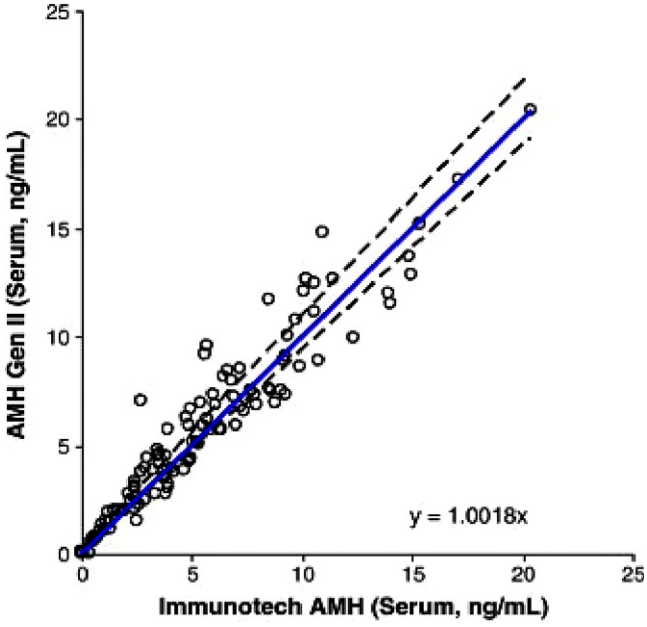

Figure 1 Passing and Bablok regression analysis between anti-Müllerian hormone (AMH) concentrations (ng/ml) obtained with Immunotech AMH and AMH Gen II ELISA assays for 120 serum samples. Linear regression analysis results were $r = 0.99$; $P < 0.0001$; $y = 1.0x$. Reproduced with permission from Kumar et al. (2010).

multicentre study of 271 women in accordance with Clinical and Laboratory Standards Institute guidelines (previously NCCLS). The results of the assays demonstrate good agreement with the Gen II assay; however, the Gen II assay will provide values ~40% higher than the DSL assay (**Figure 2**) (Wallace et al., 2011).

This means that when clinicians are reading the previously published literature based on the DSL kit to interpret these data in a current context, they will need to increase the values by ~40%. However, the reader should be aware that the conversion will always be inaccurate since the calculated conversion factor from one assay to the other is not constant but is higher with increasing AMH values, as demonstrated in **Figure 2** (Wallace et al., 2011).

With respect to reporting of the results, despite AMH being a glycoprotein and therefore the molecular mass is difficult to ascertain, the IOT assay has always used ng/ml, while the DSL assay used pmol/l. Although it is possible to convert from ng/ml to pmol/l by multiplying by 7.14, with the AMH Gen II assay using the IOT standards, it would seem

