ISSN: 1343-4292
Volume 140, Issue 01, April, 2022

Mid- Luteal estradiol level as predictor marker of successful pregnancy in women undergo ovulation induction in Baquba city

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ABSTRACT— The aim of the current study was to determine the levels E_2, Prolactin, TSH, LH and FSH in mid luteal phase and depended it as a good marker to early predict for pregnancy in women. A total of 58 healthy women aged 20–40 years which undergo ovulation induction included in this study from Al- Batool Teaching Hospital and private clinic in Diyala province, Iraq, during February 2021 to February 2022. Ultrasound scanning by transvaginal to all women of this study were recorded to assess ovarian efficiency, moreover blood samples got in day 12 of the cycle to determine levels of E_2, Prolactin, TSH, LH and FSH. The result of this study showed significant difference increasing ($p \leq 0.05$) serum estrogen concentration in pregnant female differentiated with non- conceived and without any significant difference for others hormones. The study recorded significant positive relationship between type of infertility with Period of infertility in nonpregnant women at the 0.05 level. Also, there is a significant positive relationship in non-pregnant women among age with Period of infertility and Period of treatment at the 0.05 level, and at the 0.01 level with the type of infertility that when the age increases the Period of infertility and Period of treatment and vice versa. Inconclusion the study showed E_2 hormone as a significant predict marker for pregnancy in women during mid luteal phase.

KEYWORDS: Pregnancy period, Estrogens, progesterone, Corpus luteum.

1. INTRODUCTION

The hormones such E_2, progesterone, FSH and LH are considered the most common factors that plat a vital role in reproduction in female human being. The progesterone hormone has major function to maintain and support implantation and stimulate the endometrium growth [1]. The crucial physiological impact to hormone of progesterone is settled by progesterone receptors (PGR). The FSH play a crucial effect on follicular growth, ripening, steroidogenesis control and increase of granulosa cells growth [2]. Anyway, the receptor of FSH hormone (FSHR) plays an important role for organization the function of this hormone in granulosa cells in ovarian follicles. Moreover, E_2 hormone encourage and support FSH action by getting larger the receptors of FSH in ovarian cells [3].

Many previous studies found about progress the hormonal status during corpus luteum period of early pregnancy detection [4- 6], anyway, hormonal profile of luteal period common later of many others protocols still make argument [7]. Previous survey recorded the level of estrogens daily and demonstrated increased of this hormone in pregnant women in comparison with non-pregnant during luteal phase [4]. This knowledge confirmed also by another study for [8] that recorded increasing the level of estrogen

hormone during initial luteal period of pregnant women in voluntary cycle. The same study recorded great concentration of serum estrogens hormone during mid corpus luteum period in conceived ladies differentiate with non-conceived. Anyway, another study got the similar concentration of estrogen hormone in both group during administration of hCG hormone [9].

Conversely, many previous studies did not register any significant difference in estrogens level between pregnant and non-pregnant women during early and mid-luteal phase in cycle [10]. There was previous research about role of progesterone to predict early pregnancy diagnosis during mid luteal phase [11], anyway another study did not find any relation between high progesterone level in mid luteal phase and high pregnancy in women. There is a lack of study about depend estrogens and FSH, LH a and prolactin during mid luteal phase to use as predict marker in pregnancy diagnosis in women, so the aim of this study to determine these hormones in day 12 in cycle to detect early conception in women.

2. MATERIALS AND METHODS

This study was conducted in Al- Batool Teaching Hospital and private clinic during the period from February 2021 to February 2022. Approval from the Scientific Research Ethics Committee in college of medicine – University of Diyala was obtained for this study. Also, written informed consent was obtained from all women participating in the study. A total of 58 women aged 20–40 years which undergo ovulation induction by clomophin citrate (clomid) start from day 2 of cycle and for five days. All women were non-smokers and in good health.

Each woman was monitored for ovarian follicular development by transvaginal sonography then patient were administered 10000 IU of HCG as a single dose when the dominant follicles reach > or equal to 18 mm, So all the laboratory test which include concentrations of hormones Estradiol, FSH, LH, TSH, and Prolactin were estimated on the day 12 of monthly cycle (on day of HCG administration).

