CODEN (USA): IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

Available online at: http://www.iajps.com *Research Article*

APPLICATION OF ANTI-MULLERIAN HORMONE AND ESTRADIOL IN PREDICTION OF OVARIAN HYPER-STIMULATION SYNDROME. A NEW APPROACH.

Afsoon Zarei[1], Leila Ghaedian[1], Mohammad Rafati Navaei[2], Lida Ghaedian[3], Mina Alimohammadzade[1], Elham Askary[*4]

[1]Infertility Research Center, Department of gynecology and obstetrics, Shiraz University of Medical Sciences, Shiraz, Iran.

[2]Department of surgery, Shiraz University of Medical Sciences, Shiraz, Iran.

[3]Department of gynecology and obstetrics, Mashhad University of Medical Sciences, Mashhad, Iran.

[4]Gynecologist, Shiraz medical university, Shooshtari hospital, Shiraz, Iran.

Abstract

Background and Objective: *Ovarian Hyperstimulation Syndrome (OHSS) is the most common iatrogenic and potentially lethal adverse effect of ovarian induction in Assisted Reproductive Technologies (ART). Recently, anti-mullerian hormone (AMH) is considered as risk factor for ovarian hyper-response in addition to estradiol in patients undergoing in vitro fertilization (IVF). The present study was aimed to assess the accuracy of anti-mullerian hormone (AMH) and Estradiol in the incidence of OHSS.*

Materials and Methods: *This is a retrospective diagnostic test study and all infertile women who undergone IVF and ICSI procedures between 2012-2013, who were <40 year old, with basic follicle stimulating hormone (FSH) <15 mIU/ml, normal serum prolactin and thyroid stimulating hormone (TSH) level, normal transvaginal sonography and normal Pap smear were entered the study. The exclusion criteria were acute infectious diseases, systemic diseases and hypothalamus or pituitary disease. The luteinizing hormone (LH), FSH, AFC, AMH and estradiol levels were recorded.*

Results: *Two hundreds and eighteen women were entered the study among whom 16 (7.3%) patients had developed to OHSS. Estradiol, AMH and AFC were significantly higher in OHSS patients (P-value< 0.05). Estradiol, AMH, AFC and FSH showed high negative predictive value (NPV> 96%) but poor positive predictive value (PPV< 40%). Estradiol showed the best accuracy and specificity. In patients with AMH≤ 4.2 (n= 164), estradiol predicted OHSS with cut-off value, sensitivity, specificity and NPV of 3441. 100%, 91.2%, 100%, respectively and in patients with AMH> 4.2, the PPV of estradiol rose to 49.5%.*

Conclusion: *Estradiol showed better sensitivity and specificity than other markers for prediction of OHSS. Classification of patients on the basis of AMH can lead to a higher sensitivity and specificity for estradiol with a higher cut-off value in OHSS prediction in patients with AMH≤ 4.2.*

Keywords: *Ovarian Hyperstimulation Syndrome, Anti-Mullerian Hormone, Estradiol.*

Corresponding Author:

Elham Askary,

Gynecologist, Shiraz medical university,
Shooshtari hospital, Shiraz, Iran
Tel: +98(9171127112)

QR code

Please cite this article in press as Elham Askary et al, ***Application of Anti-Mullerian Hormone and Estradiol in Prediction of Ovarian Hyper-Stimulation Syndrome. A New Approach****, Indo Am. J. P. Sci, 2017(Suppl); 4(03).*

INTRODUCTION:

Infertility is a relatively common disorder around the world with much higher prevalence rates in regions such as Middle East (1). Although the incidence rate of infertility remains constant in the past decades (2), there is significant improvement in the management strategies via assistive reproductive technology (ART). Such growing utility of these techniques will also lead to an increased rate of complications which need a more restrictive approach to prevent them effectively. The most common complication of ART is ovarian hyper-stimulation syndrome (OHSS) with reported incidence rate of 3-8% for in vitro fertilization (IVF) (3, 4). OHSS presented with sign and symptoms of ovarian enlargement and excess fluid in the third spaces of body due to increased vascular permeability (4). Since the management of this syndrome is almost supportive and no definite treatment for handling of underlying pathophysiologic process is not available, preventive strategies and consequently recognition of risk factors and prediction of this syndrome play an important role in the management and work up of patients who will be undergo ART (3, 4). Although various parameters such as age, serum estradiol and follicle stimulating hormone (FSH) level and antral follicle count (AFC) have been known as predictors of OHSS, however, none of these parameters can independently predict OHSS and attempts are still made to provide a more constructive approach for prediction and prevention of OHSS (4-6).

