Assessment of ovarian reserve prior to IVF

Comment by: Hossam I Abdalla, FRCOG
London, U.K.

In the female fetus oocyte numbers peak by 16 weeks of gestation, reaching up to 7 million. They decrease progressively and at the time of birth a female will have about 2 million eggs. As she approaches menarche the woman will have about 500,000. This primordial follicular disappearance continues throughout reproductive life and accelerates approximately 10 years prior to the menopause, by which time the number of eggs have fallen to a few hundred. If a woman ovulates one egg per month she will release 12 eggs per annum. Throughout her reproductive career (approximately 40 years, from the age of 10 to the age of 50) she will produce 480 eggs - yet nature has given her 480,000 eggs to work with.

At all stages of the menstrual cycle there are a number of small antral follicles present. This number changes with age, being at its highest in younger women. As the level of FSH rises at the beginning of the menstrual cycle more follicles are recruited and some are then selected for further development. One follicle, however, will be at the right condition for development and this “chosen” follicle will continue to develop till mid cycle when it ovulates. The quality of the released oocyte is related to both the age of the woman and the number of primordial follicles available in the ovary. Statistically speaking it is more likely for an egg to be of better quality if it is randomly “chosen”, for example, out of 100 competing primordial follicles than if it was randomly “chosen” out 10 competing primordial follicles.

Follicle stimulating hormone (FSH) is crucial for follicular development as it is the hormone that controls the process of recruitment, selection and finally the full development of the maturing follicle. The number of primordial follicles available in the ovary influences the level of FSH. As the follicles are recruited, however, they secrete both estrogen and inhibin, which in turn keeps the level of FSH low. As women age, the number of recruited follicles decrease. Consequently the suppression of the FSH level decreases and, as a result, the level of FSH increases with age in an attempt to continue to recruit the ever-decreasing number of eggs within the ovary. The level of FSH provides a biological marker for ovarian reserve. The higher the level, the less the ovarian reserve. Consequently fewer primordial follicles will be recruited and the situation takes place as described above, with the “chosen” follicle sought from only a few competing ones.

Oocyte quality is established early during fetal life. The first produced oocytes (less susceptible to non-disjunction) ovulate first and ‘poorer’ oocytes ovulate later. There is also evidence to suggest that there is age dependent damage in oocytes due to gradual increase in intracellular oxidative stress that also leads to increased frequency of non-disjunction. It is well documented that there is an age related reduction in fecundity due to reduction in pregnancy rate and rise in miscarriage rate. This is associated with an age related increase in aneuploidy due to non-disjunction. Age therefore results in reduction of both quality and quantity of oocytes.

As discussed, the number of eggs available in the ovary is certainly reduced and the level of FSH is elevated as women age. The question here is: ‘If a woman at the age of 30 years, for example, has a
level of FSH raised above a certain limit; do these eggs behave like those of a woman aged 40 years?"

And: ‘what is the relevance of high FSH in the context of assisted conception? Should these women have treatment with their own eggs or should they all have oocyte donation?’

Elevated levels of FSH are associated with reduction in pregnancy and live birth rate (LBR). It has been shown that this is primarily due to reduction in oocyte quantity rather than oocyte quality (Abdalla & Thum 2004). Women who are regularly cycling and who have evidence of ovulation despite reduced ovarian reserve should be offered ovulation induction for the purpose of IVF. These women, however, need to know that their chances of having a successful outcome are significantly reduced, compared to women of similar age with normal FSH. This reduction is because they will produce fewer eggs and not because the eggs are older. We should not expect a large number of follicles to develop and to be prepared to go ahead with egg collection with a lower number of follicles.

It is well established that the number of embryos available to choose from or to transfer is perhaps the second most important factor after age. The more eggs we collect, the more embryos available to make a selection of the best embryos to put back. As a result, the live birth rate significantly improves. Women with elevated FSH are associated with increased cancellation rates, as higher amounts of drugs are given to effect ovarian response and fewer eggs are collected. Whatever eggs are collected, they have the same fertilization rate - but there will be fewer embryos to choose from. Consequently the pregnancy rate will be lower, the higher the level of FSH - with fewer embryos to transfer. The level of FSH, however, does not affect the miscarriage rate. Once these women became pregnant they have the same chance of achieving a live birth as those with normal FSH. All this confirms our view that the reduction in outcome in these patients is secondary to a quantitative reduction in the eggs rather than a qualitative one. The incidence of aneuploidy is the same - regardless of the level of FSH. Aneuploidy rate, however, is significantly higher in older women with normal FSH compared to younger women with elevated FSH. (Personal experience).

