

Level of 17- β Estradiol in follicular fluid for patient undergoes IVF as correlation with pregnancy rate:

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Abstract:

Background: 17-beta-estradiol (E2) is a C-18 steroid hormone (molecular weight 272.4 Da) produced mainly by the ovary and placenta and in small amounts by adrenals and testes. Estradiol is in equilibrium with estrone, which can be converted to estriol by the liver and placenta. Like for LH-FSH-progesterone, measurement of estradiol concentration in serum, peritoneal fluid and follicular fluid is an essential biochemical tool for the investigation of fertility, tumor and sexual diseases, and disorders of hypothalamic/pituitary/gonadal axis, for example. To detect the follicular phase.

Objective: To assess E2 in early follicular phase as a predictor of pregnancy rate among females undergoing in vitro fertilization (IVF) in the Baghdad patient.

Method: The study population consisted of women undergoing in vitro fertilization and aged between 20-45 years. All women were non-smokers and had been unable to be pregnant naturally for at least one year. Pregnancies mentioned in this study were clinical pregnancies where one or more gestational sacs and heart beat were confirmed by trans vaginal ultrasound 4 weeks after embryos transfer. E2 was determined by enzyme linked immunoassay. The medical records of 56 patients infertility undergoing IVF in the program at center of Bagdad center in the period between February 2015 and July 2015, they have normal ovulatory cycles, and without any evident endometrial pathology. All patients underwent 1st cycles of IVF with long down-regulation protocol of Gonadotropin releasing hormone analogue (GnRHa) and became pregnant following one of them. The cycles, 56 in total were divided into pregnant and non-pregnant cycles and divided into subgroup (age and BMI). Data were computer analyzed using Statistical calculations were made using the Statistical Package for the Social Sciences (version 12.0, SPSS Inc. Chicago, IL, USA).

Results: The pregnant women were 11 (20%) while the non-pregnant women were 45 (80%). The mean age of the non-pregnant in this study was 30.63 years and for the pregnant group was 32.18 years. The mean of BMI of the non-pregnant in this study was 22.9 (kg/m²) and for the pregnant group was 22.55(kg/m²). The mean levels of E2 fluctuated among different age groups in follicular fluid have non-significant as shown 1061.417 \pm 535.6363 pg/ml at age \leq 25 year, level 1384.12 \pm 709.454 pg/ml at age 26-35 years and 1199.42 \pm 643.6185 pg/ml for age (year) $>$ 35 cases, (p value 0.25, 0.64, 0.25 respectively). The mean levels of E2 in relation to the BMI of the study population in blood serum and follicular fluid According to their BMI, the study population was divided into three groups: Group A (Normal) BMI 18–22 (Kg/m²), Group B (Overweight) BMI 23–25 (Kg/m²), Group C (Obese) BMI \geq 26 (Kg/m²) E2 levels in Follicular fluid was 1197.07 \pm 615.5659 for Group A (normal) BMI, 1535.20 \pm 793.9 for Group B (overweight) and 1423.33 \pm 761.35 for Group C (obese) there's no significant in all Group compared to group. IVF result showed that the chance of IVF success increased with increased age. This implies that mid 30 year women had more chance to have pregnancy through IVF. There's no significant between pregnant and non-pregnant. IVF result showed that the chance of IVF success increased with decreased BMI (p= 0.7). This implies that slim women had more chance to have pregnancy through IVF. And there's no significant between pregnant and non-pregnant with BMI. The mean level of E2 in follicular fluid The maximum mean level of E2 was observed in positive pregnancy 1404.73 \pm 331.49, followed by negative pregnancy 1289.95 \pm 286.27 and there's no significant (p $>$ 0.005).

Conclusion: E2 cannot be used in IVF programs as a success predictor of pregnancy.