After good cleaning of patient's venipuncture site, A 5.0 ml of venous blood was collected using standard collecting techniques and collected blood sample and added to gel tube, and was immediately centrifuged at 4000 r.p.m for 15 minutes to obtain serum which used to asses Estradiol, FSH, LH, TSH, and Prolactin hormones.

determination of hormones:

The VIDAS® Estradiol Hormone (E2) assay is an enzyme-linked fluorescent immunoassay (ELFA) that is performed in an automated instrument. All assay steps and assay temperature are controlled by the instrument. A pipette tip-like disposable device, the Solid Phase Receptacle, serves as a solid phase for the assay as well as a pipetting device. The SPR® is coated at the time of manufacture with mouse monoclonal anti- Estradiol antibodies. The VIDAS Estradiol (E2) assay configuration prevents nonspecific reactions with the Solid Phase Receptacle. The sealed Reagent Strips have reagents for the assay. The samples are transferred into the well that containing anti- Estradiol antibody conjugated with alkaline phosphatase. Then cycled the mixture sample/conjugate in and out of the Solid Phase Receptacle when the estradiol bind with antibodies coated on the Solid Phase Receptacle and to the conjugate forming a "sandwich". Wash steps remove unbound conjugate.

A fluorescent substrate, 4-methylumbelliferyl phosphate, is cycled through Solid Phase Receptacle. Enzyme remaining on Solid Phase Receptacle wall will catalyze the conversion of the substrate to the fluorescent product 4-methylumbelliferone. The intensity of fluorescence is measured by the optical scanner in the instrument; it is proportional to Estradiol concentration present in sample. When the VIDAS Estradiol (E2)

ISSN: 1343-4292
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assay is completed, the results analyzed automatically by the instrument, then printed a reports for samples. Also same procedure was considered in measure of the concentrations of other hormones included FSH, LH, TSH, and Prolactin. Data were calculated by SPSS for windows. Statistical analysis of data was performed using t test. Data of experimental are presented as Mean ±SD (12). Pearson Correlation (r) is used to measure the relationships between variables. P-value < 0.05 and P ≤ 0.01 is considered the level of significance.

3. RESULTS

The results obtained from this study were based on analysis of 58 women's complain of primary or secondary infertility for different periods with range one up to seven years. The causes of infertility in those women varies in frequency of distribution which included (PCOS and hyperprolactinoma, Tubal occlusion opened by HSG, RT tubal obstruction, unovulation and other unexplained causes. Majority of women included in this study between 20-40 years of age, also they undergo to treatments of ovulation induction for different periods extended to many months.

The mean values of estradiol hormone in the studied groups in current study were recorded in table (1) and figure (1). The mean values that recorded in this table were revealed that significantly higher level of estradiol hormone in group of pregnant women (443.5458± 412.711) in comparison with the group of non pregnant women (177.4857± 207.130). Also there are no any significant differences (p ≥ 0.05) between (pregnant and non pregnant) groups in the levels of other hormones included FSH, LH, TSH, and Prolactin (Table 1 and figure 2).

Table 1: concentrations of different hormones in pregnant and non pregnant women

parameters	pregnant	non pregnant	P value
Age	28.7083± 5.81213	29.7500± 7.78710	0.72
E2	443.5458± 412.711	177.4857± 207.130	0.01*
FSH	3.7750± 1.25880	3.7218± 1.04510	0.77
LH	4.3292± 2.85192	3.6729± 2.99478	0.81
Prolactin	13.9375± 7.93083	10.5139± 7.23323	0.07
TSH	2.1108± 1.07470	1.9557±.97522	0.53
* Differences are significant at the 0.05 level			