**Why do we assess ovarian reserve?**

There are many tests to assess ovarian reserve: the most common and the cheapest is the measurement of day 3 FSH as well as the level of estradiol. Clinicians may also use the measurement of Inhibin B or Anti Mullerian Hormone (AMH). The latter might be more sensitive. Dynamic tests have been devised including clomiphene challenge test and GnRH-a stimulation test (GAST). The purpose of all these tests is to assess the potential responsiveness of the ovary to stimulating agents.

The assessment of ovarian reserve can be beneficial to patients undergoing assisted conception treatment. As suggested above it helps in determining the dose of the medication to induce multiple follicular development. Patients with reduced reserve will require higher doses of medication and those with sensitive ovaries require a more measured approach. Most clinics, however, use a basal day 3 FSH level or other ovarian reserve tests as a screening tool to assess the chance of individual patients achieving a pregnancy or a live birth with IVF treatment. It has been suggested that the reason some clinics refuse to treat patients with elevated basal FSH is to maintain the clinic’s overall success rate or to improve their position on the league tables (Sharif and Afnan, 2003).

Elevated day 3 FSH levels in a previous cycle is associated with a reduction in the overall live birth rate if compared to women with normal basal FSH levels. (Abdalla & Thum 2004). Nevertheless, the live birth rate was considered reasonable, especially in cycling women under the age of 38 with FSH between 10 – 20 IU/L where the chance of a live birth is at least 20%. This is comparable to the national average LBR in the UK (HFEA Patient Guide 2001) in patients who are not considered by most assessments to be average! In fact 2 patients with FSH > 20 IU/l achieved a live birth: one in her first cycle; and the other in her second cycle, giving a cumulative LBR of 19.2%. Indeed patients of all groups of elevated levels of FSH (> 10 IU/L) where the age < 38, the LBR was in excess of 20% per single cycle with a cumulative LBR after 3 cycles of 49.3%. These results are, for a significant number of women, a far better choice than the alternatives of oocyte
It is true that if the woman has reduced ovarian reserve she may respond very poorly necessitating in some cases canceling the cycle. We do however believe that the best arbiter is to give those patients ovulation induction agents and gage their response rather than condemning them for either repeated futile testing or denying them treatment altogether on the mere potential of their response.

The practice of canceling treatment for poor responders should be questioned. A study completed by Ranieri et al 1998; showed that 27% of the patients had their treatment cycles cancelled as they were considered poor responders (< 5 follicles of 15 mm and E < 200 pmol/l). Was it right to cancel these treatment cycles and advise the patients of other modalities of treatment? Analysis of similar data in our program in patients of similar mean age and similar criteria who were not cancelled showed a live birth rate of 23% which was significantly lower than those with who had 5 or more follicles who yielded a live birth rate of 32%. Nevertheless a live birth rate of 20% is a better outcome than canceling the cycle. The problem here is not that they produce fewer live births, but whether such a lower live birth rate is acceptable to these patients. Clinicians should advise patients with reduced ovarian reserve to expect a lower pregnancy rate, due to the lower number of eggs she will produce, as compared to their counterpart of similar age who may produce a larger number of eggs. Clinicians and patients alike should also accept that patients with high FSH levels will have a poorer ovarian response and be prepared to go ahead and have egg collection when small numbers of follicles have developed.

In summary, cycling women with high basal day 3 FSH will have a lower chance of achieving a live birth, but there is still a reasonable chance of success even with FSH levels up to 20 IU/L. High levels of FSH reflects reduction in number but not quality of oocytes, while age results in reduction of both oocyte quality and number. In the current system, many of the women with elevated FSH are lead to believe that they are unsuitable for IVF treatment and would have no chance of a successful outcome. These women are forced to consider other treatment options to provide them with a chance of motherhood, although not with their own genetic child. A chance, although a reduced one, of achieving a pregnancy with their own genetic child is a precious and important opportunity for them to consider. For some woman, a lower chance is better than no chance at all - and to deny them this choice is a tragedy. Every woman in this position should have the right to choose. The level of basal FSH should not be used as a screening tool to select patients for treatment; instead it should be used as additional information to counsel patients appropriately regarding the realistic chance of conception as well as aiding the clinician in determining the appropriate dose of gonadotropins.

It is our belief that it is incumbent on the clinician to be candid with their patients and carry out their wishes, and not allow their vanity (the clinic’s success rate) to stand in the way of the wishes of the patients.

