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Predictive value of anti-Müllerian hormone and antral follicle count in ovarian stimulation response in Cameroonian women

Rôle prédictif de l'hormone antimüllérienne et du nombre de follicules antraux dans la réponse à la stimulation ovarienne chez les femmes camerounaises

Nsahlai CJF[1,2], Ngono Akam Marga V[1,3], Mboua Batoum VS[1,4], Mpono Emenguele P[1,3], Nyada SR[1,3], Ebong Ebontane C[1,5], Kasia JM[1,3]

Original Article

1. Department of Obstetrics and Gynecology, Faculty of Medicine and Biomedical Sciences, The University of Yaoundé I,
2. Essos Hospital Center, Yaoundé
3. Gynecological Endoscopic Surgery and Human Reproductive Teaching Hospital, Yaoundé
4. University Teaching Hospital
5. Yaoundé Central Hospital

Corresponding author: Christiane Jivir F Nsahlai, Faculty of Medicine and Biomedical Sciences, The University of Yaoundé I, PO Box 1364, Yaoundé. Tel : (+237) 670 217 703, Email: cnsahlai1974@gmail.com ; christiane.nsahlai@fmsb-uy1.cm

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ABSTRACT

Background: Infertility affects one in six adults globally, with assisted reproductive techniques (ART) offering tailored treatment options. Predicting ovarian response is essential to optimize outcomes and minimize risks. Anti-Müllerian Hormone (AMH) and Antral Follicle Count (AFC) are key markers of ovarian reserve. The aim of our study was to evaluate the predictive capacity of the combined use of AFC and AMH in the prediction of ovarian response in ART techniques.

Methods: This analytical cross-sectional study included 282 African women aged 20–43 undergoing IVF/ICSI at CHRACERH, Yaoundé. AMH levels were measured via electro-chemiluminescence and AFC via transvaginal ultrasound. Ovarian response was categorized as poor (<4 oocytes), normal (4–19), or hyper-response (≥20). Statistical analyses included correlation tests and multivariate regression.

Results: Normal response occurred in 70.2% of participants; 27% were poor responders. AMH and AFC showed strong positive correlations with both follicle count and mature oocyte yield ($p < 0.001$). Age and BMI were negatively associated with ovarian response. AFC remained a significant predictor of follicle count in multivariate analysis, while AMH showed borderline significance for mature oocytes. FSH and estradiol levels were less predictive.

Conclusion: AMH and AFC are reliable predictors of ovarian response in African women. AFC, being more accessible and cost-effective, may serve as a practical alternative to AMH in low-resource settings. Age and BMI significantly influence ovarian reserve and stimulation outcomes.

RESUME

Contexte : Les techniques de procréation médicalement assistée (PMA) constituent un traitement de l'infertilité. L'hormone anti-müllérienne (HAM) et le compte de follicules antraux (CFA) sont des marqueurs clés de la réserve ovarienne. L'objectif était d'évaluer la capacité prédictive de l'utilisation combinée du CFA et de l'HAM dans la prédiction de la réponse ovarienne dans les techniques de PMA.

Méthodes : Cette étude analytique transversale a porté sur 282 femmes africaines âgées de 20 à 43 ans ayant subi une FIV/ICSI au CHRACERH, à Yaoundé. Les taux de l'HAM ont été mesurés par électrochimiluminescence et le CFA par échographie transvaginale. La réponse ovarienne a été classée comme faible, normale ou hyper-réponse. Les analyses statistiques comprenaient des tests de corrélation et une régression multivariée.

Résultats : Une réponse normale a été observée chez 70,2 % des participantes ; 27 % ont présenté une faible réponse. L'HAM et le CFA ont montré de fortes corrélations positives avec le nombre de follicules et le rendement en ovocytes matures ($p < 0,001$). Le CFA est resté un prédicteur significatif du nombre de follicules dans l'analyse multivariée, tandis que l'HAM a montré une signification limite pour les ovocytes matures.