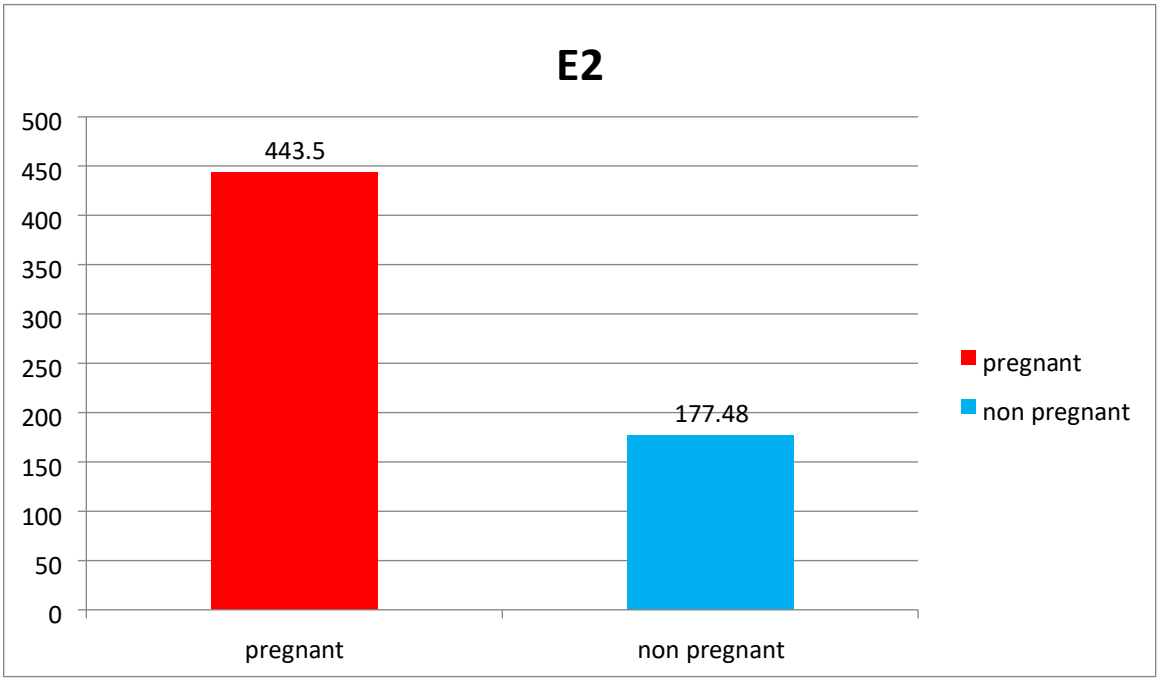

Figure (1): mean ±SD Estradiol of studied groups

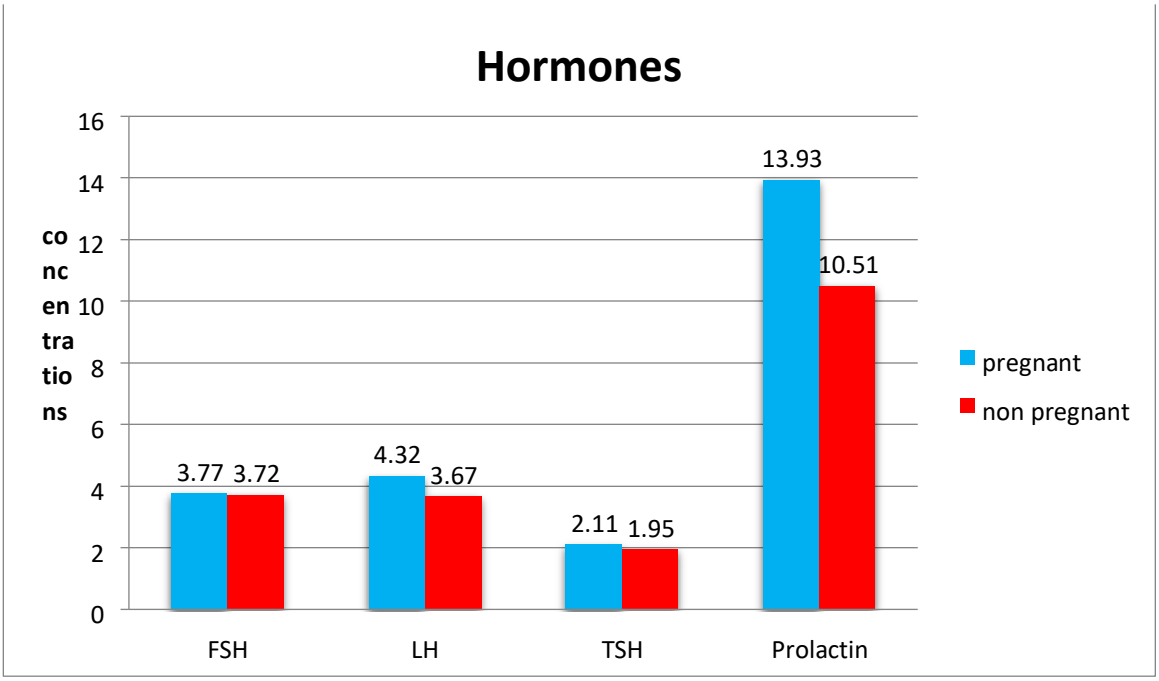

Figure (2): mean ±SD FSH, LH, TSH, and Prolactin of studied groups

Spearman correlation have been used to identify the association among studded parameters in each case, Table (2) shows the correlation coefficient among different hormones in pregnant women where the result shows a positive correlation between levels of FSH and TSH in non pregnant women, this correlationssignificant at the 0.05 level (figure 3). Additionally the outcomes shows a positive connection between FSH and LH and this correlation issignificant at 0.05 level (Table 3 and figure 4).

