Review Article

Comprehensive review of Ovarian reserve assessment techniques

ABSTRACT

Ovarian reserve assessment techniques play a pivotal role in the management of female subfertility, specifically for predicting the response to Invitro Fertilization. In this study we have discussed a plethora of static and dynamic tests in order to determine the clinically preferred marker of ovarian reserve. Nine randomized and quasi-randomized clinical trials on AMH and other Ovarian Reserve markers, published over the last 20 years were selected for review~~, from the advanced search builder of PubMed~~. Markers used in the prediction of ovarian response include age, BMI, AMH, AFC, FSH, Inhibin B, Estradiol, LH, Basal ovarian volume, CCCT, GAST and EFORT. After an in depth review of the nine studies, AMH appears to the preferred marker of ovarian reserve in the detection of both poor and hyper response to ovarian stimulation. However, combinations of markers may be superior in the prediction of ovarian response and may result in a reduced rate of cycle cancellation. In conclusion, the overall performance of AMH with regard to intra and inter cycle variability, sensitivity and specificity is superior to the other assessment techniques included in this study.

Keywords: ovarian reserve, AMH, ovulation, fertility, AFC, IVF

Comment [AaM1]: In-Vitro Fertilization (IVF)

Comment [AaM2]: Anti-Mullerian Hormone (AMH)

Comment [AaM3]: No need; You may delete it.

Comment [AaM4]: body mass index (BMI)

Comment [AaM5]: Antral Follicle Count (AFC)

Comment [AaM6]: follicle stimulating hormone (FSH)

Comment [AaM7]: luteinizing hormone (LH)

INTRODUCTION

Ovarian reserve is a description of the functional potential of the ovary and is a measure of the quality and quantity of oocytes remaining.[1] Fertility in women begins to drop from the age of 32 years and sees a rapid decline after the age of 37 years. This is evident from the decline seen in cumulative pregnancy rate being 74% in women younger than 31 years, 62% in women between the ages of 31-35 years and 52% over the age of 35 years. [2] This is attributed to the age-related increase in oocyte atresia, as well as the increase in other conditions namely leiomyomas, endometriosis, uterine surgery and systemic disorders which are associated with increased age. Studies revealed that the maternal age at first pregnancy in several European countries is reaching 30 years with multiple women becoming primiparous above the age of 35 years [3] Due to this increased incidence of postponing childbearing, there has been an increase in the number of subfertility cases and the need for assisted reproductive techniques (ART) which makes assessment of ovarian reserve prudent.

In the past 20 years, a plethora of ovarian reserve tests have been developed to predict the outcome of In-Vitro Fertilization (IVF) in terms of chances of pregnancy through oocyte yield and quality as well as in optimizing the treatment protocol in controlled ovarian stimulation. These tests have become a routine in patients opting for ART. [4] Since the inception of the development of these tests, there has been significant variation in the predictive value, sensitivity, specificity, inter and intra cycle variability of markers of ovarian reserve. Recent studies have increasingly focused on Anti-Mullerian Hormone (AMH), Follicle Stimulating Hormone (FSH) and Antral Follicle Count (AFC) as reliable markers of ovarian reserve [5]

Although various biomarkers provided immense insight into ovarian reserve, till date none of them have been suitable to be a stand-alone predictive marker to satisfy the criteria to be established as a single parameter for ART. In this study, we will focus on AMH as a marker for ovarian reserve along with the various other markers. The objective of this study will be to look

into the efficacy of AMH as an ovarian reserve marker and assess how it compares to other known markers of ovarian reserve.

METHODS

A search for randomized and quasi-randomized clinical trials on AMH and other ovarian reserve markers was made in PubMed Database over the last 20 years from 2001-2021. The advanced search builder of PubMed was implemented with the following keywords to generate citations: Ovarian reserve, oocyte count, AMH, antral follicle and assisted reproduction. Only English articles were taken into consideration for this review.

A total of 9 studies were found matching with our study criteria. The various ovarian reserve assessment techniques were compared with one another with regard to sensitivity, specificity, intra and inter cycle variability.

