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Ovarian response predictive model in different controlled ovarian stimulation protocols for IVF/ICSI treatment

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Summary

Purpose of investigation: Assesment of biomarkers of the ovarian reserve for ovarian response prediction using different controlled ovarian stimulation (COS) treatments. *Materials and Methods:* A retrospective cohort study included 363 patients who underwent assisted reproduction at the Clinic of Gynecology and Obstetrics, Belgrade, Serbia. Antral follicle count (AFC), serum AMH, inhibin B, follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), and progesterone were measured on the second cycle day prior to stimulation commencement. Three types of ovulation stimulation protocols were used. The number and quality of obtained oocytes were used for evaluation of the ovarian response. *Results:* Patients' age, number of antral follicles, AMH level, and FSH/LH ratio were confirmed as predictors of the number of obtained oocytes. The AFC was the main parameter that influenced the number of obtained oocytes regardless of selected stimulation protocol. *Conclusion:* The individualization of stimulation protocols may be further improved by using both AFC- and AMH-tailored approach.

Key words: Controlled ovarian stimulation (COS); Ovarian reserve; anti-Müllerian hormone (AMH); Antral follicle count (AFC); Ovarian response.

Introduction

Controlled ovarian stimulation (COS) with gonadotropins for in vitro fertilization (IVF/ICSI) treatment is used to obtain an adequate number of competent oocytes with the minimum risk for the woman [1]. Individual variability in ovarian response to a given dose of gonadotropins is well recognized and significant efforts have been made to identify clinical parameters that can imply the ovarian response and also improve efficacy and safety outcomes of COS [2, 3]. Biomarkers of the functional ovarian reserve, such as basal follicle-stimulating hormone (FSH), inhibin B, estradiol (E2), anti-Müllerian hormone (AMH) and antral follicle count (AFC) assessed by transvaginal ultrasound have been suggested as predictors of ovarian response and clinical outcome [4, 5]. Several studies demonstrated that AMH is an accurate predictor in both high and low ovarian response during GnRH agonist treatment [2, 6-8], as well as during GnRH antagonist treatment [9, 10], suggesting that it would be an ideal marker for the individualization of COS strategies. On the other hand, clinicians often use patient characteristics, such as female age, body mass index (BMI), cycle length, results from previous IVF/ICSI cycles, cause of infertility, as well as infertility type to select a treatment protocol [11]. The accuracy of biomarkers in ovarian response prediction for gonadotropin hormone (GnRH) antagonist protocols may differ from that in GnRH agonist treatments concerning a difference in the early follicle recruitment and synchronization of follicular development, leading to a difference in number of oocytes retrieved [12]. The question therefore remains whether biomarkers of the functional ovarian reserve, as well as which one, are able to correctly predict ovarian response with accuracy independent of selected COS treatment.

The aim of the present study was to investigate the potential predictors of ovarian response using different COS treatments: short GnRH agonist protocol, long GnRH agonist protocol, and GnRH antagonist protocol, and to construct a predictive model of ovarian response.

Materials and Methods

A total of 363 female patients who underwent assisted reproductive techniques (ART) as infertility treatment within National program at the Clinic of Gynecology and Obstetrics, Clinical Center of Serbia during the period of 18 months, were included in this retrospective cohort study. Written informed consent was provided from each of the subjects prior to enrolment in the program and the Institutional Review Board approved the study.

Age, BMI, cause of infertility, and the infertility type were determined for all patients. All female patients were younger than 40 years, since it is one of conditions for National program. Infertility cause was categorized as tubal, ovarian, endometriosis, immunological, mild male, unknown or combined. Patients were divided according infertility type in the groups with primary or

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secondary infertility. BMI was defined as weight (kilograms) divided by the square of height (square meters).

