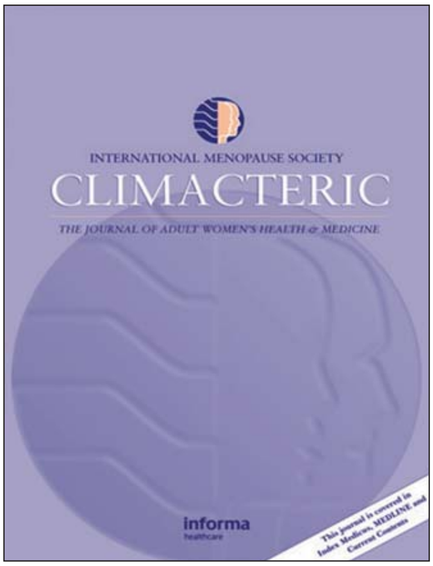
informa
healthcare

Just Accepted by *Climacteric*



Elevated LH levels draw a stronger distinction than AMH in premature ovarian insufficiency

S. Sahmay, T. A. Usta, T. Erel, N. Atakul and B. Aydogan

10.3109/13697137.2013.870149

Abstract

Objectives: A significant number of individuals have high serum follicle-stimulating hormone (FSH) level but do not meet the criteria for diagnosis of premature ovarian insufficiency (POI) due to ongoing menstruation. We compared a group of women with elevated FSH levels (EFSH) and POI with a control group in terms of biochemical markers.
Methods: In this cross-sectional retrospective study, 38 POI cases and 48 EFSH cases were compared to 89 individuals in a control arm in terms of biochemical markers. The receiver operating characteristics curve calculated to assess the utility of AMH levels to discriminate women with elevated FSH levels accompanied by POI from those women with elevated FSH levels but not definable as POI.
Results: A multiple regression analyses revealed that only AMH level was significantly different for the discrimination of control and EFSH groups. AMH and estradiol (E2) levels were found to be statistically significant for the discrimination of control and POI cases. However, only luteinizing hormone (LH) found to be significant for distinguishing between women with EFSH and POI, interestingly excluding the serum AMH level in this context.
Conclusions: AMH was the most important and superior marker to differentiate both POI cases and patients with an elevated FSH level from the controls; however, it did not show the same resolution for differentiating POI cases from the elevated FSH group. Moreover, we conclude that serum LH levels is the most useful marker for differentiating POI cases from women with EFSH levels

Elevated LH levels draw a stronger distinction than AMH in premature ovarian insufficiency

S. Sahmay[a], T. A. Usta[b], T. Erel[a], N. Atakul[a] and B. Aydogan[a]

[a]Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey. [b]Department of Obstetrics and Gynecology, Bagcilar Training and Research Hospital, Bagcilar, Istanbul, Turkey

Short title: LH and AMH levels In Premature Ovarian Failure

Corresponding author at: Department of Obstetrics and Gynecology, Bagcilar Training and Research Hospital, Bagcilar, Istanbul, Turkey
E-mail:tanerusta74@yahoo.com (T.A.Usta)

Abstract

Objectives: A significant number of individuals have high serum follicle-stimulating hormone (FSH) level but do not meet the criteria for diagnosis of premature ovarian insufficiency (POI) due to ongoing menstruation. We compared a group of women with elevated FSH levels (EFSH) and POI with a control group in terms of biochemical markers.

Methods: In this cross-sectional retrospective study, 38 POI cases and 48 EFSH cases were compared to 89 individuals in a control arm in terms of biochemical markers. The receiver operating characteristics curve calculated to assess the utility of AMH levels to discriminate women with elevated FSH levels accompanied by POI from those women with elevated FSH levels but not definable as POI.

Results: A multiple regression analyses revealed that only AMH level was significantly different for the discrimination of control and EFSH groups. AMH and estradiol (E2) levels were found to be statistically significant for the discrimination of control and POI cases. However, only luteinizing hormone (LH) found to be significant for distinguishing between women with EFSH and POI, interestingly excluding the serum AMH level in this context.

Conclusions: AMH was the most important and superior marker to differentiate both POI cases and patients with an elevated FSH level from the controls; however, it did not show the same resolution for differentiating POI cases from the elevated FSH group. Moreover, we conclude that serum LH levels is the most useful marker for differentiating POI cases from women with EFSH levels

Key words: Premature Ovarian Failure, AMH, Elevated LH, Elevated FSH.