Recently, serum anti-mullerian hormone (AMH) level has been recognized as a valuable marker for evaluation of ovarian follicular reserve before ART (7, 8). Since AMH is produced by granulosa cells of small antral follicles, lower serum AMH level is in favor of a weaker response to ovarian stimulation. Regarding this direct dose-dependent response, some investigators suggested that higher levels of basal serum AMH would also related to higher possibility of ovarian hyper-stimulation (9-11). This study was performed to evaluate the role of AMH alone and in combination with other known risk factors in the prediction of OHSS.

METHODS AND MATERIALS:

Patient selection

We retrospectively studied patients who underwent ovarian stimulation in our fertility center due to IVF or intra-cytoplasmic sperm injection (ICSI) between March 2012 to March 2013. Patients with age< 40 years, basal FSH at 3rd day of menstruation cycle< 15 mIU/ml, normal serum thyroid stimulating hormone (TSH) and prolactin, normal transvaginal sonography and normal Pap smear were included. The exclusion criteria were acute infectious diseases, systemic diseases and hypothalamus or pituitary disease. Cases with incomplete follow up or laboratory data, were excluded from the analysis as well. The study was approved by the local ethic committee of Shiraz University of Medical Sciences. Since the retrospective nature of the study and lack of research purposes during management of patients, no informed consent was obtained from the patients.

Protocol of ovarian stimulation

For all patients who scheduled to undergo ovarian stimulation, serum AMH, FSH and luteinizing hormone (LH) levels in the 2nd or 3rd day of menstruation cycle were measured once within 3 months before the ovarian stimulation. For ovarian stimulation, long-term gonadotropin releasing hormone (GnRH) agonist protocol was applied. According to this protocol, 1 mg/day leuprolide was started from 21th day of menstruation cycle and then tapered to 0.5 mg/day from 3rd day when gonadotropin (150-255 mg) was started. Patients were monitored with serum estradiol level and transvaginal sonography for evaluation of the count and size of antral follicle. When equal or more than 3 antral follicles reached to 18 mm in transvaginal sonography, gonadotropin was discontinued and 5000 unit human chorionic gonadotropin (hCG) was administered, unless possibility of complications such as OHSS was suspected. The diagnosis of OHSS was made on the basis of clinical, sonographic and laboratory data. Basal AFC and serum estradiol level at the day of hCG administration were recorded. After 24-36 hours, ovum was taken and then, fetus was transferred during next 72 hours. The patients were followed for outcome of treatment and incidence of pregnancy via phone call or medical databases.

Statistical analysis

Statistical analysis was performed with SPSS software (SPSS Statistics, version 17.0. Chicago: SPSS Inc.). The quantitative continuous variables are expressed as mean ± standard deviation (SD), and the categorical variables are presented by numbers (percentages). The Kolmogorov-Smirnov test was used to assess normality of distribution. Comparison of quantitative variables between the subgroups was calculated with Mann-Whitney U test. Receiver-operating curves (ROC) for prediction of OHSS were generated by MedCalc software for Windows, version 8.0 (MedCalc Software, Ostend, Belgium). A P-value< 0.05 was considered statistically significant in all the analyses.

RESULTS:

Considering inclusion and exclusion criteria, 218 patients were included, among whom 16 patients (7.3%) developed to moderate or severe clinical OHSS. The mean ± SD of age, FSH, LH AMH, estradiol and AFC of patients are presented in Table 1. Comparing these parameters between the two groups, mean value of AMH, AFC and estradiol were significantly higher in OHSS patients and FSH was significantly lower in these patients.