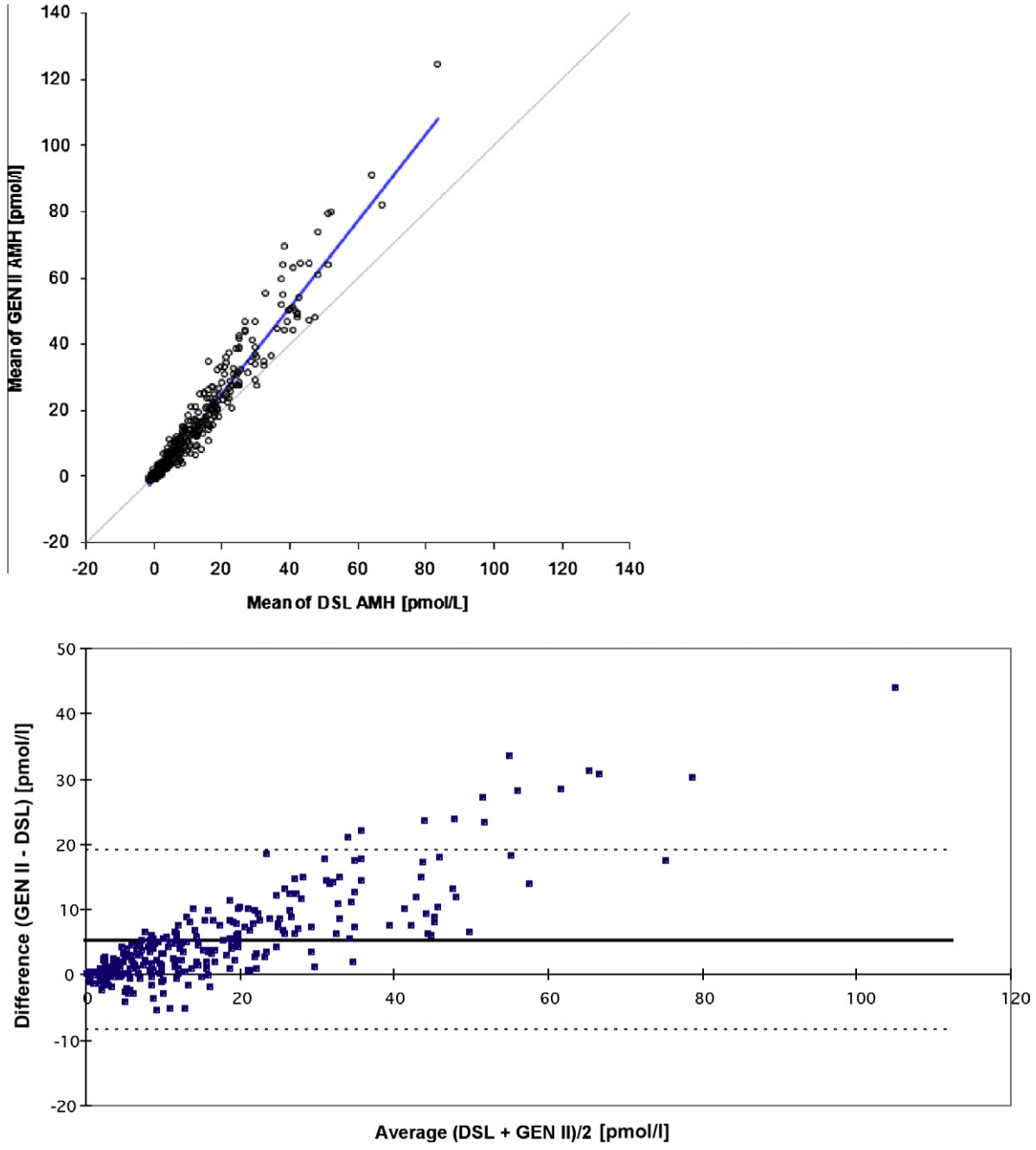

Figure 2 Multicentric evaluation of Diagnostic Systems Lab (DSL) anti-Müllerian hormone (AMH) assay and Beckman Coulter AMH Generation II (GenII) assay, for 271 samples across the assay range. Top panel = Passing and Bablok regression (intercept 0.62, 95% CI −1.51–0.28, SE 0.46; slope 1.39, 95% CI 1.35–1.44, SE 0.020). Bottom panel = Bland Altman plot (solid line = mean difference; dotted lines = 95% CI). Reproduced with permission from Wallace et al. (2011). CI = Confidence interval; SE = standard error.

appropriate for the scientific literature to move towards standardization and papers to report in ng/ml. Although some may be attracted by the prospect of g/l, this would be a complete shift in units with limited adoption of this to date. Use of ng/ml would also allow for all future papers to be easily placed in the context of the historical IOT work.

Normal values — the ongoing search for age-specific normograms

Given the recognition that AMH declines prior to the menopause and is associated with the date of the final menstrual period (Anderson et al., 2006; La Marca et al., 2005; Soto et al., 2009; Sowers et al., 2008; van Rooij et al., 2004), there has been considerable interest in being able to place any given individuals AMH in context and predict her reproductive lifespan. Thus many clinicians and patients are keen to know not only what their actual AMH is but how this relates to women of a similar age i.e. an age-specific AMH centile. The potential widespread utility of this would be that if it was low it would suggest expediting the pursuit of a family either naturally or through assisted conception or considering oocyte freezing, as it is clear that with increasing age, her ovarian reserve and AMH will only decrease. In addition, given that AMH and age are independent predictors of oocyte yield (Nelson et al., 2007), an age-specific AMH should allow more accurate modelling of anticipated response to ovarian stimulation, improving individualization of treatment strategy and management of a patient's expectations.