AFC was expressed as a the total number of follicles with a diameter between two and ten mm in both ovaries on the second cycle day before the start of stimulation, as measured by transvaginal ultrasound by expert sonographers. Serum AMH, inhibin B, FSH, LH, E2, and progesterone levels were measured on the second cycle day prior to stimulation commencement. Blood samples were collected into tubes and centrifuged according manufacturer's recommendations to obtain serum samples.

Serum AMH (one ng/ml = 7.14 pmol/l; Gen II ELISA ref. no. A79765) and inhibin B (Gen II ELISA ref. no. A81303) were measured by enzyme-linked immunosorbent assay (ELISA). The AMH and inhibin B assays have a limit of detection of 0.08 ng/ml and 2.6 pg/ml, respectively, and these assays exhibit within run and total imprecision of less than 3.7% and 4.4%, and 2.67% and 4.70%, across the assay range, respectively. AMH levels were categorized as low (less than one ng/ml), normal (one to four ng/ml) and high (above four ng/ml). Levels of FSH, LH, E2, and progesterone were analyzed by a chemiluminescent immunoassay on Access 2 immunoassay system. The Access FSH, LH, E2, and progesterone assays have a measurement range of 0.2-200 mIU/ml (IU/L), with a limit of detection of < 0.2 mIU/ml (IU/L), 0.2-250 mIU/ml (IU/l), with a limit of detection of <0.2 mIU/ml (IU/L), 20-48000 pg/ml (73-17,621 pmol/l) with a limit of detection of < 20 pg/ml (73 pmol/l), and 0.10-40.0 ng/ml (0.32-127.20 nmol/L), with a limit of detection of < 0.10 ng/ml (<0.32 nmol/L), respectively. FSH and LH assays exhibit within run and total imprecision of less than 5% and 10%, and 6% and 10% across the assay range, respectively. The Access E2 and progesterone assays exhibit total imprecision of 12% at concentration of 120 pg/ml (438 pmol/L) and less than 12% across the assay range, respectively. The ratio of FSH and LH levels was calculated and the obtained measure was used for final analysis.

Three types of ovulation stimulation protocols were used. In the long GnRH agonist treatment, pituitary suppression was initiated with 0.1 mg/d of triptorelin seven days prior the start of next cycle and continued until the end of stimulation. Gonadotropin administration, started on cycle day 2 and the dose was fixed at 225 IU/d for the first five days of COS followed by individual dose adjustments. In the short GnRH agonist treatment, triptorelin administration started on cycle day 2 and continued until the end of stimulation that started on cycle day 3 with a fixed dose of 150 IU/d for the first five days. In the GnRH antagonist trial, treatment with a daily dose of 150 IU/d of gonadotropin started on day 2 of the menstrual cycle and was fixed for the first five days of COS. Treatment with 0.25 mg/d of cetrorelix was initiated on stimulation day 6 and continued until the end of COS. In all trials, the criteria for application of hCG was development of at least three follicles with a diameter > 17 mm. Oocyte retrieval was done 34 – 36 hours later, followed by IVF or ICSI and embryo transfer.

The outcome measure was ovarian response that was assessed by the number of obtained oocytes. The authors used the most common definitions for low, adequate, and high ovarian response: a low response was defined as obtaining four or less oocytes, while a high response was considered as obtaining more than 15 oocytes. Therefore, an adequate ovarian response was defined as obtaining four to 15 oocytes. Additionally, the quality of obtained oocytes was assessed through their maturation level and percentage of immature oocytes (more or less than 20%).

Finally, the authors registered the occurrence of ovarian hyperstimulation syndrome (OHSS).