REVIEW FINDINGS

Anti Mullerian Hormone and its physiology

Comment [AaM8]: AMH

AMH is a dimeric glycoprotein produced by the gonads and secreted into the circulation. Production in females begin as early as 36 weeks in utero, peaking approximately at 25 years after which AMH levels remain almost constant until the initiation of follicular reserve exhaustion. [15] In females, AMH is exclusively produced by the granulosa cells of the primary, pre antral and small antral follicles of the ovary. Peak production is at the small antral follicular stage at 6-7mm, after which AMH levels decline and FSH dependent follicular growth begins. [16]

The mystery of somatic sex differentiation was resolved by Professor Alfred Jost in the 1940s, when he proved the role of AMH, formerly known as Mullerian inhibiting substance in the regression of the Mullerian duct and the formation of the Wolffian ducts, urogenital sinus and the external male genitalia during fetal development [17] Subsequently, the absence of AMH causes

the embryo to develop into a female with the development of the Mullerian ducts into the fallopian tubes, uterus, cervix and upper one third of the vagina. [18]

AMH plays a role in the conservation of ovarian reserve inhibiting both, the early stages of follicular recruitment, thereby preventing over recruitment of follicles from the primordial follicular pool, [19] as well as reducing cyclical recruitment by decreasing the sensitivity of primordial follicles to FSH after puberty, influencing the age of onset of menopause. [20]

AMH in the assessment of ovarian reserve

With the global increase in focus placed on career, more women are putting off childbearing, leading to an increase in the subfertility rates and the subsequent need for ART.[21] A crucial step in ART is to obtain an optimal ovarian response for controlled stimulation. Poor response may result in cycle cancellation and a lower probability of pregnancy. Hyper response may also result in cycle cancellation, as well as ovarian hyperstimulation syndrome (OHSS). [22] This optimal response is achieved through the measurement of markers of ovarian reserve and the subsequent individualization of gonadotropin stimulation. [23]

The success of ART is primarily dependent on the quality of the recruitable cohort of oocytes. Oocyte quality and quantity follows an age dependent decline due to the gradual accumulation DNA damage and reduced capacity of DNA repair. AMH emerged as a quantitative and qualitative measure of oocyte yield and ovarian response in many studies. Hiedar et al. reported normal ovarian responders in the age category of 20-25 to have an AMH level between 0.11-7.64ng/ml, 26-30 year olds having AMH levels between 0.10 – 6.96ng/ml, 31-35 year olds having AMH levels between 0.095 – 6.44ng/ml and 36- 43 year olds having levels between 0.08-5.95ng/ml. [19][24][25] Doubts however have also been raised in the literature of AMH as an indicator of oocyte and embryo quality. Lie Fong et al found that AMH did not correlate with both embryo morphology and chromosomal competency, concluding that a direct relationship between oocyte quantity and embryo quality was absent. [26] Another study by Alexopoulou et

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Hegazy (2014) added that AMH is produced in the early embryonic development from the testes in XY embryo inhibiting development of Mullerian duct in males.

Hegazy A. Clinical embryology for medical students and postgraduate doctors. Lap Lambert Academic Publishing; 2014.

al concluded similar morpho kinetic properties and cleavage patterns of day 2 embryo quality in both normal and poor ovarian response based on AMH levels, indicating a low role of AMH as a predictor of embryo quality. [27] However AMH still remains the preferred marker for functional ovarian reserve due to its superiority as a proxy for the quantitative aspect of ovarian reserve

AMH fluctuations during intra and inter cycle

AMH as opposed to other markers of ovarian reserve stays relatively constant throughout and between menstrual cycles and therefore can be measured at any time of the cycle. [19][28][29] This is a result of AMH levels not being influenced by dominant follicular growth in the late follicular phase of the menstrual cycle unlike other markers of ovarian reserve such as E2, FSH and Inhibin B. [20] A study by ~~J~~van Disseldorp et al in 2010 comparing two of the main markers of ovarian reserve – AMH and AFC found that the inter and intra cycle variability of AMH was significantly less than AFC. [30] This is corroborated by Anderson et al. (2011) who concluded that the intercycle variability was lowest with AMH when compared to AFC, FSH, E2, Inh B, LH and total ovarian volume in both groups studied. However Broekmans ~~FJ~~ et al and Gracia ~~CR~~ suggested that there was substantial variability in AMH levels during and between menstrual cycles, increasingly seen amongst younger women, reflecting the function of AMH in the regulation of follicular growth and the development of a dominant follicle. [22][29] This was supported by John F Randolf who found that at low AMH levels there was no variance across the cycle, while at higher levels a mid follicular increase is observed, followed by a mid cycle decrease and another rise in levels during the mid luteal phase. [31] The variance across the cycle is important, as if levels do fluctuate significantly across the cycle, the optimal time of measurement becomes cardinal.

