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Correlation between Anti Mullerian Hormone (Amh), Antral Follicule Count and the Response to Ovarian Stimulation in Infertile Women in Douala

Bilkissou Moustapha[1], Tatah Humphry Neng[2], Nancy Elage Munge[1], Yannick R. Onana[3], Yaneu Junie Ngaha[1], Diane Kamdem[4], Jean Marie Alima[4], Alphonse Ngalame[2], Georges Mangala[1], Astrid Ndolo[1], Gervais Mounchikpou[1], Michelle Mendoua[1], Aimée Timnou Djokam[5], Tchounzou Robert[2], Michel Roger Ekono[1], Henri Essome[1], Charlotte Nguefack Tchente[1]

[1]Faculty of Medicine and Pharmaceutical Sciences, University of Douala, Douala, Cameroon
[2]Faculty of Health Sciences, University of Buea, Buea, Cameroon
[3]Faculty of Medicine and Biomedical Sciences, University of Ngaoundere, Ngaoundere, Cameroon
[4]Faculty of Medicine and Pharmaceutical Sciences, University of Dschang, Dschang, Cameroon
[5]Faculty of Medicine and Biomedical Sciences, University of Yaounde, Yaounde, Cameroon
Email: bilkissoumoustapha@yahoo.fr

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Abstract

Background: The use of assisted reproductive technique (ART) is becoming more common in infertility. During ART most patients undergo ovarian stimulation. In this study we study the correlation between ovarian reserve markers: Anti-Mullerian hormone (AMH) and antral follicle count (AFC), and the response to ovarian stimulation at *in vitro* fertilization (IVF) centres in Douala Cameroon. **Methods**: This was a hospital based cross-sectional sectional analytic study carried out over a period of 3 years, 4 months at Clinique de l'Aéroport, Clinique Odyssée and Clinique Urogyn. Inclusion criteria were: Female partners of infertile couples undergoing ovarian stimulation for an *in vitro* fertilization cycle, patients who had both ovaries and had done either AMH, AFC or both before ovarian stimulation. Patients were divided into three groups based on the number of oocytes retrieved: low ovarian response for ≤3 oocytes, normal ovarian response for 4 - 15 oocytes and high ovarian response for >15 oocytes. Data obtained was analyzed by SPSS version 25.0. **Results:** The ages of participants ranged from 20 - 4 7 years, with a mean age of 34.11 ± 5.11 years. Most of them had secondary infertility (57.9%). The GnRH antagonist protocol was mainly used, and ovulation was triggered using HCG predominantly. On Multivariate analysis, age and history of PCOS were

significantly associated with ovarian response in the low and high ovarian response groups, respectively. **Conclusion**: AMH has a better predictive value than AFC, however, it is less sensitive but more specific than AFC.

Keywords

Ovarian Stimulation, AMH, AFC, Ovarian Reserve, Correlation and Prediction Value

1. Introduction

According to the World Health Organization, infertility as a disease of the male and female reproductive system defined by the failure to achieve a pregnancy after 12 months or more of regular unprotected sexual intercourse. It has become a global health issue affecting millions of people of reproductive age worldwide, estimated data shows that one in six people worldwide experiences infertility once in their lifetime [1]. The lifetime prevalence of infertility is 17.8% in high-income countries and 16.5% in low and middle-income countries [2]. In Cameroon 20% - 30% of couples suffer from infertility and the prevalence varies from region to region [3]. Egbe *et al.* carried out a hospital-based study in 2016 and reported a 19.2%, the prevalence of infertility in Douala [4]. The Groupe Interafricain de Recherche et d'Application sur la Fertilite (GIERAF) carried a study in 2010 that identified the main cause of female infertility (where) to be tubal stenosis secondary to poorly treated/untreated sexual transmitted diseases [5]. Other causes of female infertility include endometriosis, uterine abnormalities, cervical causes and ovulation disorders. Ovulatory disorders account for approximately 25% of diagnosed infertility, while 70% of women with anovulation have polycystic ovarian syndrome (PCOS) [3]. FIGO classifies ovulatory disorders into four types; type 1, corresponds to hypothalamic causes; type 2, pituitary causes; type 3, ovarian causes and type 4, PCOS [4].

The assessment of ovarian reserve to determine the strategy for female infertility treatment has become essential. Ovarian reserve is defined as the number of oocytes remaining in the ovary or oocyte quantity [5]. This parameter is actively used in pregnancy planning and in assisted reproductive technology applications [6]. The fecundity of a woman decreases with age due to a decrease in the quantity and quality of oocytes, primarily as a result of a continuous process of oocyte atresia [7]. The total number of oocytes is thought to peak in fetal life at 20 weeks of gestation with approximately 5 - 7 million, this number decreases to about 400,000 - 500,000 follicles by puberty [8].

Traditionally, follicle-stimulating hormone (FSH) levels, oestradiol (E2) levels, and antral follicle count (AFC) by ultrasound investigation at the early follicular phase have been used for evaluation of ovarian reserve [9]. Recently, identification of Anti Mullerian hormone (AMH) levels became important as it is considered a

more reliable maker in the assessment of ovarian reserve [10]. Anti-Mullerian hormone is a member of the transforming growth factor beta family, it is secreted by the pre-antral and antral follicles and incurs little intra and inter cycle variations, therefore the levels of AMH indirectly represent the quantity of these follicles over time [10] [11].

An antral follicle is a resting follicle and appears as a small fluid sac which contains an immature egg. Antral Follicular Count (AFC) is a procedure where a transvaginal ultrasound is used to identify follicles with a mean diameter ranging from 2 to 10mm [12]. AFC is frequently assessed in women of reproductive age for various reasons; it is helpful in infertility and ART work up in predicting ovarian response to gonadotropin stimulation. The ovarian reserve is considered to be adequate when the number of antral follicles identified in each ovary is greater than or equal to five in each ovary [7].