Conclusion : L'HAM et le CFA sont des prédicteurs fiables de la réponse ovarienne chez les femmes africaines. Le CFA, plus accessible et plus rentable, peut constituer une alternative pratique à l'HAM dans les milieux à faibles ressources.

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Introduction

Infertility is currently considered a global public health issue according to the World Health Organization (WHO) with approximately 17.5% of adults—about one in six people globally—struggling with infertility, highlighting the critical need to expand access to affordable, high-quality fertility care for those affected (1). Management depends largely on etiology and ranges from medical treatments to surgical procedures to assisted reproductive techniques (ART). The main objective in the treatment of infertility by ART is to tailor each woman to the most appropriate treatment based on her specific clinical and paraclinical characteristics, thus maximizing the chances of pregnancy while eliminating avoidable iatrogenic risks, such as those from ovarian stimulation. Therefore, personalization of treatment in ART should be primarily based on the prediction of ovarian response for each woman, keeping in mind that decreasing success rates of ART due to aging are primarily linked to the gradual reduction in ovarian reserve (2). To this end, several tests for predicting ovarian response have been proposed including FSH level at cycle day 3, inhibin B, antral follicle count (AFC) and anti-Müllerian hormone (AMH). However, none of these markers taken individually is unanimously predictive of a better response to ovarian stimulation, so current concepts aim to combine several of them for greater efficacy (3,4). Recent research suggests that AMH and AFC are highly effective tests for assessing ovarian reserve and predicting ovarian response to controlled ovarian stimulation (5,6). Anti-Müllerian hormone is a glycoprotein belonging to the transforming growth factor β family. During male fetal development, it plays a crucial role in the regression of the Müllerian ducts. Additionally, AMH is produced by growing ovarian follicles in women throughout their reproductive years (7). Equally, antral follicle count refers to the process of counting resting follicles present in both ovaries at the start of the proliferative phase of the menstrual cycle using transvaginal ultrasonography. These follicles, typically measuring 2–6 mm in size, have been identified in recent studies as potential indicators of ovarian response during controlled ovarian stimulation (8). Although studies have shown that AMH varies according to race, ethnicity and environmental factors, few studies have been undertaken in black African women (9). The aim of our study was to evaluate the predictive capacity of the combined use of AFC and AMH in the prediction of ovarian response in ART [intracytoplasmic sperm injection (ICSI)/in vitro fertilization (IVF)] techniques and subsequent outcomes in black women.

Materials and Methods

We carried out an analytical cross-sectional study with both retrospective and prospective data collection at a university-associated facility called Gynecological Endoscopic Surgery and Human Reproductive Teaching Hospital (CHRACERH), that is also a public hospital with IVF facilities in Yaoundé-Cameroon. Our subjects were patients who underwent ovarian stimulation from December 2016 to May 2019. The research and ethics committee of the facility and faculty of medicine and biomedical sciences of the University of Yaoundé I approved the study protocol, and informed consent was obtained from all participants.

Our inclusion criteria were as follows: we included any patient undergoing ovarian stimulation for IVF/ICSI and patients who had documented results of both anti-Müllerian hormone (AMH) levels and antral follicle count (AFC).

Our exclusion criteria included patients with ovarian cysts, those with incomplete ovarian stimulation records, and any case with missing medical records.

Sampling and sample size

We used exhaustive and consecutive sampling and obtained 282 subjects that met our inclusion criteria within the study period. We collected ovarian stimulation data from patients' medical records at CHRACERH archives. The prospective segment involved consecutive sampling of patients corresponding to our inclusion criteria and evaluating their response to ovarian stimulation.

Hormonal assay and antral follicle count

All study participants had undergone a hormonal work-up at CHRACERH that included an AMH level. AMH was measured using the electro-chemiluminescence technique (Cobas Roch) and results noted in ng/ml. Antral follicle count was performed between days 3 and 5 of participants' menstrual cycle using a Siemens Acuson 150 version 2.5 transvaginal 7.5-megahertz ultrasound probe by different health care providers.

