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Predictability of consecutive measurements of serum Anti-Mullerian Hormone (AMH) during In Vitro Fertilization (IVF)

Saman Hussein Noori / MBChB, MSc, PhD Clinical Biochemistry

University of Sulaimani / College of Medicine / Department of Biochemistry

Abstract

Background : In recent years, many studies had been carried out on the basal AMH level and its association with controlled ovarian hyperstimulation (COH) outcome. Only a few studies have been conducted on the correlation between COH outcomes and AMH levels measured on different stimulation days and these studies did not show a comparison between different measurement timings over the entire period of the COH cycle.

Objectives: the current study aim is to assess the predictive values of basal and consecutive serum AMH levels during COH cycle.

Methods and Results*:* One hundred women were involved in this study, scheduled for IVF program in Dwarozh Fertility Center in Sulaimanyiah , between December 2015 until January 2017, blood samples were collected for measurement of AMH, Estradiol, and FSH on day 2 of menstrual cycle, and the subsequent samples on day 4,6,8 and 10 were taking after the stimulation of ovaries with gonadotrophin. All hormones being analyzed by using electrochemiluminescence methods (Cobas 411 by Roche) as a single batch, each patient was given a unique numerical identifier, which issued in data analysis. P values <0.05 were considered significant.

Results: the patients' characteristics , basal serum estradiol (E2), FSH and AMH levels at day 2 of cycle and subsequent days after stimulation . As expected significant differences were observed for total dosage of FSH, peak E2 levels and duration of stimulation between short and long GnRH agonist groups. The ROC curve was used to assess the AMH values in different days (day 2, 4, 6, 8, and 10) for prediction of IVF outcomes (implantation, abortion, preganancy and live birth). All the days showed significant area under the curve (AUC) ($p<0.05$). However, when all the ROC curve were comapred to each others there were no significance differences bwteeen them ($p>0.05$)

Conclusions*:* The present study concluded that measurement of serum AMH at any time after stimulation still predictive of the IVF outcomes which will reinforce the already known value of AMH in clinical practice.

Introduction

Anti-Mullerian hormone (AMH) is a member of the transforming growth factor-β superfamily (1). Recently, it has been suggest that AMH acts as direct biochemical marker of ovarian reserve as well as a regulator of folliculogenesis and oocyte maturation (2).

Many studies had been carried out on the basal AMH level and its association with controlled ovarian hyperstimulation (COH) outcome. It has been shown that the basal AMH level is correlated with antral follicle count (AFC), total dose of gonadotrophins used, duration of COH, estradiol level on hCG day, the number of mature follicles on hCG day and the number of oocytes retrieved (3-5). Moreover, the AMH level was found to be positively related to pregnancy in COH cycles (6, 7). It had been also suggested that the serum AMH level could predict poor response and ovarian hyperstimulation syndrome for IVF cycles (8, 9). Serum AMH levels show no fluctuation throughout the menstrual cycle, and the AMH level had been shown to be correlate with ovarian response to gonadotrophin stimulation independent of the days of the menstrual cycle (8, 10).

Several provocative tests had been developed to indirectly assess ovarian reserve and identify patients who might not be define by basal hormone screening alone. However, whether these indirect provocative tests are more informative of ovarian reserve than basal hormone remains controversial. Furthermore, neither basal hormonal measurements nor those dynamic tests are capable of providing direct manifestation concerning the outcomes of the exogenous gonadotropin used in ovarian stimulation for ART (11-15).

To clarify these issues, studies had been perform on changes in serum AMH levels during COH cycle, however; most of these studies were conducted only during GnRH agonist cycles and either follicular or luteal phases of the COH cycle (16-20). To the best of our knowledge, there were no studies on AMH changes over the entire COH period, including days before human chorionic gonadotrophin (HCG) administration, and during GnRH antagonist cycles. Therefore, the current study aim is to assess the predictive values of basal and consecutive serum AMH level during IVF stimulation.

Serum AMH levels show no fluctuation throughout the menstrual cycle, and the AMH level had been shown to be correlate with ovarian response to gonadotrophin stimulation independent of the days of the menstrual cycle (8, 10). In another study, using different

measurement timing, the AMH levels during the menstrual cycle showed a correlation with the outcomes similar to that observed following COH cycles (21). However, during COH cycles, the serum AMH level changes throughout the cycle; thus, this correlation with COH outcomes could depend on the timing of the measurement. Only a few studies have been conducted on the correlation between COH outcomes and AMH levels measured on different stimulation days (16, 22, 23), and these studies did not show a comparison between different measurement timings over the entire period of the COH cycle. Therefore, the current study aim is to assess the predictive values of basal and consecutive serum AMH levels during COH cycle.