In 2015, Ludmila *et al* reported that AMH and AFC are preferred methods for predicting ovarian reserve with a varied degree in precision [9] and also the type of response to ovarian stimulation. The quantity and quality of oocyte are known to decline with age, however, large variations of oocyte reserves exist between individual patients, as do ovarian responses to gonadotrophin stimulation even among women of the same group. Ovarian stimulation is defined as a pharmacological treatment with the intention of inducing the development of ovarian follicles, it is used in ART to obtain multiple oocytes for follicular aspiration [12]. Fleming *et al.* in 2013 reported that women with low ovarian reserve tend to have low AMH levels and thus respond to ovarian stimulation poorly and may require greater management of their expectations for outcome success while at the other end of the spectrum women with high ovarian response are at risk of excessive ovarian response that can lead to ovarian hyperstimulation syndrome. Similarly, patients presenting over 10 antral follicles in each ovary during AFC will tend to have a high ovarian response to stimulation [7]. A study in Cameroon by Kasia *et al.* in 2020 suggested that a threshold value of 1.1 ng/l was a predictive value for an acceptable response during ovarian stimulation [11]. AMH has been correlated highly with baseline AFC as the two most reliable and valuable markers of ovarian responses, however, there is a paucity of data of this correlation in our country. To ensure safe and efficient ovarian stimulation, our goal during this study is to better understand the correlation between AMH, AFC and the response to ovarian stimulation during IVF in the city of Douala.

2. Methodology

It was a descriptive and analytic cross-sectional study with a retrospective and prospective data collection. The general objective was to determine the relation between AMH, AFC and the response of ovarian stimulation in IVF centres in Douala. Our study period was 3 years and 4 months, from January 2021 to April 2024. The target population included patients who had undergone ovarian stimulation during IVF in the above-mentioned clinics from January 2021 to April 2024. Our Inclusion Criteria where

- Infertile women who had undergone ovarian stimulation during an IVF cycle;
- Patents who had done either AMH serum test, AFC, or the both before ovarian stimulation;
- The presence of bilateral ovaries was confirmed by transvaginal ultrasound.

We excluded infertile patients who had undergone ovarian stimulation for a different ART method (such as artificial insemination) and incomplete patient records. We used Cochran's Formula for the sampling size's = $\frac{Z^2(P)(1-P)}{d^2}$. This gave us an approximate sample size of 238 patients.

Z is the statistic corresponding to the level of confidence = 1.96;

P is expected prevalence = 0.192 (a hospital based study carried out by Egbe *et al.* in 2016 in Douala Cameroon reported a prevalence of 1nfertility of 19.2%) carried in Cameroon;

d is precision (corresponding to effect size) = 0.05 n is sample size.

After approval of the protocol by the research panel of the Faculty of Medicine and Pharmaceutical Sciences Douala, ethical clearance was obtained from the Institutional Review Board of the Faculty of Medicine and Pharmaceutical Sciences, University of Douala (IRB/UD). Administrative approval was obtained from the managing Director of Clinique de l'Aeroport, Clinique Odyssee and Clinique Urogyn. Patients' files were selected following inclusion criteria above and data was collected for a period of 4 months using questionnaires established by the researcher. The files of patients diagnosed with infertility who had undergone ovarian stimulation for an IVF cycle at Clinique de l'Aeroport, Odyssee and Urogyn were reviewed. We approached the archivist or a nurse at the gynecological unit to obtain the files of patients. Patients' information was collected using pre-designed questionnaires. Data concerning epidemiology, demography, relevant past history, evaluation of ovarian reserve markers, indication of treatment or management (medical) and evolution was collected.

- Sociodemographic variables such as; age, marital status, occupation, level of education, religion, residence and region of origin was collected.
- Gynaecological and Obstetrical Past History included; gravidity, parity, type of infertility, duration of infertility, history of pelvic inflammatory disease, PCOS, Endometriosis, history of pelvic surgery, previous ovarian stimulation.
- Clinical profile; type of protocol, type and dosage of gonadotropin, molecule used to induce ovulation, duration of stimulation, number of oocytes retrieved, and number of fertilised embryos.
- Ovarian reserve markers; AFC recorded on day 2 - 3 of the menstrual cycle was taken from patients files. Values of AMH level where taken directly from patients files. However, it is important to note that the technique used to analyze serum AMH in two of study sites (Clinique Aeroport and Clinique odyssee) was imunofluroscence using automated AMH assay. AMH was recorded in ng/ml.
- The ovarian stimulation protocol was selected according to the patient's age, basal endocrine level and number of antral follicles.

Patients were divided into three groups based on the number of oocyte retrieved

after ovarian stimulation:

Low response = ≤3 oocytes;

Normal response = 4 - 15 oocytes;

High response = >15 oocytes.

Data was collected using the ODK collect application, entered into SPSS version 26 for analysis. Binary logistic regression analysis/chi-square test was used to test association between dependent variable and independent variables and reported as Crude odd ratios with 95% confidence intervals. A two-tailed p-value less than 0.05 was considered statistically significant. Pearson correlation was used to determine the correlation between AMH, AFC and number of retrieved oocytes. ROC analysis curve was used to determine the predictive value of both AMH and AFC. Confusion matrix was used to calculate specificity and sensibility of AMH and AFC.

Both the ethical clearance and the administrative approval documents were used to obtain an approval to carry out this study in the referred clinics. Patient's confidentiality was respected.

3. Result

For the study, we went through all the records of patients who did ovarian stimulation during an IVF cycle from January 2021 to April 2024 at Clinique de l'Aeroport, Clinique Odyssee and Clinique Urogyn (Figure 1).