Ovarian stimulation protocol

In most cases we used a short agonist protocol for ovarian stimulation. This protocol consisted in administering a minidose estrogen-progestin pill for the first 12 days of the previous menstrual cycle, then stopping the pill. Two to three days after stoppage, blood was drawn to measure pituitary hormones (follicle stimulating hormone (FSH) and luteinizing hormone (LH) and ovarian hormones (progesterone (P4) and estradiol (E2)). The patient was eligible to begin ovarian stimulation if the following conditions were met: E2 level < 50 pg/ml, P4 level <1 IU/L and endometrium less than 4mm. Stimulation began on cycle day 2 with a subcutaneous injection of LH-GnRH (Gonadotropin Releasing Hormone) analogue followed on day 3 by a follicle stimulating hormone (FSH) agonist (Human Menopausal Gonadotropin (HMG) or Gonal-F). Doses administered were decided based on AMH levels: if AMH was < 1.5 ng/ml, a dose of 300 IU was administered; if AMH

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was between 1.5 and 4.5 ng/ml, a dose of 225 IU was administered, if AMH was > 4.5 ng/ml, a dose of 150 IU was administered (sometimes with the choice of an antagonist protocol, which consisted of the injection of 150 IU gonadotropins (HMG) the day after bleeding onset and an injection of GnRH antagonist from day 6 of stimulation).

Regardless of the protocol utilized, we evaluated hormones P4 and E2 on day 4 of gonadotropin administration, followed by ultrasound monitoring of follicular growth. An increase in E2 levels, indicative of follicular growth, enabled stimulation to be continued, maintaining doses until a follicular diameter of at least 14 mm was reached, with a minimum number of 4 follicles for both ovaries and an average E2 level of 200 pg/mL mature follicle. Any deviation from these criteria not only heightened concerns about ovarian hyperstimulation syndrome (OHSS) but was also worrisome about a poor response. Consequently, adjustments were made, including dose reductions, dose increases, or extensions in the duration of stimulation.

For patients with diminished ovarian reserves, such as, in patients with a history of endometriosis or being a previous poor responder, a long protocol would be undertaken. This involved the administration of a sustained release (SR) GnRH analogue (Decapeptyl SR 3mg) between days 18 and 21 of the preceding cycle, menstruations followed 8 to 10 days later. One day after bleeding onset, the start-up assessment was carried out. If the start-up criteria were met, daily gonadotropin administration was started until the induction criteria were attained. Another protocol used was the short agonist/antagonist protocol. This was performed on a natural cycle or after treatment with estrogen-progestogen pills. Prior to stimulation, a hormone assay (FSH, E2 and P4) and pelvic ultrasound were performed to ensure ovarian quiescence.

Oocyte retrieval occurred 36 hours after induction and was performed transvaginally via ultrasound guidance and under general anesthesia. The products retrieved were sent directly to the IVF laboratory for analysis and follicle decoronization. Quantitative response to ovarian stimulation was assessed according to the number of follicles punctured and the number of mature oocytes. These were classified as follows:

- Poor responders: <4 follicles (oocytes)
- Normal responders: between 4 and 19 follicles, with sub-optimal responders between 4 and 9 follicles and optimal responders between 10 and 19 follicles.
- Hyper responders: ≥ 20 follicles (oocytes)

Our study's primary outcome measures included: the number of oocytes retrieved and the corresponding ovarian response, and a comparison of AMH and AFC as predictors of ovarian response.

Statistical analysis

Data collected were analyzed using SPSS version 23.0 and Epi info version 3.5.4. Tables were drawn using Microsoft Office Excel and Word 2016. The Chi-squared statistical test was used to compare variables, and the Student's t test to compare means and their standard deviations. The coefficient correlation was calculated between serum AMH level and number of punctured follicles, and between AFC and number of punctured follicles. Multivariate logistical regression analysis was used to test the association between poor response and normal response with measured parameters. The threshold of significance, allowing us to affirm that the difference between the samples will be negative or not, was set at 5%, i.e. 0.05.