Correlation to Response

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The correlation of AMH with ovarian reserve is well established and supported by the elevated levels reported in Ovarian tumors [32] and PCOS [33][34] and the diminishing to undetectable levels in the setting of prevalence of primordial follicular exhaustion by approximately 5 years before menopause [35] and in its use as a marker of premature ovarian failure. [34]

Although a consensus threshold in the decline of ovarian reserve is yet to be agreed upon, much of the literature has used an AMH cut off level of 0.98ng/ml. [28] Low responders have been defined as <6 oocytes obtained, while high responders have been defined as >18 oocytes obtained. [22]

IVF cut off values for poor and hyper response have varied greatly in practice. Salmassi et al. described the cut off for poor response as <0.61ng/ml [36a] and Nardo et al. described poor response as <1ng/ml [37] A study by Satwik et al. found upto a 20 fold variability from 0.1-2ng/ml in the cut off levels for poor response. [38] The nine RCTs reviewed in this article reflect this high variability in choosing a cut off for poor response. The cut off values for AMH in the prediction of poor response used by Penarrubia et al. 0.69ng/ml (sensitivity of 53%; specificity of 96%). However both Kwee et al. (2008) and Arce et al. quoted cut off values for poor response significantly higher at 1.4ng/ml (sensitivity of 76%; specificity of 86%) and 1.82ng/ml (sensitivity of 66%; specificity of 80%) respectively.

The comparatively high specificity compared to sensitivity demonstrated in the cut off levels for poor response of Kwee et al (2008), Arce et al. (2013) and Penarrubia et al. reflect the significant social implication of correctly identifying the poor responder and minimizing the proportion of false negative results, thus reducing the possibility of rejecting a woman with potential for a good response from an IVF program. However increased specificity comes at the cost of a compromised sensitivity. From our review, Penarrubia et al had the highest specificity at 96% in the prediction of poor response at a cut off level of 0.69ng/ml ml and a sensitivity of 53%. The low sensitivity implies that an increasing number of true poor responders would be incorrectly picked for IVF. The wide range of values associated with the identification of the poor responder

indicates the large overlap between poor and normal responders. Having said that AMH levels should not be used to absolutely exclude a candidate from ART. Instead, it may be used in the counselling for poor response and with caution in the individualization of stimulation dose.

The determination of an appropriate cut off level of AMH for hyper response stems from the risk OHSS and cycle cancellation. The risk of developing severe OHSS increases beyond 15 mature follicles. [39] There is a mild variation in the upper cut off levels across literature, value by Vembu et al - 4.85ng/ml (sensitivity 85.7%; specificity of 89.7%) [40]; Choi MH et al - 3.55ng/ml (sensitivity 94%; specificity 81%) [41]; Arce et al - 3.92ng/ml (sensitivity 78%; specificity 76%); Kwee et al. at 5ng/ml (sensitivity of 53%; specificity of 67%).

Table 1. AMH

Study	Poor Response			Hyper Response		
	Result (ng/ml)	Sensitivity	Specificity	Result (ng/ml)	Sensitivity	Specificity
Salmassi et al.	<0.61	-	-	-	-	-
Nardo et al.	<1	-	-	-	-	-
Satwik et al.	0.1-2	-	-	-	-	-
Penarrubia et al.	0.69	53%	96%	-	-	-
Kwee et al.	1.4	76%	86%	5	53%	67%
Arce et al.	1.82	66%	80%	3.92	78%	76%
Vembu et al.	-	-	-	4.85	85.7%	89.7%
Choi MH et al.	-	-	-	3.55	94%	81%

Existing literature defines poor response from anywhere between 3-6 oocytes aspirated.[38] Arce et al.- <3 oocytes; Magnusson et al.- <5 oocytes; both Anderson et al. and Kwee et al. - <6 oocytes. Poor response ideally indicates the level below which there would be no chance of pregnancy.

IVF programmes are becoming increasingly wary of the possibility of OHSS. Satwik et al. defines hyper response as an aspiration of >15 oocytes. Anderson et al. and Kwee et al. chose the cut off of 18 and 20 oocytes aspirated respectively, with Kwee et al. having a sensitivity of 53%.[38] Arce et al. and Magnusson et al., being more recent, defined hyper response as >12 oocytes aspirated, with Arce et al. quoting a higher sensitivity of 78%. This reflects the increasing attempts made by IVF programs in ensuring the true hyper responder is not picked, in order to avoid OHSS.