Material and methods

One hundred women were involved in this cohort study, scheduled for IVF program, between December 2015 until January 2017, blood samples were collected for measurement of AMH, Estradiol, and FSH on day 2 of menstrual cycle, and the subsequent samples on day 4,6,8 and 10 were taking after the stimulation of ovaries with gonadotrophin.

Venous blood samples (5-10ml) were collected in tubes containing no anticoagulant. Samples were allowed to clot at 37˚C; then centrifuged at 3000 rpm for more than10 minutes to separate the cellular component. Sera were removed and stored in aliquots at -20 C until analyses. Lipemic or hemolyzed samples were eliminated. All hormones being analyzed by using electrochemiluminescence methods (Cobas 411 by Roche) as a single batch, each patient was given a unique numerical identifier, which issued in data analysis.

Written consent for the treatment by IVF/ICSI and enrollment for research were taken from each patient, inclusion criteria were a patient age under 46 years, normal pretreatment hormonal values, presence of two ovaries, gynecological ultrasound results, and cervical smears. The exclusion criteria included none of the women had received sex steroids or any drug known to affect ovarian function for at least 6 months, no previous ovarian surgery, acute or chronic infectious diseases of the woman or her partner, severe psychiatric illnesses, no endocrine abnormalities including hyperprolactinaemia or the presence of other concomitant chronic conditions such as genetic syndromes, celiac disease, renal disease, liver disease, thyroid disorder, diabetic mellitus, cancer, ischemic heart diseases, nephrotic

syndrome they were not included and excluded from the current study. Menstrual cycles were consider as irregular if they were longer than 35 days or shorter than 25 days or when the length difference between two successive cycles was greater than 7 days.

Statistical analysis:

Data is translate into codes using a specially designed coding sheet, and then converted to computerized database. An expert statistical advice was taken and statistical analyses were done using SPSS (Statistical Package for Social Science) (version 19 Chicago, USA) and MedCalc (version 12.2.1.0, Mariakerke, Belgium) computer software. The variables were assessed by the chi square test, D'Agostino-Pearson and Kolmogorov-Smirnov test for normal distribution. The receiver operative characteristic (ROC) curve was use for predicting the IVF outcomes paired T test was use for comparing two variables. P values <0.05 were considered significant.

Results

The frequency distribution, Kolmogorov-Simirnov test and D'Agostino-Pearson test of all the variables show that they were normally distributed (P<0.05) (data not shown), therefore parametrical methods were applied for subsequent statistical analyses.

Table 1 showed the patients' characteristics , basal serum estradiol (E2), FSH and AMH levels at day 2 of cycle and subsequent days after stimulation . As expected significant differences were observed for total dosage of FSH , peak E2 levels and duration of stimulation between short and long GnRH agonist groups

The reciver operative charactertic (ROC) curve was used to assess the AMH values in different days (day 2, 4, 6, 8, and 10) for predction of IVF outcomes (implantation, abortion, preganancy and live birth). All the days showed significant area under the curve (AUC) (p<0.05) figures 1to 4. However, when all the ROC curves were comapred to each other there were no significance differences bwteeen them (p>0.05) tables 2 to 5.

Discussion

In recent years, many studies had been performing on the basal AMH level and its association with COH outcomes. Only few studies have been conducted on the correlation between COH outcomes and AMH levels measured on different stimulation days (16, 22, 23). However, these studies neither show a comparison between different measurement timings of serum AMH over the entire period of the

COH cycle nor timing effectiveness on IVF outcomes.

The present study results showed no significant changes in serum AMH at basal and subsequent days after stimulation and that serum AMH levels significantly predict ($P<0.05$) the IVF outcomes (implantation, abortion, preganancy and live birth) at basal and sequential measurements after gonadotrophin stimulation. Data available in the literature with respect to this issue are limited. The current study results are in keeping with the results of Eldar-Geva1T. *et al* study who found that early follicular and mid-luteal serum AMH levels were very similar (17) as well as in agreement with Elgindy E. A *et al*. study, by using different measurement timing, found that the midluteal and early AMH levels were statistically significant predictors of clinical pregnancy (21). Even so, the present study results diverge from preliminary data reported by others (17, 19) that showed decline in serum AMH levels during COH cycles.