Data analyses were performed for the entire group as well as regarding the ovarian stimulation protocol type. The authors applied methods of descriptive and analytical statistics. Data for continuous variables are presented as medians with interquartile ranges (IQR), due to non-normal distributed data. Categorical data were presented as absolute numbers with percentages. Differences in assessed parameters regarding the type of used protocols were analyzed by Kruskal-Wallis non-parametric test with Mann Whitney test for post hoc comparisons. Univariate and multivariate logistic regression (Stepwise method) was applied to test which of the examined parameters could be predictors of ovarian response when different ovarian stimulation protocols were used (for long and short GnRH agonist protocol, antagonist GnRH protocol model was not assessed due to insufficient number of patients). Receiver operating characteristic (ROC) curves were constructed to demonstrate the prognostic accuracy of AMH and the number of antral follicles as predictors of high, adequate, and low ovarian response. The corresponding area under the curve (AUC) was calculated for all responses in different protocols in order to express the overall accuracy. In order to illustrate the clinical usefulness of potential predictors, the sensitivity and specificity were determined for optimal cut-off values that were derived from the ROC analysis. The level of significance was $p < 0.05$. Obtained data were analyzed using the SPSS 21.0 software.

Table 1. — *Baseline clinical characteristics in the study population.*

Variable	Med (IQR)
Age, years	35.0 (32.0−37.0)
BMI, kg/m^2	22.0 (20.4−24.2)
AFC, n.	12 (8−18)
AMH level, ng/ml	2.04 (0.80−3.80)
AMH class, n. (%)	
Low	100 (27.5%)
Normal	210 (57.9%)
High	53 (14.6%)
E2, pg/ml	58.2 (40.4−102.0)
Progesterone, ng/ml	1.02 (0.50−2.40)
FSH/LH, mIU/ml	1.47 (1.06−1.97)
Inhibin B, pg/ml	25.25 (15.90−51.20)

AFC: antral follicle count, AMH:anti-Müllerian hormone, BMI: body mass index, E2: estradiol, FSH: follicle-stimulating hormone, GnRH: gonadotropin-releasing hormone, LH: luteinizing hormone, n.: number.

Results

The study included 363 women whose baseline clinical characteristics are presented on Table 1. Among them, 266 (73.3%) had primary, and 97 (26.7%) had secondary infertility type. The primary treatment diagnosis was mild male factor infertility (31.4%) or unexplained infertility (24.0%). They were divided in three groups according to used stimulation protocol: short GnRH agonist protocol (n=158), long GnRH agonist protocol (n=181), and GnRH antagonist protocol group (n=24) (Table 2). Differences in baseline clinical characteristics according to used stimulation protocol are shown in Table 3. Post hoc comparisons showed that women who were treated with long GnRH agonist protocol were younger [35.0 (32.7-38.0) *vs.* 34.0 (31.0-36.0) *vs.* 35.5 (33.2-37.0) years; $p < 0.001$)] and had higher antral follicle

Table 2. — *Frequency of infertility type and factor regarding the ovarian stimulation protocol.*

Categories	Total n.	Total %	Short GnRH agonist protocol	GnRH antagonist protocol	Long GnRH agonist protocol
Infertility type					
Primary	266	73.3	118	13	136
Secondary	97	26.7	40	11	45
Infertility factor					
Male	114	31.4	48	5	61
Tubal	84	23.1	33	3	48
Ovarian	15	4.1	8	0	7
Endometriosis	12	3.3	4	1	7
Immunological	4	1.1	2	0	2
Unexplained	87	24.0	46	8	33
Combined	47	12.9	17	7	23

number (11.5 (7.0-16.25) *vs.* 13.0 (9.0-20.0) *vs.* 10.5 (7.25-12.75); $p < 0.001$)] and AMH level [1.65 (0.69-3.28) *vs.* 2.60 (1.0-4.75) *vs.* 1.15 (0.25-1.95); $p < 0.001$)] than those who were treated with short GnRH agonist and GnRH antagonist protocols, respectively. Furthermore, AMH level was also significantly higher in short GnRH agonist protocol group than in GnRH antagonist group. On the other hand, there were no significant differences regarding the used protocol in patients BMI ($p = 0.419$) as well as E2 ($p = 0.648$), progesterone ($p = 0.258$), inhibin B ($p = 0.961$) levels, and FSH/LH ratio ($p = 0.575$). Yet, ovarian stimulation lasted significantly longer in long GnRH agonist protocol group than in the other two groups.