INTRODUCTION

Premature ovarian insufficiency (POI) is the most extreme phenotype of diminished ovarian reserve at a young age [1]. POI occurs at an age less than two standard deviations below the mean established for the reference population, but is defined as menopause before the age of 40 years in practice [2]. POI affects 1% of women <40 years and 0.1% of women<30 years [3]. Incipient ovarian failure (IOF) or late reproductive aging according to the Stages of Reproductive Aging Workshop classification describes another subgroup characterized by elevated follicular-phase FSH levels along with a regular menstrual cycle [4]. IOF precedes the onset of cycle irregularity and hence the menopausal transition by 3–10 yr and may be considered an early sign of advanced ovarian aging in young women [5].

Various markers such as FSH levels or antral follicle count have been used to measure ovarian insufficiency. Serum levels of FSH, estradiol (E2) or inhibin B are of limited value for predicting the presence of an ovarian reserve in patients with POI, as most of these markers only indicate advanced ovarian senescence [6]. Women with POI may develop follicles up to antral stage and serum Anti-Mullerian Hormone (AMH) level might be a good indicator of follicular presence [7]. Serum AMH is negatively correlated with age and positively correlated with antral follicle count on ultrasound [8,9]. Serum AMH levels show an age-related decrease after 30 years, whereas other markers associated with ovarian aging do not change during this time [10].

Women in western society are delaying having children until later in life [1]. As a result, significant numbers of women present with elevated FSH levels suggestive of decreased ovarian reserve, with or without cycle abnormalities [11]. We have encountered many women who have elevated serum FSH levels but not fulfill the definition of POI. One study compared patients with secondary amenorrhea and controls and identified a high percentage of very low AMH levels in patients with POI [10]. Another small study identified low AMH levels as a marker of diminished ovarian reserve in patients with IOF with consistent elevated FSH levels [12].There is insufficient evidence about whether serum AMH measurements are sufficient for distinguishing between POI cases and women with only EFSH. In light of this, we hypothesized that the determination of serum AMH level and other serum markers in differantiating the patient with elevated serum FSH level from POI patients.

METHODS

Participants

The study was conducted at the Reproductive Endocrinology and Infertility Clinic of Department of Obstetrics and Gynecology, Cerrahpasa School of Medicine, Istanbul University between January 2008 and January 2010. The files were reviewed retrospectively to evaluate the utility of serum AMH levels in women with elevated serum FSH levels (EFSH) and a POI group. Inclusion criteria for POI were; Age 25-39yr secondary amenorrhea, concomitant vasomotor symptoms, normal karyotype, no endocrinopathy and no history of radiotherapy, chemotherapy or ovarian surgery. Spontaneous absence of menses for at least 4 months in combination with FSH levels >40 IU/L before age 40 years is accepted as a definition of POI [1]. POI group included 38 patients. The EFSH group included 48 patients who had elevated FSH levels (above 10.2 IU/L and below 40 IU/L) but not fulfill the criteria for POI definition. The control group included 89 patients in the same age group who were admitted to our department as routine controls. The control group had regular menstrual cycles, no signs of hyperandrogenemia, and normal sonographic appearance of the ovaries. Potential participants were excluded if they were smokers, pregnant, breastfeeding or had history of cardiovascular, liver, kidney or respiratory disease, uncontrolled hypertension, diabetes or malignancy. None of the study participants reported the use of any medications in the previous 3 months that could have interfered with normal functioning of the hypothalamic–pituitary–gonadal axis. Informed consent was obtained from all women, and approval from the Human Ethics Committee of Istanbul University was obtained. All study procedures were performed in accordance with the Declaration of Helsinki.

Biochemical Measurements

Blood samples in women with regular menstrual cycles (26-32 d) were collected from the early follicular phase of menstrual cycle whereas samples were obtained at random in the same laboratory using the same assays from women without a regular cycle. Serum levels of AMH, FSH, LH, E2 were analyzed in each group. All blood samples for AMH measurement were collected in a lithium-heparin tube. Serum AMH concentrations were measured with an enzymatically amplified two-sided immunoassay [DSL-10-14400 Active Mullerian Inhibiting Substance/AMH enzyme-linked immunosorbent assay (ELISA) kit, Diagnostic Systems Laboratories (DSL), Webster, TX, USA]. The theoretical sensitivity of the method was 0.006 ng/mL, the intra-assay coefficient of variation for low values was 3.3%, and the inter-assay coefficient of variation for high values was 6.7%. The levels of serum FSH, LH, and E2 were measured with Roche E-170 automated immunoassay analyzer (Roche Diagnostics, Mannheim, Germany). The inter-batch coefficient of variation for these assays was 10 %.