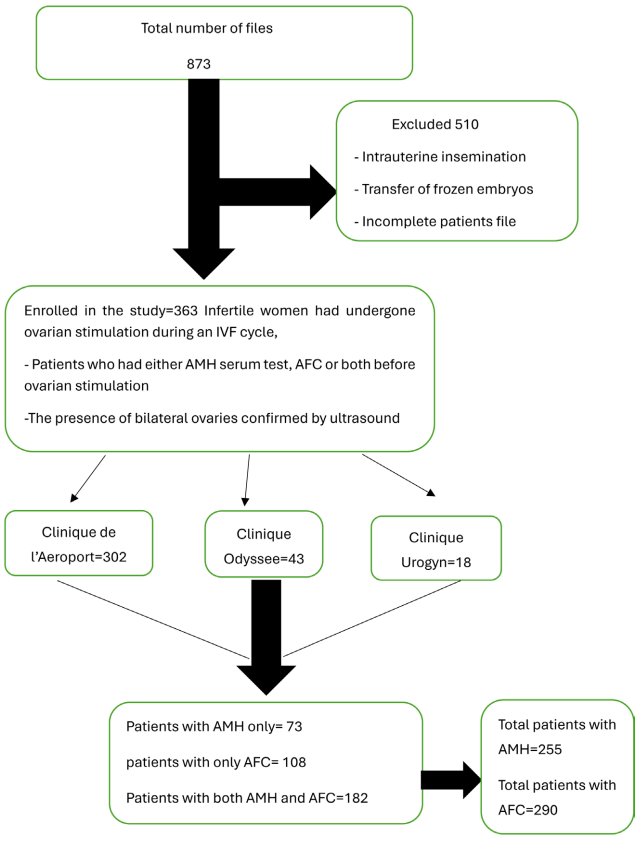

Figure 1. Recruitment flow chat.

3.1. Sociodemographic Profile

Past History

There was a predominance of 206 pauciparous women (56.7%), 210 women had secondary infertility (57.9%), 56.7% had a history of pelvic inflammatory disease (PID), while 19.3% had a history of PCOS. History of tubal surgery was found in 24% of women, while 32.5% had previously undergone ovarian stimulation. Averagely most women had been dealing with infertility for about 5.28 ± 3.622 years. The most common cause of infertility was mixed causes (60.6%) (Table 1).

Table 1. Gynecological past history.

Variable	Category	Frequency (N)	Percentage (%)
Gravidity	Nulligravida (0)	143	39.4
	Paucigravid (1 - 4)	206	56.7
	Multigravida (>4)	14	3.9
Type of infertility	Primary	153	42.1
	Secondary	210	57.9
Duration of infertility (years)	1 to 5	242	66.7
	6 to 10	98	27.0
	11 to 15	16	4.4
	>15	7	1.9
History of PCOS	Yes	70	19.3
	No	293	80.7
History of PID	Yes	206	56.7
	No	157	43.3
History of pelvic surgery	Tubal	87	24.0
	Ovarian	17	4.7
	Myomectomy	51	14.0
	Other abdominopelvic	48	13.2
	None	160	44.1
Etiology of infertility	Female factor	93	25.6
	Male factor	47	12.9
	Mixed causes	220	60.6
	Unexplained cause	3	0.8
Previous ovarian stimulation	Yes	118	32.5
	No	245	67.5

3.2. Different Protocols Used during Ovarian Stimulation

311 women (85.7%) were stimulated using the antagonist protocol while 51 women were stimulated using either the long (8.0%) or short agonist (6.1%) protocol. Recombinant FSH gonadotropin was the most frequently used gonadotropin with a percentage of about 88.4%. HMG was used for ovarian stimulation in 11.6% of cycles, nearly always in combination with FSH. Ovulation was induced 93.9% of the times with HCG. Duration of stimulation ranged from 8 - 16 days (Table 2).

Table 2. Protocols used during ovarian stimulation.

Variables	Category	Frequency (N)	Percentage (%)
Type of protocol	Antagonist	311	85.7
	Long-agonist	29	8.0
	PPOS	1	0.3
	Short agonist	22	6.1
Type of gonadotropin	Highly Purified HMG	4	1.1
	Urinary gonadotropin (HMG)	38	10.5
	Recombinant gonadotropin (FSH)	321	88.4
Posologie of gonadotropin (IU)	≤150	151	41.6
	151 - 225	192	52.9
	>225	20	5.5
	>225	16	4.4
Molecule used to trigger oocyte maturation	GnRH agonist	22	6.1
	Human chorionic gonadotropin (HCG)	341	93.9
Duration of stimulation (days)	<10	8	2.2
	10 to 12	262	72.2
	13 to 15	84	23.1
	>15	9	2.5

Age

The ages of participants ranged from 20 - 47 years, with a mean age of 34.11 ± 5.11 years. The median age of the population was 34 years, with a predominant age range of [30 - 40] (66.6%) (Table 3).

Table 3. Distribution according to age.

	Mean	Standard deviation	Median	Min	Max
Age	34.11	5.11	34	20	47
Age group		Frequency		Percentage (%)	
<25		10		2.8	
[25 - 30]		56		15.4	
[30 - 35]		121		33.3	
[35 - 40]		121		33.3	
≥40		55		15.2	
Total		363		100.0	

3.3. Factors Associated with Ovarian Response

3.3.1. Low Ovarian Response

On bivariate analysis: From the odd ratios displayed women within the age groups [25 - 35] were less likely to have a low ovarian response as compared to women above 40 years. Women who had PCOS where less likely to have a low ovarian response to stimulation compared to women who did not have PCOS. In addition, women who had been previously stimulated had lower chances of responding poorly to ovarian stimulation as compared to women who had not been previously stimulated. Furthermore, patients who had been stimulated for 10 - 12 - 15 days were less likely to have a poor response than patients who were stimulated for less than 10 days. After multi-regression analysis, we found out that older age, not having PCOS and previous ovarian stimulation were independent predictor variables of low ovarian response (Table 4, Table 5).

Table 4. Bivariate analysis of factors associated with low ovarian response.

