The Egyptian Journal of Hospital Medicine (October 2017) Vol.69(1), Page 1646-1654

Comparative Study between Measuring FSH, LH and E2 on Day Two and on Any Day of the Cycle

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ABSTRACT

Background: evaluation of ovarian reserve has become an essential part of the treatment assessment of woman to undergo assisted reproductive technique. **Aim of the Work:** this work was conducted to measure FSH, LH and E2 at any day of the whole menstrual cycle compared to day2 with measurement and establishing an actual negative correlation between FSH & E2. **Patients and methods:** observational analytical prospective study on 50 women attending the Gynecology Outpatient Clinics of Maternity Hospital, Ain-Shams University in 2016. **Results:** there was a significant negative correlation between E2/FSH on the 3rd, 5th and 10th days of the cycle. Also there was insignificant negative correlation on day 21. **Conclusion:** there was negative correlation between basal day2 E2/FSH which was equivalent or similar to that ratio on days (5,10,21) so there was no need to wait for hormonal analysis to the next cycle (day 2 or 3) to save time specially in patients >35years; for them there was importance of cycle day3 for evaluation of ovarian reserve and prior ovulation induction and subsequent pregnancy potential during the infertility work up. **Recommendations:** during the infertility work up, rigid adherence to cycle day3 collection, no longer seems necessary ,no need to wait for hormonal analysis to Estradiol and FSH to the next cycle as there was equivalent negative correlation between E2/FSH on menstrual cycle days (3,5,10,21) aiming for saving time.

Key words: FSH, LH, E2, menstrual cycle.

INTRODUCTION

The concept of ovarian reserve as assessed by follicle stimulating hormone (FSH) measurement has been proven useful in predicting pregnancy outcome [(1)]. Determination of cycle day 3 FSH has evolved as the standard for predicting oocyte quality and the likelihood of conception in assisted reproductive technology programmers. The information obtained from cycle day 3 FSH testing is invaluable in counselling patients as to their chances of achieving a pregnancy and deciding on options for stimulation protocols [(2)].

Muasher *et al.* [(3)] demonstrated that basal cycle day 3 concentrations of FSH reflected the reproductive potential of that menstrual cycle. This could be applied further to discriminate between patients who would be more likely to respond to ovarian stimulation and those who would not [(4)]. Cycle day 3 testing has emerged as a dictum from this study because most stimulation protocols were initiated on cycle day 3, 4 or 5 [(5,6)]. The validity of testing on other days has not yet been explored. Early follicular phase oestradiol concentrations may reflect die stage of follicular development, with higher concentrations associated with asynchrony of follicular development. An abrupt early rise of oestradiol may be a subtle sign of the shortened follicular phase often seen prior to menopause. The purpose of this study was to evaluate the intra- and inter-cycle variability of serum values of FSH and oestradiol in the early follicular phase (cycle days 2-5) [(7)].

The importance of cycle day 3 FSH for evaluation of ovarian reserve and subsequent pregnancy potential has recently been emphasized in women >35 years during the infertility workup [(1)]. Basal FSH values have been utilized to decide on treatment protocols and to counsel patients as to their potential pregnancy success [(4)]. Many authors attest to the importance of cycle day 3 testing [(8, 9,10)]. The emphasis on cycle day 3 testing seems to have evolved in part from convenience, based on the cycle day 3 starts of most stimulation protocols [(5)]. According to **Hodgen's work** [(11)], early follicular growth and recruitment occur in the beginning of the cycle prior to cycle days 5-7. By day 7 the one follicle destined to ovulate has been selected. Since the objective in ovarian hyperstimulation for assisted reproductive technology is to recruit more than one dominant follicle, stimulation must be initiated prior to the loss of this multipotentiality of the follicles. Before the widespread use of GnRHa, stimulation protocols traditionally began on cycle day 3 or 4. Thus, basal testing had to be performed by cycle day 3. According to the data of **Hansen *et al***. [(12)] testing FSH on any of cycle days 2-5 will give equivalent results, regardless of patient age.

Ovarian reserve tests (ORT) help to predict the response to exogenous gonadotrophin stimulation and the likelihood of success with IVF and they are widely accepted as an essential element of the evaluation of IVF [(13)].