REFERENCE


Hossam I Abdalla, FRCOG
Director of Lister Fertility Clinic
The Lister Hospital
Chelsea Bridge Road
London SW1W 8RH
UK

Comment by: Aboubakr M. Elnashar, M.D.
Benha, Egypt.

Ovarian reserve is a term used to describe the functional potential of the ovary and is currently defined as the number and quality of the follicles left in the ovary at any given time. Various
methods are currently used in the assessment of ovarian reserve in order to predict the outcome in assisted reproduction. There is currently no clinically useful predictive test sufficiently accurate and distinct to assess ovarian reserve accurately. Female age alone is a rough parameter for assessing ovarian reserve. The basal follicle stimulating hormone level is not adequately sensitive to predict poor outcome and the same is true for other basal parameters, including basal estradiol, the follicle stimulating hormone/luteinizing hormone ratio, and inhibin-B levels.

Most IVF units use basal FSH levels as an indicator of ovarian responsiveness, even though the evidence to support its efficacy as a routine test is weak. There is some evidence to support the predictive value of FSH in a population of women at high risk (women >40 years of age, women with poor response to ovarian stimulation and women who have failed to conceive in previous cycles) in terms of the likelihood of achieving pregnancy through assisted reproduction. In contrast, the role of day 3 FSH in the evaluation of young healthy women is extremely limited (1). Basal FSH is simple to perform but does not diagnose poor ovarian reserve until high thresholds are used. As a test, it does not predict pregnancy and should not be used to exclude people from assisted reproduction technology (ART), especially regularly cycling young women.

A fall in day 3 inhibin-B levels may predict poor ovarian reserve before the expected rise in day 3 FSH. However, other studies do not support its use as a predictive marker in IVF (2). Inhibin-B levels are influenced by the amount of fat in an individual, suggesting that the follicles of obese women do not produce as much inhibin-B as those of lean women.

Anti-Müllerian hormone (AMH) has been suggested as a predictor of ovarian response. AMH is the only marker of ovarian reserve that can be tested in follicular as well as luteal phase, although the threshold levels in both phases need to be standardized (3). AMH levels have been found to be two to three times higher in PCOS women, making it difficult to find a threshold value for poor ovarian reserve without a significant overlap with normal values. Although it is the most sensitive and specific indicator of ovarian response (thresholds 25 pg/l), compared with other available tests, it does not predict pregnancy. AMH may be a better marker of ovarian responsiveness than inhibin B, as it may reflect the size of the larger resting pool of pre-FSH-dependent follicles. AMH appears to have less inter-cycle variability than other markers of ovarian reserve.

A systematic review has demonstrated the superiority of antral follicle count (AFC) over basal FSH in the prediction of poor ovarian response (4). Although AFC is the single best available predictor of response to ovarian stimulation with exogenous gonadotrophins, the precise definition of what constitutes an antral follicle is variable, with cited diameters ranging between 2–10 and 2–5 mm. Moreover, different thresholds for defining low AFC are used in different studies. Inter-cycle variability appears to be more significant in young women and in women with high AFC. Hence, a low AFC in young, infertile but ovulatory women should be interpreted cautiously, as this may not indicate poor ovarian reserve. The performance of AFC for predicting failure to achieve pregnancy is poor. This is because while AFC determines the number of oocytes, a clinically relevant outcome (pregnancy or live birth) depends on oocyte quality as well as quantity. A correlation was found between ovarian volume and reproductive success in ART cycles; however, the likelihood ratio of a positive test with regard to pregnancy was 1.0–1.4, suggesting that its value is limited (5). Moreover, there is a wide range in the definition of normal ovarian volume in the reproductive age group.

All the dynamic tests are more expensive, invasive and associated with the side effects of administered stimulation regimens. Recent meta-analysis has shown that the clomiphene citrate challenge test (CCCT) is no better than basal FSH in predicting a clinical pregnancy (6). Gonadotropin agonist stimulation test (GAST) did not perform better (4). In addition, its predictive ability towards ongoing pregnancy is poor. It is clear that none of the above tests fulfill the criteria for a good screening test as opportunistic screening or to develop a mass screening program.