	Low ovarian response ≤ 3 oocytes retrieved					(CI)	
Variables	**Yes**	%	**No**	%	**P value**	OR	**Inf**	Sup
Age								
<25	2	20.0	8	80.0	0.374	0.5	0.1	2.5
[25 - 30]	4	7.1	52	92.9	**0.001**	**0.1**	**0.0**	**0.5**
[30 - 35]	15	12.4	106	87.6	**0.001**	**0.3**	**0.1**	**0.6**
[35 - 40]	21	17.4	100	82.6	0.013	0.4	0.2	0.8
≥40	19	34.5	36	65.5	1			
History of PCOS								
Yes	**3**	4.3	**67**	95.7	**0.005**	**0.3**	**0.1**	**0.6**
No	58	19.8	235	80.2	1			

Continued

Etiology of infertility								
Male factor	8	17.0	39	83.0	0.399	1.5	0.6	4.1
Mixed causes	42	19.1	178	80.9	0.121	1.8	0.9	3.6
Unexplained cause	0	0.0	3	100.0	0.999	0.0	0.0	
Female factor	11	11.8	82	88.2	1			
Previous ovarian stimulation								
Yes	10	8.7	105	91.3	**0.006**	**0.4**	**0.2**	**0.8**
No	**51**	20.6	**197**	79.4	1.000			
Type of protocol								
Long-agonist	**6**	20.7	**23**	79.3	0.654	1.2	0.5	3.2
PPOS	0	0.0	1	100.0	1.000	0.0	0.0	
Short agonist	**1**	4.5	**21**	95.5	0.151	0.2	0.0	1.7
Antagonist	54	17.4	257	82.6	1.000			
Type of gonadotropin								
Highly Purified HMG	0	0.0	4	100.0	0.999	-	-	-
Recombinant gonadotropin	55	16.4	280	83.6	0.284	0.6	0.2	1.6
Urinary gonadotropin	6	25.0	18	75.0	1			
Dosage of gonadotropin								
≤150	31	19.255	130	80.745	0.584	0.7	0.2	2.4
151-225	26	13.978	160	86.022	0.243	0.5	0.1	1.6
>225	4	25	12	75	1			
Duration of stimulation								
10 to 12	44	16.8	218	83.2	**0.028**	**0.2**	**0.0**	**0.8**
13 to 15	12	14.3	72	85.7	**0.020**	**0.2**	**0.0**	**0.8**
>15	1	11.1	8	88.9	0.103	0.1	0.0	1.5
<10	4	50.0	4	50.0	1			

Table 5. Multivariate logistic regression of predictor variables with low ovarian response.

Variable	P. value	OR	(CI) 95%	
			Inf	Sup
History of PCOS	0.025	0.250	0.074	0.841
Previous ovarian stimulation	0.003	0.318	0.150	0.673

Continued

Duration of stimulation	0.0099	0.857	0.713	1.030
Age (years)	**0.001**	**1.122**	**1.053**	**1.195**

3.3.2. High Ovarian Response

On bivariate analysis age, history of PCOS and duration of stimulation were significantly associated with high ovarian response. From the odd ratio displayed, women within the age groups [25 - 30] years were four times more likely to have a high ovarian response than women above 40 years. Women who had PCOS were 11 times more likely to have a higher ovarian response than those who did not have PCOS. Women who had been stimulated for 13 - 15 days were 3 times more likely to have a high ovarian response than those stimulated for 10 – 12 days. After multivariate logistic regression analysis, we found out that being younger, having of PCOS were independent predictors of high ovarian response (Table 6, Table 7).

Table 6. Independent predictors of high ovarian response.

	High response > 15						(CI)	
Variables	**Yes**	**%**	**No**	**%**	**P value**	**OR**	**Inf**	**Sup**
Age								
<25 years	3	30.0	7	70.0	0.124	3.5	0.7	17.3
[25 - 30]	20	35.7	36	64.3	**0.003**	**4.5**	**1.7**	**12.4**
[30 - 35]	25	20.7	96	79.3	0.122	2.1	0.8	5.5
[35 - 40]	14	11.6	107	88.4	0.898	1.1	0.4	2.9
≥40	6	10.9	49	89.1	1			
History of PCOS								
Yes	39	55.7	31	44.3	**0.001**	**11.45**	**6.2**	**21.0**
No	29	9.9	264	90.1	1			
History of PID								
Yes	36	17.5	170	82.5	0.482	0.8	0.5	1.4
No	32	20.4	125	79.6				
Etiology of infertility								
Male factor	11	23.4	36	76.6	0.166	1.9	0.8	4.6
Mixed causes	44	20.0	176	80.0	0.209	1.5	0.8	3.0
Unexplained cause		0.0	3	100.0	0.999	0.0	0.0	
Female factor	13	14.0	80	86.0				

Continued

Previous ovarian Stimulation								
Yes	25	21.7	90	78.3	0.318	1.3	0.8	2.3
No	43	17.3	205	82.7				
Type of protocol								
Long-agonist	5	17.9	23	82.1	0.859	0.9	0.3	2.5
PPOS	1	100.0		0.0	1.000	0.0	0.0	
Short agonist	2	9.1	20	90.9	0.251	0.4	0.1	1.8
Antagonist	60	19.2	252	80.8				
Type of gonadotropin								
Recombinant gonadotropin	2	50.0	2	50.0	0.201	0.5	0.2	1.4
Highly Purified HMG		0.0	14	100.0	0.418	2.4	0.3	20.8
Urinary gonadotropin (HMG)	66	47.6	279	52.4	1			
Dosage of Gonadotropin (IU)								
≤150	29	18.0	132	82.0	0.942	1.0	0.3	3.6
151 - 225	36	19.4	150	80.6	0.953	1.0	0.3	3.8
>225	3	18.8	13	81.3	1			
Duration of stimulation (days)								
<10	0	0.0	8	100.0	0.999	0.0	0.0	
>15	0	0.0	9	100.0	0.999	0.0	0.0	
13 to 15	7	8.3	77	91.7	**0.004**	**3.3**	**1.5**	**7.6**
10 to 12	61	23.3	201	76.7	1			

Table 7. Multivariate analysis.

