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Serum anti-Müllerian hormone as a predictor of metaphase II oocyte yield during controlled ovarian stimulation

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Abstract

Background: The potential to predict metaphase II (MII) oocyte yield in controlled ovarian stimulation (COS) has not been elucidated, despite being a mandatory predictor of fertilization potential. Hence this area is marred by the diversity of approaches to ovarian stimulation and the deficiency of standards.

Objective: To determine the role of serum anti-Müllerian hormone (AMH) as a predictor of MII oocyte yield during controlled ovarian stimulation at a private fertility clinic in Nairobi.

Methods: A retrospective descriptive cohort study design was employed in which MII outcomes during COS were compared among women aged 18-45 years with normal or low serum AMH levels. Data were collected sequentially until the desired sample size was achieved. Data were analyzed using the IBM statistical package for social sciences (SPSS) version 24.0 and STATA version 15. The level of significance was set at p<0.05.

Results: Approximately one-third of women above 35 years of age had normal serum AMH levels, while nearly three-quarters had low serum AMH levels (OR=0.1; 95% CI (0.1–0.4); p-value<0.001). By day five, hyperresponse was more preponderant among those with normal AMH levels (39.0% vs. 3.8%; OR=15.9; CI (2.0-126.0); p-value<0.001) and remained consistent. Almost three-quarters of women with low AMH levels had a low MII yield compared to nearly one-quarter of those with normal AMH levels (OR=0.3; 95% CI (0.1-0.8); p-value=0.014), even after controlling for age. This trend was consistent with that of the total oocyte count. The sensitivity, specificity, and positive and negative predictive values of serum AMH level as a predictor of MII oocyte yield were 86.0%, 54.3%, 72.96%, and 73.1%, respectively.

Conclusion: Normal serum anti-Müllerian hormone levels is associated with increased follicular count and total oocyte harvest during controlled ovarian stimulation and is a good predictor of MII oocyte yield.

Keywords: anti-Müllerian hormone, ovarian stimulation, follicular count, follicular harvest, metaphase II oocytes

Introduction

Ovarian stimulation (OS) targets follicular development and oocyte maturation to achieve pregnancy in women with difficulties in conception (1). Worldwide, infertility affects approximately 20% of couples (2,3). Assisted reproductive technology (ART) is the pinnacle of care where other treatments are impossible or fail. However, it is not universally available because of many factors, including high treatment costs, lack of policy guidelines, limited free cycles, lack of a skilled workforce, and ART facilities, especially in developing countries (4,5). In addition, the high frequency of cycle cancellation and poor success rates remain discouraging (6). A major limiting factor has been the inability to predict oocyte quality outcomes, from which the cascade of fertilization, pregnancy, and eventually live birth rates (LBRs) emanate (7-10). Despite the practical limitations of biomarkers such as anti-Müllerian hormone (AMH) and antral follicular count (AFC), correlations with ovarian reserve and the number of oocytes retrieved, including fertilization rates, have been observed (9,11-16). However, guidelines for their clinical application are lacking. Fertilizable oocytes are present in metaphase II (MII) (17). Accurate prediction of this potential should focus on MII counts and proportions, not sperm quality. MII oocytes are formed by the extrusion of the first polar body, thereby converting it into a haploid gamete (17). Therefore, this study sought to determine whether serum AMH levels could predict MII oocytes in controlled ovarian stimulation.

Methods

Study design

This was a retrospective descriptive cohort study. The primary outcome of interest was the number and proportion of MII oocytes in women with high or low serum AMH levels. The secondary outcome of interest was the number of follicles observed during stimulation monitoring on days five and seven.

Study setting

The study site was the Nairobi Fertility Clinic, a private clinic that recruits patients with infertility and referrals for in vitro fertilization (IVF). Follow up after treatment is done at the facilities where the patients were seen. The clinic serves patients with similar socioeconomic statuses.

Study population

The study population was women between 18-45 years who had undergone OS using the long agonist protocol and were either diagnosed with primary or secondary infertility or were ovum donors, with serum AMH values assayed at Medipath laboratories using the VIDAS (BioMerieux, France) kit. Women under 18 or above 45 years with endocrine disorders, those who had been subjected to gonadotropin-releasing hormone (GnRH) antagonist protocol or ultra-short agonist protocol, or had high serum AMH values (>6.80 ng/mL) were excluded. The study population was divided into two groups based on the serum AMH values. The first group was women with normal serum AMH levels (1.00-6.80 ng/mL), whose response was predicted to be good, while the second group included women with low serum AMH levels (0.20-0.99 ng/mL), whose response was predicted to be low.