ORT can roughly be divided into three groups: [(14)]

Received:10 / 6 /2017 DOI: 10.12816/0040114
Accepted: 19 /6 /2017

These tests measure early follicular phase hormones level and they include: Female age; cycle day 3 serum FSH concentration; cycle day 3 serum estradiol (E2) concentration; cycle day 10 serum progesterone(P) concentration; cycle day 3 serum inhibin B concentration; Serum Anti-Mullerian Hormone (AMH) concentration and ovarian biopsies.

Basal FSH has been reported to be better predictor of ovarian response in IVF cycles stimulated with gonadotrophins than age [(15)].

As women ages, FSH becomes elevated in an attempt to force the aging ovary to respond. It starts to increase because of reduced inhibin-B and E2 production by the diminished cohort of growing follicles. This event takes place a few years before the actual menopause [(16)].

The cycle day 3 FSH level is one of the most commonly used tests for predicting success in IVF treatment. This was first described by **Muasher *et al.*** [(3)] who demonstrated that women with an elevated cycle day 3 FSH had reduced ovarian reserve. **Abdalla and Thum** [(17)] have shown that women with an elevated FSH level, independent of age, have a poor response to ovarian stimulation, leading to a lower pregnancy rate with assisted reproductive technique (ART).

The basal level of serum FSH is used as a screening test for patients undergoing IVF. It is well documented that a high day 3 basal level of FSH is associated with a lower pregnancy rate. Indeed, some units have been used this test to screen patients with a lower chance of a pregnancy in view of maintaining high clinic success rates [(18)].

In reproductive endocrinology, basal FSH is measured in order to detect women with ovarian failure. FSH measured in serum on day 3 of the menstrual cycle is probably the most widely used test to determine reproductive potential in women [(19)]. From a pathophysiological point of view, large inter-cycle variations in basal FSH remain a frequent problem. Appropriate timing of FSH measurement is difficult for women with irregular periods, such as those with PCOS. Despite appropriately timed methods of sample collection, inter-cycle variations and inter-sample variations within assay and between assays may result in disparate FSH measurements. A wide range (4–25 IU) in threshold values has been used for abnormal levels of basal [(20)].The value of cycle day 3 E2 levels in the prediction of ovarian reserve is still debatable. No data are available on the relationship between day 3 estradiol values and fecundity in spontaneous cycles. Elevated basal estradiol may predict the poor response even when basal FSH is normal. In regularly menstruating women between the ages of 24 and 50 years, no differences in basal estradiol levels have been demonstrated according to age. No relationship has been found between serum estradiol levels and pregnancy rates [(20)].

In patients with normal FSH levels, basal E2 has been shown to predict high cancellation rates and low oocyte yield in IVF. In another study, cancellation rates did correlate with basal E2 levels, but did not correlate with pregnancy outcome in those patients who were not cancelled. Pregnancy rates have also been shown to be higher in a group of women undergoing in vitro maturation. The predictive ability of basal E2 is improved in patients of advanced reproductive age, especially when combined with basal FSH [(21)].

Early follicular phase serum concentrations of FSH and estradiol are generally recognized as markers for ovarian reserve [(22)]. Measurement of basal E2 in addition to FSH might improve the ability to predict fertility potential compared to basal FSH and chronological age alone. Cycle day 3 E2 of <80 pg/ml with normal FSH concentration in women of 38–42 years of age gives a good prognosis of successful treatment [(23)].This study aimed to measure FSH, LH and E2 at any day of the whole (menstrual cycle) compared to mandatory day 2 measurement and establishing an actual negative correlation between FSH &E2.

PATIENT AND METHODS

Study Design:

Observational analytical prospective study.

Study population

This study was included 50 women attending the Gynecology Outpatient Clinics of Maternity Hospital, Ain-Shams University in 2016.

Sample size calculation and justification:

Sample size was calculated using PASS 11.0 sample size calculation program and according to the study carried out by **Leslie *et al*.** [(12)]. The sample size would be increased to include **50** healthy women to measure prospectively follicle stimulating hormone (FSH) and oestradiol between cycle days 2 and 5 as sample size of 8 achieves 82% power to detect the mean of paired differences of 26.6 with a known standard deviation of differences of 26.2 and with a significance level (alpha) of 0.05000 using a two-sided paired z-test and based on the finding that serum oestradiol concentrations began to increase by cycle day 4 such that the mean value on cycle day 4 (202.9 ± 99.3 pmol/ml) was significantly greater than on cycle day 2 (172.4 ±81.0 pmol/ml. *P* - 0.005).