The sources of conflict between these studies and the present study may include characteristics of the study population, variations in the study design, and differences of statistical methods as well as the smaller sample sizes used by previous studies, which may affect the accuracy of their results. Another factor that might be involved in the ontogenesis of AMH is different stimulation protocols used in each study.

The present study concluded that measurement of serum AMH at any time after stimulation still predictive of the IVF outcomes, which will reinforce the already known value of AMH in clinical practice. Moreover, this new finding may resolved missed basal AMH measurement due to any various reasons and will be more convenient to the patient and the clinician

Variables	Short GnRH agonist (n= 77)	Long GnRH Agonist (n= 23)	P-value

Table 1: Clinical and biochemical characteristics of the study patients

Age (years)	**36.23 ±7.0**	**35.50±6.93**	**0.66**
BMI (kg/m2)	**25±3**	**26±2**	**0.13**
Estradiol (pg/ml)	**44.02±30.51**	**35.26±18.55**	**0.19**
FSH (IU/I)	**8.57±3.36**	**6.45±3.62**	**0.01**
AMH Day 2 (ng/ml)	**2.01±1.67**	**1.75±1.27**	**0.49**
Duration of infertility (years)	**8.85±5.19**	**10.62±7.69**	**0.07**
Types of infertility			
Primary infertility	**65 (84.4%)**	**19 (82.6) %**	**0.8**
Secondary infertility	**12 (15.6%)**	**4 (17.3%)**	**0.85**
Cause of infertility			
Male factor	**5 (6.5 %)**	**2 (7.1%)**	**0.72**
Female factor	**10 (12.9%)**	**2 (7.1%)**	**0.73**
Male and female factor	**53 (68.8%)**	**17 (78.6%)**	**0.6**
Unexplained factor	**9 (11.7%)**	**2 (7.1%)**	**0.89**
IVF outcomes			
Duration of stimulation (days)	**11.20±0.96**	**9.29±5.08**	**0.002**
Total dosage of FSH (IU)	**2924.22±986.51**	**3369.64±1203.77**	**0.07**
Peak estradiol (pg/ml)	**1554.88± 933.74**	**937.96±758.49**	**0.004**
Implantation	**65 (84.3%)**	**19 (82.6%)**	**0.75**
Clinical pregnancy	**15 (20.3%)**	**8 (34.7%)**	**0.85**
Live birth	**8 (10.9%)**	**3 (13.1 %)**	**0.33**
Abortion	**7 (9%)**	**5 (21.7 %)**	**0.79**
AMH (ng/ml) Day 4	**2.1±1.63**	**1.77±1.3**	**0.37**
AMH (ng/ml) Day 6	**2.01±1.56**	**1.64±1.76**	**0.33**
AMH (ng/ml) Day 8	**2.07±1.55**	**1.89±2.3**	**0.66**
AMH (ng/ml) Day 10	**2.05±1.54**	**1.91±2.1**	**0.72**