Sampling

Despite extensive literature searches, no relevant similar studies were found. Therefore, two assumptions were made. In the first group, the response was expected to be better; the MII oocyte yield rate was presumed at 50%. For the second group with low AMH levels and lower expected MII oocyte yield, a presumptive value of 25% of MII oocyte yield was made. Based on the formula by Kelsey et al. (18), the calculated sample size was 59 patients in each group.

Data collection and management

Data were collected using a questionnaire. General reproductive characteristics, day five and seven follicular counts, total oocyte harvest, and MII oocyte yield data were collected sequentially from the clinic records of patients from November 2019 downwards until the targeted sample size was achieved.

Data analysis

Data were analyzed using the IBM statistical package for social sciences (SPSS), version 24.0 and STATA version 15. A p-value of <0.05 was considered statistically significant.

Ethical consideration

Ethical approval for this study was obtained from the Kenyatta National Hospital and University of Nairobi Ethical Research Committee (registration number KNH-ERC/A/299). Data were kept confidential, and the patients' identifiable data were de-identified. Since this was a clinical audit and data collection was retrospective, patient informed consent was not required.

Results

Eighty-five patient records were included in this study. Forty-four percent of the women (n=26) had normal AMH levels, were between 30-35 years

compared to 11.5 % (n=3) of those with low serum AMH levels (OR=6.3; 95 % CI (1.7-23.2); p-value=0.003). A minority, 28.8 % (n=17) with normal AMH levels were above 35 years compared to 73.1% (n=19) of those who had low serum AMH levels (OR=0.1; 95 % CI (0.1-0.4); p-value<0.001). There were no significant differences with the type of infertility, cause of infertility, or history of previous stimulation between the two populations (**Table 1**).

On day five, normal response (5-14 follicles) predominated in both groups. Still, it was more preponderant among those with normal AMH levels 57.6 % (n=34) compared with 65.4 % (n=17) among those with low AMH levels (OR=0.7; 95 % CI (0.3-1.9); p-value<0.001). Hyperresponse was reported in 39.0 % (n=23) and 3.8 % (n=1) in those with normal and low serum AMH levels, respectively (OR=15.9; 95 % CI (2.0-126.1); p-value<0.001). However, normal and hyperresponse combined constituted 96.6% (n=57) of those with normal AMH levels compared to 69.2 % (n=18) of those with low AMH levels. Overall, the low response was more common among those with low AMH levels, 30.8 % (n=8) than among those with normal AMH levels, 3.4 % (n=2) (OR=0.1; 95 % CI (0.0-0.4); p-value<0.001. Follicular count on day seven showed similar trends (p-value<0.001 for all categories) (**Table 2**).

For the total oocyte harvest, less than five follicles were predominant in women with low serum AMH levels than those with normal serum AMH levels, 61.5 % (n=16) and 18.6 % (n=11), respectively (OR=0.1; 95 % CI (0.1-0.4); p-value<0.001). Those with normal response (5-14 oocytes) were 40.7 % (n=24) and 34.6 % (n=9) in the normal and low serum AMH categories, respectively (p-value=0.597). However, those who had 15 oocytes and above were 40.7% (n=24) and 3.8 % (n=1) for the normal and low serum AMH categories, respectively (OR=17.0; 95 % CI (2.2-135.2); p-value<0.001). When normal and high oocyte harvest is combined, the proportion rises to 81.4% (n=48) for the normal AMH category compared to 38.4 % (n=10) among the low AMH category, indicating better response prediction with normal AMH. It shows hyperresponse, thereby reducing the number of patients in the normal category creating a misnomer of apparent good response among those with low serum AMH (**Table 3**).

Grouped data on MII oocyte yield by serum AMH category showed that 73.1 % (n=19) of the low serum AMH category had MII oocyte yield of fewer than five oocytes compared to 27.1 % (n=16) of the normal serum AMH category (OR=0.3; 95 % CI (0.1-0.8); p-value=0.014). MII oocyte yield of 5-14 was higher in the normal serum AMH group 50.8 % (n=30) compared to 26.9 % (n=7) in the low serum AMH group (OR=2.8; 95 % CI (1.0-7.7); p-value=0.040). MII oocyte yield of 15 or more was 22.1 % (n=13) in the normal serum AMH group compared to no yield in the low serum AMH group (**Table 4**).