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Volume 140, Issue 01, April, 2022

Table 2: Correlations among different hormones in pregnant women

		Age	E2	FSH	LH	Prolactine	TSH
Age	Pearson Correlation	1	-.013	.057	-.132	-.308	-.099
	Sig. (2-tailed)		.948	.776	.513	.119	.622
	N		27	27	27	27	27
E2	Pearson Correlation		1	-.076	-.301	-.098	-.270
	Sig. (2-tailed)			.708	.128	.626	.172
	N			27	27	27	27
FSH	Pearson Correlation			1	.333	.092	.449*
	Sig. (2-tailed)				.090	.648	.019
	N				27	27	27
LH	Pearson Correlation				1	-.030	.133
	Sig. (2-tailed)					.882	.507
	N					27	27
Prolactin	Pearson Correlation					1	.000
	Sig. (2-tailed)						1.000
	N						27

*. Correlation is significant at the 0.05 level (2-tailed).

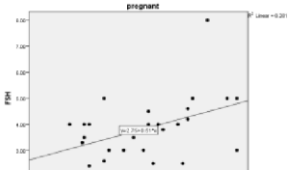

Figure (3): Pearson Correlation between FSH and TSH concentrations in pregnant women

Table 3: Correlations among different hormones in non pregnant women

		E2	Age	FSH	LH	Prolactin	TSH
E2	Pearson Correlation	1	.116	-.052	-.259	.137	-.166
	Sig. (2-tailed)		.534	.783	.160	.463	.372
	N		31	31	31	31	31
Age	Pearson Correlation		1	.164	-.294	-.166	-.241
	Sig. (2-tailed)			.377	.109	.372	.192
	N			31	31	31	31
FSH	Pearson Correlation			1	.607**	-.020	.046
	Sig. (2-tailed)				.000	.917	.804
	N				31	31	31
LH	Pearson Correlation				1	-.035	-.201

	Sig. (2-tailed)					.854	.277
	N					31	31
	Pearson Correlation					1	.207
	Sig. (2-tailed)						.264
Prolactin	N						31
**. Correlation is significant at the 0.01 level (2-tailed).							

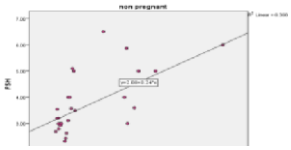

Figure (4): Pearson Correlation between FSH and LH concentrations in non pregnant women

Table (4) showed a significant positive relationship between type of infertility with Period of infertility in non pregnant women at the 0.05 level. Also, there is a significant positive relationship in non pregnant women among age with Period of infertility and Period of treatment at the 0.05 level, and at the 0.01 level with the type of infertility that when the age increase lead to increase the Period of infertility and Period of treatment and vice versa (Table 4). On another side the results showed there are no any significant relationship in pregnant women among age with type of infertility, Period of infertility and Period of treatment at the 0.05 level (Table 5).

Table 4: Correlations among different factors with estradiol hormone in non pregnant women

		Age	E2	Period of infertility	Period of treatment	Type of infertility
	Pearson Correlation	1	.104	.429*	.402*	.571**
	Sig. (2-tailed)		.597	.023	.034	.001
Age	N		28	28	28	28
	Pearson Correlation		1	.164	.091	-.077
	Sig. (2-tailed)			.403	.645	.696
E2	N			28	28	28
	Pearson Correlation			1	.253	.442*
	Sig. (2-tailed)				.195	.019
Period of infertility	N				28	28
	Pearson Correlation				1	.275
	Sig. (2-tailed)					.157
Period of treatment	N					28
*. Correlation is significant at the 0.05 level (2-tailed).						
**. Correlation is significant at the 0.01 level (2-tailed).						