Results

Our study involved 282 women aged 20 and 43 years, with an average age of 33.6 ± 4.9 years. The most represented age group was 35-39 (34%). Of these patients, 96.8% were Cameroonian, while 3.2% belonged to other African nationalities, 71.6% were married and 53.3% had a tertiary level of education. The mean duration of infertility was 6.26 ± 4.1 years, and secondary infertility was more frequent in 60.3% of cases.

Of the 282 cycles studied, 277 (98.2%) were complete cycles and 5 (1.8%) cycles were cancelled, 3 (1.06 %) for unresponsiveness and 2 (0.74%) for ovarian hyperstimulation.

In terms of protocol used, the agonist protocol was used in 278 (98.6%) cycles: 270 (95.8%) short agonists versus 8 (2.8%) long agonists. The antagonist protocol was used in 4 (1.4%) cycles. The mean duration of stimulation was 10.4±2.1 days, with a minimum of 6 days and a maximum of 18 days. Gonadotropin doses ranged from 150 IU to 300 IU. Most patients (56.4%) received 300 IU of gonadotropins.

In determining the distribution of participants according to ovarian stimulation, we recorded that only mature oocytes retrieved determine the quality of response to ovarian stimulation. Thus, in terms of mature oocytes obtained, 27% of patients had a poor response. Most patients (70.2%) had a normal response, with 55.3% sub-optimal and 14.9% optimal. Hyper responders represented 2.8% of patients (Table 1).

We recorded overall mean values for ovarian reserve parameters and then dichotomized our participants by age (less than 38 years and 38 years or more). The overall mean AMH level was 2.56 ± 1.87. Women younger than 38 have significantly higher AMH levels (2.92 ± 1.98) compared to those 38 and older (1.46 ± 0.85), with a p-value of 0.00, indicating a statistically significant difference. The overall mean AFC is 10.79

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± 7. Younger women have a higher AFC (12.12 ± 7.22), while older women have a significantly lower count (6.79 ± 4.31), with a p-value of 0.01, indicating a statistically significant difference. The overall mean FSH level is 7.6 ± 7.6. Younger women have lower FSH levels (7.2 ± 5.77) compared to older women (9.28 ± 10.01). However, the p-value is 0.06, suggesting a marginal difference that is not statistically significant. Follicles punctured, and mature oocytes retrieved show statistically significant differences between age groups, with younger women having consistently higher values as shown on Table 2.

Table 1: Distribution of patients according to their response to ovarian stimulation

	Poor responders	Normal responders		Hyper-responders
	n (%)	Sub-optimal response n (%)	Optimal response n (%)	n (%)
Follicular puncture	42 (14.9)	129 (45.6)	90 (32)	21 (7.5)
Mature oocytes	76 (27)	153 (55.3)	41 (14.9)	7 (2.8)

Table 2: Comparison of ovarian reserve parameters according to age

Variables	All subjects (N= 282) Mean ± SD	< 38 ans (N = 212) Mean ± SD	>=38ans (N= 70) Mean ± SD	P-value
AMH	2.56 ± 1.87	2.92 ± 1.98	1.46 ± 0.85	0.00
AFC	10.79 ± 7	12.12 ± 7.22	6.79 ± 4.31	0.01
FSH	7.6 ± 7.6	7.2 ± 5.77	9.28 ± 10.01	0.06
Follicules punctured	9.38 ± 6.39	10.22 ± 6.79	6.86 ± 4.14	0.00
Mature oocytes retrieved	6.56 ± 4.71	7.08 ± 5	5 ± 3.32	0.00

Table 3: Comparison of ovarian reserve parameters in normal and poor responders

Variables	Poor responders N= 42	Normal responders N= 218	P-value
Age	33.73 ± 4.86	35.69 ± 4.86	0.015
AMH	1.14 ± 0.97	2.65 ± 1.80	0.000
AFC	6.64 ± 4.65	10.86 ± 6.55	0.000
FSH	7.23 ± 3.40	7.93 ± 7.64	0.603
E2 (at stimulation onset)	1652.22 ± 2427	3321.86 ± 4665.23	0.006
Day of stimulation	10.35 ± 2.13	10.30 ± 1.98	0.890