Combinations of various markers (AFC, AMH and inhibin-B) have been tried, and a joint scoring system has been developed which predicts a poor
response to gonadotrophin stimulation at best with 87% sensitivity and 80% specificity and a positive likelihood ratio of 4.36%. However, they have not been tested for prediction of pregnancy (7). From these data it seems that compared to other ORTs, multifactor models do not create a definite improvement in predictive capacity. All the ovarian reserve tests (ORTs) described so far test oocyte quantity. None of the available tests or combination of tests, of ovarian reserve has been shown to predict pregnancy or live birth with sufficient accuracy. Therefore, the clinical utility of each test in isolation would be insufficient for them to be recommended in routine IVF practice. However, measurements may be useful in patients with a high pre-test probability of poor response and/or cycle cancellation due to reduced ovarian reserve. Whether this information should be used to discourage women from entering IVF treatment is debatable, because ovarian reserve testing is better at predicting a reduced response to stimulation than the possibility of pregnancy. This information can be used as a basis for discussion of the likelihood of a poor response to stimulation and possible reduced chance of success with IVF. As long as patients are aware that their chances of success are reduced, it seems reasonable to offer them the chance of pregnancy. Ovarian reserve testing in these women may help with treatment decisions and counseling about prognosis but should not be used to stop access to treatment.

A systematic review by Broekmans et al. (8) concluded that the role of routine ORT is limited. The value of ORT prior to IVF for individual couples strongly depends on the prevalence of IVF failure as well as on the valuation of the false positive (incorrect withholding IVF) or false negative (incorrect performing IVF) outcomes. Variations in the pregnancy rate of the IVF program have an important effect on the value of the ORTs. If the pregnancy rate increases from 20 to 50%, the test accuracy of ORT has to improve very strongly. It should be noted that the use of pregnancy as outcome parameter for the assessment of ovarian reserve status may be insufficient if only one exposure cycle is taken into account. As such, the possibility of misjudgment on the basis of currently known ORTs is hard to rule out. This implies that the use of the test as a method to deny treatment to assumed ovarian aged women should be declined and, as a consequence the test should not be applied on a regular basis and should only be used for counseling or screening purposes. Treatment of all couples without testing was found to generate less distress than testing for ovarian reserve. Based on the decision analysis, where current test accuracy and preference inventory among patients and physicians were used, testing for ovarian reserve seems not useful for current IVF programs.

In the assisted conception population, the first cycle of IVF still remains the most informative test in terms of how a woman will respond to ovarian stimulation. Recent work has shown that only the combination of an abnormal ORT and a poor ovarian response in the first cycle (the expected poor response case) indicates very low prospects in subsequent cycles (9). As poor ovarian response will provide some information on ovarian reserve status, especially if the stimulation is maximal, entering the first cycle of IVF without any prior testing seems to be the preferable strategy. Once a poor response is obtained, the question arises whether this finding is based on depleted ovaries or other causes, like underdosing for instance, based on the presence of certain FSH receptor polymorphisms. A repeat cycle with adequate, maximal stimulation or a post hoc-performed ORT [basal FSH or AFC] may correctly classify the poor responder patient having an aged ovary (4) and may correctly suggest that they refrain from further treatment (9). In conclusion: use of any ORT for outcome prediction cannot be supported and entering the first cycle of IVF without any prior testing seems to be the preferable strategy. Regularly cycling women should not be excluded from IVF on the basis of abnormal results following ORTs.

REFERENCES


Aboubakr Mohamad Elnashar, M.D.
Prof. of obstetrics and gynecology
Benha university hospital
E-mail: elnashar53@hotmail.com

Comment by: Raja Al-Karaki, M.D.
Amman, Jordan

Ovarian reserve is one of the prognostic factors that determine IVF outcome. It is defined by the number of follicles and the quality of oocytes in the ovary and describes its functional potential. Ovarian reserve screening in IVF identifies women with poor ovarian response to stimulation and diminished chance of achieving pregnancy. Currently, one of the issues being debated revolves around which tests should be used for its assessment and how much is their significance. In most ART programs, several parameters known as ovarian reserve markers have been tested to estimate the functional state of the ovary. These include hormonal markers and ultrasonographic parameters.

