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DEBATE

The current status of anti-Müllerian hormone measurement in assisted conception

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Anti-Müllerian hormone has attained a major prominence as the main marker of ovarian reserve in recent years and its measurement has been utilised in most aspects of assisted reproduction, including (a) diagnosis of patients with diminished ovarian reserve, (b) screening for polycystic ovary syndrome, (c) triaging of patients into or out of cycles of assisted conception and (d) individualisation of controlled ovarian stimulation protocols in IVF/ICSI (7,6,13). It is therefore more important than ever that reliability of the methods for the accurate and reproducible measurement of AMH is ensured.

Measurement of AMH using an enzyme-linked immunosorbent assay (ELISA) was first described two decades ago, following the development of various "in-house" non-commercial immunoassays (2,4). The introduction of commercially available first generation immunoassays by Immunotech-Beckman Coulter (IOT) and later by Diagnostic Systems Laboratory (DSL) during the early part of the last decade has resulted in a significant surge in research and clinical applications of the test (1,5). More recently, a second generation immunoassay, the Gen II AMH ELISA, manufactured by Beckman Coulter has, in the UK at least, replaced both first generation assays and is now the only commercially available immunoassay for the measurement of AMH (8). The ultimate aim of the introduction of a new immunoassay should be to obtain more accurate, reproducible and robust AMH measurements. Initial validation studies showed that the Gen II AMH assay was more sensitive and stable, however more recent assessment of the performance of the assay in clinical samples suggests that the assay may provide less reliable measurements compared to the first generation DSL assay. In this paper, we discuss the limitations and pitfalls of the currently available Gen II AMH assay with reference to the reliability and fitness for purpose in the current form.

The AMH Gen II assay was developed using a pair of monoclonal antibodies that target the mature region of AMH, which is believed to ensure the measurements are not affected by proteolysis. The initial validation study conducted by the manufacturer confirmed that the Gen II assay was stable when serum samples were stored either unfrozen (2–8 °C) or at −20 °C for up to 7 days (3). Consequently, many clinics have adopted a simplified mode of transportation of samples between centres; including sending unprocessed, unfrozen samples by post over long distances. However, recent studies conducted by our group suggest that both storage of samples at room temperature and the freezing of samples at −20 °C give rise to significant sample instability resulting in average increases in measurable AMH concentrations of 58% and 23%, respectively (10). Furthermore, contrary to the manufacturer's current data, the dilution of samples also appears to disrupt the measurement of AMH, causing an approximate doubling of the AMH concentration in some samples when using the Gen II assay (10). Linearity of dilution in any assay is of fundamental importance in the assessment of assay quality and ensures that measurements in clinical samples that have higher concentrations than the working range of the assay can be achieved. This disproportionality of AMH levels following dilution indicates that there is an underlying problem with the stability of the AMH molecule in the sample. In view of these observed anomalies, it is plausible to conclude that this sample "instability" can have a very significant impact on AMH results in clinical samples which have been assessed both by (a) comparing the Gen II assay clinical results to those obtained using first generation assay methods and by (b) measurement of within-patient, sample-to-sample AMH variability (10).

The AMH Gen II assay has been calibrated to the IOT immunoassay standards and therefore it should provide similar AMH results to the IOT assay and higher values when compared to levels obtained using the DSL assay. An initial study by the manufacturer and subsequent independent validation confirmed that when the assays were compared, using analysis of paired aliquots obtained from the same sets of samples, the Gen II assay provided, on average, AMH values that were 40% higher than those obtained on the same sample using the DSL assay (3,12). These findings should be reproducible when sequential clinical samples are compared provided the samples are stable during processing and storage.

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However, when paired Gen II and DSL samples from 330 women were compared, using age-adjusted regression analysis, we found that Gen II AMH results were on average 20% lower than those obtained with the DSL assay (10). Such a significant difference between a single laboratory based, within- and between-sample comparison of the two assay methods questions the applicability of the results of laboratory-based studies to actual clinical practice. Anti-Müllerian hormone is produced by steadily growing pre-antral and antral follicles and therefore it is believed that the serum concentration of the hormone does not change significantly between repeated samples. However, estimation of within-subject variability found that there is a significant variation in the AMH concentration between repeated Gen II measurements (CV 59%), considerably higher than that seen in the DSL assay (CV 32%) (10). In view of these observed anomalies in the stability studies and the significant discrepancy between assay methods we believe this variability is mainly due to poor performance of the Gen II AMH assay. Conflicting reports on the validity of the Gen II assay have prompted further evaluation of its performance. In collaboration with another research group from the UK, we reproduced the results of within-patient sample-to-sample variability of Gen II AMH and confirmed that our findings were independent of the assaying laboratory. On the basis of our personal communications, we can confirm that other groups have also found that linearity of dilution in the Gen II AMH assay does not hold. Having concluded that there are issues with the stability of Gen II measured AMH in samples, it is important to establish the underlying cause of these anomalies so that accuracy, reproducibility and stability of existing and future assays can be improved. Curiously, our experiments on both the stability of AMH measured after periods of storage under different conditions and linear dilution of samples resulted in an increase in Gen II measured AMH concentration, rather than a decrease. Furthermore, our dilution test showed that there was no significant further increase in the AMH concentration following an approximate doubling of the AMH levels. This has led us to speculate that these procedures may lead to breakdown of the covalent bond of the AMH homodimer, which in some way provides an additional binding site for the antibodies, resulting in a higher signal. This was observed in the early in-house assays and was believed to be preventable with proteolytic inhibitors (9,11), although we do not think that this is the whole story as one should not expect increased proteolysis merely on dilution of the sample in assay buffer. Nevertheless, these recent developments pose two important questions to clinicians and service providers: (1) can we trust the research evidence obtained from clinical studies that used the Gen II assay? and (2) can we rely on Gen II assay measurements in the management of individual patients, especially when these AMH readings may assign a patient to an inappropriate treatment group?