A secondary analysis of IVF patients undergoing a GnRH antagonist protocol concluded that AMH was a good predictor of both low and high ovarian response. [42] Kwee et al. (2008) concluded that AMH performed similarly to FSH, AFC, Clomiphene citrate challenge test (CCCT) and Inhibin B increment in Exogenous follicle stimulating hormone ovarian reserve test (EFORT) in the prediction of poor response. However, the more recent studies by Arce et al. (2013) and Anderson et al. (2011) preferred AMH as a predictor of low ovarian response. Arce et al. concluded that AMH had a high accuracy in the prediction of low response, with FSH, AFC and Inhibin B performing statistically significantly lower compared to AMH. Similarly, Anderson et al. concluded that the only statistically significant predictor of poor response was AMH and smoking. Kwee et al. (2008) and Anderson et al. (2011) concluded that AMH, FSH, AFC and Inhibin B increment in EFFORT were all statistically significant in the prediction of hyper response. However the more recently published study by Arce at al. (2013) concluded that AMH had a better accuracy in the prediction of high ovarian response.

Conflicts in correlation

Several studies have suggested that AMH is more useful in conditions under hyperstimulation and thus is a better predictor of ovarian response rather than ovarian reserve. A study conducted by AZ Steiner et al. amongst a cohort of women in the older reproductive age group attempting to conceive naturally, found that low AMH was not associated with a reduced probability of conception. [43] A RCT done by Shvetha MZ et al. also concluded that both low and high AMH

values showed no correlation to fecundability in natural conception. [44] In another study by Knauff E et al. AMH was found to be a more accurate predictor of ovarian reserve in candidates with elevated FSH, suggesting that it may be a more beneficial in cases with diminishing ovarian reserve. [45]

There are also a few studies that disagree over the superiority of AMH in assisted reproduction and have found other markers to be similar to or even superior to AMH as a marker of ovarian reserve in ART. Yangyang Zhang found that AFC was a better predictor of poor ovarian response compared to AMH as it is a direct measure of the cohort of recruitable follicles [46a] A study by Yubin Lee found that AMH and AFC had a similar predictive value of clinical pregnancy as well as of live birth. [47] However a study done by Rosen claimed that although AMH is a more cost effective measure of ovarian reserve, AFC is more accurate. [48]

A drop in AMH levels has been reported to be the first marker in ovarian decline [28] This promotes it's use as an important marker of ovarian reserve as ovarian decline is more biological than chronological and is highly variable in onset. A review by SL Broer in 2014, confirmed that AMH is the current most accurate measure of the follicular reserve under hyper stimulated conditions. [35]

Other Markers of Ovarian Reserve

Several other markers of ovarian reserve have been discovered and extensively studied in the past to show correlation with ovarian reserve in the setting of assisted reproductive therapy. These markers are classified as static and dynamic tests.

STATIC TESTS OF OVARIAN RESERVE

Static tests assess either biochemical or ultrasound parameters and are as follows

1. Age
2. Body Mass Index (BMI)

3. AFC
4. FSH
5. Inhibin B
6. Estradiol
7. LH
8. Basal Ovarian Volume
9. AMH [1]

Comparison of AMH with other markers of Ovarian Reserve

AGE

Ovarian function is well known to decline with age and several studies claim that age is the primary determinant of the prediction in the success of IVF. [49] However there is a large variance in the onset of ovarian decline as well as the age of menopause. [48] A multivariate analysis found only AMH to be statistically significantly predictive, leading to the conclusion that age is a sufficient predictor till a direct marker of ovarian reserve is applied. [50] Although Freiesleben et al. (2010) and Arce et al. (2013) showed the presence of a significant correlation between age and AMH, Pennarrubia et al. (2005), Kwee et al.(2008) and Anderson et al. (2011) concluded that AMH is a clinically superior marker of ovarian reserve than age.

BMI

A study by Malhotra et al. investigating the effect of obesity on ovarian reserve concluded that obesity directly compromised Inhibin B and thus ovarian reserve. [51] Freiesleben et al. conducted a study on intrauterine insemination (IUI) and found that AMH was not superior to BMI. However, this can be explained by the different stimulation protocol for IUI verses conventional IVF studies. Penarrubia et al. (2005) concluded that AMH was superior to BMI as a marker of ovarian reserve. Thus, in cases of IUI, BMI is an important factor in achieving the

optimal serum FSH and therefore results, whereas in IVF AMH is a better reflection of ovarian reserve.