Inclusion criteria

- Women who were eligible to participate in this study were healthy women in the childbearing period with age range (18-49 years) at gynecology clinics.

Exclusion Criteria

- Extremes of age not in childbearing period
- Suspected pregnancy.
- Women under hormonal treatment (Steroid hormones) or ovulation induction or drugs affecting hormonal levels.

METHODOLOGY

For each woman, a study sheet was fulfilled and it included proper history taking, proper examination and ultrasound findings.

Serum samples were obtained on menstrual cycle days 3,5 mid follicular (day 9) and day 21. FSH serum concentrations were determined through a solid phase FSH coated immunoradiometric assay (FSH coat -count, IRMA). Eestradiol determinations were performed using radioimmunoassay (RIA) kit (BioLab-Cairo), the analysis of the hormone was done by single reading and two kits were used in the study.

Ethical committee and confidentiality

Written informed consent was obtained from all participants after explanation of the purpose and the procedure of the study with a strict confidentiality of data. Approval was obtained from the Ethical Committee of Obstetrics and Gynecology Department, Faculty of Medicine, Ain-Shams University.

Conflict of interest

The researcher declares no conflict of interest. No funding was received for this study.

We do not have any commercial association that might pose a conflict of interest in connection with the manuscript. We certify that neither this manuscript nor one with substantially similar content under our authorship has been published or is being considered for publication elsewhere.

Statistical analysis

Data Management and Analysis:

The collected data were revised, coded, tabulated and introduced to a PC using statistical package for social sciences (SPSS 20.0 for windows; SPSS Inc, Chicago, IL, 2001). Data were presented and suitable analysis was done according to the type of data obtained for each parameter.

I- Descriptive Statistics:

1. Mean, standard deviation (+ SD) and range for parametric numerical data, while median and interquartile range (IQR) for non parametric data were considered.
2. Frequency and percentage of non-numerical data. were considered.

II- Analytical Statistics

1- Pearson Correlation Coefficient (r): correlation was used as a measure of the strength of a linear association between two quantitative variables. The Pearson correlation coefficient, r, can take a range of values from +1 to -I. A value of 0 indicates that there was no association between the two variables. A value greater than 0 indicates a positive association; that is, as the value of one variable increases, so does the value of the other variable. A value less than 0 indicates a negative association; that is, as the value of one variable increases, the value of the other variable decreases.

2- Paired t-test was used to compare mean estradiol level at baseline and in selected days after that (compare measurement before and after)

- P>0.05: Non significant (NS)
- PO.05: Significant (S)
- P<0.01: Highly significant (HS)

RESULTS

Table 1: frequency distribution of the studied patients as regard their ages

Age categories	No	%
Less than 30	30	60.0%
More than 30	20	40.0%
Age	**Mean + SD**	**Range**
	28.90 + 5.77	(18.00-41.00)

Table 3 and figure 2 showed that 60.0% of the studied patients were less than 30 years; while 40.0% were more than 30 years. In addition, the mean age was 28.90 + 5.77 and it ranged between 18.00-41.00.

Table 2: frequency distribution of the studied patients as regard history of PCO

History of PCO	No	%
Negative	37	74.0%
Positive	13	26.0%

Table 4 and figure 3 showed that 74.0% of the studied patients were negative PCO, while 26.0% showed positive PCO.

Table 3: frequency distribution of the studied patients as regard history of infertility

History of Infertility	No	%
Negative	19	38.0%
Positive	31	62.0%

Table 5 and figure 4 showed that 74.0% of the studied patients had negative history of cycle irregularity, while 26.0% had positive history of cycle irregularity.

Table 4: frequency distribution of the studied patients as regard cycle irregularity

History of Cycle irregularity	No	%
Negative	37	74.0%
Positive	13	26.0%

Table 6 and figure 5 showed that 74.0% of the studied patients had negative history of cycle irregularity, while 26.0% had positive history of cycle irregularity.

