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Validation of a Clinical Model to Predict Starting Initial Gonadotropin Dose in Women Undergoing IVF

Ashraf Aleyassin[1], Shahede Khansari[1,2*], Marzieh Aghahosseini[1], Fatemeh Sarvi[1], Sedigheh Hosseinimousa[1], Ayda Najafian[1], Salman Khazaei[3]

1. *Department of Obstetrics and Gynecology, School of Medicine, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran*
2. *Department of Obstetrics and Gynecology, School of Medicine, Fatemieh Hospital, Hamadan University of Medical Sciences, Hamadan, Iran*
3. *Research Center for Health Sciences, Hamadan University of Medical Sciences, Hamadan, Iran*

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Corresponding Information:
Shahede Khansari,
Department of Obstetrics and Gynecology, School of Medicine, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran

Email: dr_sh_kh@yahoo.com

ABSTRACT

Background & Objective: The selection of the initial gonadotropin dose in assisted reproductive techniques, particularly in In-Vitro Fertilization (IVF), can enhance patient outcomes and minimize the risks associated with over- or under-stimulation during IVF cycles. The initial gonadotropin dose can be predicted through variables such as age, Anti-Müllerian Hormone (AMH), and Follicle-Stimulating Hormone (FSH). The present study was conducted with aim to validate the La Marca nomogram for predicting the initial gonadotropin dose in women undergoing IVF.

Materials & Methods: This cross-sectional observational study was conducted on 187 women undergoing IVF at the infertility center of Shariati Hospital, affiliated with Iran University of Medical Sciences in Tehran, Iran, in 2024. Data regarding age, Body Mass Index (BMI), Antral Follicle Count (AFC), AMH, FSH, oocyte count, oocyte quality, and initial gonadotropin dose were extracted from patients' medical records. The validity and performance of the La Marca nomogram were assessed through cross-validation. The multivariate linear regression was used for analysis of the data.

Results: The mean age of women was 34.37±4.78 years. Multivariate analysis across all datasets revealed that age (β=0.04), AMH (-0.16), and FSH (0.09) were significant determinants of the initial gonadotropin dose (P-value<0.05). A 10-fold cross-validation showed similar performance of classifiers in both training and test datasets, indicating minimal overfitting. In the test data, regression coefficients for age, AMH, and FSH were 0.05, -0.16, and 0.10, respectively

Conclusion: The present study provides evidence regarding the appropriate performance of the La Marca nomogram for predicting the initial gonadotropin dose in women undergoing IVF.

Keywords: Assisted reproductive techniques, Starting gonadotropin dose, prediction, Validation, La marca nomogram

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1. Introduction

The World Health Organization (WHO) considers infertility as a public health issue and defines it as "a disease of the reproductive system characterized by the inability to conceive after 12 months or more of regular unprotected sexual intercourse" **(1)**. The prevalence of infertility is quite challenging, with studies indicating that one in four women experiences infertility issues at some point in their lives. Recent studies report infertility prevalence rates ranging from 3.5% to 16.7% in developed countries and between 6.9% and 9.3% in less developed countries **(1)**. Evidence suggests that infertility rates continue to rise due to the factors such as couples choosing to have children at older ages, increased obesity rates, tobacco and alcohol use, and heightened stress levels among couples **(1,2)**.

As women aging, both the quantity and quality of oocytes decline **(3)**. At birth, human ovaries contain one to two million follicles, but only a small fraction mature during a woman's reproductive years. The process of nuclear maturation in oocytes occurs alongside cytoplasmic maturation, indicating that oocytes progress from prophase I during recruitment and follicular development to metaphase II during final maturation and subsequent ovulation **(4,5)**. Recently,

live birth and fertility rates per ovarian retrieval cycle as the key performance indicators for ART have garnered significant attention **(6,7)**.