Hormonal markers

Much of the ovarian reserve literatures focus on basal FSH levels (measured on day 3 of menstrual cycle) as an indicator of ovarian responsiveness to stimulation. Basal FSH elevation can predict fewer recruitable eggs, cycle cancellation and poor pregnancy potential. Although the usefulness of FSH as a routine test in ovarian reserve estimation in IVF programs has been questioned, there is some evidence to support its predictive value in women with poor chance of achieving pregnancy (women over 40 years of age, women with poor response to ovarian stimulation and women who failed to conceive in previous cycles) (1). The clinical value of measuring FSH depends on the choice of the cutoff level. The mostly used FSH assays suggest that modest FSH elevations are in the range of 10-20 IU/L while marked elevations are above 20 IU/L. In reality, there is no level that can absolutely predict a failed outcome after treatment. In case of elevated FSH > 15 IU/L, patients should be told that although the prospects of conceiving is low, first trial treatment is justified. Patients with mildly elevated levels (10-15 IU/L) seem to have a good probability of getting pregnant. Furthermore FSH level should be regarded in view of other variables including age and cycle regularity. Some studies reported that a moderately elevated FSH level in IVF population has a limited predictive value in young women and age seemed to be a more important predictive factor for the occurrence of pregnancy than FSH (2). In contrast, it has recently been shown that younger patients with elevated FSH above 10 IU/L performed as poorly as the older patients after IVF (3).

To use day 3 FSH in a better way, the inclusion of cycle regularity as an additional measure of ovarian reserve was investigated. Some groups concluded that it is not justified to exclude patients with regular cycles from treatment on the basis of FSH value alone (4). For patients with FSH levels above 15 IU/L and cycling regularly, ongoing
pregnancy rate is quite acceptable. On the other hand, low results were reported in another study which included patients with irregular cycles in addition to elevated FSH (5). Cycle irregularity reflects more advanced stage of reproductive ageing leading to much less favorable outcome.

Concerning further tests, it is still debatable whether inhibin B and estradiol levels are useful predictors for ovarian reserve. Some reported that women with low inhibin B levels (< 45 pg/ml) demonstrated a poorer ovarian response than women with higher levels (6), while others did not support its use as a predictive marker in IVF (7).

Regarding day 3 estradiol, no correlation has been detected between elevated levels ≥ 50 pg/ml and IVF outcome (8). In contrast, this level was associated with higher cancellation and lower pregnancy rates independent of FSH levels (9). Moreover, elevated day 3 estradiol may predict poor ovarian response even when basal FSH is normal (10) which reflects that estradiol level on day 3 could be of added value for counseling patients with normal FSH concerning their reproductive potential.

Anti-Mullerian hormone (AMH) has been suggested as an indicator of ovarian response (threshold = 25 pg/L) and been found to decline with advanced female age (11). However some of data showed that AMH can not predict pregnancy (12).

Other markers of ovarian reserve are dynamic tests which involves The clomiphene citrate challenge test (CCCT), exogenous FSH ovarian reserve test (EFORT) and GnRH agonist stimulation test (GAST).

An abnormal CCCT is defined by an elevated FSH on day 10 after administration of 100 mg clomiphene citrate on days 5-9. It has a high predictive value for cycle cancellation due to poor ovarian response and failure to conceive (13). The test may be superior to basal FSH screening because it appears to be more sensitive and unmasks patients who might not be detected by basal FSH screening alone. However, it is recommend to screen patients with both day 3 and day 10 FSH levels.

EFORT measures basal FSH, estradiol and estradiol response one day after 300 IU FSH administration on day 3. It has been studied for detecting poor responders (14) while it was not tested for prediction of pregnancy in IVF population.

GAST evaluates the change in estradiol level from cycle day 2 to 3 after administration of 1 mg of leuprolide acetate. While elevation of estradiol may be associated with good ovarian response, this test did not show better clinical value of ovarian reserve when compared with basal day 3 FSH, antral follicle count and inhibin B (15).

Ultrasonographic markers

Imaging techniques may further help in predicting poor ovarian response in patients undergoing IVF with normal ovarian functional tests. The measurements of ovarian volume, antral follicle count (AFC) and ovarian stromal blood flow with color Doppler seem to be helpful markers. It was demonstrated that in women with low ovarian volume (< 3 cm3), poor response to stimulation is evident with lower pregnancy rate (16). Recently, antral follicle count has been evaluated extensively as a test of ovarian reserve in patients with limited ovarian response. The superiority of AFC over basal FSH in the prediction of poor ovarian response was illustrated while its significance for predicting failure to achieve pregnancy is poor (17). Issues to be addressed are the possible inter-cyclic variability and the lack of consensus regarding the definition of low AFC which necessitate careful interpretation. Ovarian stromal blood flow is another parameter measured to assess ovarian reserve. It has been reported that ovarian response during IVF is significantly correlated with mean peak systolic velocity (PSV) which is higher in normal responders than in poor responders (18).