Table 1: The mean ± SD of age, FSH, LH AMH, estradiol and AFC of study population

	Total n = 218	OHSS Positive n = 16 (7.3%)	OHSS Negative n = 202 (92.7%)	P value
Age (year)	31.2±5.5	29.1±6.1	31.4±5.5	0.131
Estradiol (pg/ml)	2151±1094	3557±553	2039±1048	*0.000
AMH (ng/ml)	3.10±3.21	6.14±3.63	2.86±3.06	*0.000
AFC	16±7	24±11	15±6	*0.015
FSH	6.66±3.01	4.26±1.68	6.85±3.02	*0.000
LH	5.17±4.00	4.94±2.64	5.19±4.09	0.597

Data are presented as mean ± standard deviation
* is statistically significant
OHSS, Ovarian Hyper Stimulation Syndrome; AMH, Anti-Mullerian Hormone; AFC, Antral Follicle Count; FSH, Follicular stimulating Hormone; LH, Luteinizing Hormone.

The ROC curve for prediction of OHSS by AMH, estradiol, AFC and FSH with confidence interval (CI) of 95% are illustrated in Figure 1. As presented in Table 2, estradiol has the best accuracy (Area under the curve (AUC): 0.90) with specificity and sensitivity of 93.7% and 84.2%, respectively, at the cut-off point of 3291 pg/ml which was significantly higher than those of FSH and AFC. Although the AUC for AMH, AFC and FSH (0.76, 0.64 and 0.76, respectively) were not statistically different, AMH revealed a more favorable sensitivity (75.0%) and specificity (79.5%) at the cut-off point of 4.2 ng/ml which was also not statistically different with estradiol (Table 3). Negative predictive value for estradiol, AMH, AFC and FSH were good whereas positive predictive value were low (Table 2).

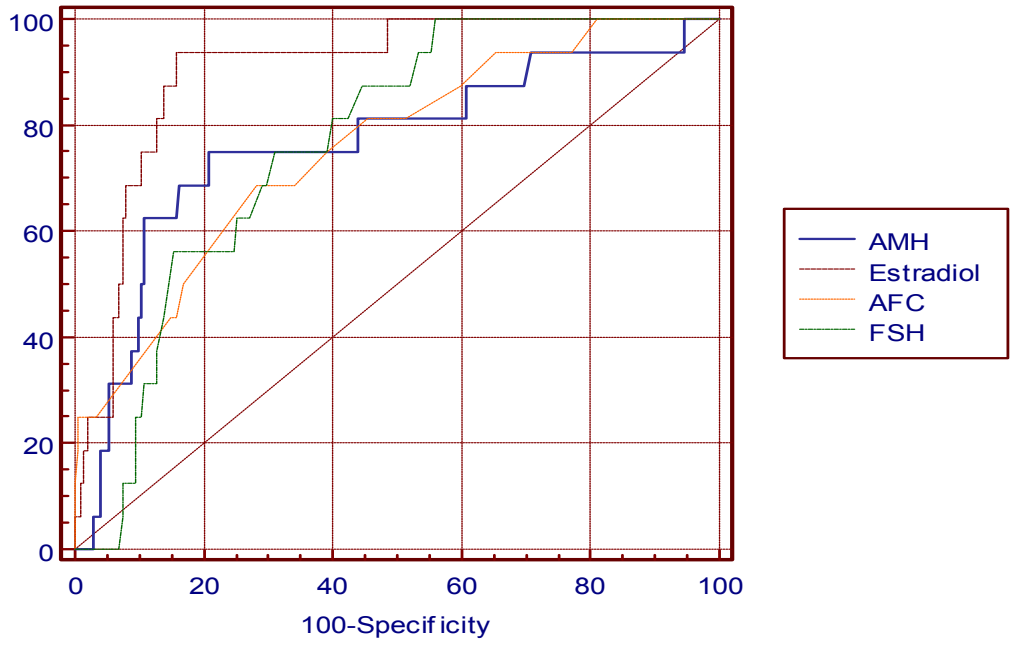

Fig1: ROC curves of AMH, Estradiol, AFC and FSH for prediction of OHSS in study population.