AFC

AFC is a measure of the sum of antral follicles in both ovaries in the early follicular phase of the menstrual cycle. As the cohort of growing follicles correlates with the size of the primordial follicular pool, the number of follicles measuring <10mm on ultrasound is considered a good reflection of the ovarian reserve. A study by Practice Committee of the American Society for Reproductive, 2015 found AFC to be highly specific at 73-100% but less sensitive 9-73% with the cut off points of 3-4 follicles, concluding against the use of AFC as a single criteria for prediction in ART. [52]

Amongst the RCTs reviewed, Kwee et al. (2007) observed a cut off number of <6 total follicles in both ovaries of appropriate size to be accurate in the prediction of poor response. This observation was associated with high (95%) specificity at the cost of a compromised (41%) sensitivity. An AFC > 16 has been recorded to be sufficient in the prediction of hyper response. [53] Kwee et al. (2007) implemented a cut off >14 follicles (specificity 89% and sensitivity 89%) in the prediction of hyper response.

A study assessing the characteristics of AMH and AFC, concluded that AFC is prone to a large amount of inter- and when a single operator is employed, even intra operator variability. [54] A recent paper by Gracia CR stated that the high correlation between AFC and AMH, has led to AMH being considered as a potential surrogate marker to AFC in the diagnosis of PCOS. [29] However in 3 separate RCTs, Magnusson et al. (2017), Freiesleben et al. (2010) and Arce et al. (2013) concluded that AMH correlated strongest with AFC compared to other markers of ovarian reserve.

Nelson SM et al. compared the two predictors of ovarian reserve: AMH and AFC, and concluded that AMH was superior compared to AFC in terms of correlation with oocyte yield [55] From the

RCTs reviewed, Arce et al (2013) and Anderson et al. (2011) and Freiesleben et al. (2010) also corroborated that AMH was a better marker of ovarian response in IVF than AFC. However, Kwee et al. (2008) disagreed, stating that AMH reasonably predicts ovarian response but is in fact not clinically superior to AFC as a marker of ovarian reserve. Nonetheless, due to its measurability throughout the cycle, AMH is the most applicable in general practice.

FSH

FSH is an indirect measure of ovarian reserve as its levels are influenced by Estradiol and Inhibin B. Early cycle FSH is highest as the inhibitory actions of Estradiol and Inhibin B are at their lowest. [56] Despite FSH being the most widely known marker of ovarian function, it is known to exhibit both inter and intra cycle variability. [20] A study by Hehelcamp WJ found FSH to exhibit a higher variability in comparison to AMH limiting the reliability of FSH. [57] FSH is known to fluctuate across the menstrual cycle, which is why FSH is used as it is a good indicator of basal levels. Freiesleben et al. (2010) and Arce et al. (2013) both agreed that FSH correlated with AMH as a marker for ovarian reserve, but Arce et al. (2013) also noted that this correlation was weaker than that of AMH with AFC and age, while Freiesleben et al. (2010) claimed that the correlation of FSH to AMH was similar to that of age and ovarian volume but less than AFC. Freiesleben et al. (2010) stated that AMH was not superior to FSH as a marker of ovarian reserve. However, four other RCTs that were considered in this review, Pennarrubia et al.(2005), Kwee et al. (2008), Arce et all (2013) and Anderson et al. (2011) disagreed and concluded that AMH was in fact superior to FSH as a maker of ovarian reserve.

FSH is also considered inadequate as a marker for diminishing reserve as abnormal levels are only detected at later stages of reserve depletion. FSH may be furthermore considered as an inappropriate marker due to levels being easily influenced by oral contraceptive pills, pituitary tumors, PCOS and hormone therapy, in contrast, AMH, is independent of the hypothalamic-pituitary feedback mechanism. [20] The sensitivity of FSH in poor response prediction with the cut-off points of 10-20IU/L was 10-80%, whereas its specificity was recorded at 83-100%,

resulting in majority of the women tested including those with a diminished reserve having falsely normal FSH values. [52] Nicole DU, et al. stresses on the importance of simultaneously measuring high Estradiol levels in cases of normal FSH. In cases of diminishing ovarian reserve, initial low estradiol causes a rise in FSH which subsequently cause a high estradiol and a drop in FSH that may be interpreted as normal FSH for that age group. [56]

The cut off value for the prediction of poor ovarian response has been recorded in the literature to range from 10-15IU/L. [53] Kwee et al. (2006) described a range of cut off levels with sensitivity and specificity. At levels more than 10IU/L sensitivity is 35% and specificity is 96%. At levels more than 12 IU/L sensitivity is 24% and specificity is 100%).