Overall, the number of oocytes and embryos available for transfer are critical factors influencing fertility and live birth rates. Recent reports indicate that a higher number of retrieved oocytes can contribute to increased overall fertility and live birth rates **(8-10)**. However, if the number of retrieved oocytes is excessive, there may be risks associated with Ovarian Hyperstimulation Syndrome (OHSS) and increased costs **(11)**. To achieve the target number of oocytes, it is essential to start with an appropriate dose of gonadotropins and provide sufficient Controlled Ovarian Stimulation (COS) duration so that the effect of FSH secretion remains stable and more oocytes can develop **(12)**.

"Ovarian reserve" refers to the functional potential of the ovaries, indicating the quantity and quality of possible oocytes. Antral Follicle Count (AFC) refers to the number of follicles measuring 2 to 10 millimeters in diameter during a phase of the menstrual cycle known as the "early follicular phase." This measure serves as a marker for ovarian reserve and can be assessed through transvaginal ultrasound. Transvaginal ultrasound is a high-quality imaging technique that uses an electrode (or probe) inserted into the vagina to provide accurate images of the ovaries and related structures **(13)**. In ovarian stimulation protocols, human Chorionic Gonadotropin (hCG) is typically used to induce oocyte maturation. The hCG hormone is produced by placental trophoblasts and helps facilitate final oocyte maturation similarly to LH hormone. hCG can be urinary-derived (uhCG) or recombinant (rhCG), with recombinant being more commonly used in ovarian stimulation **(14,15)**.

GnRH agonists are employed not only to inhibit the hypothalamic-pituitary axis but also to induce oocyte maturation in cases where GnRH antagonists have been used, thereby preventing complications associated with overstimulation **(15)**, which results from an excessive response to hormones used in ovarian stimulation. Over the past decade, using GnRH agonists as triggers for oocyte maturation instead of hCG has led to reduced severity and incidence of OHSS in women at risk for this complication. Using GnRH agonists as triggers is the best option for preventing ovarian hyperstimulation while maintaining better reproductive outcomes **(16)**. A study in Japan aimed to introduce a calculation algorithm adjusting gonadotropin starting doses based on patient age and AMH levels as predictors for achieving target ovarian counts **(17)**. For this purpose, reference was made to La Marca et al., who developed a normal distribution chart for determining gonadotropin starting doses. In La Marca et al.'s chart, using a GnRH agonist protocol for COS, multiple regression analysis was performed with AFC counts per gonadotropin starting dose as the target variable along with AMH levels, age, and baseline FSH levels from patient data **(18)**. The ovarian sensitivity index, defined as the number of retrieved oocytes per total gonadotropin dose, is utilized as a dynamic marker for ovarian response during controlled ovarian stimulation **(19)**. While AMH levels and AFC can predict ovarian reserve during controlled ovarian stimulation, they do not always accurately forecast ovarian response. The ovarian sensitivity index reflects ovarian response using retrieved oocyte counts relative to total gonadotropin dosage **(19)**. This index correlates with age, AMH levels, AFC counts, and Body Mass Index (BMI) **(20)**. To improve treatment outcomes and reduce potential treatment-related complications for patients and subsequently enhance treatment quality within the Iranian population, the present study was conducted with aims to develop an algorithm capable of adjusting gonadotropin starting doses within antagonist protocols.

2. Materials and Methods

This cross-sectional study was conducted on 187 women who underwent In Vitro Fertilization (IVF) and visited the infertility clinic at Shariati Hospital in 2024. Based on a similar article by Kobanawa **(17)**, three variables of age, AMH level, and FSH can predict up to 59% of the variations in the ovarian sensitivity index. To ensure adequate sample size, a minimum correlation coefficient of 0.18 was considered. With a power of 80% and a significance level of 0.05, the sample size was calculated to be 187 women. The estimated sample size is allocated 75 percent for the first phase of the study and 25 percent for the second phase. The two-phase approach allows for the development of the predictive model in the first phase and its validation in the second phase. This method helps to assess the model's reliability and generalizability. The performance of La Marca nomogram in two datasets is compared for predicting the initial dose of gonadotropin.