It is clear that, across the female lifespan, AMH increases initially in the neonatal period then declines before rising again during prepubertal development, peaking during adolescence and then again declining gradually to non-detectable concentrations approximately 5 years prior to the menopause (Hagen et al., 2010). Although this general trend has been observed in all studies to date, development of age-specific nomograms have been fraught with use of small cohorts and discussions regarding which patients should be included and whether a nomogram derived from infertility patients would be applicable to fertile populations. The limitation of small cohorts ($n = 81-136$) has recently been emphasized, as many of these had reported a linear decline in AMH with age (Mulders et al., 2004; Nardo et al., 2007; Sowers et al., 2008; van Rooij et al., 2005), with only two small cohorts ($n = 50$ and 144, respectively) suggesting a non-linear decline (Anderson et al., 2006; de Vet et al., 2002). The biological feasibility of a linear decline was, however, questionable, as circulating AMH relates to the continuous, non-cyclic growth of small follicles in the ovary (Cook et al., 2000; La Marca et al., 2004, 2006a,b), which is known to decrease in a non-linear manner. Detailed statistical analysis of 9178 European women relative to the described models of decline in primordial follicle number, including linear, bi-exponential models (Faddy et al., 1992), decay-curve models (Faddy, 2000; Faddy and Gosden, 1996), power models (Hansen et al., 2008) and quadratic models in adult life (Wallace and Kelsey, 2010), demonstrated that the decline in AMH was optimally modelled by a quadratic equation (Nelson et al., 2011b) (**Figure 3**). Subsequent external validation in 15,834 US women confirmed that the optimal model was a quadratic decline (Nelson et al., 2011b). Although a non-linear decline had been suggested previously (Anderson et al., 2006; de Vet et al., 2002), the major advantage of using large datasets is the ability to examine all possible models and perform internal and then subsequently external validation ensuring generalizability to unseen data. This latter stage is particularly important as it is recognized that internal validation may systematically give a too optimistic impression about the quality of the predictions and that external validation is essential for further assessment of model performance (Harrell et al., 1996). The initial analysis, although derived principally from infertility patients, did also show that the model fitted AMH values derived from 423 regularly menstruating women with evidence of ovulation and a normal pelvic ultrasound, but their partners had severe oligospermia, suggesting that such a model would fit a normal population (Nelson et al., 2011b). Notably the good fit observed in the external validation study with a US population would further support this claim (Nelson et al., 2011a). The approach used in these two studies may be a road map for future nomogram development, with the clear size of the datasets and the use of a well-phenotyped cohort for validation overcoming the problem of contamination with asymptomatic women with polycystic ovaries (Hart et al., 2010; Hickey et al., 2011). The major limitation of these studies is that this nomogram is for the DSL assay, which, as noted, gives values ~40% less than the current new AMH Gen II assay and therefore its clinical lifespan will be limited, although it will allow all historical results to be placed in context.

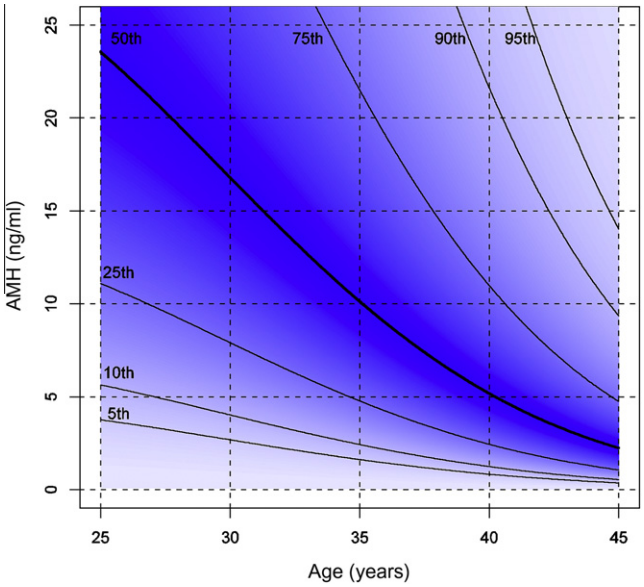

Figure 3 Anti-Müllerian hormone (AMH) nomogram for values obtained by the Diagnostic Systems Lab assay, based on a quadratic model of log(AMH) on age, showing predicted AMH value versus age and reference centiles of the distribution. Reproduced with permission from Nelson et al. (2011b).