The mean age of poor responders is 33.73 ± 4.86 years, while normal responders are slightly older at 35.69 ± 4.86 years, with a p-value of 0.015 indicating a statistically significant difference. Poor responders have a significantly lower AMH level (1.14 ± 0.97) compared to normal responders (2.65 ± 1.80) and the p-value of 0.000 confirms a significant difference, reinforcing AMH as a strong predictor of ovarian response. On examination of AFC, poor responders show a lower AFC (6.64 ± 4.65) compared to normal responders (10.86 ± 6.55), with a p-value of 0.000 confirming that this difference is statistically significant, emphasizing AFC as another key indicator of ovarian reserve. FSH levels are slightly lower in poor responders (7.23 ± 3.40) compared to normal responders (7.93 ± 7.64) but the p-value of 0.603 suggests that there is no statistically significant difference, indicating that FSH might not be a reliable predictor of ovarian response in this dataset. Estradiol (E2) levels at stimulation onset are significantly lower in poor responders than normal responders, 1652.22 ± 2427 and 3321.86 ± 4665.23, respectively. The p-value of 0.006 suggests a significant difference, indicating that E2 levels at stimulation onset may be linked to ovarian response. The stimulation duration is similar between groups (10.35 ± 2.13 vs. 10.30 ± 1.98) and the p-value of 0.890 shows no significant difference, suggesting that stimulation duration does not vary much between poor and normal responders.

Table 4: Correlation between the number of oocytes with measured parameters

Variables	Number of follicles punctured		Number of mature oocytes	
	Correlation coefficient	p-value	Correlation coefficient	p-value
Age	-0.327	0.00	-0.0269	0.00
AMH	0.477	0.00	0.443	0.00
FSH	-0.097	0.14	-0.047	0.49
AFC	0.510	0.00	0.441	0.00

Age shows a significant negative correlation with both the number of follicles punctured (-0.327, p=0.00) and mature oocytes retrieved (-0.269, p=0.00), indicating a decline in ovarian response with age. AMH and AFC exhibit strong positive correlations with follicle count and oocyte yield, reinforcing their role as key predictors. Conversely, FSH shows weak negative correlations (-0.097, p=0.14 for follicles punctured; -0.047, p=0.49 for oocytes), with no statistical significance, suggesting it may not be a reliable indicator of ovarian response.

Table 5: Multiple regression analysis of factors predicting ovarian response (follicles punctured)

	Coef (6)	std err	t	P>|t|	95% CI
const	14.234	2.965	4.801	0.000	8.388 - 20.080
BMI	-0.223	0.094	-2.377	0.018	-0.408 - 0.038*
AMH (ng)	0.111	0.241	0.459	0.647	-0.364 - 0.586
FSH (IU/L)	0.007	0.071	0.098	0.922	-0.134 - 0.148
AFC	0.153	0.062	2.447	0.015	0.030 - 0.276*

** = Significant; BMI = Body Mass Index; AMH = Anti-mullerian hormone; FSH = Follicle stimulating hormone; AFC = Antral follicle count*

A multiple linear regression was used to evaluate the relationship between selected clinical predictors and the number of follicles punctured. The predictors included body mass index (BMI), anti-mullerian

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hormone (AMH), follicle stimulating hormone (FSH), and antral follicle count (AFC). The model revealed that both BMI and AFC were statistically significant predictors. A one-unit increase in BMI was associated with a decrease of approximately 0.22 number of follicles punctured (β = −0.223, 95% CI: −0.408 to −0.038, p = 0.018). Conversely, a one-unit increase in antral follicle count was associated with an increase of 0.15 in follicles punctured (β = 0.153, 95% CI: 0.030 to 0.276, p = 0.015). AMH and FSH were not statistically significant in this model (p > 0.05).