CONCLUSION

We would like to recommend that the ovarian reserve should be assessed in all IVF patients prior to stimulation to predict those who will respond poorly.

The real clinical value of measuring ovarian reserve markers prior to IVF is debatable, therefore careful interpretation of the results are needed
because this will influence the decisions in patients' management.

The benefit of the available tests of ovarian reserve is counseling women regarding poor prognosis but none of these tests have been shown to predict ongoing pregnancy or live birth with sufficient accuracy. Abnormal test values can predict those who will respond poorly and reflects low chances for conception. However, these tests should not be used to exclude patients from treatment especially regularly cycling young women.

REFERENCES

2. Esposito MA, Contifiani C, Barnhart KT. A moderately elevated day 3 FSH concentration has limited predictive value, especially in younger women. Hum Reprod 2002; 17:118-23.

Raja Al-Karaki, M.D.
Assisted Reproduction Technology Unit
Al- Amal Maternity Hospital
Amman, Jordan

Comment by: Samir M. Al-Halawat, FRCOG
Riyadh, Saudi Arabia
Hesham Al-Inany, M.D, PhD
Cairo, Egypt

The ovarian reserve, constituted by the size of the ovarian follicle pool and the quality of the oocyte therein, decline with increasing age, resulting in the decrease of a woman’s reproductive function (1). At birth, about one
million oocytes are present. This number decreases during childhood, resulting in a primordial follicle pool of 300,000-500,000 follicles at menarche, as a result of atresia of the majority of the growing follicles (2). After puberty, under the effect of follicle stimulating hormone (FSH), only one follicle is selected to become the dominant follicle, which will ovulate under the influence of luteinizing hormone (LH) (3). This process continues throughout life until the primordial follicle pool is exhausted, resulting in menopause. Menopause is preceded by a transition period, during which fertility decreases and menstrual cycle become irregular. This menopausal transition period precedes menopause by a fixed time interval (4-6). The median age of menopause is variable according to ethnic origins. However, there is considerable individual variation in the age of menopause and, subsequently, also in the age of subfertility (5, 6). Hence, chronological age is a poor predictor of reproductive aging and thus the ovarian reserve.

To assess the ovarian reserve, early follicular serum levels of FSH, inhibin B and estradiol (E2) have been measured. With the decline of the follicle pool, serum levels of inhibin B and E2 decrease and serum FSH levels rise (7). The changes of serum levels of these hormones occur relatively late in the reproduction aging process (8). The assessment of the antral follicle count (AFC), best predicts the quantitative aspect of ovarian reserve (9). However, measurement of the AFC requires an additional transvaginal ultrasound examination in the early follicular phase.

Therefore a serum marker that reflects the number of follicles that have made the transition from the primordial pool into the growing follicle pool, and that is not controlled by gonadotrophins, would benefit both patients and clinicians. In recent years, accumulated data indicate that anti-Mullerian hormone (AMH) may fulfill this role.

**Anti-Mullerian Hormone (AMH)**

In the middle of the 20th century, Alfred Jost showed that a testicular product different from testosterone was responsible for the regression of Mullerian ducts in the male fetus. It is produced by Sertoli cells of the fetal testis (10, 11). In the absence of AMH, Mullerian ducts of both sexes develop into the uterus, fallopian tubes and upper part of vagina (12, 13).

AMH is a homodimeric disulfide-linked glycoprotein with a molecular weight of 140 kDa. The gene is located on the short arm of chromosome 19 in humans, band 19p 13.3 (14). AMH is strongly expressed in Sertoli cells from testicular differentiation up to puberty and to a much lesser degree in granulosa cells form birth up to menopause (15, 16).

Granulosa cells of primary follicles show homogenous AMH expression; in larger follicles, AMH is mainly produced in cells near the oocyte and in few cells surrounding the antrum. AMH continues to be expressed in the growing follicles in the ovary until they have reached the size of differentiation state at which they are to be selected by the action of FSH 23. In mouse this occurs at the early antral stage in small growing follicles (17), and in the human in antral follicle of size 4-6 mm (18). AMH is not expressed in atretic follicle and theca cells (18-21).

The findings of Salmon et al (22) study on the oocyte regulation of AMH expression in granulosa cells in mice suggest that oocyte regulation of granulosa cell gene expression occurs during extended periods of follicle development, and that oocyte regulation of AMH expression may play a role in intra-and interfollicular coordination of follicle development.

**Control of primordial follicle by AMH**

The activation of primordial follicles and the pace of follicular development are regulated by both positive and negative factors. AMH is considered as a negative regulator of the early stages of follicular development (23).