Significant differences were also found in outcome measure of ovarian response according to used stimulation protocol. Women treated with long GnRH agonist protocol had significantly higher total number of oocytes retrieved [11 (6-15) *vs.* 6 (3-9) *vs.* 6 (4-8); $p < 0.001$)] than women treated with short GnRH agonist and GnRH antagonist protocols, respectively. Moreover, they also had significantly higher adequate ovarian response (62.8% *vs.* 60.5% *vs.* 52.2%; $p < 0.001$), without significant difference in maturity level between oocytes from different stimulation protocol groups ($p = 0.399$). On the other hand, the occurrence of OHSS was also significantly higher in long GnRH agonist protocol group (17.7%; $p < 0.001$) (Table 4).

Applying the univariate logistic regression, the authors constructed a model for prediction of the adequate and excessive number of oocytes that can be obtained in ART procedure using different ovarian stimulation protocols. Patients' age,

Table 3. — *Baseline clinical characteristics of the study population according to used stimulation protocol.*

Variable	Short GnRH agonist protocol (n=158) Med (IQR)	Long GnRH agonist protocol (n=181) Med (IQR)	GnRH antagonist protocol (n=24) Med (IQR)	*p*	Post-hoc
Age, years	35.0 (32.7−38.0)	34.0 (31.0−36.0)	35.5 (33.2−37.0)	<0.001	S-L;L-A
BMI, kg/m^2	21.6 (20.2−23.9)	22.0 (20.5−24.4)	22.1 (21.2−24.0)	0.419	
AMH level, ng/ml	1.65 (0.69−3.28)	2.60 (1.0−4.75)	1.15 (0.25−1.95)	<0.001	S-L;L-A;S-A
AMH class, n(%)					
Low	47 (29.7%)	43 (23.8%)	10 (41.7%)		
Normal	97 (61.4%)	100 (55.2%)	13 (54.2%)		
High	14 (8.9%)	38 (21.0%)	1 (4.2%)		
AFC, n.	11.5 (7.0−16.25)	13.0 (9.0−20.0)	10.5 (7.25−12.75)	<0.001	S-L;L-A
E2, pg/ml	55.2 (39.6−95.1)	55.2 (39.6−95.1)	54.1 (41.7−80.8)	0.648	
Progesterone, ng/ml	1.03 (0.50−2.50)	1.13 (0.54−2.50)	0.76 (0.38−1.52)	0.258	
FSH/LH, mIU/ml	1.47 (1.05−2.00)	1.46 (1.07−1.96)	1.68 (1.15−1.96)	0.575	
Inhibin B, pg/ml	30.6 (15.8−49.9)	24.9 (16.7−51.5)	27.8 (13.0−57.5)	0.961	
Stimulation days, n.	9 (8−10)	11 (10−12)	9 (9−10)	<0.001	S-L;L-A

AFC: antral follicle count, AMH: anti-Müllerian hormone, BMI: body mass index, E2: estradiol, FSH: follicle-stimulating hormone, GnRH: gonadotropin-releasing hormone, LH: luteinizing hormone, n.: number.

Table 4. — *Ovarian response according to used stimulation protocol in studied population.*

Variable		Short GnRH agonist (n=158) n. (%)	Long GnRH agonist (n=181) n. (%)	GnRH antagonist (n=24) n. (%)	*p*	post-hoc
Obtained oocytes*		6 (3−9)	11 (6−15)	6 (4−8)	<0.001	S-L;L-A
Obtained oocytes	few (< 5)	50 (32.9%)	26 (14.4%)	10 (43.5%)	<0.001	S-L;L-A
	adequate No	92 (60.5%)	113 (62.8%)	12 (52.2%)		
	numerous (> 15)	10 (6.6%)	41 (22.8%)	1 (4.3%)		
Immature oocytes > 20%		39 (25.7%)	51 (28.3%)	9 (39.1%)	0.399	
OHSS		5 (3.2%)	32 (17.7%)	1 (4.2%)	<0.001	S-L

*Data are presented as median with interquartile range; n.: number.