Statistical Analyses

The data analysis was performed using SPSS for Windows, version 11.5 (SPSS Inc., Chicago, IL, USA). Whether the distributions of continuous variables were normal or not was determined by the Kolmogorov-Smirnov test. Continuous variables are shown as mean ± standard deviation (SD) or median (range), where applicable. Mean differences between groups were compared by Student's *t* test. The Mann-Whitney *U*-test was applied for comparisons of the median values between the case and control groups. The Kruskal-Wallis test was used to assess differences in median values among groups when the number of independent groups was more than two. When the p-value from the Kruskal-Wallis test was statistically significant, Conover's non-parametric multiple comparison test was used to identify which group differed from the others. The optimal cutoff points for levels of AMH, FSH, LH and E2 to distinguish among the groups were evaluated by receiver operating characteristic (ROC) analyses after calculating the area under the curve (AUC) given the maximum sum of sensitivity and specificity (i.e. Youden Index) for the significance test. Sensitivity, specificity, positive predictive value(PPV), and negative predictive value(NPV) were calculated for each clinical measurement at the best cutoff points. Multiple logistic regression analyses were used to identify the predictive ability of the clinical measurements after adjustment. Odds ratios(OR) and 95% confidence intervals (CIs) were also calculated for each independent variable. A p-value <0.05 was considered significant.

RESULTS

No differences were found for age or body mass index (BMI) between the groups (Table 1). Serum AMH and E2 levels were significantly lower in the EFSH and POI groups than in the control group. FSH and LH levels were significantly higher in EFSH and POI groups compared to controls (p<0,001). The serum LH levels (with the serum FSH) were significantly higher in POI group than in the EFSH group (p<0,001).

The area under the curves (AUC) for serum AMH, FSH, LH and E2 significantly discriminated between controls and the EFSH group. The highest AUC value was for AMH (OR,0.963; 95%CI , 0.936-0.989) and the lowest AUC value was for E_2 in the analysis of EFSH and control groups. (OR,0.769; 95%CI, 0.676-0.862) (Table 2, Figure 1). The highest PPV was for FSH (84.3%) and the highest NPV was for AMH (98.7%). The best AMH cut-off point was 0.955 ng/mL for the EFSH group. AMH had the highest sensitivity (97.9%) for discriminating between the two groups and FSH had the highest specificity (91.0%) among the four biochemical markers.

The AUCs for serum AMH, FSH, LH and E_2 were significantly discriminated between the POI group and controls (Figure 2). The highest AUC value was for LH in the analysis of POI-versuscontrols (OR,0.999; 95% CI, 0.997-1.001) and the lowest AUC value was for E2 (OR,0.860; 95% CI , 0.777-0.944) (Figure 2). The best cut-off point for AMH was 0.945 ng/mL and it was 12.85 IU/L for LH in the POI group. Among four biochemical markers, LH had the highest sensitivity (97.4%), specificity (100%), positive predictive (100%) and negative predictive (98.9%) values for discriminating between the POI and controls.

The AUCs for LH and E2 significantly discriminated between the EFSH and POI groups (Table 2, Figure 3). That for AMH was not significant. The ROC analysis for FSH could not be performed because the groups were segregated according to FSH level.

Only serum AMH and FSH levels significantly discriminated between EFSH and controls in multiple logistic regression analyses. AMH level was more statistically decisive than FSH. (Table 3). On the other hand AMH and E2 significantly discriminated between controls and POI patients, with AMH being statistically decisive (Table 3). FSH and LH could not be included in the regression models, because of the odds ratios could not be calculated for the best cut-off values of these markers. Only serum LH level was statistically significant when serum LH and E2 levels were evaluated together for discriminating between the EFSH and POI groups (Table 3).