Durlinger et al (24) investigated whether the effects of AMH were directly on the primordial follicles. 2-day-old AMH null mice ovaries were cultured in vitro in the presence of AMH. After 2 days of in vitro exposure to AMH, about 50% of growing follicles were found, showing that AMH may directly affect the primordial follicles themselves. Similar results were obtained in in-vitro experiments on the bovine ovarian cortex (25). These studies suggest that the presence of
AMH acts as a brake on the activation of primordial follicles and the growth of preantral follicles.

The effects of AMH on the response of ovarian follicles to FSH during cyclic recruitment in mice was demonstrated in both in-vitro and in-vivo studies. Durlinger et al. (26) study demonstrated that follicles are more sensitive to FSH in the absence of AMH. AMH was found to inhibit the FSH dependent follicle growth in a time dependent manner (26). This effect of AMH was mainly the result of reduced granulosa cell proliferations and is consistent with another in-vitro study, in which it was shown that exogenous AMH reduced aromatase expression and the number of LH receptors in cultured granulose cells (27).

The earlier study of Ueno et al (28) showed that AMH inhibits the first meiotic division of diplotene oocytes in immature rats. However, this has not been confirmed by others (29). In addition, AMH blocked the proliferation of human granulose-luteal cells in vitro (30), and the concentration of AMH in follicular fluid was inversely proportional to mitotic indices of granulosa cells in vivo (31). Thus, AMH appears to have an autocrine role in the maturation of normal follicles. Salmon et al. (22) has recently shown that oocytes up-regulate AMH expression in granulosa cells in a way that is dependent upon the development stage of the oocyte.

Recently, an ultrasensitive enzyme-linked immuno-sorbent essay (ELISA) for AMH was developed (32, 33) with sensitivity as low as 0.1 ng/ml. More recently, a double-antibody ELISA for AMH has been developed with a detection limit of less than 0.078 ng/ml (34).

Clinical utility of AMH measurement

AMH levels in women are lower than in men throughout life. In infant and child females AMH serum levels are almost undetectable at birth (19), with a subtle increase within the first 2-4 years of age, then AMH appears to be stable until adulthood. Later it decreases as a sign of follicular reserve exhaustion (35). Serum AMH level have been measured at different times during the menstrual cycle, suggesting extremely subtle or non existent fluctuation (36, 37). Minimal fluctuations in serum AMH levels may be consistent with continuous noncyclic growth of small follicles. Hence, AMH is relatively convenient to determine, especially as it seems to exhibit a fairly stable expression during the menstrual cycle, making it an attractive determinant of ovarian activity (38). However, published data (36, 38) are lacking with regard to day-to-day fluctuation and pulstility.

AMH as a marker of ovarian aging

As discussed above, the quantitative aspect of ovarian aging is reflected by decline in the size of the primordial follicle pool. Direct measurement of the primordial follicle pool is impossible. However, the number of primordial follicles is indirectly reflected by the number of growing follicles (39). Hence, a factor primarily secreted by growing follicles will reflect the size of the primordial follicle pool. Since AMH is expressed by growing follicles up to selection (40), and can be detected is serum (41, 42), it is a promising candidate. Recent studies suggest Serum AMH levels show a reduction throughout reproductive life (42). Undetectable AMH levels after spontaneous menopause have been reported (35,43,44,77). Ovariectomy in regularly cycling women is associated with disappearance of AMH in 3-5 days, demonstrating that circulating AMH is exclusively of ovarian origin (33, 44).

In young normal ovulatory woman, early follicular phase hormone measurement at 3 years interval revealed that serum AMH levels decline significantly whereas serum levels of FSH and inhibin B and the AFC do not change during this interval (42).

Stratification for age revealed that both serum AMH levels and the AFC decline with age. Importantly, a strong correlation of serum AMH levels with AFC was observed. This positive correlation was later confirmed by (45), who showed a strong correlation between serum AMH levels and AFC than between AMH and serum levels of inhibin B, FSH, and E2 on cycle day 3.

The results of deVert et al (76) also suggest that changes in serum AMH levels occur relatively early in the sequence of events associated with ovarian aging. Substantially elevated serum levels
of FSH are not found until cycles have already become irregular (8). Therefore, a marker that already shows a considerable change when cyclicity is still normal would better identify women with declining fertility. In a similar study done by Rooij et al (43), it has been shown that AMH gave the highest accuracy to predict occurrence of menopausal transition when compared with other markers. Furthermore, compared to other ovarian markers, only AMH was the only marker of ovarian reserve sharing a mean longitudinal decline over time (44).