Table 5. — *Predictors of ovarian response in studied stimulation protocols.*

Variable		Univariate logistic regression			Multivariate logistic regression		
	p	RR	95%CI for RR		*p*	RR	95% CI for RR
Long GnRH agonist - adequate number of obtained oocytes	Age	0.003	0.808	0.701−0.931			
	AFC	<0.001	1.488	1.265−1.750	<0.001	1.488	1.265−1.750
	AMH level ng/ml	0.001	1.557	1.190−2.036			
	FSH/LH mIU/ml	0.009	0.533	0.333−0.854			
Long GnRH agonist - numerous number of obtained oocytes	Age	0.003	0.863	0.784−0.950			
	AFC	<0.001	1.282	1.164−1.412	<0.001	1.282	1.164−1.412
	AMH level ng/ml	<0.001	1.304	1.153−1.476			
	FSH/LH mIU/ml	0.044	0.567	0.327−0.986			
Short GnRH agonist - adequate number of obtained oocytes	Age	0.009	0.872	0.786−0.967			
	AFC	<0.001	1.301	1.182−1.432	<0.001	1.301	1.182−1.432
	AMH level ng/ml	0.007	1.363	1.087−1.709			
	FSH/LH mIU/ml	0.022	0.633	0.428−0.936			

AMH: anti-Müllerian hormone, CI: confidence interval, FSH: follicle-stimulating hormone, GnRH: gonadotropin-releasing hormone, LH: luteinizing hormone, RR: relative risk.

Table 6. — *ROC analyses of AMH and AFC in prediction of number of obtained oocytes.*

Variable	Protocol	Obtained oocytes, n.	Area	Cut-off	Sn	Sp
AMH serum level ng/ml	Short GnRH agonist	Adequate	0.683	1.5	63%	64%
	Long GnRH agonist	Adequate	0.728	1.9	67%	69%
		Numerous	0.717	3.3	71%	69%
AFC	Short GnRH agonist	Adequate	0.814	9.5	75%	66%
	Long GnRH agonist	Adequate	0.874	9.5	84%	85%
		Numerous	0.787	14.5	83%	65%

AMH: anti-Müllerian hormone, AFC: antral follicle count, GnRH: gonadotropin-releasing hormone, n.: number, ROC: receiver operator characteristics curve.

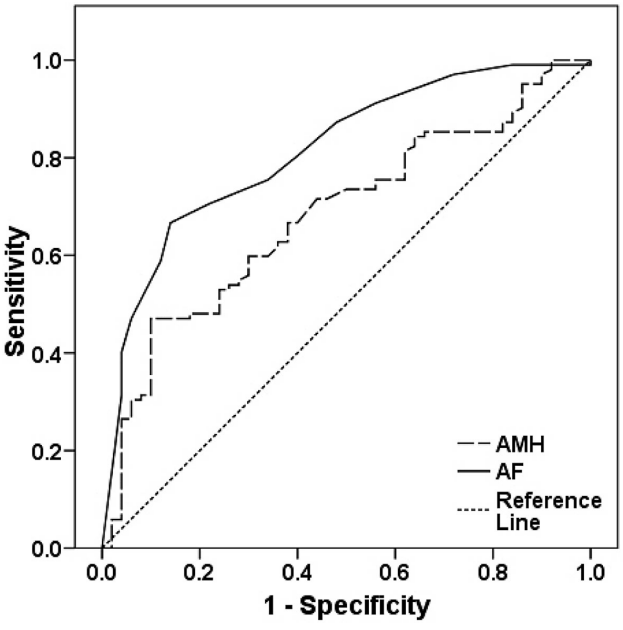

Figure 1. — AMH and AFC reliability in predicting the adequate oocyte number using short GnRH agonist protocol. AMH: anti-Müllerian hormone (ng/ml), AFC: antral follicles count.