Table 5: frequency distribution of the studied patients as regard Parity

Parity	No	%
Negative	23	46.0%
Positive	27	54.0%

Table 7 and figure 6 showed that 46.0% of the studied patients had negative parity, while 54.0% had positive parity.

Table 6: description of the studied hormone values

Variables	Minimum	Maximum	Mean + Std. Deviation
FSH_A	2.80	13.60	6.75+2.33
FSH_B	3.30	14.00	7.26+2.37
FSH_C	4.20	13.60	8.48+2.33
FSH_D	3.20	13.60	5.69+1.93
LH_A	2.17	19.50	5.33+2.99
LH_B	2.70	21.20	5.94+3.28
LH_C	3.20	24.00	7.46+2.32
LH_D	2.50	9.60	4.91+1.92
E2_A	21.80	76.30	43.46+14.58
E2_B	40.50	89.60	62.68+13.51
E2_C	51.80	190.60	107.44+28.83
E2_D	85.30	223.90	129.41+34.59
E2/FSH_A	2.22	21.29	7.51+2.41
E2/FSH_B	3.78	20.48	9.77+2.41
E2/FSH_C	4.98	41.80	13.91+3.37
E2/FSH_D	7.27	46.91	25.05+10.18

Table 8 showed that the mean of FSH A was 6.75+2.33, while the mean of FSH B was 7.26+2.37; the mean of FSH C was 8.48+2.33 and the mean FSH D was 5.69+1.93).The mean of LH A was 5.33+2.99; the of mean LH B was 5.94+3.28 .The mean of LH C was 7.46+4.32 and the mean of LH D was 4.91+1.92. In addition to that; the mean of E2 A was 43.46+14.58; the mean of E2 B was 62.68+13.51; the mean of E2 C was 107.44+28.83 and the mean of E2 D was 129.41+34.59. Moreover, the mean of E2/FSH A was 7.51+4.41, while the mean of E2/FSH B was 9.77+4.41; the mean of E2/FSH C was (13.91+6.37) and the mean of E2/FSH D was 25.05+10.18.

Table 7: comparison between E2/FSH Levels at the 1st, 2nd, 3rd and 4th samples

E2/FSH levels		Paired Differences					Wilcoxon sign rank test	P-value
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference			
					Lower	Upper		
Pair 1	**E2/FSH _A - E2/FSH _B**	-2.25786	1.50710	.21314	-2.68617	-1.82954	-5.980	.001**
Pair 2	**E2/FSH _A - E2/FSH _C**	-6.39769	6.87714	.97257	-8.35215	-4.44323	-5.614	.001**
Pair 3	**E2/FSH _A - E2/FSH _D**	-17.53753	9.35626	1.32318	-20.19655	-14.87851	-6.144	.001**
Pair 4	**E2/FSH _B - E2/FSH _C**	-4.13984	6.97747	.98676	-6.12281	-2.15686	-4.407	.001**
Pair 5	**E2/FSH _B - E2/FSH _D**	-15.27968	9.13327	1.29164	-17.87532	-12.68403	-6.125	.001**
Pair 6	**E2/FSH _C - E2/FSH _D**	-11.13984	10.23789	1.44786	-14.04942	-8.23027	-5.256	.001**

Table 7 showed that there was a highly statistically significant difference between the values of **E2/FSH** at 1st and 2nd samples, 1st and 3rd samples, 1st and 4th samples, 2nd and 3rd samples, 2nd and 4th samples and 3rd and 4th samples (P<0.01).

Table 8: correlation between E2/FSH Levels (1st sample) and age

			E2/FSH_A	Age
Spearman's rho	**E2/FSH_A**	**Correlation Coefficient**	1	-.269
		Sig. (2-tailed)		.059
	Age	**Correlation Coefficient**	-.269	1
		Sig. (2-tailed)	.059	
*. Correlation is significant at the 0.05 level (2-tailed)				
**. Correlation is significant at the 0.01 level (2-tailed)				

Table 8 showed that there was a statistically insignificant negative correlation between E2/FSH at 1st sample and patients' Age (P>0.05).