I. Introduction:

A woman is said to be infertile if she is unable to conceive while a man is infertile if he is unable to impregnate. A couple is said to be infertile if either a woman or a man is infertile. There are two types of infertility; primary and secondary infertility. Primary infertility is when the couples have never had a child while secondary infertility means that the couples have children but a medical, emotional or physical condition is currently making conception difficult or not possible. Infertility may also refer to the state of a woman who is unable to carry a pregnancy to full term. Pregnancy is the result of a complex chain of events. In order for a woman to get pregnant: Firstly, a woman must release an egg from one of her ovaries (ovulation). This egg must

go through a fallopian tube and enter the uterus (womb). Secondly a man's sperm must penetrate and fertilize the egg along the way. The fertilized egg must attach to the inside of the uterus^(1, 2).

In vitro fertilization (IVF) is a procedure in which eggs (ova) from a woman's ovary are collected. They are fertilized with sperm in the laboratory, and then the fertilized egg (embryo) is returned to the woman's uterus⁽³⁾. Preparation Once a woman is determined to be a good candidate for IVF, she will generally be given "fertility drugs" to stimulate ovulation and the development of multiple eggs. These drugs may include gonadotropin releasing hormone agonists (GnRHa) and human chorionic gonadotropin (HCG). The maturation of the eggs is then monitored with ultrasound tests and frequent blood tests. If enough eggs mature, the physician will perform the procedure to collect them. The woman may be given a sedative prior to the procedure. A local anesthetic may also be used to reduce discomfort during the procedure^(3, 4).

Follicular fluid (FF) provides a very important microenvironment for the development of oocytes. FF is a product of both the transfer of blood plasma constituents that cross the blood follicular barrier and of the secretory activity of granulosa and thecal cells⁽⁵⁾. It is reasonable to think that some biochemical characteristics of the FF surrounding the oocyte may play a critical role in determining oocyte quality and the subsequent potential to achieve fertilization and embryo development. The analysis of FF components may also provide information on metabolic changes in blood serum, as the circulating biochemical milieu may be reflected in the composition of FF⁽⁶⁾.

It is well known that a predominantly intra-follicular estrogenic environment is associated with good follicular-growth and has anti-atresia effects. In addition, E2 enhances the cytoplasmic maturation of oocytes via a direct non-genomic action at the plasma membrane level, in turn inducing extracellular calcium influx into the cell and a specific pattern of Ca²⁺ oscillations⁽⁷⁾. Elevated E2 and E2/P ratio in FF indicate a more advanced stage of oocyte maturation and have been repeatedly found to be associated with a higher chance of achieving pregnancy. This observation, however, was not confirmed by other studies]

The role of estradiol in in vitro fertilization (IVF) is well known up to the fertilization stage, while its role beyond that stage remains controversial. Some groups report that high estradiol levels are associated with major oocyte production, which is accompanied by suppression of implantation and endometrial receptivity and decreased pregnancy rates, while others report no influence of estradiol in these processes⁽⁸⁾.

We conclude that the highest implantation and pregnancy rates observed in previous study resulted from the concerted action of estradiol (1001–2000 pg/ml, serum) and (FF)). Outside this concentration range these hormones exert negative influence on embryo quality and IVF outcome. By modulating estradiol-dependent embryo quality,. The conditional hormonal IVF effects observed suggest that estradiol interact coordinately in a concentration-dependent manner. Further studies are needed to substantiate and clarify the mechanism of proposed interaction between the two hormonal systems⁽⁹⁾.

Follicular fluid estradiol is an important determinant of IVF success because it is abundant for estradiol. While total follicular estradiol does not exert any positive or negative influence on IVF outcome, estradiol per mature follicle and retrieved oocytes do have an impact. Pregnancy rate is better when E2/fol is between 200 and 299.99 pg/ml. Also, increasing serum E2/fol positively correlates with better oocytes and embryo quality. In contrast, E2/O negatively correlates⁽¹⁰⁾.