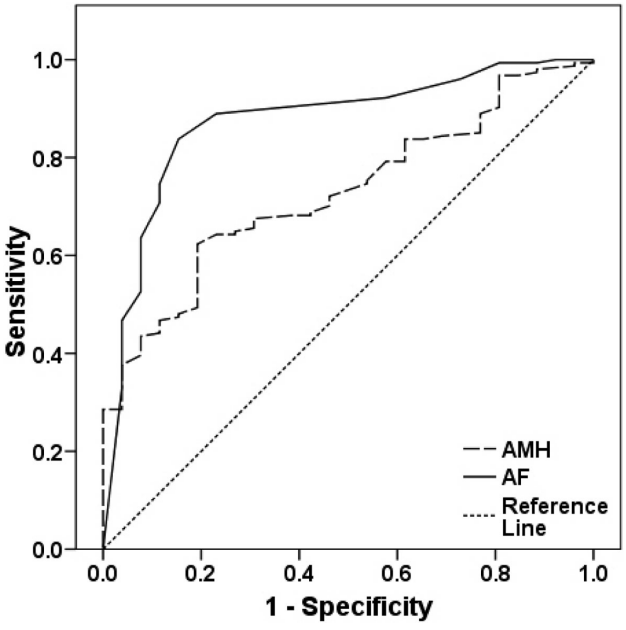

Figure 2. — AMH and AFC reliability in predicting the adequate oocyte number using long GnRH agonist protocol. AMH: anti-Müllerian hormone (ng/ml), AFC: antral follicles count.

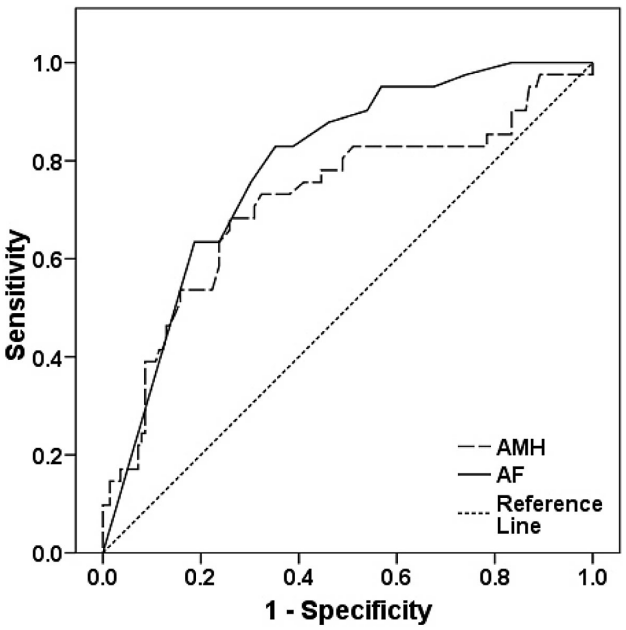

Figure 3. — AMH and AFC reliability in predicting the excessive oocyte number using long GnRH agonist protocol. AMH: anti-Müllerian hormone (ng/ml), AFC: antral follicles count.

number of antral follicles, AMH level, and FSH/LH ratio were confirmed as predictors of the number of obtained oocytes (Table 5). Additionally, they demonstrated that number of antral follicles was the main parameter that influenced the number of obtained oocytes regardless of stimulation protocol (RR 1.488, 95% CI 1.265–1.750; $p < 0.001$ for adequate number and RR 1.282, 95% CI 1.164–1.412; $p < 0.001$ for excessive number of oocytes in long GnRH agonist protocol group, and RR 1.301, 95% CI 1.182–1.432; $p < 0.001$ for adequate number of oocytes in short GnRH agonist protocol group).

Results of ROC analysis using AMH levels and number of antral follicles for prediction the number of obtained oocytes using different COS protocols are presented in Table 6. In examined population, sensitivity, as well as the AUC was higher for the number of antral follicles than for AMH, especially for adequate ovarian response, regardless of selected stimulation protocol.