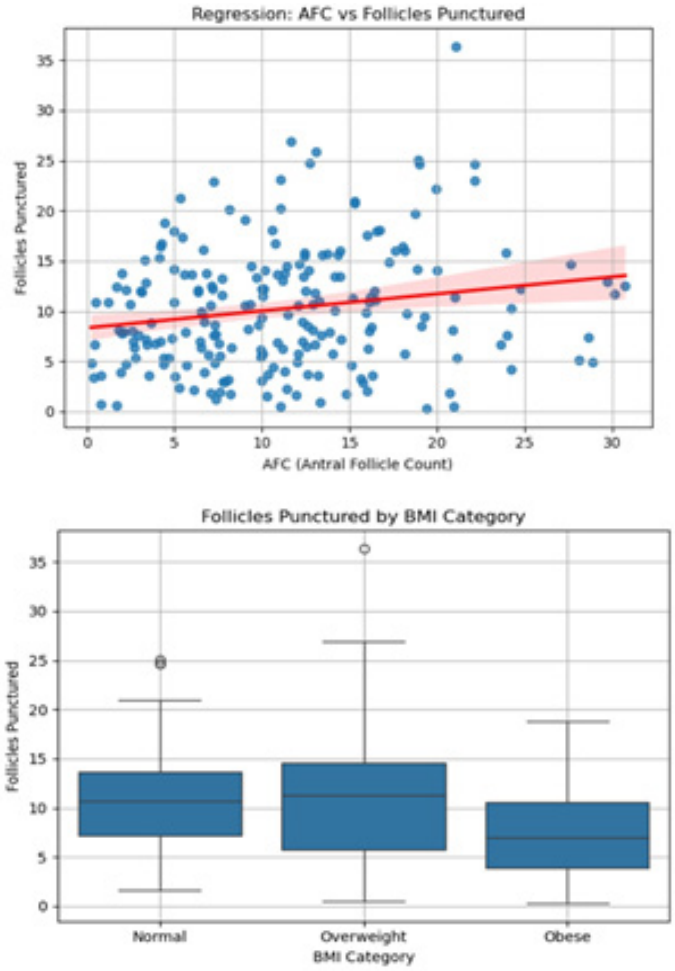

Figure 1: Graphical representation of ovarian response to AFC and BMI

Table 6: Predicting ovarian response (mature follicles) based on the BMI, AMH, FSH, and AFC

	Coef (6)	Std err	t	P>|t|	95% CI
const	8.232	2.129	3.866	0.000	4.034 - 12.431
BMI	-0.078	0.067	-1.155	0.249	-0.211 - 0.055
AMH (ng)	0.335	0.173	1.938	0.054	-0.006 - 0.676
FSH (IU/L)	-0.010	0.051	-0.190	0.850	-0.111 - 0.091
AFC	0.038	0.045	0.848	0.398	-0.050 - 0.127

BMI = Body Mass Index; AMH = Anti-mullerian hormone; FSH = Follicle stimulating hormone; AFC = Antral follicle count

A multiple linear regression was conducted to determine the influence of ovarian reserve markers and anthropometric indices on the number of mature follicles. We included BMI, AMH, FSH, and AFC as predictors. The results show that AMH had a borderline significant positive association with the number of mature follicles (β = 0.34, 95% CI: −0.006 to 0.676, p = 0.054). None of the other variables, BMI (β = −0.078, p = 0.249), FSH (β = −0.010, p = 0.850), or AFC (β = 0.038, p = 0.398) were statistically significant predictors of mature follicle count (Table 6).

Discussion

This study evaluated the predictive capacity of the antral follicle count (AFC) and anti-Müllerian hormone (AMH) in determining ovarian response to controlled ovarian stimulation among black African women undergoing assisted reproductive techniques (ART) at the Center for Research and Application in Endoscopic Surgery and Human Reproduction (CHRACERH). Our findings reinforce the utility of AMH and AFC as reliable markers of ovarian reserve and response, while also highlighting age and body mass index (BMI) as influential factors.