Variable	P value	OR	(CI) 95%	
			Inf	Sup
Age (years)	0.004	0.913	0.858	0.972
History of PCOS	**0.000**	**11.242**	**5.906**	**21.400**
Duration of stimulation	0.040	0.786	0.625	0.989

3.4. Correlation Analysis between AMH, AFC and the Response to Ovarian Stimulation

In our study 65.01% of our population had a normal ovarian response, 19.56% had

a high ovarian response while 15.3% were low responders. Using Pearson correlative analysis, the number of oocytes had a highly significant correlation with AFC (r = 0.545, P = 0.001) (r = 0.643, P = 0.001) than with AMH (r = 0.519, P = 0.001) (r = 0.572, P = 0,001) in both the low and high ovarian response group respectively. The number of oocytes had a higher statistically more significant correlation with AMH (r = 0.490, P = 0.001) than with AFC (r = 0.430, P = 0.001) in patients with normal response (see Table 13). Furthermore, Age had a statistically significant inverse correlation with number of oocytes (r: −0.261, P: <0.001), AMH (r = −0.247, P < 0.001) and AFC (r = −0.452, P < 0.001) (see Table 8, Table 9).

Table 8. Number of oocytes retrieved.

Number of oocytes retrieved	Number of patients	Percentage
Low response = ≤3	56	15.43
Normal response = 4 - 17	236	65.01
High response = ≥15	71	19.56
Total	363	100.0

Table 9. Correlation between the number of oocytes, AMH and AFC using pearson correlation.

	Frequency	Mean	Number of oocytes	Coefficient	P-value
AMH	38	0.89 ± 0.12	≤ 3	0.519	0.001
	173	2.31 ± 0.13	4 - 15	0.490	0.001
	44	6.69 ± 0.64	>15	0.572	0.001
Total	255	2.85 ± 0.18			
AFC	45	9.13 ± 0.61	≤ 3	0.545	0.001
	192	13.01 ± 0.35	4 - 15	0.430	0.001
	53	20.28 ± 0.67	>15	0.643	0.001
Total	290	13.74 ± 0.34			

3.4.1. Predicting Low Ovarian Response

AMH had the highest accuracy for predicting low ovarian response (AUC: 0.856, 95%CI: 0.79 - 0.92, P < 0.001) and was significantly better than AFC (AUC: 0.774, 95%CI: 0.71 - 0.84, P < 0.001). An AMH cut-off value of ≤0.5 ng/ml had a sensitivity of 52.60% and a specificity of 98.61%. Corresponding values for an AFC of ≤3 were 62.50% and 85.81% (Table 10, Table 11).

3.4.2. Predicting High Ovarian Response

AMH had the highest accuracy for predicting high ovarian response (AUC: 0.917, 95%CI: 0.88 - 0.95, P < 0.001) and was significantly better than AFC (AUC: 0.874,

95%CI: 0.83 - 0.92, P < 0.001). An AMH h cut-off value of ≥6.4 ng/ml had a sensibility of 61.29% and a specificity of 88.84%. Corresponding values for an AFC of ≥21 were 84.21% and 86.34% (Table 12, Table 13).

Table 10. Confusion matrix for AMH in predicting low response.

	Predicted values		
Observed values	0	1	Total
0	214 (TN)	3 (FP)	217
1	20 (FN)	18 (TP)	38
Total	234	21	255

Where; TN = True negative; FP = False positive; FN = False negative; TP = True positive. Specificity; VN/VN + FP= 98.62 %; Sensitivity; 100 − (VP/VP + FN) = 52.60%.

Table 11. Confusion matrix for AFC in predicting low response.

	Predicted values		
Observed values	0	1	Total
0	234	3	237
1	37	16	53
Total	271	20	290

Specificity = 85.81%, Sensitivity = 62.50%.

Table 12. Confusion matrix for AMH in predicting high ovarian response.

	Predicted values		
Observed values	0	1	Total
0	199	12	211
1	25	19	44
Total	224	31	255

Specificity = 61.29%, Sensitivity = 88.84%.

Table 13. Confusion matrix for AFC in predicting high ovarian response.

	Predicted values		
Observed values	0	1	Total
0	234	3	237
1	37	16	53
Total	271	20	290

Specificity = 84.21%, Sensitivity = 86.34%.

4. Discussion

We carried out a hospital based descriptive and analytic cross-sectional study which set out to identify the correlation between AMH, AFC and the response to ovarian stimulation. Our study population consisted of patient files with 510 patient files excluded, and 363 patient files included and analyzed.

4.1. Sociodemographic and Clinical Profile

4.1.1. Age

The mean age of our study was 34.11 ± 5.11 years, the age of participants ranged from 20 - 47 years, with a predominant age of 30 - 40 years (66.6%). This is consistent with the findings of Vuong *et al.* [13], who found their mean age to be 34.3 ± 5.2 years in a hospital based prospective study in Vietnam in 2015. Permadi *et al.* [14] also had a mean age of 34.8 ± 2.8 years in a hospital based retrospective study in 2021. Himabindu *et al.* [15] in India had a mean age of 34.61 ± 3.62 years. This similarity can be explained by the fact that female ovarian reserve declines progressively with increasing chronological age. Above 30 years there is a significant decline in the quality and quantity of oocytes which decreases the probability of a spontaneous conception. By 35 years a woman's fecundity declines by 50%, which is why optimal fertility is accepted to be between late teens and late 20 s.

4.1.2. Type of Infertility

Majority of our patients had secondary infertility with 57.9%, which was similar to the findings of Kasia *et al.* [11] in 2020 where 62.9% of patients had secondary infertility in a hospital based retropective study in Cameroon. These findings were contrary to that of Siddiqui *et al.* [16] and Vuong *et al.* [13] who found that majority of their patients had primary infertility (68.3% and 55.4%). Our findings can be explained by the fact that women in low-income countries have a high rate of PID, practice unsafe abortions and inadequate maternity care leading to post-abortive and postpartum infections. Due to the lack of adequate medical treatment these infections tend to cause significant damaged to reproductive organs.