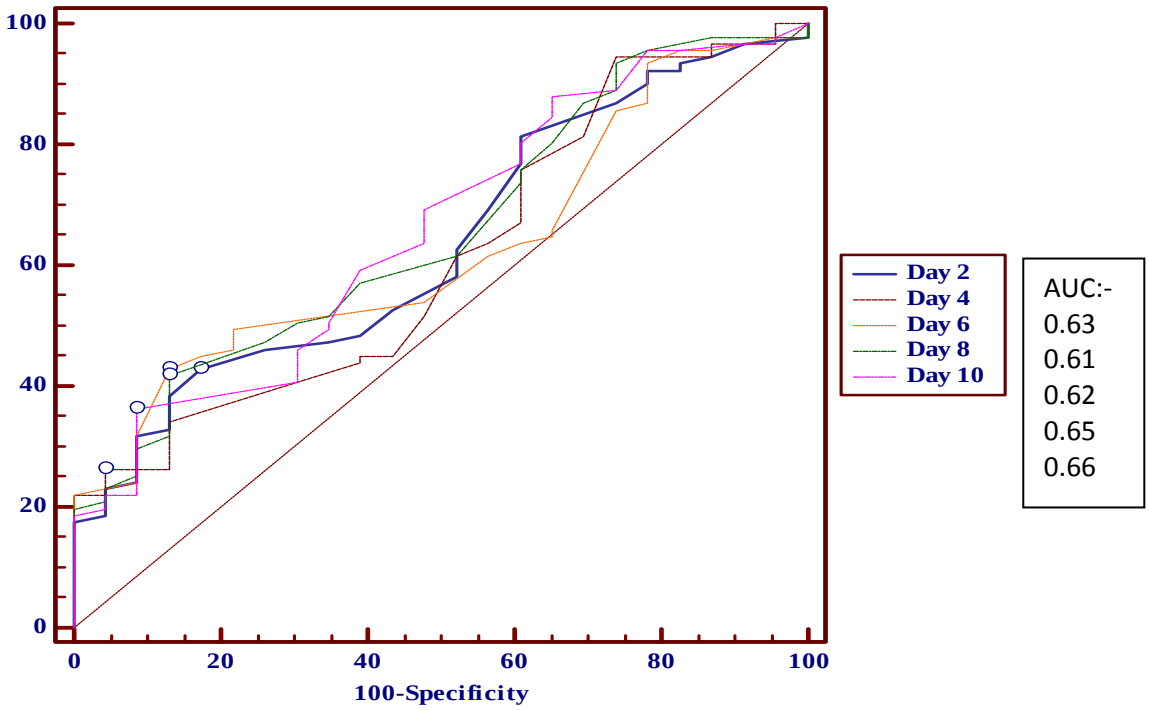

Figure 1: ROC curve of AMH value of different days in prediction of implantation.

Table 2: Pairwise comparison of various AMH ROC curves of different days in favor of implantation

Paired ROC curves	Paired T test	P value
Day 2 Vs Day 4	0.73	0.46
Day 2 Vs Day 6	0.27	0.78
Day 2 Vs Day 8	0.66	0.50
Day 2 Vs Day 10	0.60	0.54
Day 4 Vs Day 6	0.55	0.57
Day 4 Vs Day 8	1.4	0.13
Day 4 Vs Day 10	1.3	0.17
Day 6 Vs Day 8	1.7	0.08
Day 6 Vs Day 10	1.08	0.27