The study was approved by the Iran University of Medical Sciences (Ethics code: IR.TUMS.SHARIATI.REC.1403.053), and informed consent was obtained from all participants. Patients with hypothalamic disorders, endometriosis, a history of ovarian surgery, or previous chemotherapy or pelvic radiotherapy were excluded from the study. The study was conducted on patients receiving ovarian stimulation treatment using the gonadotropin-releasing hormone antagonist protocol. The variables including age, Body Mass Index (BMI), Antral Follicle Count (AFC), Anti-Müllerian Hormone (AMH), Follicle-Stimulating Hormone (FSH), duration of stimulation (in days), number of retrieved oocytes and starting gonadotropin dose were measured.

Data were analyzed using R 4.4.1 SPSS software (version 27). Data were expressed using mean±standard deviation ad number (percentage). The univariate linear regression analysis was performed to examine the effect of each exploratory variable on the

outcome studied individually. Subsequently, multivariate analysis assessed relationships among exploratory variable and starting gonadotropin dose. P<0.05 was considered statistically significant.

3. Results

A total of 143 participants were included in the phase 1 of the study. The mean age of the participants was 34.36±4.76 years, the mean Body Mass Index (BMI) was 25.34±3.46 kg/m², the mean AMH level was 2.99±3.04 ng/ml, the mean Antral Follicle Count (AFC) for follicles measuring 2 to 10 mm was 7.37±4.37, the mean Follicle-Stimulating Hormone (FSH) level was 7.13±3.61, and the mean initial gonadotropin dose was 3.85±0.97 IU (Table 1).

The impact of age, AMH, and FSH (components of La Marca) on the initial gonadotropin dose in both univariate and multivariate analyses in sample phase 1 is presented in Table 2. In the univariate analysis, both AMH and FSH showed significant effects (P<0.05). In the multivariate analysis, all three variables were significant, indicating that for each unit increase in age, AMH, and FSH, the mean initial gonadotropin dose is expected to change by 0.04, -0.16, and 0.08 units, respectively. The developed model can be expressed as follows:

Gonadotropin starting dose=0.04 (age)-0.16 (AMH)+0.08 (FSH)

The predictive nomogram designed based on the multivariate model for the sample phase 1 is illustrated in Figure 1. For an individual aged 38 years, with an AMH level of 6 and an FSH level of 6, the total score (Points) is approximately calculated as 20+60+20=100. The corresponding initial dose associated with a total point's score of 100 is approximately 3.25 IU. Given that the model designed based on the sample phase 1 may be optimistic and prone to overfitting, the next step involved evaluating the model in the independent sample. The differences between regression coefficients in the sample 1 and sample 2 were minimal, indicating consistent model performance under various conditions. The developed model for predicting the initial gonadotropin dose in the sample phase 2 can be expressed as follows:

Gonadotropin starting dose=0.05 (age)-0.13 (AMH)+0.10 (FSH)

The predictive nomogram designed based on the multivariate model for sample 2 is illustrated in Figure 2. For instance, for an individual aged 35 years, with an AMH level of 6 and an FSH level of 6, the total score (Points) is approximately calculated as 38+52+30=120. The corresponding initial dose associated with a total point's score of 120 is about 3.75 IU.

In the second-phase sample (Table 3), the regression coefficients for age, AMH, and FSH were 0.05, -0.13, and 0.10, respectively, all of which were statistically significant (P<0.05). This pattern was similar to the results from phase one. Regression analysis on phase one data showed that the initial dose of gonadotropin has an inverse relationship with the number of oocytes, while the number of days receiving the medication has a direct relationship with the number of oocytes (Table 4).