FSH has been scarcely mentioned in literature in the prediction of hyper response. Kwee et al. (2006) observed that at the highest accuracy of 86%, the cut off level for the prediction of hyper response was <4IU/L (sensitivity 18% and specificity 99%)

ESTRADIOL

Estradiol is never measured as a solo marker of ovarian reserve as it has multiple sources of production apart from the ovary, namely adipose cells, the liver, the adrenals, breast and neural tissue. [20] Also several studies have reported no difference in Estradiol levels in women with and without a diminished ovarian reserve. [52] Pennarubia et al. (2005) found that AMH was clinically superior to E2 but was observed to have similar predictive properties to E2. Serum Estradiol levels must be checked in the event of a normal FSH as FSH may be falsely interpreted as normal in low ovarian reserve as mentioned before. Therefore, Estradiol and FSH measurements are generally done together. [56]

INHIBIN B

Inhibin B, similar to AMH, is secreted by the granulosa and theca cells of the developing preantral and early antral follicles and thus is a direct marker of the small GnRH reactive follicular pool. [20] The drop in Inhibin B with approaching menopause leads to a rise in FSH. Circulating levels show high inter and intracycle variability and are prone to be highest during the mid follicular phase [45][52] and therefore levels are assessing early in the cycle- around day 2-5 for basal values. Arce et al. (2013) found a low, but positive correlation between AMH and Inhibin B, but noted it to be relatively lower than the correlation between AMH and AFC and AMH and Age, finally concluding that AMH was superior to Inhibin B in the prediction of ovarian reserve.

BASAL OVARIAN VOLUME

Basal ovarian volume is the sum of the volumes of both ovaries and is measured by calculating length x width x depth x 0.52 of each ovary. [58] Freiesleben et al. found basal ovarian volume to have significant association with AMH. Anderson et al. and Kwee et al. (2008) both agree that AMH is superior to basal ovarian volume at predicting ovarian response.

LH

LH is a glycoprotein produced and secreted by the anterior pituitary gland and is yet another indirect measure of ovarian reserve, meaning levels rely on the effect of levels of another hormone through feedback mechanisms. LH exhibits a great deal of fluctuation across the menstrual cycle- increasing across the first half of the menstrual cycle and reaching its highest level at ovulation, decreasing its reproducibility. [20] Hehelcamp WJ proved that LH, similar to FSH showed more variability than AMH [57] A study by Tal R et al on AMH as a predictor of implantation and pregnancy concluded that AMH has a higher sensitivity and specificity at predicting ovarian reserve in comparison to LH. Of the RCTs reviewed, Penarrubia et al. (2005) concluded that AMH is clinically superior to LH.

DYNAMIC TESTS OF OVARIAN RESERVE

Dynamic tests were developed based on the theory that baseline values are not reflective of the functionality of endocrine organs, and thus measure the change of hormone concentrations in response to ovarian stimulation and include

1. Clomiphene citrate challenge test (CCCT)
2. GnRh agonist stimulation test (GAST)
3. Exogenous follicle stimulating hormone ovarian reserve test (EFORT). [1]

CLOMIPHENE CITRATE CHALLENGE TEST (CCCT)

CCCT is a dynamic test for ovarian reserve. CCCT measures the change in FSH levels after 100mg of clomiphene citrate is administered through days 5-9 of the cycle. In the case of normally functioning ovaries, clomiphene citrate will increase secretion of Inhibin B and Estradiol from the cohort of growing follicles and subsequently cause a drop in FSH levels. In the case of diminished reserve, the generation of Estradiol and Inhibin B is stunted, resulting in higher concentrations of FSH. Care should be taken when administering 100mg of clomiphene citrate as it may trigger OHSS in patients with PCOS. A low dosage or shorter duration of treatment with clomiphene citrate is explicitly recommended in such patients to prevent OHSS. [59] A drawback of CCCT is the intercycle variability of the stimulated levels of FSH. [52] A paper by La Marca, A. et al in 2012 stated that CCCT has limited use, having no statistically significant difference from basal FSH measurements, and therefore should be used only in specific cases. [60] Kwee at al. (2008) concluded that AMH is superior to CCCT as a marker of ovarian reserve.