DISCUSSION

POI is a common condition that is of growing concern to the general population due to the increasing trend of delaying pregnancy [13]. Women with POI are at risk for not only infertility but also urogenital problems, low bone density, earlier onset of osteoporosis and fractures, earlier onset of coronary heart disease and increased cardiovascular mortality [14]. They are prone to those risks at an early age. The causes of POI are largely unknown and it is generally irreversible [15]. After a diagnosis of POI, patients have limited options. Unfortunately, most of the time, oocyte donation, embryo donation or adoption seem to be the only options for such patients to have children. Therefore, the anticipatory guidance of these patients and the clinical approaches are critical.

The most frequently applied definition of POI is the spontaneous absence of menses for at least 4 months in combination with FSH levels >40 IU/dl before age of 40 years [1]. Although often used as synonyms, POI and menopause are not the same entities as many patients with POI can exhibit intermittent ovarian function and ovulation. Moreover, 5-10% may conceive [16]. The only feature of patient history, this is helpful in determining the etiology of ovarian failure is a positive family history [17].

Early predictors of ovarian ageing are challenging to identify. So far, no biological markers have been identified that are able to distinguish between women with EFSH and POI. But we demonstrated that serum LH level as the most important parameter and AMH didn't show any superiority to differentiate those two groups from one another. In this sense our results is very important indicating the status of women with ovarian ageing.

A series of ovarian reserve tests have been used to predict ovarian ageing or poor ovarian reserve. The main purpose of these tests is to determine reduced fertility at a stage when appropriate treatment can be preemptively applied. AMH levels decrease, while other factors associated with perimenopausal status such as FSH, inhibin B or E2 levels do not change significantly in ageing females [8,18]. Serum levels of FSH, E2 or inhibin B are of limited value for predicting the presence of an ovarian reserve in patients with POI [6,7]. Elevated serum levels of FSH is an undeniable hormonal hallmark of reproductive aging; however, many studies have shown that it is a relatively late predictor of completed menopausal transition [9,19,20,21]. In contrast AMH reported to be a useful marker for the ovarian follicular pool [9]. AMH appears in serum after birth, increases until puberty, and progressively decreases in parallel with ovarian aging [8,9]. It has been suggested that POI and early ovarian ageing are associated with very low or undetectable serum AMH levels [1,10,12]. It was demonstrated that when AMH levels fall to undetectable levels, menopause will be observed within 5 years [21]. Several studies have demonstrated that serum AMH level is a better marker of ovarian reserve than age alone or other markers such as basal serum FSH, estradiol and inhibin B levels [22]. The main challange is to capture the condition early enough to identify an 'ongoing' or rather insidious menopausal transition. From this point of view, serum levels of AMH seem to be the best marker for declining ovarian age [23]. AMH measurements have practical advantages with respect to other methods that are used for follicular evaluation. One of the most appealing advantages of using serum levels of AMH is that it is stable under various influences such as hormonal contraception, the menstrual cycle, and pregnancy, and measurements can therefore be made anytime during the menstrual cycle [17,23,24,25].

Serum LH measurement is a commonly used diagnostic test; however, knowledge on its practical implications is limited. The two-cell/two-gonadotropin model states that LH stimulates the conversion of cholesterol into androgens in theca cells and in parallel, FSH stimulates the aromatization of androgens into estrogen in granulosa cells. The lack of the stimulating gonadotropins or the lack of response of the target ovaries results in hypogonadism. While in some patients only FSH is elevated, in some others elevated LH levels, low serum estradiol levels and deprivation symptoms of it, accompanying secondary amenorrhea may be observed. In this study, multiple regression analysis revealed that serum AMH level was the most prominent parameter in differentiating the POF and the EFSH groups from the control group, whereas serum LH level was the most important marker in demarcating the POF cases from women with EFSH. Accounting for the two-cell/two-gonadotropin model; regarding that a healthy functioning ovary needs the collaboration of the theca and granulosa cells, we hypothesize that the EFSH group may represent an early stage of POI when there are only insufficient granulosa cells and that is progress as the elevation of LH due to malfunctioning theca cells, which is then definable as ‘POI’. Data regarding the significance of serum LH levels in patients with POI are very limited. The value of LH measurements for discriminating between POI and EFSH cases has not been evaluated in any study, so far.