The usefulness of serum AMH levels as a measurement of ovarian reserve was recently shown in young women after treatment for childhood cancer (46). Chemotherapy and radiotherapy treatment may result in loss of primordial follicles. Cancer survivors, consequently have partial loss of ovarian reserve which is reflected by increased FSH levels and decreased ovarian volume. Unexpectedly, the numbers of small central follicles is unchanged (47), a finding that may reflect a low accuracy and observed dependency of AFC measurements. Nevertheless, serum AMH levels were decreased in these patients, supporting the use of AMH as an early predictor of ovarian reserve.

**AMH as a marker of ovarian responsiveness**

AMH levels are also seen to decline gradually during FSH administration as part of controlled ovarian hyperstimulation (COH) (37, 48).

The decrease in AMH in FSH-treated women might be the result of growth stimulation by FSH of the follicles that enlarge, thereby losing their AMH expression (2, 23).

Throughout COH, serum AMH levels correlated positively with the number of small but not larger antral follicles, and with inhibin B serum levels (45). It was concluded that serum AMH levels decline gradually during multiple follicular maturation. This was confirmed in a recent study in which AMH levels in follicular fluid were evaluated (49). Small follicular (8-12 mm in diameter) secreted AMH at levels that were approximately three times as high as those of larger follicles (16-20 mm in diameter).

AMH is produced by the growing antral follicles in the human ovary up to the selection stage (4-6 mm) (18). It may serve as a serum marker of ovarian reserve for women undergoing in-vitro fertilization (IVF). Serum AMH showed an excellent to correlation with AFC (42).

Van Rooij et al. (50) conducted a study showing that the use of AMH serum levels as a measure of ovarian reserve was tested in women undergoing IVF treatment. The study has shown that the ovaries of normal responding women contained more growing antral follicles than ovaries of women with a poor response to IVF treatment. In addition, AMH serum levels were lower in the poor responders than in normal responders. Serum AMH levels correlated strongly with AFC, the number of follicles retrieved, age, inhibin B and FSH. In addition, logistic regression analysis for prediction of poor response showed that serum AMH levels had a better prediction value than serum levels of FSH, inhibin B and E2 (51), and that the prediction values for AMH and AFC were almost identical (50,52). Serum AMH levels on day 3 may also be of same value in predicting clinical pregnancy outcome in IVF cycles (51). However, in a recent perspective study (53) it was reported that AMH serum levels obtained in day 3 during COH for IVF are better predictors of ovarian response than day 5. However, AMH does not seem to be useful in the prediction of pregnancy. In a large prospective study done by Tremellen et al. (54) on 238 women undergoing IVF, it was concluded that AMH assessment was shown to predict ovarian reserve using a cut-off value of 1.13 ng/ml, with 80% sensitivity and 85% specificity.

AMH levels have also shown to be 10 folds lower in the cancellation cycles compared with patients who had a completed IVF cycle (55). AMH seems to be a better marker in predicting a cancelled cycle compared with FSH or inhibin B. Using a cut-off of 0.1 ng/ml, AMH had 87.5% sensitivity and 72.2% specificity in the prediction of cancellation (23).

**CONCLUSION**

The studies described here indicate that serum AMH levels decrease with age in premenopausal women. In addition, they suggest that AMH levels
reflect the size of primordial follicle pool. The relative stability of AMH serum levels indicate that AMH could be used as a marker for ovarian aging and for ovarian response to controlled ovarian stimulation. Compared to other ovarian tests, AMH seems to be the best marker reflecting the decline of reproductive function.

REFERENCES


5. te Velde ER, Dorland M, Broekmans FJ. Age at menopause as a marker of reproductive ageing. Maturitas 1998; 30; 119-125.


23. LaMarce Volpe A. Anti-Mullerian hormone (AMH) in female reproduction: is measurement of circulating AMH useful? Clinical endocrinology 2006; 64: 603-610.


49. Van Rooij IA, Broekmans FJ, te Velde ER, Fauser BC, Bancsi LF, Jong FH and Themmen AP. Serum anti-Müllerian hormone levels: a novel measure of ovarian


Samir M. Al-Halawat FRCOG
Hesham Al-Inany, M.D, PhD
Women’s Specialized Hospital,
King Fahad Medical City
P.O. Box 49065 Riyadh, Saudi Arabia 11525