EXOGENOUS FOLLICLE STIMULATING HORMONE OVARIAN RESERVE TEST (EFORT)

The exogenous FSH Ovarian Reserve Test is based on the increment in Inhibin B and Estradiol levels after the administration of 150IU of FSH on the 3rd day of the cycle, with an increment in

both hormone levels indicating a good predictive performance. Individualization of the dose of FSH can substantially reduce the risk of OHSS. [61] Because of differences in dosages and preparations of FSH used, the hormones tested for and the timings of hormonal testing, there is mixed evidence on the diagnostic accuracy of EFORT in its use in ART. [1] Kwee at al. (2008) concluded that AMH is not superior to Estradiol and Inhibin B increment in EFORT.

GnRH AGONIST STIMULATION TEST (GAST)

The gonadotropin analogue stimulation test measures the increase in FSH, LH and Estradiol twenty four hours after the administration of GnRH. However La Marca, A. et al, in 2012 discusses the controversies in literature on the use of GAST as a test of ovarian reserve, with many of them quoting either a similar or poorer performance clinically when compared to basal Inhibin B and AFC. [60] A study by Hendricks DJ et al. found GAST to have a good ability to predict the poor responder but also concluded that the predictive accuracy was not superior to AFC and basal Inhibin B [62]

IMPLICATIONS AND RECOMMENDATIONS

Misconceptions concerning fertility continue to be present in modern day. Schmidt et al concluded that many women are still not fully aware of the fact that delaying childbearing increases the risk of infertility. There is also an overestimation in the ability of IVF to assist in pregnancy with over 80% of participants believing that ART can overcome the age-related decline in fertility. [63]

Estrogen not only influences reproductive health, but also cardiovascular, mental and skeletal health. Conversely, infertility results in an immense burden on the woman, reflecting not only psychologically, but also medically. Ovarian testing can give women answers about fertility, menopause and other reproductive conditions [63] AMH is widely used by general practitioners in counselling women on the topic of reproductive health and in helping couples make informed decisions on parenthood. In clinical practice, the use of markers of ovarian response depends on

the accuracy of response prediction and their use in individualized dosage of stimulation. It is imperative to realize that a high false positive of either low or high response will result in adjustments made to the stimulation regimes. A reduction in the dose of stimulation in a falsely predicted high responder may result in a low response, and conversely an increased dose in a falsely predicted low responder may cause a hyper response or OHSS. Cancellation rates and costs must be considered in the assessment of the value of individualized treatment in ART. [22]

Nelson et al, 2007 showed that live birth rate rose with increased levels of AMH. This was only true only where levels were <1.1ng/ml. [64] ART should not be withheld even with low levels of AMH as pregnancies have been reported even in cases of undetectable levels of AMH [28] Counselling should be started early to prepare the mother for the possible prediction of poor response. [19] Furthermore, as AMH cannot definitively predict live birth, this should be discussed with the patient prior to the commencement of ART. [24] Also, it should be kept in mind that age and AMH are independently associated with live birth and therefore the use of AMH should be focused in predicting and evaluating the efficacy of treatment. [65]

Comment [AaM14]: Low level of AMH is alarming for low ovarian reserve. This may require the woman to try to conceive or to postpone menopause (Hegazy, 2020). It has been suggested that ovarian cryopreservation can be used to delay menopause in women and even preserve fertility (Hegazy, 2021).

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CONCLUSION

In recent years, AMH has become increasingly important in the management of female subfertility. It has established itself as a reliable marker of ovarian reserve and is commonly used in prediction of ovarian response in IVF. Other markers that have also been employed in fertility centers, include Age, BMI, AFC, FSH, Inhibin B, Estradiol, LH, Basal ovarian volume, CCCT, GAST and EFORT. Although over the years several studies have widely disputed the superiority of one marker over the others, as per the various RCTs and studies considered in this review, AMH appears to be the preferred marker of ovarian reserve. Combinations of markers may prove to be even more superior in the prediction of ovarian response and can result in a reduced rate of cycle cancellation.

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