The sensitivity and positive predictive value of serum AMH as a predictor of total oocyte harvest were high at 82.8 % and 81.4 % , respectively. On the other hand, the specificity and negative predictive value were relatively low at 59.3 % and 61.5 % , respectively (**Table 5**).

The sensitivity, positive predictive value, and negative predictive value of serum AMH as a predictor of MII oocyte yield were high (86.0 % , 72.96 % , and 73.1 % , respectively). However, the specificity was relatively low at 54.3 % (**Table 6**).

Discussion

This study shows that serum AMH is a good predictor of MII oocyte yield. The study exploited the performance milestones that reflect levels of efficiency in the sequence that leads to MII oocyte yield during ovarian stimulation. Sequential follicular count and growth appraisal and oocyte harvest confer promise on the possibility of success and enable gonadotrophin dosage adjustment (19). This study's findings show a preponderance of higher follicular count among women with normal serum AMH levels throughout the cycle than women with low serum AMH levels. Therefore, the results of this study depict the obvious advantage conferred by normal AMH levels, which persists throughout the stimulation cycle. This was significant for days five and seven (p<0.001). Therefore, these observed differences are critical surrogate indicators of better ability to produce fertilizable oocytes among those with normal serum AMH. A similar hypothesis was made in a study that related AMH to live births, qualitative oocyte yield, and embryos (19).

The objective of OS is to achieve a pregnancy (8), which can be deterred by cycle cancellation. This study revealed very high discontinuation rates among women with low serum AMH levels of up to nearly 50%, indicating high specificity of low serum AMH levels. On the other hand, cycle cancellation rates of up to 5% among those with normal serum AMH were due to hyperresponse during ovarian stimulation. The overall inference of this observation is that serum AMH is a good predictor of ovarian response, the prerequisite of good MII oocyte yield. However, this hyperresponse is not excessive, an indication of a high margin of safety when serum AMH is normal while at the same time giving the advantage of obtaining enough oocytes. Thus, it can be inferred that the likelihood of oocyte harvest is much higher in women with normal serum AMH levels. Similar findings were reported by Jayaprakasan K et al. (11), who found a high predictive value of AMH and antral follicle count to COS. However, the need is discernible for the development of clinical policies

Table 1: Selected general and reproductive characteristics of the study population by normal versus low serum AMH levels

Characteristic	AMH level		OR (95%CI)	P-value
	Normal (N=59) No. (%)	Low (N=26) No. (%)		
Age (Completed yrs)				
<30	16(27.1)	4(15.4)	1.9(0.5-6.6)	0.27
30-35	26(44.1)	3(11.5)	6.3(1.7-23.2)	0
>35	17(28.8)	19(73.1)	0.1(0.1-0.4)	<0.001
Type of infertility				
Primary	10(16.9)	3(11.5)	1.6(0.4-6.2)	0.52
Secondary	10(16.9)	7(26.9)	0.5(0.2-1.3)	0.15
Not indicated	33(55.9)	16(57.7)	0.8(0.3-2.0)	0.63
N/A (Donors)	6(10.2)	0	-	-
Cause of infertility				
Tubal factors	35(59.2)	12(46.2)	1.4(0.6-3.6)	0.45
Ovarian factors	2(3.4)	5(19.2)	0.1(0.0-0.8)	0.01
Uterine factors	3(5.1)	2(7.7)	0.2(0.0-2.3)	0.16
Male factors	10(16.9)	7(26.9)	0.8(0.3-2.2)	0.6
Age factor	2(3.4)	1(3.8)	0.9(0.1-9.9)	0.91
Unexplained infertility	11(18.6)	5(19.2)	1.3(0.4-4.0)	0.67
Previous stimulation cycles				
One	10(16.9)	4(15.4)	1.1(0.3-3.9)	0.88
Two	1(1.7)	1(3.8)	0.4(0.0-7.0)	0.54
Three	-	-	-	-
>3	1(1.7)	1(3.8)	0.4(0.0-7.0)	0.54
Nil	47(79.7)	20(76.9)	1.2(0.4-3.6)	0.75

Table 2: Follicular count on days five and seven by normal versus low serum AMH levels