Table 9: correlation between E2/FSH Levels (1st sample) and FSH, E2 (1st sample)

			E2/FSH_A	FSH_A	E2_A
Spearman's rho	**E2/FSH_A**	Correlation Coefficient	1	-.721	.798
		Sig. (2-tailed)		.001**	.001**
	FSH_A	Correlation Coefficient	-.721	1	-.337
		Sig. (2-tailed)	.001**		.017*
	E2_A	Correlation Coefficient	.798	-.337	1
		Sig. (2-tailed)	.001**	.017*	
*. Correlation is significant at the 0.05 level (2-tailed).					
**. Correlation is significant at the 0.01 level (2-tailed).					

Table 9 showed that there was a highly statistically significant negative correlation between E2/FSH at 1st sample and FSH at 1st sample (P<0.01). In addition to that, there was a highly statistically significant positive correlation between E2/FSH at 1st sample and E2 at 1st sample (P<0.01). Moreover; there was a statistically significant negative correlation between E2 at 1st sample and FSH at 1st sample (P<0.05).

Table 10: correlation between E2/FSH Levels (2nd sample) and FSH, E2 (1ST sample)

			E2/FSH_B	FSH_A	E2_A
-+ 35Spearman's rho	**E2/FSH_B**	Correlation Coefficient	1	-.795	.678
		Sig. (2-tailed)		.001**	.001**
	FSH_A	Correlation Coefficient	-.795	1	-.337
		Sig. (2-tailed)	.000**		.017*
	E2_A	Correlation Coefficient	.678	-.337	1
		Sig. (2-tailed)	.001**	.017*	
*. Correlation is significant at the 0.05 level (2-tailed)					
**. Correlation is significant at the 0.01 level (2-tailed)					

Table 10 showed that there was a highly statistically significant negative correlation between E2/FSH at 2nd sample and FSH at 1st sample (P<0.01). In addition to that, there was a highly statistically significant positive correlation between E2/FSH at 2nd sample and E2 at 1st sample (P<0.01). Moreover, there was a statistically significant negative correlation between E2 at 1st sample and FSH at 1st sample (P<0.05).

Table 11: correlation between E2/FSH Levels (3rd sample) and FSH, E2 (1ST sample)

			E2/FSH_C	FSH_A	E2_A
Spearman's rho	**E2/FSH_C**	Correlation Coefficient	1	-.337	.032
		Sig. (2-tailed)		.017*	.824
	FSH_A	Correlation Coefficient	-.337	1	.417
		Sig. (2-tailed)	.017*		.003**
	E2_A	Correlation Coefficient	.032	.417	1
		Sig. (2-tailed)	.824	.003**	
*. Correlation is significant at the 0.05 level (2-tailed)					
**. Correlation is significant at the 0.01 level (2-tailed)					

Table 11 showed that there was a statistically significant negative correlation between E2/FSH at 3rd sample and FSH at 1st sample (P<0.05). In addition to that, there was a statistically insignificant positive correlation between E2/FSH at 3rd sample and E2 at 1st sample (P>0.05). Moreover, there was a highly statistically significant negative correlation between E2 at 1st sample and FSH at 1st sample (P<0.01).

Table 12: correlation between E2/FSH Levels (4th sample) and FSH, E2 (1ST sample)

			E2/FSH_D	FSH_A	E2_A
Spearman's rho	E2/FSH_D	Correlation Coefficient	1	-.214	.322
		Sig. (2-tailed)		.136	.022*
	FSH_A	Correlation Coefficient	-.214	1	-.337
		Sig. (2-tailed)	.136		.017*
	E2_A	Correlation Coefficient	.322	-.337	1
		Sig. (2-tailed)	.022*	.017*	
*. Correlation is significant at the 0.05 level (2-tailed).					
**. Correlation is significant at the 0.01 level (2-tailed).					

Table 12 showed that there was a statistically insignificant negative correlation between E2/FSH at 4th sample and FSH at 1st sample (P>0.05). In addition to that, there was a statistically significant positive correlation between E2/FSH at 4th sample and E2 at 1st sample (P>0.05). Moreover, there was a statistically significant negative correlation between E2 at 1st sample and FSH at 1st sample (P<0.05).