4.1.3. Duration of Infertility

In our study, 66.7% of our patients had been diagnosed with infertility for about 1 - 5 years. The mean infertility years was 5.28 ± 3.622 years which was similar to the findings of Vuong *et al.* [13] where their mean infertility years was 5.28 ± 4.133.

4.1.4. Etiology of Infertility

The majority of our study population had mixed factors as the predominant etiology of infertility with a frequency of 60.6%, followed by female factors with a frequency of 25.6% which was not in accordance with the findings of Kasia *et al.* [11] where female factors were the predominant cause of infertility with a frequency of 52.9%. This difference in results may be attributed to the patient selection bias in the various study sites.

4.2. Types of Protocol Used during Ovarian Stimulation

4.2.1. Type of Protocol

The antagonist protocol was the most prescribed ovarian stimulation protocol with a frequency of 85.7%, followed by the long protocol with a frequency of 8.0%. This finding was similar to that of Baker *et al.* [17], in this study the antagonist protocol had a frequency of 86% while the long agonist had a frequency of 9%. This can be explained by the observation that the antagonist protocol is associated with a lower risk of ovarian hyperstimulation.

4.2.2. Type of Gonadotropin

For ovarian stimulation, 88.4% of cycles used FSH. HMG was used for ovarian stimulation in 11.6% of cycles, nearly always in combination with FSH. This was similar to the studies done by Baker *et al.* [17] in the United States of America in 2018, where 95% of cycles used FSH. The similarity in both studies can be explained by the fact that recombinant FSH results in shorter duration of treatment, lower gonadotropin dose and better follicular characteristics on the day of the ovulation trigger shot.

4.2.3. Molecule Used to Trigger Ovulation

To induce ovulation, 93.9% of cycles used human chorionic gonadotrophin. GnRH agonist (6.1%) was mostly used to induce ovulation in patients who had risk factors of developing ovarian hyperstimulation syndrome. This finding was very similar to that of Vuong *et al.* [13], where 93.1% of cycles used HCG to trigger oocyte release while 6.9% used GnRH agonist. This is because HCG induced cycles have been associated with higher rates of implantation, clinical pregnancy, lower rates of early pregnancy loss and higher risk of OHSS due to their prolonged half-life which is responsible for higher LH activity. However, GnRH has a lower LH activity due to its short half-life of 60 mins hence lower risk of OHSS and high rates of pregnancy loss.

4.3. Factors Associated with Ovarian Response to Stimulation

4.3.1. Age

Our study revealed that there was a significant association between age and number of oocyte. Women within the age group [25 - 30[years (OR: 0.1, 95%CI: 0.0 - 0.5, P = 0.001), and 30 - 35 [years (OR: 0.3, 95%CI: 0.1 - 0.6, P = 0.001) were less likely to have a low ovarian response as compared to women above 40 years. Women within the age group [25 - 30 [years (OR: 4.5, 95%CI: 1.7 - 12.4, P = 0.004) were more likely to have a higher ovarian response than women above 40 years. These findings can be explained by the fact that a woman's ovarian reserve gradually decreases with age.

4.3.2. History of PCOS

History of PCOS was significantly associated with the number of oocytes. Patients who had PCOS were 11 times more likely to have a high response to ovarian stimulation than those who did not have PCOSPCOS is known to be a risk factor of

excessive ovarian response.

4.4. Correlation Analysis between AMH, AFC and Number of Oocyte

Our study revealed that number of oocytes was positively and significantly correlated With AFC and AMH in the low and high ovarian response groups. However AFC had a stronger correlation coefficient, This is in accordance with the findings of, Permadi *et al.* [14], Himanbindu *et al.* [15], Tsakos *et al.* [16] and Sun *et al.* [17] who found out that number of oocytes had a stronger correlation with AFC. This can be explained by the fact that AFC has less variability than AMH and also it is a direct ultrasound measurement of the number of antral follicles in the ovaries in contrast to AMH which indirectly estimates ovarian reserve [ACOG]. These findings were in contrary to those of Nelson *et al.* [18], Siddiquie *et al.* [16] and Vuong *et al.* [13] who found out that AMH had a stronger correlation coefficient than AFC. These differences can be attributed to the difference in our study population and size since AMH level can be influenced by factors such as age, variability in assay results, hormonal changes and ethnicity. [ACOG]. In addition, the Pearson correlation coefficient showed that age was significant but inversely correlated with number of oocytes, AMH and AFC. This is in accordance with the findings of Himanbindu *et al.* [15], Baker *et al.* [17] and Vuong *et al.* [13] who reported a statistically significant and inverse correlation between age and number of oocytes. This is understandable as there is a progressive decline in the quality and quantity of oocyte as age increases.

Predicting Ovarian Response

AMH had the highest accuracy for predicting ovarian response (AUC: 0.856, 95%CI: 0.79 - 0.92, P < 0.001); (AUC:0.917, 95%CI: 0.88 - 0.95, P < 0.001) and was significantly better than AFC (AUC: 0.774, 95%CI: 0.71 - 0.84, P < 0.001); (AUC: 0.874, 95%CI: 0.83 - 0.92, P < 0.001) in the low and high ovarian response group respectively. This was accordance to the findings of Vuong *et al.* [13] where AMH was a better predictor of ovarian response than AFC. Also Nelson *et al.* [20] in a multicenter analysis, assessed the relative capacity of AMH and AFC for predicting oocyte yield which demonstrated that AMH dominated the model: AMH, R = 0.29 and 0.23; AFC: R = 0.07 and 0.07 for long GnRH agonist and GnRH antagonist trials respectively. In contrast Himabindu *et al.* [15], Tsakos *et al* [16] and Sun *et al.* [17], in single center studies found out that AFC was a better predictor of ovarian response. Our findings can be explained by the observation that AMH can detect smaller follicles at earlier stages of follicular development, providing a wider range of detection and potentially a stronger predictive value. Also AMH is less operator dependent compared to AFC, which requires ultrasound assessment and can be influenced by operator expertise and ultrasound technology [18]-[20].