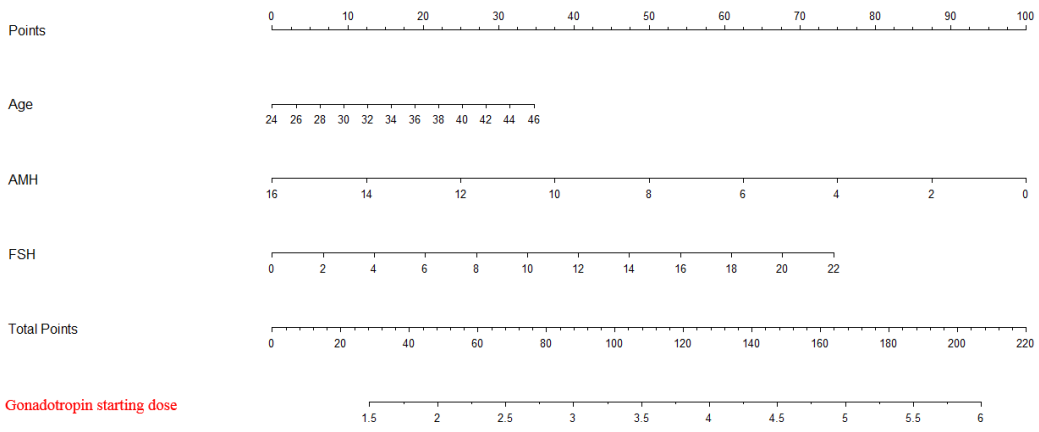


Figure 1. Predictive nomogram for gonadotropin starting based on age, AMH and FSH in sample 1

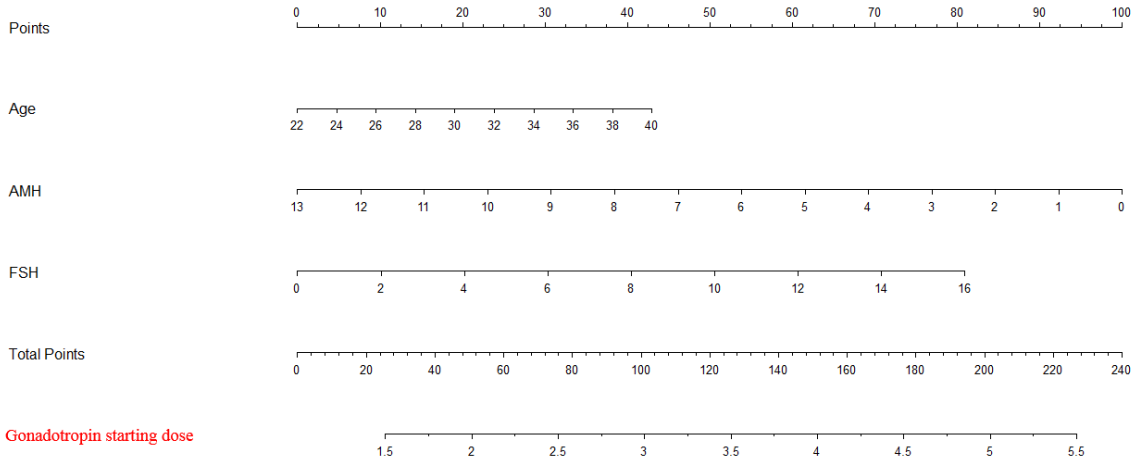


Figure 2. **Predictive nomogram for gonadotropin starting based on age, AMH and FSH in the sample 2**

Table 1. **Descriptive statistics of the studied variables in sample 1**

Variables	Sample 1 (n=143)		Sample 2 (n=44)	
	Mean	standard deviation	Mean	standard deviation
Age, years	34.36	4.76	34.40	4.89
BMI, kg/m²	25.34	3.46	26.20	3.12
AFC	7.37	4.37	7.88	4.44
AMH, ng/ml	2.99	3.04	2.84	2.57
FSH, IU/l	7.13	3.61	7.15	3.58
Oocyte number of oocyte	10.12	9.29	12.04	10.07
Quality of oocyte	7.00	6.86	7.90	6.48
Gonadotropin starting dose	3.85	0.97	3.85	0.83
Receiving drug (days)	10.40	1.43	10.34	1.42