Table 13:comparison between E2/FSH Ratio at 1st, 2nd, 3rd and 4th samples as regard history of PCO

E2/FSH Ratio	History of PCO				Mann Whitney U test	P-value
	Negative (n=37)		Positive (n=13)			
	Mean	Standard Deviation	Mean	Standard Deviation		
E2/FSH_A	7.68	2.56	7.04	2.07	235.000	0.903
E2/FSH_B	10.09	2.57	8.85	3.94	201.000	0.382
E2/FSH_C	14.07	3.88	13.44	4.79	232.000	0.851
E2/FSH_D	24.33	4.67	27.09	8.69	193.000	0.293

Table 13 showed that there is a statistically insignificant difference between E2/FSH Ratio at 1st, 2nd, 3rd and 4th samples as regard history of PCO (P>0.05).

Table 14: comparison between E2/FSH Ratio at 1st, 2nd, 3rd and 4th samples as regard history of Infertility

E2/FSH Ratio	History of Infertility				Mann Whitney U test	P-value
	Negative (n=19)		Positive (n=31)			
	Mean	Standard Deviation	Mean	Standard Deviation		
E2/FSH_A	7.35	2.50	7.61	2.93	283.000	0.818
E2/FSH_B	9.72	2.29	9.80	2.02	268.000	0.596
E2/FSH_C	14.43	4.68	13.59	5253	282.000	0.803
E2/FSH_D	24.62	4.53	25.31	4.71	270.000	0.624

Table 14 showed that there was a statistically insignificant difference between E2/FSH ratio at 1st, 2nd, 3rd and 4th samples as regard history of infertility (P>0.05).

Table 15: comparison between E2/FSH Ratio at 1st, 2nd, 3rd and 4th samples as regard cycle irregularity

E2/FSH Ratio	Cycle Irregularity				Mann Whitney U test	P-value
	Negative (n=37)		Positive (n=13)			
	Mean	Standard Deviation	Mean	Standard Deviation		
E2/FSH_A	7.28	2.54	8.17	2.11	188.000	0.246
E2/FSH_B	9.46	2.34	10.64	2.67	203.000	0.407
E2/FSH_C	13.79	3.98	14.24	4.37	213.000	0.543
E2/FSH_D	23.77	4.73	28.67	7.66	150.000	0.045*

Table 15 showed that there is a statistically insignificant difference between E2/FSH ratio at 1st, 2nd, 3rd samples as regard cycle irregularity (P>0.05). However, there was a statistically significant difference between E2/FSH ratio at 4th sample (P<0.05).

Table 16: comparison between E2/FSH Ratio at 1st, 2nd, 3rd and 4th samples as regard Parity

E2/FSH Ratio	Parity				Mann Whitney U test	P-value
	Negative (n=23)		Positive (n=27)			
	Mean	Standard Deviation	Mean	Standard Deviation		
E2/FSH_A	8.41	2.87	6.75	2.90	236.500	0.150
E2/FSH_B	10.80	2.97	8.89	2.74	247.000	0.216
E2/FSH_C	15.15	3.08	12.85	3.21	203.000	0.036*
E2/FSH_D	27.12	4.23	23.28	9.99	237.000	0.152

Table 16 showed that there is a statistically insignificant difference between E2/FSH ratio at 1st, 2nd, 4th samples as regard parity (P>0.05). However, there was a statistically significant difference between E2/FSH ratio at the 3rd sample (P<0.05).

DISCUSSION

This current observational analytical prospective study was conducted at the Gynecology Clinic and Assisted Reproductive Technology Unit , Ain Shams University Maternity Hospital including 50 women .They were in the childbearing period with age range 18-49)years old; extremes of age, women who were under hormonal treatment, ovulation induction ,drugs affecting hormonal levels were excluded. Serum samples were obtained on menstrual cycle days 3,5,10,21 for each woman.

This study consumed or was for long time for obtaining these 4 samples from each woman without missing any of sample as regard patient compliance for follow up with the woman 4 times per month and there were many serum samples loss because of long time for the study. But this long time also had its benefits via long time in contact with laboratory physicians and patients.
Serum samples were analyzed in the Royal Lab and Laboratory Research Unit of Faculty of Medicine, Ain Shams University.

The aim of the study was to compare the correlation between the hormonal levels E2/FSH on different menstrual cycle days 3, 5, 10, 21 if it is equivalent or different.

The finding results will add no more adherence to cycle (day 3) collection no longer more necessary and will add flexibility for both patients and physicians in evaluation of infertile couple regarding premature ovarian failure, menopausal transition period and ovarian reserve that allow saving time specially to patients undergoing assisted reproductive technology (ART) because of time is extremely important, already was consumed for previous investigations.