The purpose of this study was to confirm our previous results from a retrospective study on the differences in luteal phase E2 secretion between conception (CC) and non-conception (NC) cycles following in vitro-fertilization/ (IVF) treatment in a prospective study design, analyses the predictive role of mid-luteal phase for CC and (iii) validate the role of luteal phase E2 as predictors of (ongoing) pregnancy⁽¹¹⁾.

II. Methods:

Study population

The study population consisted of women undergoing *in vitro* fertilization and aged between 20-45 years. All women were non-smokers and had been unable to be pregnant naturally for at least one year. Pregnancies mentioned in this study were clinical pregnancies where one or more gestational sacs and heart beat were confirmed by trans vaginal ultrasound 4 weeks after embryos transfer. The medical records of 56 patients infertile undergoing IVF in the program at center of Bagdad center in the period between February 2015 and July 2015, they have normal ovulatory cycles, and without any evident endometrial pathology. All patients underwent 1st cycles of IVF with long down-regulation protocol of Gonadotrophin releasing hormone analogue (GnRHa) and became pregnant following one of them. The cycles, 56 in total were divided into pregnant and non-pregnant cycles.

The inclusion criteria included:

Female infertility with a duration of 1-8 years; Age 20-45 years old; BMI 20-30kg/ m²; 1st cycle IVF; Have two ovaries; Regular cycle; No Poly Cystic Ovarian Disease (PCOD); No pelvic masses or diseases (e.g.; fibrosis, pelvic inflammatory disease, endometriosis); No history of medical disorders (e.g.: thyroid

dysfunction, hypertension, liver disease, DM, renal disease); Non smoker or alcohol consumer and exclude azoospermia as a cause of male infertility.

III. Ovulation induction and IVF procedure:

Ovulation induction (OI) and IVF procedure was performed according to the standard long protocol as described previously⁽¹²⁾. Briefly, in the mid-luteal phase of the preceding cycle, a gonadotropin releasing hormone (GnRH) agonist (Decapeptyl 0.1 mg s.c. daily, Ferring, Kiel, Germany or Synarela 0.4 mg intranasal, Pfizer/Pharmacia, Erlangen, Germany) was applied. Pituitary down-regulation was confirmed by vaginal bleeding and oestradiol serum concentration 5110 pmol/l followed by ovarian Stimulation within 14 days later. Ovarian stimulation was performed with recombinant FSH preparations (Gonal F, Merck Serono, Darmstadt, Germany or Puregon, MSD, Germany) at a standard dose of 150 IU that could be adjusted according to the expected ovarian response. Criteria for OI with either 10 000 IU urinary hCG (Choragon, Ferring) or 250 mg recombinant hCG (Ovitrelle, MerckSerono) were fulfilled in patients with at least three follicles ≥ 17 mm. Embryo quality following oocyte retrieval and IVF/ICSI was assessed with a scoring system by Steer et al.⁽¹³⁾. Preceding ETs on day 2 or 3, LPS was commenced on the day after FP with vaginal P only with two different formulations that had been previously proven to be comparable in terms of clinical and ongoing pregnancy⁽¹⁴⁾. Pregnancy was detected by hCG measurement on ET \pm 14, and transvaginal ultrasonography (TVUS) was performed 1 week later and onwards. Only clinical pregnancies with detection of an intrauterine fetal sac were counted and followed for at least 20 weeks. All clinical pregnancies lost until that time were considered as miscarriage

Sampling Collection

Follicular fluid samples from individual follicles were pooled and centrifuged for 10 min at 500 g and the supernatants were stored at -20 C until analyzed further. Fractions of FF with massive blood contamination were excluded. E2, were carried out at the Medical Relief Laboratory.

Estimate of BMI :

Body Mass Index (BMI) Formula and Table Body Mass Index (BMI) is a number calculated from a person's weight and height. BMI is a screening method that may indicate underlying health issues⁽¹⁵⁾

Estradiol assay

E2 level was determined according to Tietz (1995)⁽¹⁶⁾ method using TECO Diagnostics ELISA kit for E2.