Day 8 Vs Day 10	0.90	0.90

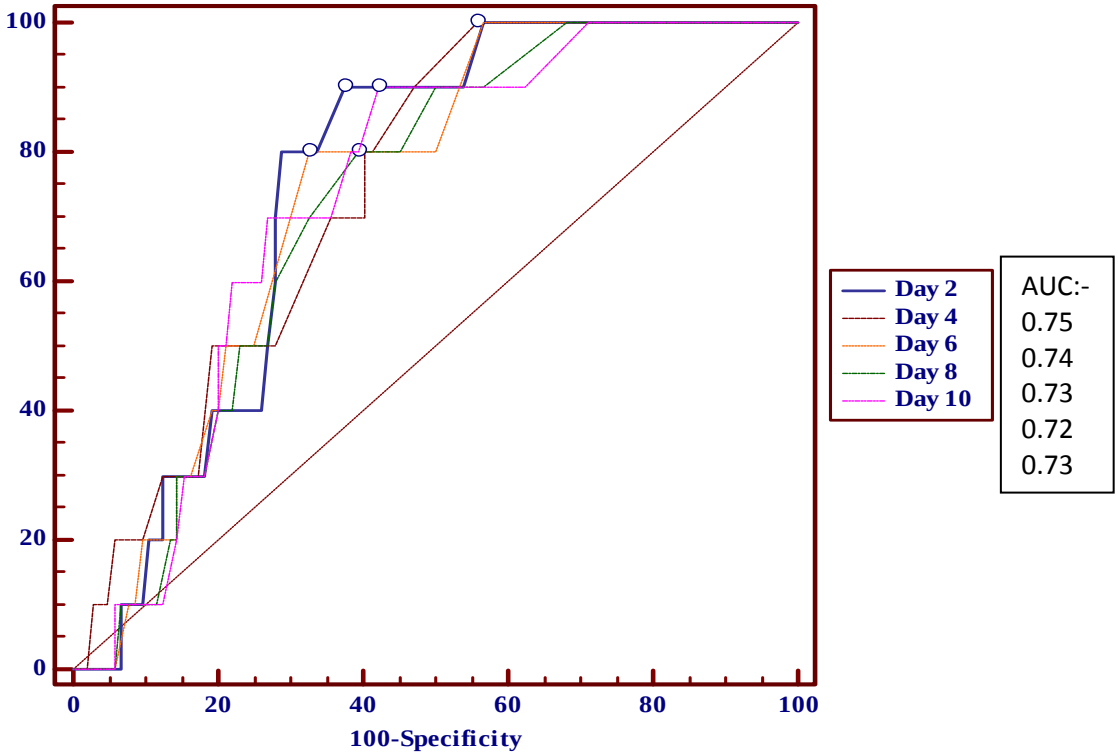

Figure 2: ROC curve of AMH value of different days in prediction of abortion.

Table 3: Pairwise comparison of various AMH ROC curves of different days in favor of abortion

Paired ROC curves	Paired T test	P value
Day 2 Vs Day 4	0.16	0.87
Day 2 Vs Day 6	0.59	0.54
Day 2 Vs Day 8	1.5	0.11
Day 2 Vs Day 10	0.43	0.66
Day 4 Vs Day 6	0.18	0.85
Day 4 Vs Day 8	0.67	0.50
Day 4 Vs Day 10	0.24	0.80
Day 6 Vs Day 8	1.15	0.24
Day 6 Vs Day 10	0.17	0.85

Day 8 Vs Day 10	0.38	0.69

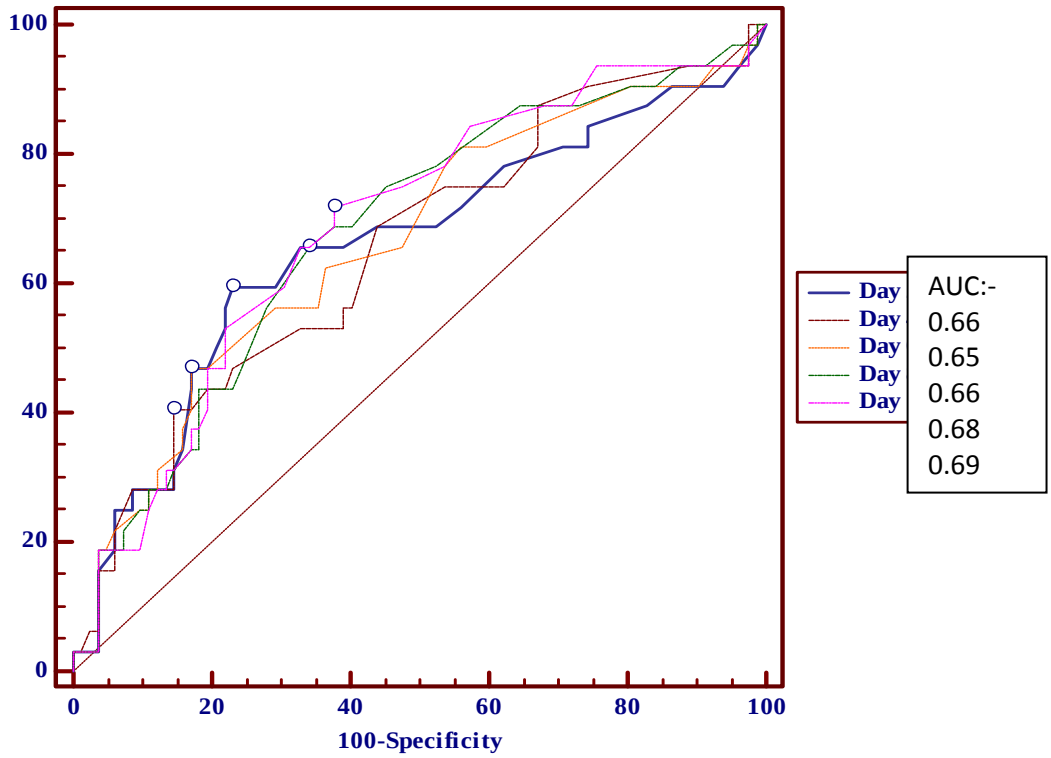

Figure 3: ROC curve of AMH value of different days in prediction of pregnancy.