Hormones from the pituitary and ovaries are largely responsible for the follicular and development and understanding of the cyclic changes in these hormones throughout the normal menstrual cycle be of use in monitoring hormonal levels in women undergoing assisted reproduction [(24)].

FSH and estradiol are measured from cycle day2 through day5 in order to evaluate the ovarian reserve and to investigate the variability between the menstrual cycles in normally cycling women. Day2 and day3 measurements of FSH and estradiol combined with maternal age are useful for predicting pregnancy outcome these measurement are standard practice for predicting oocyte quality and likelihood of conception in assisted reproductive technologies basal cycle

day3 FSH and LH have a strong predictive value that in useful for choosing a cycle for controlled ovarian hyperstimulation [25].

In a study of patients undergoing (IVF) pregnancy rates decreased significantly at FSH levels greater than (15 mIU/ml) at cycle day3 [26].

In another study of 592 patients undergoing controlled ovarian hyperstimulation pregnancy rate was the highest in patient with the lowest FSH and Estradiol levels [27].

In results based on **Siemens study** [28] conducted on 29 normally cycling women during the early follicular phase showed FSH levels:

Cycle day

Day	FSH(mIU/ml)
2	5.7 ± 1.9
3	5.9 ± 2.1
4	6.3 ± 2.4
5	6.3 ± 2.5

According to **Hansen *et al.*** [7] study the purpose of their study was to evaluate the intra and inter cycle variability of serum values of FSH and estradiol in the early follicular phase (cycle day 2-5) to determine if greater flexibility in testing days was possible. Their results showed that mean FSH and Estradiol values obtained on cycle days 2-5 no significant difference was noted and estradiol values were not constant through the early follicular phase.

Serum estradiol concentrations was began to increase by cycle day4 (202.9+99.3 pmol) mean value was significantly greater than on cycle day2(172+88pmol) or cycle day3 (172+77 pmol); the mean value on cycle day5(229.5+125 pmol) was significantly greater than that on cycle day4 ,no correlation between estradiol and FSH was found statistically significant only on day 5 and no difference in FSH values on all 4 cycle days in a total of 19 women their FSH & estradiol values on day 2 through day5 showed no difference.

Results of **Ali *et al.*** [29] showed a significant negative correlation between E2&FSH and other hormones (FSH,LH) with a significant direct correlation in E2 on day 3 of the cycle.

The gonadal (E2/FSH) ratio was likely to reflect more closely the degree of readiness of the follicular cohort and enhanced ability to predict the outcome before initiating the ovarian stimulation in ART [30].This opinion supports our current study data that showed that there was a significant negative correlation between E2/FSH on day 3 $p<0.05$.

In addition, according to our data there was a highly statistically significant negative correlation between E2/FSH on cycle day5.Also there was a statistically significant negative correlation between E2/FSH on cycle day 10.

According to our data there was insignificant statistical negative correlation between E2/FSH on cycle day 21.Also, there was a statistical insignificant difference between E2/FSH ratio as regard history of PCO and history of infertility. However, there was statistical significant difference between E2/FSH on day 21 as regard cycle irregularity with statistical significant difference between E2/FSH on cycle day 10 as regard the parity.

Finally, according to results of our as regard E2/FSH correlation on days 3,5,10 and 21 of the cycle give equivalent results that were add greater flexibility for both patient physicians in evaluation of infertile couples without consuming time.

CONCLUSION

The final conclusion of results of this study showed that there was negative correlation between basal day2 E2/FSH) which was equivalent or similar to that ratio on days(5,10,21) so there is no need to wait for hormonal analysis to the next cycle (day 2 ,or 3) to save time specially in patients >35years;for them there is importance of cycle day3 for evaluation of ovarian reserve and prior ovulation induction and subsequent pregnancy potential during the infertility work up.

RECOMMENDATIONS

During the infertility work up , rigid adherence to cycle day3 collection ,no longer seems necessary ,no need to wait for hormonal analysis to estradiol and FSH to the next cycle as there is equivalent negative correlation between E2/FSH on menstrual cycle days 3,5,10 and 21 aiming for saving time.

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