Data analysis:

The following parameters were also evaluated: age of patients, body mass index (BMI), total amount of administered recombinant, E2.

To determine whether FF markers could distinguish the patients who became pregnant from those who did not, FF of E2 measurements were compared between pregnant and non-pregnant patients following ART. Statistical calculations were made using the Statistical Package for the Social Sciences (version 12.0, SPSS Inc., Chicago, IL, USA). For continuous variables Student's t-test and for categorical variables Chi-squared test and Fisher's exact test were used, where applicable. Results are expressed as means \pm SD or percentages (counts) as appropriate. Statistical significance was defined as a value of $P < 0.05$

IV. Result

The study population comprised 56 females; all of them have a problem in the process of pregnancy and were seeking in vitro fertilization at Bagdad center in Bagdad. Results for couples characteristics. The pregnant women were 11 (20%) while the non-pregnant women were 45 (80%). The mean age of the non-pregnant in this study was 30.63 years and for the pregnant group was 32.18 years. The mean of BMI of the non-pregnant in this study was 22.9 (kg/m²) and for the pregnant group was 22.55 (kg/m²) .

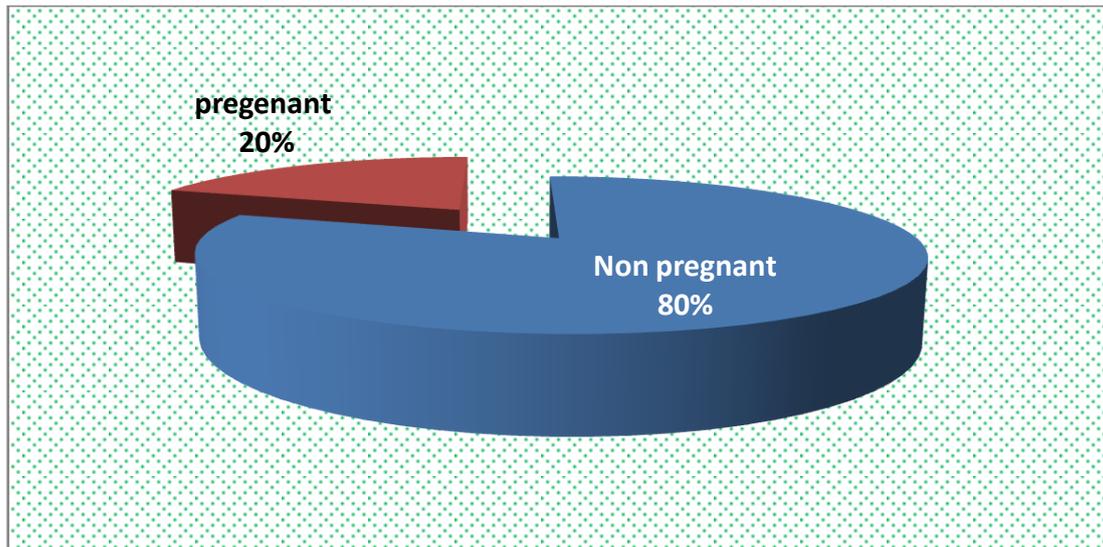


Figure (1) Showed that pregnancy rate was 20%.