Table 2. Results of Receiever operating curve for estradiol, AMH, AFC, FSH.

	Cut-off value	AUC	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)
Estradiol (pg/ml)	3291	0.90	93.7	84.2	99.4	31.9
AMH (ng/ml)	4.2	0.76	75.0	79.2	97.5	22.2
AFC	15	0.64	75.0	55.0	96.5	11.6
FSH(mIU/ml)	7.1	0.76	100.0	44.1	100.0	12.4

OHSS, Ovarian Hyper Stimulation Syndrome; AMH, Anti-Mullerian Hormone; AFC, Antral Follicle Count; FSH, Follicular stimulating Hormone.

Table 3. Pairwise comparison of ROC curves

	Difference between areas	Standard error	95% Confidence interval	P value
AMH vs Estradiol	0.141	0.080	-0.015 to 0.298	0.077
AMH vs AFC	0.006	0.075	-0.142 to 0.153	0.939
AMH vs FSH	0.003	0.081	-0.155 to 0.161	0.971
Estradiol vs AFC	0.147	0.072	0.007 to 0.287	*0.040
Estradiol vs FSH	0.138	0.058	0.025 to 0.252	*0.017
AFC vs FSH	0.009	0.086	-0.160 to 0.177	0.920

* is statistically significant
OHSS, Ovarian Hyper Stimulation Syndrome; AMH, Anti-Mullerian Hormone; AFC, Antral Follicle Count; FSH, Follicular stimulating Hormone.

Applying the best cut-off value for AMH (4.2 ng/ml), patients were categorized as group with AMH≤4.2 and group with AMH> 4.2. Then, the ROC curves were generated for estradiol in these two groups, separately (Figure 2). As shown in Table 4, the AUC, sensitivity, specificity and negative predictive value of estradiol in patients with AMH≤4.2 were rose to 0.93, 100%, 91.2% and 100%, respectively in cut-off value of 3441. In patients with AMH>4.2, positive predictive value was increased to 49.9%, however, the other parameters were slightly decreased (Table 4).