Table 4: Pairwise comparison of various AMH ROC curve of different days in favor of pregnancy

Paired ROC curves	Paired T test	P value
Day 2 Vs Day 4	0.14	0.88
Day 2 Vs Day 6	0.26	0.79
Day 2 Vs Day 8	0.91	0.36
Day 2 Vs Day 10	1.02	0.30
Day 4 Vs Day 6	0.37	0.70
Day 4 Vs Day 8	0.88	0.37
Day 4 Vs Day 10	1.0	0.31
Day 6 Vs Day 8	1.1	0.26
Day 6 Vs Day 10	1.2	0.22

Day 8 Vs Day 10	0.5	0.60

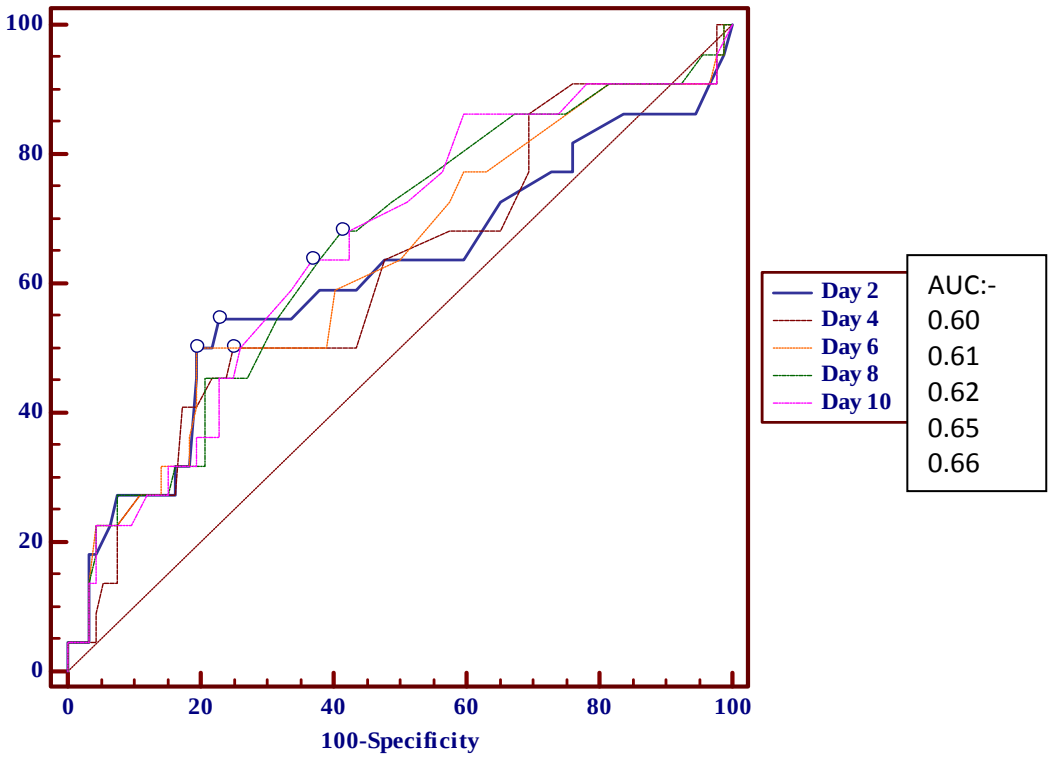

Figure 4: ROC curve of AMH value of different days in prediction of live birth.

Table 5: Pairwise comparison of various AMH ROC curve of different days in favor of live birth

Paired ROC curves	Paired T test	P value
Day 2 Vs Day 4	0.005	0.99
Day 2 Vs Day 6	0.7	0.44
Day 2 Vs Day 8	1.5	0.11
Day 2 Vs Day 10	1.3	0.18
Day 4 Vs Day 6	0.5	0.57
Day 4 Vs Day 8	1.17	0.23
Day 4 Vs Day 10	1.06	0.28

Day 6 Vs Day 8	1.47	0.14
Day 6 Vs Day 10	1.11	0.26
Day 8 Vs Day 10	0.03	0.97

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قدرة التنبؤ للقياسات المتتابعة لهرمون ضد المولاري خلال الاخصاب الخارجي

المدرس الدكتور سامان حسين نوري

الخلفيـــة : اجريـــت عـــدت دراســـات فـــي الســـنوات الاخيـــرة علـــى مســـتوى الاساســـي لهرمـــون ضـــد المـــوليري وعلاقتـــة بمخرجـــات تحفيـــز المبـــايض المســـيطر عليـــه. بينمـــا القليـــل مـــن الدراســـات قـــد اجريـــت غلـــى علاقـــة هـــذا الهرمـــون مـــع مخرجـــات التحفيـــز للمبـــايض خـــلال الايـــام التـــي تلـــي التحفيـــز، وان هـــذه الدراســـات لـــم تبـــين وجـــود فروقـــات فـــي اوقـــات القيـــاس المختلفـــة خـــلال دورة تحفيـــز المبـــايض المســـيطر عليه.