Figures 1 and 2 illustrate the comparison of AMH serum level and the number of antral follicles reliability in predicting the adequate number of oocytes acquired regardless of used protocol, and the excessive number of oocytes using long GnRH agonist stimulation protocol (Figure 3). It can be observed that overall number of antral follicles was somewhat more reliable predictor than AMH.

Discussion

This study assessed ovarian response predictors that took into consideration three currently most common ovarian stimulation protocols. Younger patients' age, higher AMH level, lower FSH/LH ratio, higher number of antral follicles, and longer stimulation increase the number of obtained oocytes. The number of antral follicles was the main parameter that influenced the ovarian response regardless of stimulation protocol. Finally, the authors determined the cut-off levels of AMH serum level and AFC for adequate and excessive ovarian response regarding different stimulation protocol.

AFC and AMH are two biomarkers that have consistently provided the best prediction of ovarian response to gonadotropins [5, 13]. Several studies have suggested that AMH and AFC have the same level of accuracy for ovarian response prediction [14, 15]. On the contrary, a few other studies have suggested either AFC [16] or AMH [17] as being a better predictor. Limitations for both biomarkers, number of follicles, as well as AMH, are used to explain the marked contrast in these results. AFC may overestimate the number of follicles that will be sensitive to gonadotropins because of possible inclusion of atretic follicles in the total count; also, the performance of AFC measurement may be affected by intra- and interoperator variability [18] and technical aspects of ultrasound equipment [19]. AMH assays are also associated with intra- and interindividual imprecision and a potential risk of complement interference [20].

Two meta-analyses indicated that AMH and AFC have similar levels of accuracy and clinical value for the prediction of poor [14], as well as excessive response [15]. In contrast, recent trials in IVF/ICSI patients of good prognosis concluded that AMH was a better predictor of ovarian response than AFC in GnRH agonist [21] and antagonist [9, 10] cycles, regarding the number of oocytes retrieved as well as categorization of low and high responders. Furthermore, recent analysis of individual study center data from two large multicenter trials, showed that AMH was a stronger predictor of ovarian response to gonadotropin therapy than AFC in both long GnRH agonist and GnRH antagonist protocols, and furthermore, inclusion of AFC in the prediction models provided no added predictive value beyond AMH [22].

Contrary to majority of literature data, in the present study population, just like in few others, AFC was better predictor of the number of obtained oocytes regardless of applied protocol [16]. This was especially true for adequate ovarian response. Finally, neither parameter is highly reliable for low response. Furthermore, aside from AMH, AFC, stimulation protocol type, and patients' age, no other investigated parameters were significant for oocyte number prediction.

Ideally, a test for ovarian response prediction would identify all women with a high or low response and exclude all women with a normal response. In previous prospective and retrospective trials, a reduction in the incidence of low and high

response to stimulation was observed when assigning patients with low or high AMH levels to the GnRH antagonist protocol, with high and low gonadotropin doses, respectively, while assigning the patients with normal AMH levels to the long GnRH agonist protocol [7, 23]. Since no difference in pregnancy rates was found between the high, normal, and low response groups, it may indicate that predicting a low response is clinically less relevant as opposed to predicting a high response, as here patient safety issues also play a role [9]. Therefore, it remains crucial to individualize IVF treatment in order to decrease the incidence of OHSS. Based on the present results, since women treated with long GnRH agonist protocol had higher AMH levels and AFC, as well as the incidence of OHSS, than women treated with other COS protocols, it can be concluded that long GnRH agonist protocol is not a choice for women with high AMH levels and high AFC.

Conclusion

The current study demonstrated that the individualization of stimulation protocols may be further improved by using both AMH- and AFC-tailored approaches. AFC and AMH are very reliable predictors of both adequate and excessive ovarian response, regardless of selected stimulation protocols.

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