The mean levels of, E2 in relation to the age of the study population in follicular fluid are illustrated in Table(1.) According to their age, the study population was divided into three groups: ≤25, 26-35 and >35 years. The mean levels of E2 fluctuated among different age groups showing the levels of 1061.417 ±535.6363 pg/ml at age ≤25year , level 1384.12 ±709.454 pg/ml at age 26-35 years and 1199.42± 643.6185 pg/ml for age (year)>35 cases,(p value 0.25, 0.64, 0.25 respictivally) shown figure(2,3,4).

hormone	Age (year) ≤25	Age (year)26-35	Age (year)>35
E2 (pg/ml)	1061.417 ±535.6363	1384.12 ±709.454	1199.42± 643.6185
P value	0.25	0.64	0.25

Table(1.) Illustrated the mean levels of, E2 in relation to the age of the study population in follicular fluid All values are expressed as mean ±SD. p> 0.05: not significant, p<0.05: significant

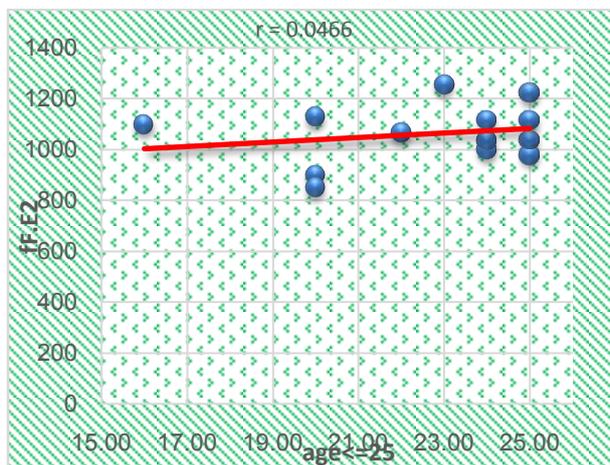


Figure 2:illustrate Negative significant correlation between FF.E2 withage <=25

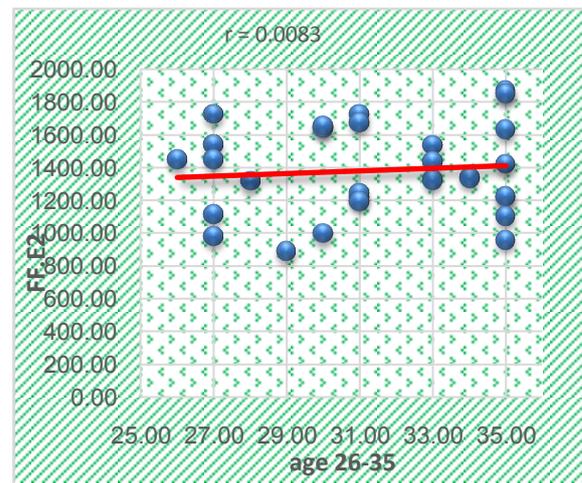


Figure 3:illustrate Negative significant correlation between FF.E2 with age 26-

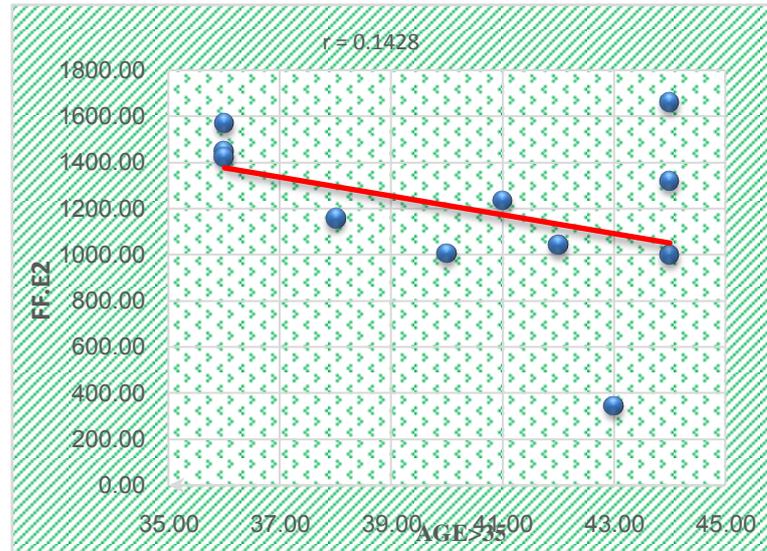


Figure 4: illustrate Negative significant correlation between S.E2 with age>35