An AMH cut-off value of ≤0.5 ng/ml had a sensitivity of 52.60% and a specificity of 98.61% in predicting low ovarian response. Corresponding values for an AFC of ≤3 was 62.50% and 85.81%. Our findings were within the limits of the Bologna

criteria, which states that an AMH levels < 0.5 ng/ml and AFC < 5 is a predictor of poor ovarian response to stimulation (POR ≥ 3 oocyte). This is in contrast with Poseidon's classification, where an AMH level <1.2 ng/ml is a predictor of poor ovarian stimulation (POR ≥ 3). Also Vuong *et al.* [13], found an AMH cut off value of ≤1.25 ng/mL with sensitivity of 86.7% and specificity of 84.8%. Corresponding values for AFC was ≤5, sensibility 78.8% and specificity 86.0%. These differences can be explained by the lack of an international standardization assay for AMH causing inter-assay variabilities. It can also be attributed to marked sonographer-dependent variability across centers. Furthermore different study designs and difference in ethnicities between study populations may contribute to these variations.

In our study an AMH cut-off value of ≥6.4 ng/ml had a sensibility of 61.29% and a specificity of 88.84% in predicting high ovarian response. Corresponding values for an AFC of ≥21 was 84.21% and 86.34% respectively. Our cut-off value for AFC was similar to that of ASRM [4], which states that AFC > 24 is a predictor of high ovarian response (>15 - 18 oocyte retrieved), whereas AMH > 3.4 ng/ml is indicative of a high ovarian response, in contrast to our findings. Also Vuong *et al.* [13] found that an AMH cut off value of >3.57 ng/mL had sensitivity of 83.7%, and a specificity of 79.8%. Corresponding values for an AFC of ≥12 were 79.2% and 81.7% respectively for predicting high ovarian response. The difference in results may be due to the lack of an international standardized definition of ovarian response. Difference in Ethnicity and study designs may contribute to these variations.

5. Strength

It is one of the few studies in Cameroon that has effectively evaluated the sensitivity, specificity and cut off values of ovarian reserve biomarkers in predicting ovarian response to stimulation in couples attending the infertility clinics in the reproductive age group and selected for IVF treatment. Moreover, the study was conducted in a hospital setting, which allows for access to medical records and patient information that provided a comprehensive understanding of ovarian response to stimulation.

6. Limitations

As this was a multicenter study, one major concern is the lack of international standardization hence variability in AMH results as different assays may have a distinct sensitivity and specificity leading to variabilities in results. However we use a conversion factor to adjust values obtained from different assays to ng/ml to ensure comparability across studies and reduce discrepancies. While our study was limited to a specific population we took steps to increase the generality of our findings by recruiting from multiple sites. This study may be subject to selection bias since it was not a probability sampling.

7. Conclusions

Majority of our study population had secondary infertility, with mixed factors

being the most frequent cause of infertility. The mean duration of infertility was 5.28 ± 3.622 years.

In our study, the most prescribed ovarian stimulation protocol was the antagonist protocol, and recombinant FSH was the preferred gonadotropin used during stimulation. Ovulation was induced 93.9% of the time with HCG. GnRH agonist was mostly used to trigger ovulation in patients with high risk of ovarian stimulation. Duration of stimulation ranged from 8 - 16 days.

On multivariate logistic analysis age and history of PCOS were significantly associated with ovarian response in both the low and high ovarian response groups.

The number of oocytes was significantly and positively correlated with AMH and AFC in both low and high ovarian groups. Age was significantly but negatively correlated with number of oocytes, AMH and AFC. Both AMH and AFC were independent predictors of ovarian response to stimulation. AMH cut-off values of ≤0.5 ng/ml and ≥6.3 ng/ml were more specific but less sensitive in predicting low and high ovarian response respectively. Whereas, AFC cut-off values of ≤3 and ≥21 were less specific and more sensitive in predicting low and high ovarian response. Although, AMH was a better predictor of ovarian response to stimulation, due to its lack of an international standardized assay, AFC combined with it may be a more accurate predictor of ovarian response.

8. Recommendation

Patients should seek early consultations to mitigate age-related infertility effects and timely initiation of fertility treatments, such as IVF, after a year of unsuccessful natural conception. Regular monitoring of ovarian reserves through AMH and AFC testing can help adjust fertility plans accordingly. Physicians are encouraged to promote early fertility evaluation in patients' early to mid-30 s, recommend egg freezing for those concerned about age-related infertility, and utilize both AMH and AFC to improve the accuracy of predicting ovarian response considering their sensitivities and specificities, with AMH's higher specificity aiding in ruling out poor responders, and AFC's higher sensitivity helping identify potential high responders. Personalized treatment should be based on combined AMH and AFC information, tailored to each patient's needs, including parameters such as BMI, contraceptive use, and smoking history. For the government, ensure AMH and AFC testing is widely available and accessible, especially in fertility clinics and ART centers, and promote education and training for healthcare providers on interpreting these results. For researchers, conduct further studies to optimally combine AMH and AFC results, improving and validating predictive models that include other relevant factors to enhance the accuracy of predicting ovarian response.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] WHO (2023) 1 in 6 People Globally Affected by Infertility 2023. https://www.who.int/news/item/04-04-2023-1-in-6-people-globally-affected-by-infertility

[2] WHO (2023) Infertility Prevalence Estimates, 1990-2021. https://www.who.int/publications/i/item/978920068315

[3] Carson, S.A. and Kallen, A.N. (2021) Diagnosis and Management of Infertility: A Review. *JAMA*, **326**, 65-76. https://doi.org/10.1001/jama.2021.4788

[4] (2024) The FIGO Ovulatory Disorders Classification System. https://europepmc.org/article/MED/35983674

[5] Practice Committee of the American Society for Reproductive Medicine (2020) Electronic Address: asrm@asrm.org, Practice Committee of the American Society for Reproductive Medicine. Testing and Interpreting Measures of Ovarian Reserve: A Committee Opinion. *Fertility and Sterility*, **114**, 1151-1157.