Table 5: Correlations among different factors with estradiol hormone in pregnant women

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		Age	E2	Period of infertility	Period of treatment	Type of infertility
Age	Pearson Correlation	1	.034	-.023	.032	-.162
	Sig. (2-tailed)		.874	.913	.880	.449
	N		24	24	24	24
E2	Pearson Correlation		1	-.300	-.185	.291
	Sig. (2-tailed)			.155	.387	.168
	N			24	24	24
Period of infertility	Pearson Correlation			1	.338	.108
	Sig. (2-tailed)				.106	.615
	N				24	24
Period of treatment	Pearson Correlation				1	-.081
	Sig. (2-tailed)					.706
	N					24

4. Discussion

The active and functional corpus luteum considers one of the most common reasons for wealthy implantation by secretion distinct hormones. Most of previous studies confirmed of rescuing of oocyte after 7-8 days by corpus luteum and, that may be indicated to early marker for pregnancy and could be depended as a diagnostic tool for successful conception [5]. The previous studies approve embryonic together with maternal E_2 releasing pathway function together, independently and to develop successful initial implantation as well as increasing concentration of E_2 hormone [13].

The current study tries to find another new marker such as E_2, Prolactin, LH, and FSH serum concentration during mid luteal phase to depended it as new diagnostic hormone for successful pregnancy in women.

Our current study showed increasing serum levels of E_2 in pregnant compared with non-pregnant women on day 12 of cycle. The level of serum estrogens was significantly higher ($p \leq 0.05$) in pregnant women sample and this result agreed with many previous studies [4], [14], [15], and this outcome could belong CL recover followed implantation. The implantation has occurred during the days 9-10 days of cycle and increasing estrogen level in serum of pregnant samples may be due to reason of the presence two-cell concept, two-gonadotropin. The high impact of two hormones, LH and FSH on the theca and the granulosa cells in follicle develops significant increasing of estrogens. Moreover, the progressive stimulation of LH hormone after ovulation on tiny cells of luteal tissue that originated from ovarian theca excrete androgen and huge cells that stem from ovarian granulosa cells develop and aromatization androgens hormone to E_2.

Moreover, increase of estrogen concentration during corpus luteum period could related with secretion of hCG hormone level that derived from early embryo [16].

The current study showed decreased in estrogen for non-pregnant women in mid luteal phase and may be explained because reversion and disappearing of CL from the ovary.

A previous study for [17] confirmed the results of the current study, and demonstrated that concentration of estrogen during luteal period going to down in non-conceive ladies and still in same time the level of estrogen to increasing in women that got chance to be pregnant.

Also, previous research concluded about any disorder affect implantation and pregnancy may lead to sharp decreasing in estrogens serum in mid luteal phase in non-pregnant women [18]. Moreover, previous survey recorded greater pregnancy percentage with upsurge of estrogen concentration during passed luteal period and reduction of estrogen hormone may be indicated to poor conception rate and bad outcome [5].

Most of previous studies confirmed the importance of luteal phase progesterone for maintained early embryo and considers the most common markers to diagnosis pregnancy in women [19]. Anyway, our current study did not achieve any analysis for this hormone because already progesterone hormone level will be higher in pregnant serum samples.

Table (1) in this study showed clearly that there are no clear significant statistical differences in blood concentration of FSH, LH and TSH between pregnant and non-pregnant women sample in mid luteal phase in cycle.

This result could be clarified by scientific truth, good implantation of new fetus, continue of active corpus luteum on ovary is prolonged due to coming new essential stimulation hCG, which support and recovery the steroidogenesis process in luteal tissue until the placental support and take role for maintained pregnancy. Prognostic worth to estrogen hormone in mid luteal period for clinical, biochemical and pregnancy diagnosis and estrogen concentration develop a good opinion about good correlation beta hCG concentration [20].

The hCG, a replacement for LH hormone, escalate aromatase process action and followed steroidogenesis operation in the luteal and granulosa tissue. The elevation of concentration of estrogen hormone develops additional inhibition for FSH level that secreted by pituitary gland in pregnant ladies [16].

The decreasing of FSH level in non-pregnant women may disagree with other previous fact. As soon as the implantation process not succeed to occur during luteal period, activity of the ovarian CL begins fast decreasing in 9 - 11 days that followed ovulation process. Decreasing of estrogen blood concentration, beside the inhibin factor led to cut off opposed effect on of the concentration FSH serum that pituitary gland secretes it, which develop elevation of FSH hormone in the middle phase of luteal period and stimulate new follicle waves of following cycle [16].

This study concluded that blood estrogen level during mid luteal period enhance the prognostic tools and diagnostic efficiency for early prediction and good expectation of early conception in pregnant women.

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