The mean levels of, E2 in relation to the BMI of the study population in follicular fluid are illustrated in Table (2). According to their BMI, the study population was divided into three groups: Group A (Normal) BMI 18–22 (Kg/m²), Group B (Overweight) BMI 23– 25 (Kg/m²), Group C (Obese) BMI ≥26 (Kg/m²). E2 levels in Follicular fluid was 1197.07 ±615.5659 for Group A (Normal) BMI, 1535.20±793.9 for Group B (Overweight) and (1423.33±761.35) for Group C (Obese) there's no significantly in all Group compared to group. see figure (5,6,7)

	Group A (Normal) BMI 18–22 (Kg/m ²)	Group B (Overweight) BMI 23–25 (Kg/m ²)	Group C (Obese) BMI ≥26 (Kg/m ²)
E2 (pg/ml)	1197.07±615.5659	1535.20±793.9	1423.33±761.35
P value	0.022	0.1	0.7

Table 2: Illustrated the mean levels of, E2 in relation to the BMI of the study population follicular fluid. All values are expressed as mean ±SD. p> 0.05: not significant, p<0.05: significant

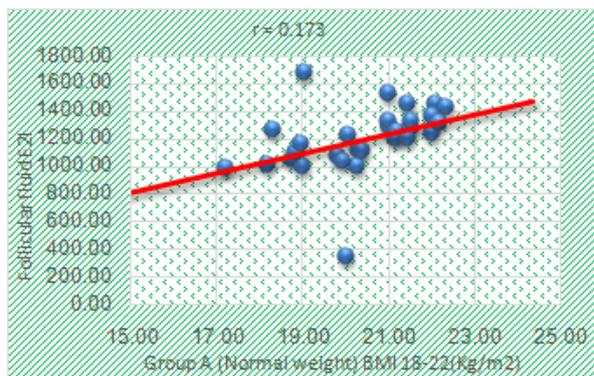


Figure 5 illustrate Negative significant correlation between BMI and FF.E2 in BMI group A (normal)

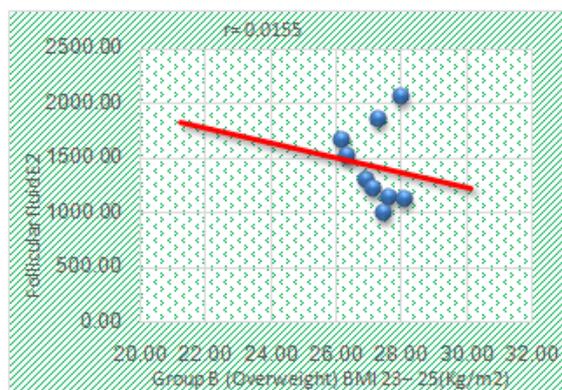


Figure 6 illustrate Negative significant correlation between FF.E2 and BMI (group B)

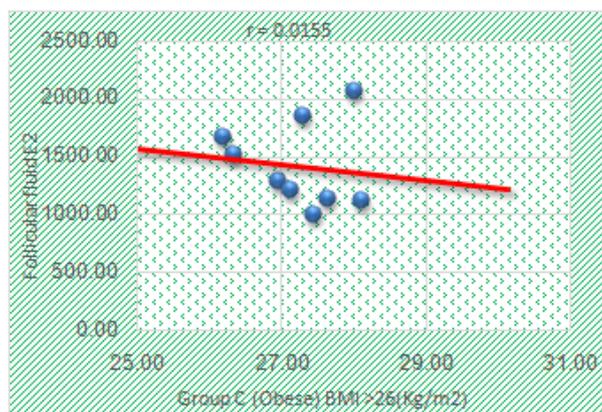


Figure 7 illustrate Negative significant correlation between BMI and S.E2 in Group C (Obese) >26(Kg/m2)