At present, a nomogram for the new Gen II AMH assay has yet to be developed; however, the standards used for the older IOT assay have been maintained in the Generation II assay and, consequently, as noted above, similar values

Table 1 Anti-Mullerian hormone nomogram for values obtained by the Immunotech assay throughout the reproductive period. Reproduced with permission from La Marca et al. (2010b).

Centile	*Age (years)*				
	≤30	*31–35*	*36–40*	*41–45*	*46–50*
2.5	0.52 (0.11–0.96)	0.35 (0.11–0.79)	0.33 (0.26–0.51)	0.26 (0.25–0.39)	0.22 (0.18–0.31)
5	0.87 (0.5–1.27)	0.64 (0.11–1.12)	0.48 (0.26–0.6)	0.36 (0.25–0.51)	0.32 (0.2–0.43)
10	1.27 (0.96–1.68)	0.93 (0.64–1.66)	0.6 (0.48–1.37)	0.42 (0.38–0.6)	0.42 (0.35–0.5)
25	2.5 (1.7–3.26)	1.88 (1.63–2.69)	1.71 (1.25–2.42)	0.78 (0.6–0.99)	0.76 (0.53–0.91)
50	4.10 (3.61–4.98)	3.46 (2.87–4.08)	3 (2.65–4.46)	1.38 (1–1.75)	1.1 (0.8–1.41)
75	6.3 (5.45–7.58)	6.08 (4.65–7.65)	5.3 (4.59–6.67)	3.56 (1.96–5.39)	2.8 (2–3.6)
90	8.68 (7.88–10.89)	8.19 (7.63–9.97)	7.89 (6.67–13.9)	7.70 (4.68–12.29)	5.5 (3.2–9.7)
95	10.98 (8.92–12.09)	9.97 (8–34–12.44)	8.67 (7.97–12.95)	8.29 (6.55–12.61)	6.92 (4.98–8.7)
97.5	12.01 (10.27–13.08)	11.84 (10.14–12.44)	9.68 (8.45–12.75)	8.78 (7.2–12.6)	6.34 (3.9–7.5)

Values are ng/ml (90% confidence intervals).

are generated. The only nomogram for the IOT assay, was developed on a small cohort of women ($n = 272$) from the general population and, although showing a non-linear decline, will require validation in external larger datasets (La Marca et al., 2010a) (**Table 1**). Although groups may be tempted to convert their DSL values and redefine their nomogram using one of the several linear equations published comparing the DSL and IBC assays, simple linear transformation across the range of values would be inappropriate.

Future development in the applications of AMH in reproductive medicine

To date, the clinical utility of AMH has primarily been centred on assisted conception because of its strong association with oocyte yield after ovarian stimulation (La Marca et al., 2010b). Consequently AMH is capable of predicting an individual's risk of a poor, normal or excessive ovarian response (La Marca et al., 2010b). This identification of extremes of response prior to ovarian stimulation will inevitably allow improved patient counselling prior to their first treatment cycle (Nelson et al., 2009). Furthermore, accurate identification of individuals at risk of ovarian hyperstimulation syndrome (OHSS) prior to their first treatment cycle will allow known strategies which reduce the risk of OHSS to be implemented, e.g. antagonist cycles (Mathur et al., 2007). Furthermore, given that classically the starting FSH dose for the first IVF cycle has been selected on the basis of age, despite its weak associations with oocyte yield, this should be able to be improved upon by using AMH either alone or in combination with age and body mass index. We have recently shown in prospective study of almost 600 patients that directing the stimulation strategy based on AMH alone significantly reduced the risk of OHSS, reduced treatment burden, reduced cycle cancellation and increased clinical pregnancies (Nelson et al., 2009). Although this study had limitations including a non-randomized design, it clearly demonstrated that a single AMH assay may be used to individualize treatment strategies and optimize the overall efficacy of an IVF treatment programme. Although there are proponents of 'just do the IVF and then assess response irrespective of the AMH' approach, this is potentially dangerous and detrimental, particularly as adoption of an agonist approach for all will be associated with a high incidence of OHSS. Conversely, if a mild antagonist approach is used as standard in all patients undergoing IVF, the live birth per fresh cycle treatment will be lower and there will be fewer cryopreserved embryos per oocyte harvest, further reducing the overall efficacy of a single stimulated cycle (Fauser et al., 2010; Heijnen et al., 2007). Although the argument for equivalence across repeated cycles with antagonist cycles is frequently cited (Fauser et al., 2010), at a time of reduced state funding for assisted conception in many European countries, maximal efficacy per cycle is required. Inevitably the currently proposed cut-offs for poor and hyper-response will continue to be refined and similarly the optimal treatment strategies for the various groups of patients will be clarified. However, it is clear that there is a necessity for individualized ovarian stimulation and one size does not fit all.