[6] Moiseeva, A.V., Kudryavtseva, V.A., Nikolenko, V.N., Gevorgyan, M.M., Unanyan, A.L., Bakhmet, A.A., *et al.* (2021) Genetic Determination of the Ovarian Reserve: A Literature Review. *Journal of Ovarian Research*, **14**, Article No. 102. https://doi.org/10.1186/s13048-021-00850-9

[7] Lonegroa, N., Nápolia, N., Pesceb, R. and Chacóna, C. (2017) Antral Follicle Count as a Predictor of Ovarian Response. https://www.semanticscholar.org/paper/Antral-follicle-count-as-a-predictor-of-ovarian-Lonegroa-N%C3%A1polia/6c4368669a9fae3114d21591127bc4b71b8e2d5e

[8] Peck, J.D., Quaas, A.M., Craig, L.B., Soules, M.R., Klein, N.A. and Hansen, K.R. (2015) Lifestyle Factors Associated with Histologically Derived Human Ovarian Non-Growing Follicle Count in Reproductive Age Women. *Human Reproduction*, **31**, 150-157. https://doi.org/10.1093/humrep/dev271

[9] Barbakadze, L., Kristesashvili, J., Khonelidze, N. and Tsagareishvili, G. (2015) The Correlations of Anti-Mullerian Hormone, Follicle-Stimulating Hormone and Antral Follicle Count in Different Age Groups of Infertile Women. *International Journal of Fertility and Sterility*, **8**, 393-398.

[10] Moolhuijsen, L.M.E. and Visser, J.A. (2024) Anti-Müllerian Hormone and Ovarian Reserve: Update on Assessing Ovarian Function. *The Journal of Clinical Endocrinology & Metabolism*, **105**, 3361-3373. https://pubmed.ncbi.nlm.nih.gov/32770239/

[11] Kasia Jean Marie, K.J., Cyrille, N.N.C., Vanina, N.A. and Etienne, B. (2020) Threshold Value of AMH Correlated with an Acceptable Response to Ovarian Stimulation in Patients over 38 Years Old at the Hospital Center for Research and Application in Endoscopic Surgery and Human Reproduction. *Health Sciences & Disease*, **21**. http://www.hsd-fmsb.org/

[12] European Society of Reproductive Medicine (2024) Ovarian Stimulation for IVF/ICSI. https://www.eshre.eu/Guidelines-and-Legal/Guidelines/Ovarian-Stimulation-in-IVF-ICSI

[13] Vuong, T.N.L., Vo, M.T. and Ho, T.M. (2015) Predictive Value of AMH, FSH and AFC for Determining Ovarian Response in Vietnamese Women Undergoing Assisted Reproductive Technologies: A Prospective Study. *Journal of Fertilization in Vitro—IVF-Worldwide Reproductive Medicine Genetics & Stem Cell Biology*, **3**, Article ID: 1000151.

[14] Permadi, W., Wahyu Ferdian, M., Tjahyadi, D., Ardhana Iswari, W. and

Djuwantono, T. (2021) Correlation of Anti-Mullerian Hormone Level and Antral Follicle Count with Oocyte Number in a Fixed-Dose Controlled Ovarian Hyperstimulation of Patients of *in Vitro* Fertilization Program. *International Journal of Fertility and Sterility*, **15**, 40-43.

[15] Himabindu, Y., Sriharibabu, M., Gopinathan, K., Satish, U., Louis, T. and Gopinath, P. (2013) Anti-Mullerian Hormone and Antral Follicle Count as Predictors of Ovarian Response in Assisted Reproduction. *Journal of Human Reproductive Sciences*, **6**, 27-31. https://doi.org/10.4103/0974-1208.112377

[16] Tsakos, E., Tolikas, A., Daniilidis, A. and Asimakopoulos, B. (2014) Predictive Value of Anti-Müllerian Hormone, Follicle-Stimulating Hormone and Antral Follicle Count on the Outcome of Ovarian Stimulation in Women Following GNRH-Antagonist Protocol for IVF/ET. *Archives of Gynecology and Obstetrics*, **290**, 1249-1253. https://doi.org/10.1007/s00404-014-3332-3

[17] Sun, X., Xiong, W., Liu, L., Xiong, J., Liao, C., Lan, Y., *et al.* (2022) Comparison of the Predictive Capability of Antral Follicle Count Vs. the Anti-Müllerian Hormone for Ovarian Response in Infertile Women. *Frontiers in Endocrinology*, **13**, Article 862733. https://doi.org/10.3389/fendo.2022.862733

[18] Nelson, S.M., Klein, B.M. and Arce, J. (2015) Comparison of Antimüllerian Hormone Levels and Antral Follicle Count as Predictor of Ovarian Response to Controlled Ovarian Stimulation in Good-Prognosis Patients at Individual Fertility Clinics in Two Multicenter Trials. *Fertility and Sterility*, **103**, 923-930.e1. https://doi.org/10.1016/j.fertnstert.2014.12.114

[19] Baker, V.L., Gracia, C., Glassner, M.J., Schnell, V.L., Doody, K., Coddington, C.C., *et al.* (2018) Multicenter Evaluation of the Access AMH Antimüllerian Hormone Assay for the Prediction of Antral Follicle Count and Poor Ovarian Response to Controlled Ovarian Stimulation. *Fertility and Sterility*, **110**, 506-513.e3.

[20] Alviggi, C., Andersen, C.Y., Buehler, K., Conforti, A., De Placido, G., Esteves, S.C., *et al.* (2016) A New More Detailed Stratification of Low Responders to Ovarian Stimulation: From a Poor Ovarian Response to a Low Prognosis Concept. *Fertility and Sterility*, **105**, 1452-1453. https://doi.org/10.1016/j.fertnstert.2016.02.005