The most important limiting factor in our study was the limited number of cases. Due to the low prevalence of POI in society, published POI studies include relatively few patients.. Studies that include a larger number of patients must be conducted multicentrally and even internationally so as to achieve higher statistical power for the results.

AMH is a promising tool for many fields of gynecology. Our findings suggest that among biochemical parameters; AMH is the most important and superior marker for distinguishing between controls and patients with both EFSH and POI however AMH is no more superior than other markers in discriminating women with POI from those with elevated FSH level. In this situation, we found serum LH level as the most important parameter for discriminating between POI and EFSH. To the best of our knowledge, this is the first study which states the significance of LH for discriminating between POI and EFSH cases.. Our conclusions draw attention to the significance of LH for evaluating and characterizing of cases with seemingly limited ovarian reserve who are more likely to be ‘POI’. However, due to the low number of cases in this study, we cannot conclusively recommend LH as a marker for POI discerning from the EFSH cases. Further studies with larger patient numbers will probably clarify our hypothesis.

Conflict of interest

The authors report no conflicts of interest.

Funding

None.

References

1. Knauff EA, Eijkemans MJ, Lambalk CB et al.; Dutch Premature Ovarian Failure Consortium. Anti-Mullerian hormone, inhibin B, and antral follicle count in young women with ovarian failure. J Clin Endocrinol Metab. 2009;94(3):786-92.
2. International Menopause Society. http://www.imsociety.org/menopause terminology.php, accessed 25 july 2013.

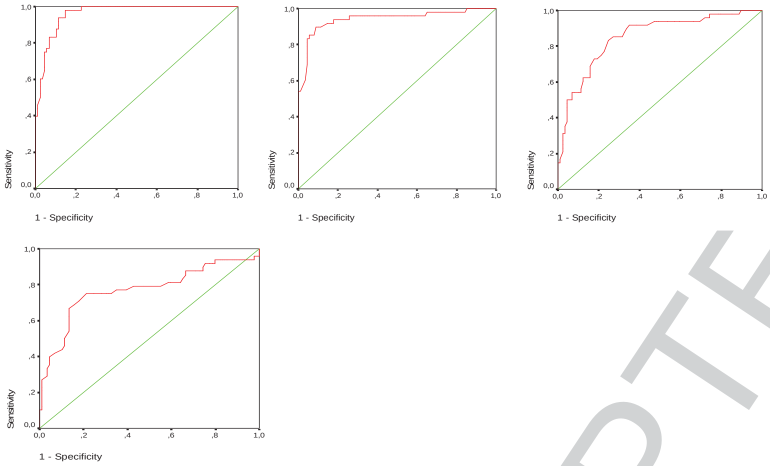
3. Coulam CB, Adamson SC, Annegers JF. Incidence of premature ovarian failure. Obstet Gynecol 1986;67:604–6.
4. Soules MR, Sherman S, Parrott E et al. Executive summary: stages of reproductive aging workshop (STRAW). Fertil Steril. 2001;76:874–878
5. Sherman BM, West JH, Korenman SG The menopausal transition: analysis of LH, FSH, estradiol, and progesterone concentrations during menstrual cycles of older women. J Clin Endocrinol Metab. 1976;42:629–636
6. Massin N, Gougeon A, Meduri G et al. Significance of ovarian histology in the management of patients presenting a premature ovarian failure. Hum Reprod. 2004;19(11):2555-60.
7. Méduri G, Massin N, Guibourdenche J et al. Serum anti-Müllerian hormone expression in women with premature ovarian failure. Hum Reprod. 2007;22(1):117-23.
8. de Vet A, Laven JS, de Jong FH, Themmen AP, Fauser BC Antimullerian hormone serum levels: a putative marker for ovarian aging. Fertil Steril. 2002;77:357–362.
9. van Rooij IA, Tonkelaar Id, Broekmans FJ et al. Anti-müllerian hormone is a promising predictor for the occurrence of the menopausal transition. Menopause. 2004;11(6 Pt 1):601-6.
10. La Marca A, Pati M, Orvieto R et al. Serum anti-mullerian hormone levels in women with secondary amenorrhea. Fertil Steril. 2006;85:1547–1549.
11. Broekmans FJ, Knauff EA, te Velde ER, Macklon NS, Fauser BC. Female reproductive ageing: current knowledge and future trends. Trends Endocrinol Metab. 2007;18:58–65.
12. de Koning CH, McDonnell J, Themmen AP, de Jong FH, Homburg R, Lambalk CB. The endocrine and follicular growth dynamics throughout the menstrual cycle in women with consistently or variably elevated early follicular phase FSH compared with controls. Hum Reprod. 2008;23:1416–1423.
13. Chand AL, Harrison CA, Shelling AN. Inhibin and premature ovarian failure.Hum Reprod Update. 2010;16(1):39-50.
14. Atsma F, Bartelink ML, Grobbee DE, van der Schouw YT. Postmenopausal status and early menopause as independent risk factors for cardiovascular disease: a meta-analysis. Menopause. 2006;13(2):265-79.
15. Shelling AN. Premature ovarian failure. Reproduction. 2010;140(5):633-41.
16. Vujovic S, Brincat M, Erel T et al. EMAS position statement: Managing women with premature ovarian failure. Maturitas. 2010;67(1):91-3.
17. Nelson LM, Anasti JN, Kimzey LM et al. Development of luteinized graafian follicles in patients with karyotypically normal spontaneous premature ovarian failure. J Clin Endocrinol Metab. 1994; 79,1470–1475.