Ultimately, the decisions on utilisation of available research evidence should be made at individual clinician and/or organisational level following a review of all published studies on the pitfalls of the Gen II assay. In the longer term, more work needs to be done by manufacturers to ensure their kits are accurate and reproducible and have been robustly tested prior to release. There also appears to be a need for a consensus from clinicians and scientists on appropriate and manageable processing, transport and storage of specimens. Until this happens, the tremendous promise that AMH measurement holds in various aspects of female fertility will not be realised.

Conflicts of Interest

There are no potential conflicts of interest.

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AMH – applications beyond IVF

Comment by: Ondrej Topolcan

1. Introduction

Anti-Müllerian hormone (AMH), also known as Müllerian Inhibiting Substance (MIS), is a glycoprotein dimer composed of two 72 kDa monomers (1). AMH is a member of the Transforming Growth Factor-β (TGF-β) superfamily. The AMH gene is located in the short arm of chromosome 19 (2). AMH uses two cell receptors: type I receptor (MISRI) and type II receptor (MISRII) which are present on the AMH target-tissues (gonads and Müllerian ducts) (3). The expression of AMH is restricted to the Sertoli cells of the fetal and postnatal testis in the male, and granulosa cells of the postnatal ovary in the female. AMH plays an important role in male sex differentiation as its production by the embryonic testes induces the regression of the Müllerian ducts (4). The measurement of AMH serum levels is currently a useful tool in the examination of the ovarian reserve. Many studies have been performed on the topic of ovarian reserve, ovarian aging, and on the prediction of the ovarian response to the hormonal stimulation arising from in vitro fertilization (IVF). However, IVF is not the only reason for measuring AMH.

2. AMH during the life of a woman

There is a fall in Serum AMH levels shortly after birth, with concentrations only increasing again after about two years of age. This age is called mini-puberty in neonatal girls (5). An initial smaller peak of serum concentration of AMH is observed at eight years of age followed by a fall in AMH serum levels between the ages of eight and twelve. There then follows a rise that peaks between twenty-five and twenty-seven years of age. After the age of twenty-seven AMH serum levels begin decreasing slowly until menopause. AMH is produced by early growing follicles at all stages up to the early antral stage but it is unknown which follicle class contributes most to circulating concentrations. The rising granulosa cell mass (and thus AMH production per follicle) will be balanced by progressively declining numbers of follicles at each stage of growth (6).

3. AMH and polycystic ovary syndrome (PCOS)

The diagnostic criteria of the European Society for Human Reproduction (ESHRE) and the American Society of Reproductive Medicine (ASRM) were established for the diagnostics of this syndrome. PCOS is clinically diagnosed when at least two of the following three features are present: chronic oligo- or anovulation, biochemical hyperandrogenemia or hyperandrogenism and polycystic ovarian morphology identified under ultrasound examination (PCO) (7). The common clinical manifestations of PCOS include menstruation disorders and androgen excess, hirsutism and male pattern alopecia (8). The syndrome is diagnosed in 5–10% of women of reproductive age. Polycystic ovary syndrome is also associated with metabolic disorders. The incidence of diabetes mellitus type 2 is ten times higher in women with PCOS than in healthy women and 30–50% of women with PCOS develop glucose intolerance or diabetes mellitus type 2 after the age of 30 (9). Women with PCOS have a two to six times greater number of follicles in their ovaries. AMH production was increased by up to 75% in women with PCOS compared to controls (10). According to some authors the high AMH levels in women with PCOS are attributed to the high number of small antral follicles with a diameter of 2–5 mm. AMH values correlate positively with the number of this type of follicles (11,12).

In a recent in vitro study, it was found that AMH production per granulosa cell was increased by up to 75% in women with PCOS compared to controls. According to the authors, the higher levels should be attributed to the increased number of follicles as well as to the intrinsic aberrant follicular function. AMH excess, via endocrine or paracrine paths, plays an essential role in the braking of the process of follicular maturation (13).

It is known that AMH levels decrease with age in women with normal ovulatory cycles. A similar but slower decline is observed in women with PCOS (14). High AMH levels were observed in girls aged 12–18 years with PCOS compared to healthy controls (15). However, increased AMH levels have been found in girls born of mothers with PCOS (16).

AMH concentrations in women with PCOS were independently and positively correlated with testosterone, androstendione and free androgen index (FAI) values (17). A great number of women with PCOS have insulin resistance and compensatory hyperinsulinemia. It is not yet clear whether there is a correlation between AMH levels and HOMA-IR values in women with PCOS. There have been differing results from related studies (18). Metformin administration in anovulatory patients with PCOS exerts a differential influence on ovarian AMH levels on the basis of ovulatory response. Changes in AMH levels in antral follicular fluid during metformin treatment could play a role in the local mechanisms mediating ovulatory restoration (19).

4. AMH vs. antral follicle count (AFC)

The relationship between AMH and AFC has recently been the subject of some very intense discussion. Additional parameters of ovarian age have been tested and only AFC and AMH follow the observed pattern of oocyte loss histologically. Although AMH may be more cost-effective, some