The result of IVF (positive pregnancy, negative pregnancy) in relation to age provided in Table 3 Out of the 56 women enrolled in the IVF programs, 11 (20. %) had pregnancy, 45 (80%) had no pregnancy). IVF results showed that positive pregnancy occurred in women aged 32.18±3.9 years where as negative pregnancy was 30.63± 4.4. When related to age, IVF result showed that the chance of IVF success increased with increased age. This implies that mid 30 year women had more chance to have pregnancy through IVF. There's no significant between pregnant and non-pregnant

Table 3: The result of IVF (positive pregnancy, negative pregnancy) in relation to age. All values are expressed as mean ±SD.

IVF outcome	%	Age (mean ± SD)
Positive	20	32.18±3.9
Negative	80	30.63± 4.4
p-value	0.4	

Positive: pregnancy occurred.

Negative: no pregnancy p> 0.05: not significant, p<0.05: significant

The result of IVF (positive pregnancy, negative pregnancy) in relation to BMI of cases is provided in (Table 4) Out of the 56 women enrolled in the IVF programs, 11 (20 %) had pregnancy, 45 (80%) had no pregnancy). IVF results showed that positive pregnancy occurred in women BMI 22.55 ±4.4 (kg/m2) whereas negative pregnancy was 22.9 ±5.8 (kg/m2). When related to BMI, IVF result showed that the chance of IVF success increased with decreased BMI (p= 0.7). This implies that slim women had more chance to have pregnancy through IVF. And there's no significant between pregnant and non-pregnant with BMI .

Table 4: The result of IVF (positive pregnancy, negative pregnancy) in relation to BMI .All values are expressed as mean ±SD.

IVF outcome	%	BMI (mean ± SD)
Positive	20	22.55 ±4.4
Negative	80	22.9 ±5.8
p-value	0.7	

Positive: pregnancy occurred.

Negative: no pregnancy

p> 0.05: not significant, p<0.05: significant

Table 5. Summarizes the outcome of IVF (positive pregnancy, negative pregnancy) in relation to the mean levels of E2 in blood serum and follicular fluid . The mean level of E2in follicular fluid The maximum mean level of E2 was observed in positive pregnancy 1404.73±331.49, followed by negative pregnancy 1289.95±286.27 and there's no significant (p>0.005) shown figure (8).

Table 3.6: Summarizes the outcome of IVF (positive pregnancy, negative pregnancy) in relation to the mean levels of E2 follicular fluid.

Follicular fluid			
hormone	positive	negative	p-value
E2(pg/ml)	1404.73±331.49	1289.95±286.27	0.3

All values are expressed as mean ±SD.

Positive: pregnancy occurred.

Negative: no pregnancy

p> 0.05: not significant, p<0.05: significant.