الاهـــداف : تهـــدف الدراســـة الحاليـــة الـــى التحـــري عـــن القـــيم التخمينيـــة لكـــل مـــن المســـتوى الاساســـي وامتسلسل بعد التحفيز لهرمون ضد المولاري خلال فترة التحفيز للمبايض.

طرائـــق عمـــل البحـــث : ضـــمت الدراســـة ١٠٠ امـــرأة تـــم اختيـــارهن لبرنـــامج الاخصـــاب الخـــارجي فـــي مركـــز داوروز للاخصـــاب فـــي الســـليمانية فـــي الفتـــرة مـــن كـــانون الاول ٢٠١٥ لغايـــة كـــانون الثـــاني ٢٠١٧ . جمعـــت عينـــات دم لغـــرض قيـــاس مســـتوى هرمـــون ضـــد المـــولاري ، الاســـترادايوول ، الهرمـــون المحفـــز للحجيـــرات، فـــي كـــل مـــن اليـــوم الثـــاني للـــدورة الشـــهرية كمســـتوى اســـاس ومـــاتلاه مـــن الايـــام بعـــد التحفيـــز بالهرمونـــات الكونـــادوتروبين (اليـــوم الرابـــع، الســـادس، الثـــامن و العاشـــر).

تـــم اســـتخدام طريقـــة التلالـــؤالالكتروني للتحـــري عـــن جميـــع الهرمونـــات المدروســـة فـــي البحـــث (جهـــاز كـــوبس ٤١١ اي مـــن شـــركة روش) . اعطـــي كـــل مـــريض داخـــل فـــي الدراســـة رقمـــا تعريفيـــا وادخـــل ضـــمن التحليـــل الرقمـــي. تـــم اعتبارقيمـــة الاحتماليـــة اقـــل مـــن ٥ بالمئـــة كقيمـــة معنويـــة.

النتـــائج : مـــن خـــلال جـــداول واشـــكال بيانيـــة تـــم ادراج وايضـــاح مميـــزات المرضـــى ومســـتوى هرمـــون ضـــد المـــولاري ، الاســـترادايوول ، الهرمـــون المحفـــز للحجيـــرات، فـــي كـــل مـــن اليـــوم الثـــاني للـــدورة الشـــهرية ومـــاتلاه مـــن الايـــام بعـــد التحفيـــز. بينـــت الدراســـة وجـــود فروقـــات معنويـــة لكـــل مـــن جرعـــة الهرمـــون المحفـــز للحجيـــرات و مســـتويات الاســـترادايول العليـــا وفتـــرات التحفيـــز مـــا بـــين مجمـــوعتي هرمونـــات التحـــرر الكونـــادوتروبين القصـــيرة والطويلـــة. تـــم اســـتخدام منحنـــى روك لتقـــدير قـــيم هرمـــون ضـــد المـــولاري فـــي الايـــام المختلفـــة قبـــل و بعـــد التحفيـــز (اليـــوم الثـــاني و الرابـــع و الســـادس و الثـــامن و العاشـــر) للتنبـــؤ عـــن مخرجـــات الاخصـــاب الخـــارجي (الانبـــات و الاجهـــاض و الحمـــل و الـــولادات الحيـــة). اظهـــرت الدراســـة وجـــود عينـــي لمنحنـــى روك فـــي جميـــع هـــذه الايـــام بينمـــا لـــم يلاحـــظ وجـــود فروقات عينية لمستوى الهرمون عند مقارنة منحنى روك فيما بينها.

الاســـتنتاج : ان تقـــدير مســـتوى هرمـــون ضـــد المـــولاري المصـــلي بعـــد عمليـــة تحفيـــز المبـــايض لازال يعـــــــــــــــد معيـــــــــــــــارا لمخرجـــــــــــــــات الاخصـــــــــــــــاب الخـــــــــــــــارجي