Although there has been a move towards using AMH to exclude women from having IVF treatment, the ability of AMH to dichotomize couples into having achieved a pregnancy or not is limited as it exhibits poor discrimination, as assessed by receiver–operator curves (Broer et al., 2009). Furthermore, all the studies to date have been small and the health economic evidence base for withholding treatment at different cut-offs non-existent. It has been shown that AMH may be able to predict whether the probability of a live birth is high, low or moderate (La Marca et al., 2011; Nelson et al., 2009), but again the studies are limited and much more accurately calibrated models exist (Leushuis et al., 2009), with the largest being established on 144,081 treatment cycles Nelson and Lawlor, 2011. Whether AMH will be able to enhance or replace these models will be seen in time.

Although many women may be concerned regarding their own fertility if they are identified as having a low ovarian reserve, at present there has been no relationship established with spontaneous pregnancy. However, it is clear that AMH will decline with age and that a low AMH is associated with a worse outcome during assisted conception. Conse-

quently, many clinicians will already be confident to advise patients to not wait on commencing a family or consider oocyte cryopreservation. Similarly identification of women with a low AMH may also allow oncologists to accurately target women at high risk of chemotherapy-induced amenorrhoea and initiate pre-chemotherapy oocyte preservation (Anderson et al., 2006; Giuseppe et al., 2007; Kelsey et al., 2011; Lie Fong et al., 2008; van Beek et al., 2007).

AMH has consistently been shown to be elevated in polycystic ovarian syndrome (PCOS) (La Marca et al., 2006a,b; Laven et al., 2004; Pellatt et al., 2006; Pigny et al., 2003), with a strong relationship between follicle number and AMH (Fanchin et al., 2003a,b). Consequently a high serum concentration of AMH has been suggested as a surrogate for a high follicle number (Pigny et al., 2006). AMH has also recently been shown to coaggregate with hyperandrogenism, thereby potentially allowing a merging of the Rotterdam and Androgen Excess Society criteria for PCOS. However, at present an AMH threshold which would replace the Rotterdam definition of 12 or more follicles in each ovary measuring 2–9 mm in diameter and/or increased ovarian volume (>10 ml) has yet to be attained (Rotterdam and EA-sPcwg, 2004). Previous analysis demonstrated that even when AMH was >60 pmol/l (IOT assay), it only demonstrated a 67% sensitivity and 92% specificity for the overall diagnosis of PCOS as per the Rotterdam criteria (Pigny et al., 2006). More recently, this relatively poor performance for the diagnosis of PCOS has been confirmed in adolescent girls and even its ability to predict PCO morphology questioned as an AMH of >30 pmol only demonstrated a 54.7% sensitivity and 72.7% specificity (Hart et al., 2010). Despite these current limitations, with the development of a single assay it is anticipated that researchers will be able to amalgamate their data in individual patient data meta-analyses. This will allow optimal thresholds to be derived and a clear consensus on whether AMH can be used as part of the diagnostic criteria for PCOS.

Conclusion

Since the first clinical papers on AMH in 2002 (Seifer et al., 2002; van Rooij et al., 2002), there has been an explosion of the number of reproductive medicine centres adopting its measurement into daily clinical practice. This widespread uptake is highly unusual for a diagnostic test in this field, e.g. inhibin B is yet to firmly establish a clinical role for itself despite 20 years of research. This unnerving success of AMH is due to its unique characteristics and relationship with ovarian reserve. Very soon the AMH Gen II assay will replace the two standards that clinicians have now used and become accustomed to interpreting for several years. Although this may cause concern for clinicians, hopefully it is now clear that those clinicians using the IOT assay should not be troubled since the new AMH Gen II assay has been calibrated identically to the old IOT assay. Whereas those using the DSL assay should become familiar with the IOT literature and be prepared that the new AMH Gen II assay will give values for AMH that are approximately 40% higher than they are accustomed to.

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