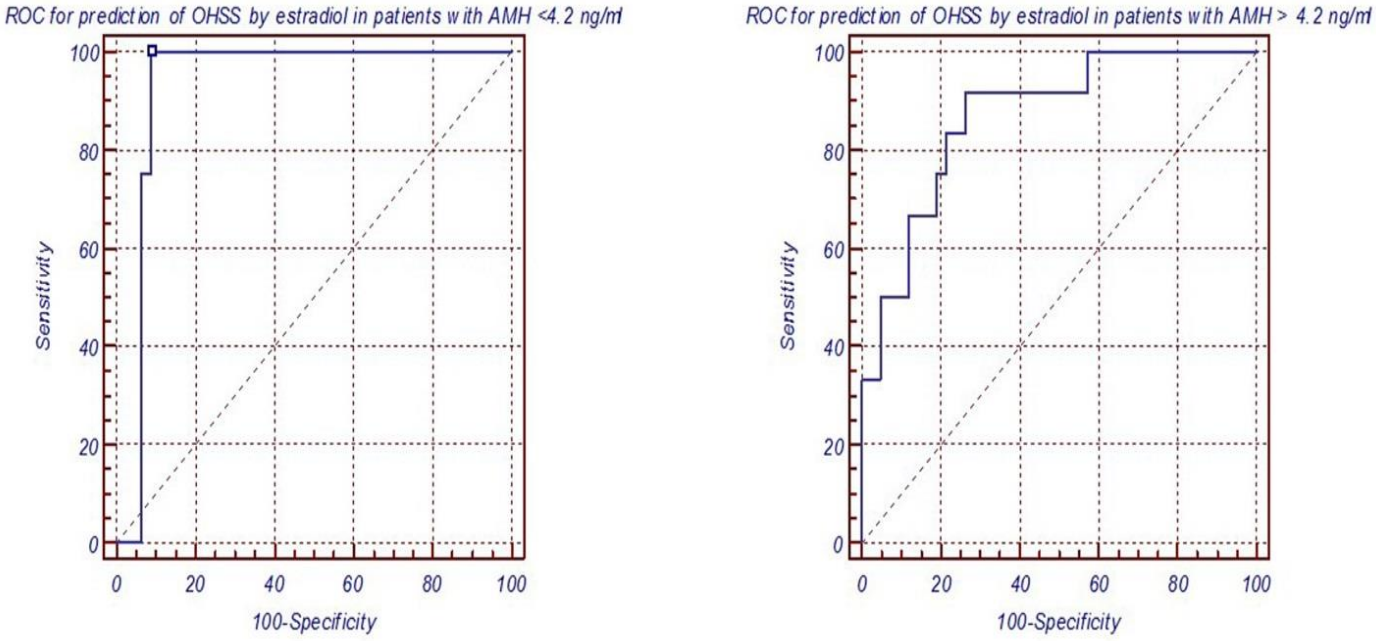

Fig 2: Roc curves of estradiol for prediction of OHSS in patients with AMH≤4.2 and AMH> 4.2.

Table 4. Results of receiver operating curve for estradiol in patients with AMH≤4.2 and AMH> 4.2.

	Cut-off value (pg/ml)	AUC	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)
AMH>4.2 (n=54)	3284	0.86	91.7	73.8	96.8	49.9
AMH≤4.2 (n=164)	3441	0.93	100.0	91.2	100.0	22.1

AMH, Anti-Mullerian Hormone; NPV, Negative Predictive Value; PPV, Positive Predictive Value.

DISCUSSION:

Our study results showed an incidence rate of 7.3 % for moderate to severe form of OHSS in IVF cycles which is in the range of 3-8 % that was reported in the previous studies. Several markers have been introduced as predictors of OHSS in the literature. Among these factors, age, FSH, AFC, and AMH as well as estradiol on the day of hCG administration are known as most important factors. In last decade, the number of researches about the association of serum AMH level and OHSS has been increased. Nakhuda et al. were one of the first who investigate the direct relation of basal serum AMH and OHSS in 2006(9). In a case-control study, they showed that mean AMH value in OHSS group is higher than control group (3.36 vs 0.63)(9). Up to that time, there were also few reports that showed the relationship of OHSS and ovarian response which indirectly indicated the predictive role of AMH in incidence of OHSS. Elder-Geva in 2005 found that mean AMH was significantly higher in the hyper-responder patients(12). There are also other studies in recent years investigating the role of AMH in prediction of OHSS, alone or in combination with other factors. Our study also showed significant differences between mean values of FSH, AFC, AMH and estradiol in both groups; The ROC-AUC for FSH, AFC, AMH and estradiol for prediction of OHSS in our study were 0.76, 0.64, 0.76 and 0.90, respectively. All markers showed a high negative predictive value with a poor positive predictive value. Estradiol and AMH also showed higher specificity than AFC and FSH.

Nelson et al. studying 340 ART cycles, showed that AMH act better than FSH and age for prediction of excessive ovarian response defined as a yield of 21 oocytes obtained at retrieval with ROC-AUC : 0.90(13). In this study, the ROC-AUC for FSH was 0.32 which is lower than that obtained in our study. These differences maybe explained, in part, by differences in the definition of excess ovarian response and OHSS as well as different ovarian stimulation protocols that used. Lee et al. prospectively investigated 262 IVF cycles with OHSS incidence rate of 8%(10). They reported that basal serum AMH can predict OHSS with sensitivity and specificity of 90.5% and 81. 3%, respectively at cut-off level of 3.36 ng/ml(10). In this study, estradiol on the day of hCG administration was also found as a good predictor of OHSS (sensitivity: 95.2; specificity: 64.6; cut-off value: 1431 pg/ml)(10). In another study by Ocal et al. different factors for prediction of OHSS including AMH, LH, FSH, AFC, inhibin B and estradiol were assessed in 695 IVF/ICSI cycles. The authors found a statistically significant difference between mean values of AMH, LH, FSH and AFC in women with OHSS (41 cases; 5.8%) and those without OHSS(7). The ROC-AUC and the sensitivity and specificity for AMH were 0.87, 90% and 71%, respectively, at cut-off value of 3.3 ng/ml and for AFC were 0.74, 78% and 65%, respectively at cut-off value of 8(7). Although our study resulted in lower values of sensitivity and specificity for AMH in prediction of OHSS as compared to the mentioned studies, however, our results is in concordance with previous studies regarding the superiority of AMH over AFC, FSH and other primary factors. Nonetheless, in a recent meta-analysis by Broer et al. the summary estimates of sensitivity and specificity of AMH for prediction of excessive ovarian response were 82% and 76%, respectively(14). They also reported sensitivity and specificity of 82% and 80% for AFC which were not statistically different from those of AMH(14). Other studies also found no significant difference between AMH and AFC for prediction of OHSS(15).