Follicular count	AMH level		OR (95%CI)	P-value
	Normal (N=59) No. (%)	Low (N=26) No. (%)		
Day 5				
Low response (<5)	2(3.4)	8(30.8)	0.1(0.0-0.4)	<0.001
Normal response (5-14)	34(57.6)	17(65.4)	0.7(0.3-1.9)	<0.001
Hyperresponse (≥15)	23(39.0)	1(3.8)	15.9(2.0-126.1)	<0.001
Day 7				
Low response (<5)	1(1.7)	6(23.1)	0.1(0.0-0.5)	<0.001
Normal response (5-14)	22(37.3)	19(73.1)	0.1(0.1-0.4)	<0.001
Hyperresponse (≥15)	36(61.0)	1(3.8)	39.1 (2.0-126.1)	<0.001

Table 3: Total oocyte harvest by normal versus low serum AMH levels

Oocyte harvest	AMH level		OR (95%CI)	P-value
	Normal (N=59) No. (%)	Low (N=26) No. (%)		
Low response (<5)	11(18.6)	16(61.5)	0.1(0.1-0.4)	<0.001
Normal response (5-14)	24(40.7)	9(34.6)	1.3(0.5-3.4)	0.6
Hyperresponse (≥15)	24(40.7)	1(3.8)	17.0(2.2-135.2)	<0.001

Table 4: Metaphase II (MII) oocyte yield by normal versus low serum AMH levels

MII oocyte yield	AMH level		OR (95%CI)	P-value
	Normal (N=59) No. (%)	Low (N=26) No. (%)		
<5	16 (27.1)	19 (73.1)	0.3(0.1-0.8)	0.01
5-14	30 (50.8)	7 (26.9)	2.8(1.0-7.7)	0.04
≥15	13 (22.1)	-		

Table 5: Sensitivity, specificity, positive predictive value, and negative predictive value of normal vs. low serum AMH as a predictor of total oocyte harvest

Serum AMH level	Total oocyte harvest		Total
	Normal/ High (≥5) (Positive)	Low (<5) (Negative)	
Normal (Positive)	48	11	59
Low (Negative)	10	16	26
TOTAL	**58**	**27**	**85**

(Sensitivity = 82.8%; Specificity = 59.3%; Positive predictive value (PPV) = 81.4%; Negative predictive value (NPV) = 61.5%)

Table 6: Sensitivity, specificity, positive predictive value, and negative predictive value of normal versus low serum AMH as a predictor of MII oocyte yield

Serum AMH level	MII oocyte yield		Total
	Normal/ High (≥5) (Positive)	Low (<5) (Negative)	
Normal (Positive)	43	16	59
Low (Negative)	7	19	26
TOTAL	**50**	**35**	**85**

(Sensitivity = 86.0%; Specificity = 54.3%; Positive predictive value (PPV) = 72.96%; Negative predictive value (NPV) = 73.1%)

that use serum AMH with or without other predictors to guide the hitherto unclear approach to OS universally and hence the diversity of OS protocols based on individual preferences and experience, with clear definitions of normal, low and hyperresponse (20-23). The overall inference in this study is that normal AMH levels are associated with better intermediate and end OS outcomes than low AMH levels. This difference is maintained after the standardization of age. This gives credence to serum AMH as a predictor of OS outcomes (19,24). Hence, the clinical use of these findings should include

counseling on the likelihood of good oocyte harvest and getting enough embryos for cryopreservation (4,5), thereby reducing the cost for repeat OS (25).

Study strengths and limitations

This study is one of the few studies to evaluate serum AMH as a predictor of MII oocyte yield, therefore, adding to the pool of knowledge in IVF, particularly in developing countries. However, it was not without limitations. Since this was a retrospective study, some data were incomplete. Also, since there were no previous similar studies, the sample size was calculated based on an assumption of 50% for the patients with normal serum AMH levels and 25% for patients with low serum AMH levels. Despite this limitation, the data was analyzable, and statistical differences were observed.

Conclusion

Normal serum anti-Müllerian hormone is associated with increased follicular count and total oocyte harvest during controlled ovarian stimulation and is a good predictor of MII oocyte yield.

Recommendations

Serum AMH can be used to predict outcomes of OS and hence provide a basis for counseling and advice on outcomes and alternative ART procedures. There is a need for more extensive studies using baseline serum AMH levels in our setting to predict with certainty metaphase II oocytes after COS using modeled receiver operating curves (ROC).

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Conflict of interests

The authors declare no conflicts of interest.

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