18. Lie Fong S, Lugtenburg PJ, Schipper I et al. Anti-müllerian hormone as a marker of ovarian function in women after chemotherapy and radiotherapy for haematological malignancies.Hum Reprod. 2008;23(3):674-8.
19. Sowers MR, Eyvazzadeh AD, McConnell D et al. Anti-mullerian hormone and inhibin B in the definition of ovarian aging and the menopause transition. J Clin Endocrinol Metab. 2008; 93:3478–3483.
20. Tehrani FR, Solaymani-Dodaran M, Azizi F. A single test of antimullerian hormone in late reproductive-aged women is a good predictor of menopause. Menopause. 2009 Jul-Aug;16(4):797-802.
21. Tremellen KP, Kolo M, Gilmore A, Lekamge DN. Anti-mullerian hormone as a marker of ovarian reserve. Aust N Z J Obstet Gynaecol. 2005;45(1):20-4.
22. La Marca A, Brozzetti A, Sighinolfi G, Marzotti S, Volpe A, Falorni A. Primary ovarian insufficiency: autoimmune causes. Curr Opin Obstet Gynecol. 2010;22(4):277-82.
23. Usta T, Oral E. Is the measurement of anti-Müllerian hormone essential? Curr Opin Obstet Gynecol. 2012;24(3):151-7.
24. Taylor AE, Adams JM, Mulder JE, Martin KA, Sluss PM and Crowley WF Jr. A randomized, controlled trial of estradiol replacement therapy in women with hypergonadotropic amenorrhea. J Clin Endocrinol Metab. 1996; 81,3615–3621.
25. Sahmay S, Atakul N, Oncul M, Tuten A, Aydogan B, Seyisoglu H. Serum anti-mullerian hormone levels in the main phenotypes of polycystic ovary syndrome. Eur J Obstet Gynecol Reprod Biol. 2013;24. pii: S0301-2115(13)00250-9. doi: 10.1016/j.ejogrb.2013.05.019. [Epub ahead of print].

Figure Legends

Figure 1

Receiver operating characteristic (ROC) curves of AMH, FSH, LH and E_2 for Clinical Measurements Discrimination of EFSH and Control Groups.



AMH ***FSH*** ***LH*** ***E2***

Figure 2

ROC analysis of AMH, LH and E_2 for clinical measurements discrimination of POI and control groups.