The other prominent difference between our results and previous studies was the difference in the best cut-off value of AFC (15 oocytes in our study) which could be due to different definitions for antral follicles and the inherent variability of sonographic results as an operator-dependent modality. In contrast to these limitations of AFC, the acceptable reproducibility for AMH and the development of high sensitive methods of measurement have made this marker more widely used in recent years.

The other investigated factor in our study was the serum estradiol level on the day of hCG administration which serve as a secondary factor in the prediction and prevention of OHSS. Our results indicate better accuracy for estradiol as compared to the FSH and AFC (estradiol-AUC: 0.90 vs FSH-AUC: 0.76 and AFC-AUC: 0.64, p value 0.040 and 0.017, respectively), confirming the previous studies. The accuracy of serum estradiol level was not statistically different from that of AMH (AMH-AUC:

0.76, p value 0.077). Considering the superiority of AMH over the other primary factors, we combined this marker with estradiol as secondary factor in prediction of OHSS in a different way. Since these two factors measured in different time points in the course of an IVF cycle we decided to apply the first factor, AMH, in defining the cut-off value for the second one i.e. estradiol on the day of hCG administration when the decision about the administration of hCG can prevent the incidence of OHSS. Applying this approach, after classification of patients on the basis of AMH cut-off value of 4.2, in the group with AMH ≤4.2 the ROC-AUC, sensitivity, specificity and cut-off value for estradiol were rose to 0.93, 100%, 91% and 3444 pg/ml, respectively. In group with AMH> 4.2, although the best cut-value of estradiol was not significantly differ from the total study population, the positive predictive value of estradiol which is usually considered as one of main limitations of this marker, were increased from 30% to 49.9% at the best cut-off value of 3284 pg/ml with sensitivity and specificity of 91.7% and 73.8 %, respectively. In other words, an early classification of patients on the basis of AMH can improve the ability of estradiol to a more useful marker in prediction of OHSS using a more appropriate cut-off value for each group. This can allow hCG administration at higher estradiol levels in patients with AMH≤4.2 with more accuracy and confidence, suggesting the incidence of OHSS in patients with AMH>4.2 with a higher positive predictive value. It is also noteworthy that applying such approach for other factors did not result in significant changes.

These results indicate the value of AMH not only as an independent predictive factor for OHSS, but also as a useful marker in combination with estradiol for risk stratification before hCG administration.

Limitations. Regarding the retrospective nature of our study in addition to rather small number of OHSS patients, it can be stated that these results can only present a primitive result on this aspect of AMH application and further prospective and larger studies with a more dedicated design are still needed to confirm these results. Our study also is incomplete about the quantity and quality of pregnancies in our patients and further investigation regarding the effect of this approach on the final outcome of ovarian stimulation is necessary to decide about the cost-effectiveness of such risk stratification.