We conducted a cross-sectional analytical study involving historical and prospective data collection from 282 in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) cycles. The patients' ages ranged from 20 to 43 years old. Poor responders accounted for 27% of our study population, while normal responders accounted for 70.2% (55.3% suboptimal and 14.9% optimal) (Table 4). Both AMH and AFC demonstrated strong positive correlations with the number of follicles punctured and mature oocytes retrieved, consistent with prior studies suggesting their superiority over traditional markers such as FSH (10).

Women with higher AMH and AFC values were significantly more likely to exhibit normal or optimal ovarian responses, while poor responders had markedly lower levels of both markers. These results align with the growing consensus that AMH and AFC, when used in combination, offer enhanced predictive accuracy for ovarian stimulation outcomes (3,4).

Interestingly, while AMH showed a significant borderline association with mature follicle count in multivariate analysis (p = 0.054), AFC did not retain statistical significance in this model. This may reflect the dynamic nature of follicular development and the influence of other physiological or environmental factors not captured in our dataset. Nonetheless, AFC remained a significant predictor of the number of follicles punctured, underscoring its clinical relevance in stimulation planning, similar to other studies reported in the literature (11,12).

Age was negatively correlated with both follicle count and oocyte yield, reaffirming its role as a critical determinant of ovarian reserve. Women aged ≥38 years had significantly lower AMH and AFC values, fewer follicles punctured, and fewer mature

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oocytes retrieved. These findings are consistent with established literature on age-related decline in ovarian function and support the need for age-stratified stimulation protocols (2,7).

BMI also emerged as a significant negative predictor of follicle count in multivariate analysis, with higher BMI associated with reduced ovarian responsiveness. Although BMI did not significantly affect mature oocyte yield, its impact on follicular development warrants further investigation, particularly in populations with high prevalence of overweight and obesity. Similar to our results, a metanalysis revealed that BMI is negatively correlated with AMH (13).

FSH levels showed weak and non-significant correlations with ovarian response, echoing concerns about its variability and limited standalone predictive value (3). Our results are consistent with studies reported by Hu et al, and Leijdekkers et al., (14,15). Likewise, while estradiol levels at stimulation onset differed significantly between poor and normal responders, their utility as early predictors remains uncertain due to wide inter-individual variability and dependence on stimulation dynamics. This is similar to Karatasiou et al., who reported similar results and went further by reporting insufficient evidence to support or deny the presence of an association between the probability of pregnancy and serum estradiol levels (16).

The current study did not demonstrate that either AFC or AMH was superior to the other in the prediction of ovarian response (p= 0.00 for both variables). This is a important observation as the AMH test is expensive and not widely available, and therefore not very affordable in Cameroon, being a low-resource setting. We can thus infer that the cheaper, more readily available AFC can be used without much bias to our patients. These results are contrary to an Indian study which clearly showed that AFC can be used as a surrogate for AMH to predict ovarian response (17).

Limitations

Several limitations should be acknowledged. First, the retrospective nature of part of the dataset may introduce selection bias. Second, AFC is an operator-dependent technique and a known limitation in these types of studies, Thirdly, environmental and genetic factors influencing AMH and AFC were not assessed. Fourth, while we focused on ovarian response, future studies should explore the predictive value of these markers for clinical pregnancy and live birth rates.

Conclusion

This study contributes valuable data on ovarian reserve markers in black African women, a population underrepresented in reproductive endocrinology research. The high prevalence of secondary infertility and prolonged infertility duration in our cohort underscores the need for timely and personalized ART interventions. Our findings support the integration of AMH and AFC, with the option of utilizing only AFC, as a cheaper biomarker, into routine pre-stimulation assessments to optimize gonadotropin dosing and reduce cycle cancellations or complications such as ovarian hyperstimulation syndrome (OHSS).

Conflict of interest : The authors declare that they have no conflicts of interest regarding the publication of this paper

Author's contributions:

Under the supervision of K.J.M, N.A.M.V. developed the research protocol, and collected and compiled clinical laboratory data. N.C.J.F. drafted the initial manuscript, performed data analysis and contributed to interpretation of the results. N.A.M.V. critically revised the manuscript for important intellectual content. All authors reviewed and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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