Table 2. **The effect of age, AMH and FSH on gonadotropin starting dose in the sample 1**

Variables	Uunivariate		Multivariable	
	β	P-value	β	-value
Age	0.09	<0.001	0.04	0.001
AMH	-0.21	<0.001	-0.16	<0.001
FSH	0.14	<0.001	0.08	<0.001

Table 3. The effect of age, AMH and FSH on gonadotropin starting in sample 2

Variables	β	P-value
Age	0.05	<0.001
AMH	-0.13	<0.001
FSH	0.10	<0.001

Table 4. The predictors of oocyte number from multivariable analysis in sample phase 1

Variables	β	P-value
Age	-0.22	0.09
AMH	1.09	<0.001
FSH	0.03	0.85
Gonadotropin starting dose	-2.50	0.004
Receiving drug (days)	1.50	<0.001

4. Discussion

In this study, the primary objective was to determine the optimal starting dose of gonadotropin based on various factors such as age, Anti-Müllerian Hormone (AMH) levels, and Follicle-Stimulating Hormone (FSH). The main findings indicated that AMH is the strongest predictor for the starting dose of gonadotropin, showing that higher AMH levels correspond to a lower required gonadotropin dose. Additionally, age and FSH were identified as the significant predictors in the multivariate model. The predictive model demonstrated good accuracy in both training and test datasets. The findings of this study align with a study by Kobanawa et al., in Japan (2022) which aimed to design a calculator for the starting dose of gonadotropin in controlled ovarian stimulation, where age and AMH were selected as predictor variables in a multivariate regression model. The equation was expressed as the number of retrieved oocytes divided by (starting gonadotropin dose×duration of stimulation in days), creating a formula for predicting the starting dose based on target oocyte count and stimulation duration **(17)**. Yubin Li et al., also selected age, BMI, serum AMH levels, baseline FSH, and AFC as the predictive factors for ovarian response during COS **(21)**. Various parameters such as age, AMH, AFC, FSH levels, and smoking have been utilized in previous studies **(18,22-24)**. A study on Japanese women demonstrated a good correlation between AMH levels and the number of oocytes in patients undergoing Controlled Ovarian Stimulation (COS) for IVF, indicating that AMH can predict the risk of ovarian hyperstimulation syndrome in these patients **(25)**.

In line with the current study's results, a similar study performed regression analysis to evaluate the correlation between AMH and oocyte count, revealing a positive correlation between AMH and oocyte count while showing a negative correlation between age and oocyte count. Furthermore, oocyte counts increased in a dose-dependent manner with FSH **(26)**. The nomogram published by La Marca et al., also utilized age, AMH, and baseline FSH to predict the optimal starting dose of gonadotropin **(18)**. Among these parameters, AMH, FSH, and age are recognized as suitable objective parameters that can be quickly assessed during outpatient consultations. Appropriate ovarian reserve indicators are fundamental for optimal ovarian response.

Reduced ovarian reserve is typically associated with a decrease in the number of available follicles or a decline in oocyte quality. Clinically, age, AMH, AFC, and FSH are commonly used as indicators to assess ovarian reserve. In recent years, AMH has been recognized as one of the most sensitive markers for evaluating ovarian reserve **(27)**. Given that age is directly related to ovarian reserve and oocyte quality, physicians should pay special attention to this factor in their treatment protocols. Some reports indicate limitations regarding increasing FSH doses; higher doses do not necessarily lead to an increase in the number of retrieved oocytes **(28)**. Regardless of the number of retrieved oocytes and patient age, some studies have shown that live birth rates decrease with increasing total FSH doses **(29)**. A subgroup analysis from a similar study limited to patients with good prognosis (age under 35 years, BMI under 30, and no reduction in ovarian reserve, endometriosis or ovulatory disorders) yielded similar results **(30)**. This may be due to the high number of fresh embryo transfers in the study that could be attributed to excessive stimulation with FSH and increased estradiol and progesterone levels affecting endometrial preparation **(30,31)**.