CONCLUSION:

According to our study, estradiol showed better sensitivity and specificity than other markers in prediction of OHSS. AMH, AFC and FSH also can be used for prediction of OHSS and their high negative predictive values can indicate the usefulness of these markers as screening tool. Our study also showed that classification of patients on the basis of AMH can lead to a higher sensitivity and specificity for estradiol with a higher cut-off value in OHSS prediction in patients with AMH≤4.2 allowing a more appropriate decision regarding the administration of hCG. In other words, as a serial approach, AMH can be served as a primary test before estradiol for better risk stratification of patients undergoing ovarian induction by enhancing the predictive value of estradiol for OHSS.

CONFLICTS OF INTEREST:

The authors declare that they have no conflict of interest.

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

REFERENCES:

1.Inhorn MC, Patrizio P. Infertility around the globe: new thinking on gender, reproductive technologies and global movements in the 21st century. Human reproduction update. 2015:dmv016.

2.Mascarenhas MN, Flaxman SR, Boerma T, Vanderpoel S, Stevens GA. National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys. PLoS Med. 2012;9(12):e1001356.

3.Aboulghar M, editor Treatment of ovarian hyperstimulation syndrome. Seminars in reproductive medicine; 2010.

4.Smith V, Osianlis T, Vollenhoven B. Prevention of Ovarian Hyperstimulation Syndrome: A Review. Obstetrics and gynecology international. 2015;2015.

5.Sousa M, Cunha M, da Silva JT, Oliveira C, Silva J, Viana P, et al. Ovarian hyperstimulation syndrome: a clinical report on 4894 consecutive ART treatment cycles. Reproductive Biology and Endocrinology. 2015;13(1):66.

6.Nastri C, Teixeira D, Moroni R, Leitão V, Martins W. Ovarian hyperstimulation syndrome: pathophysiology, staging, prediction and prevention. Ultrasound in Obstetrics & Gynecology. 2015;45(4):377-93.

7.Ocal P, Sahmay S, Cetin M, Irez T, Guralp O, Cepni I. Serum anti-Müllerian hormone and antral follicle count as predictive markers of OHSS in ART cycles. Journal of Assisted Reproduction and Genetics. 2011;28(12):1197-203.

8.Nelson SM. Biomarkers of ovarian response: current and future applications. Fertility and sterility. 2013;99(4):963-9.

9.Nakhuda GS, Chu MC, Wang JG, Sauer MV, Lobo RA. Elevated serum müllerian-inhibiting substance may be a marker for ovarian hyperstimulation

syndrome in normal women undergoing in vitro fertilization. Fertility and Sterility. 2006;85(5):1541-3.
10.Lee T-H, Liu C-H, Huang C-C, Wu Y-L, Shih Y-T, Ho H-N, et al. Serum anti-Müllerian hormone and estradiol levels as predictors of ovarian hyperstimulation syndrome in assisted reproduction technology cycles. Human Reproduction. 2008;23(1):160-7.
11.La Marca A, Sighinolfi G, Radi D, Argento C, Baraldi E, Artenisio AC, et al. Anti-Müllerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART). Human reproduction update. 2010;16(2):113-30.
12.Eldar-Geva T, Ben-Chetrit A, Spitz IM, Rabinowitz R, Markowitz E, Mimoni T, et al. Dynamic assays of inhibin B, anti-Mullerian hormone and estradiol following FSH stimulation and ovarian ultrasonography as predictors of IVF outcome. Human Reproduction. 2005;20(11):3178-83.
13.Nelson SM, Yates RW, Fleming R. Serum anti-Müllerian hormone and FSH: prediction of live birth and extremes of response in stimulated cycles—implications for individualization of therapy. Human Reproduction. 2007;22(9):2414-21.
14.Broer S, Dólleman M, Opmeer B, Fauser B, Mol B, Broekmans F. AMH and AFC as predictors of excessive response in controlled ovarian hyperstimulation: a meta-analysis. Human reproduction update. 2011;17(1):46-54.
15.Aflatoonian A, Oskouian H, Ahmadi S, Oskouian L. Prediction of high ovarian response to controlled ovarian hyperstimulation: anti-Müllerian hormone versus small antral follicle count (2–6 mm). Journal of assisted reproduction and genetics. 2009;26(6):319-25.