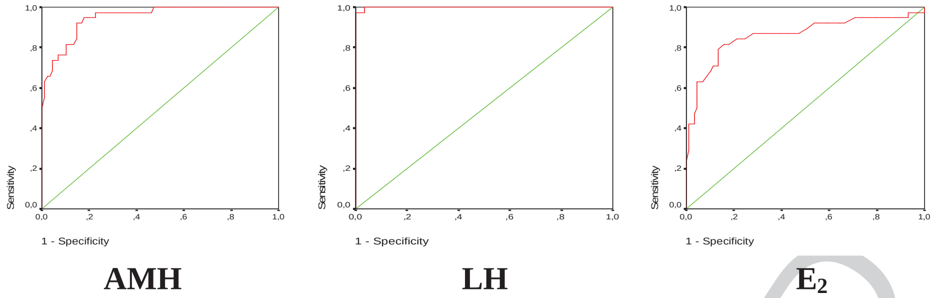
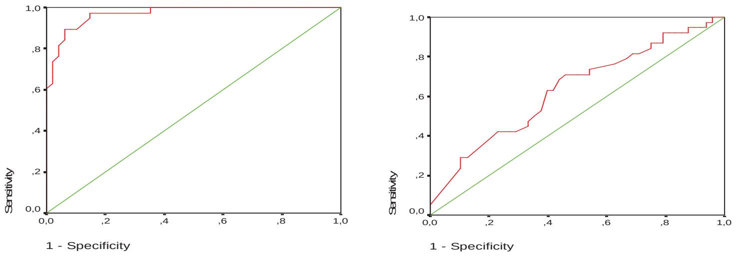


Figure 3

ROC analysis of LH and E_2 for clinical measurements discrimination of EFSH and POI groups.



LH E_2

Table Legends

Table 1

Demographical and clinical parameters regarding for groups.

Variables	Controls (n=89)	Elevated FSH (FSH <40) (n=48)	POI (FSH >40) (n=38)	p-value
Age (years)	30.7±5.5	33.0±5.5	30.4±6.5	0.055
BMI (kg/m^2)	26.4±4.9	27.4±5.7	24.4±3.5	0.208
AMH	2.1 (0.04-11.0)*, †	0.1 (0.01-1.1)*	0.03 (0.01-2.0) †	<0.001
FSH	6.2 (0.1-10.1)*, †	22.2 (10.2-39.0)*, ‡	67.0(41.0-162.0)†, ‡	<0.001
LH	2.9 (0.1-12.7)*, †	6.7 (1.6-24.0)*, ‡	31.5 (8.4-120.0) †, ‡	<0.001
E2	39.0 (9.0-108.0)*, †	22.5 (8.0-130.0)*	16.5 (4.0-110.0) †	<0.001

*****: The difference between controls and EFSH groups was statistically significant (p<0.001), †: The difference between control and POI groups was statistically significant (p<0.001), ‡: The difference between EFSH and POI groups was statistically significant (p<0.001).

Table 2

The Results of ROC Analysis for Clinical Measurements Discrimination of EFSH and POI Groups.

Statistics	Definitions	AMH	LH	E2
AUC		0.527	0.970	0.633
95%CI for AUC		0.401-0.653	0.941-1.000	0.514-0.752
p-value		0.664	<0.001	0.035
The Best Cut off Point		-	>16.18	<21.5
No. of Cases	N	-	86	86
Sensitivity	TP/(TP+FN)	-	34/38 (89.5%)	27/38 (71.1%)
Specificity	TN/(TN+FP)	-	45/48 (93.8%)	26/48 (54.2%)
PPV	TP/(TP+FP)	-	34/37 (91.9%)	27/49 (55.1%)
NPV	TN/(TN+FN)	-	45/49 (91.8%)	26/37 (70.3%)
OR (95%CI)		-	127.500	2.901
95%CI for OR		-	26.744-607.839	1.177-7.150

AUC: Area Under the Curve, CI: Confidence Interval, N: Number of cases, TP: True Positive, FN: False Negative, TN: True Negative, FP: False Positive, PPV: Positive Predictive Value, NPV: Negative Predictive Value, OR: Odds Ratio.

Table 3

Results of Multiple Logistic Regression Analyses**.**

Models	OR (95%CI)	p-value	Wald	Sensitivity	Specificity
Control vs EFSH				91.7	93.3
AMH<0.955	62.672 (6.582-596.734)	<0.001	12.952		
FSH>11.8	11.059 (2.236-54.695)	0.003	8.683		
LH>4.35	1.090 (0.203-5.846)	0.920	0.010		
E2<30.5	3.673 (0.814-16.576)	0.091	2.863		
Control vs POI				73.7	95.5
AMH<0.945	46.846 (10.828-202.678)	<0.001	26.496		
E2<28.5	14.803 (3.944-55.566)	<0.001	15.944		
EFSH vs POI				89.5	93.8
LH>16.18	144.712 (26.276-796.985)	<0.001	32.661		
E2<21.5	4.028 (0.717-22.646)	0.114	2.502		