Reports suggest that sensitivity to gonadotropins may differ between Asian and Western populations; furthermore, the distribution of FSH receptor polymorphisms may vary across these groups **(32,33)**. Therefore, using algorithms for determining starting doses of gonadotropins designed for Western populations may not be easily applicable to our population. Thus, this calculator developed based on Iranian data is considered more effective than previous studies for its applications within Iran. The calculator designed by the researchers in this study enhances the performance of Controlled Ovarian Stimulation (COS) and allows for national standardization since it enables all physicians, regardless of their years of experience,

to determine an appropriate starting dose of gonadotropin uniformly. Excessive dosing of gonadotropins can be avoided by establishing target oocyte counts at each center and calculating individual doses required to achieve this target. This will reduce stimulation days, minimize risk of complications, and lower additional costs. Moreover, this calculator can help prevent excessively low dosing, thereby reducing the likelihood of cycle cancellations and prolonging COS duration. Future prospective studies including real-world usage of this tool in clinical practice are essential to confirm the accuracy of the developed calculator presented here.

One strength of this study is its use of a multivariate predictive model that comprehensively considers various influencing variables. Additionally, validating the model using independent datasets has contributed to enhancing the reliability of results. On the other hand, one limitation is that sampling from a specific treatment center may restrict the generalizability of the findings. Secondly, although this model has been internally validated, it requires further external validation with women assessed across multiple centers.

It is suggested that broader studies consider additional factors such as duration of infertility and other hormones. Examining the impact of different gonadotropin doses on embryo quality and pregnancy success could improve treatment protocols. Conducting multicenter studies could also enhance the generalizability of findings. Furthermore, each center should develop precise and personalized algorithms tailored to their patients' specific needs to improve fertility treatment quality and increase success rates in oocyte retrievals. This approach not only optimizes treatment processes but may also lead to reduced complications and additional costs.

5. Conclusion

This study demonstrated that AMH levels, age, and baseline FSH are the strong predictors for determining the starting dose of gonadotropins. AMH was recognized as the most sensitive marker for assessing ovarian reserve; higher levels correlate with lower required doses of gonadotropins. Additionally, age and baseline FSH were identified as significant predictors within the multivariate model. These findings underscore the importance of accurately assessing these factors when determining optimal gonadotropin doses and can contribute to improving treatment outcomes for patients undergoing IVF. Physicians should seriously consider AMH levels, age, and FSH when evaluating patients' ovarian reserves to optimize treatment protocols while minimizing the risks associated with excessive dosing. Furthermore, these results led to developing a nomogram for determining starting doses based on these predictive factors. This nomogram can serve as a valuable tool for personalized dosing in COS applications.

6. Declarations

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Ethical Considerations

This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. The study protocol was reviewed and approved by the Ethics Committee of Iran University of Medical Sciences (Ethics code: IR.TUMS.SHARIATI.REC.1403.053).

Authors' Contributions

Ashraf Aleyassin: Conceptualization, Methodology, Project Administration, Writing – Review & Editing.

Shahede Khansari: Conceptualization, Investigation, Data Curation, Formal Analysis, Writing – Original Draft, Supervision.

Marzieh Aghahosseini: Investigation, Resources, Data Curation.

Fatemeh Sarvi: Investigation, Resources.

Sedigheh Hosseinimousa: Investigation, Resources.

Ayda Najafian: Investigation, Resources.

Salman Khazaei: Methodology, Formal Analysis, Software, Validation, Writing – Review & Editing.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding this study.

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None.

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