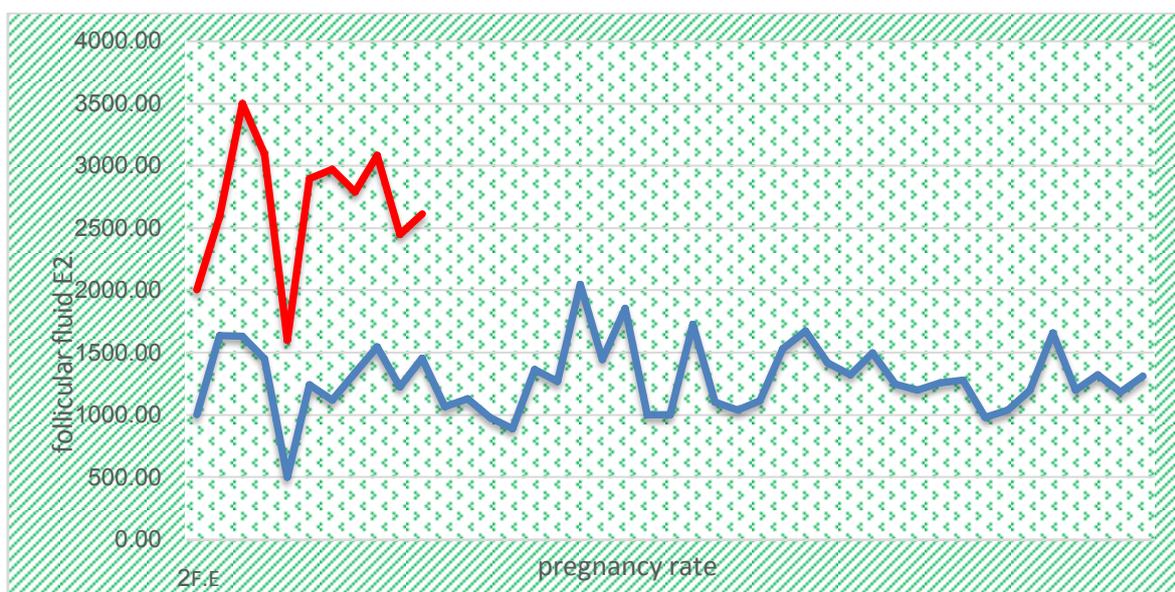


Figure (8) Negative significant correlation between FF.E2 level with IVF outcome (pregnant and non-pregnant women)

V. Discussion:

In vitro fertilization (IVF) entails egg fertilization with sperm in vitro, and then returning the fertilized egg (zygote) to the woman's uterus. This process is employed worldwide particularly in developing countries in Bagdad, women seek IVF program mostly when the man had fertility problems and the process of pregnancy is delayed. However, the present study is one to identify some clinical aspects of IVF as well as to asses E2 as a predictor of pregnancy rate in IVF candidates in Iraqi patients

Data presented in this study dealt with 56 women enrolled in IVF programs and divided into two group (pregnant and non-pregnant patient) therefor the mean age of the pregnant in the present study (32.18 years) and (30.36 year) for non-pregnant women .Compared with previous studies our studies disagreement with plastine study (28.7 year) and egeption (29 year) and Iranian(29.1year) and (28year) (Shahin, 2007⁽¹⁷⁾; Dehghani-Fieouzabadi et al., 2008⁽¹⁸⁾ , Mohammed M. Laqqan;2010⁽¹⁹⁾ .but was close to that reported from the Netherlands (33.8 years) and UK studies (30.36year) ⁽²⁰⁾HFEA’s IVF National data Statistics 1/04/2000 – 31/03/2001, (van Rooij et al., 2002 ⁽²¹⁾).

So many women choose to get pregnant later in life, waiting until their mid-30 or later to begin trying. Unfortunately, the term “biological clock” is an apt one because as a woman’s body ages her eggs age as well. It is well known and scientifically proven that a woman’s fertility decreases with age beginning at 30 years old but in our studies in the number of patient different with another studies as shown in this study and different in selected age group especially in Palestine study.at last The younger age of women seeking IVF in developing countries, including Iraq, could be explained in the context of social habits where most families have the desire to have children immediately after marriage.

The mean of BMI of the non-pregnant in this study was **22.9** (kg/m2) and for the pregnant group was 22.55, this is agreement with egeption studies (25.9 for non-preg and 23.8for pregnant) (*HalaAbd El-Fttah Ali, *Ola Mohamed Ebraheem, **SaharNaguebMohamed ;2013⁽²²⁾)

In previous study BMI (p value) was 0.25, pregnant group mean = 25.1, non-pregnant group mean = 25.0, (Tiffany et al 2005)⁽²³⁾ and not similar with our studies. In another study morbidly obese women had significantly lower clinical pregnancy rates after IVF.

In the current study we estimated E2 level in blood and follicular fluid. The mean levels of E2 in relation to the age of the study population in follicular fluid levels of E2 fluctuated among different age groups. There's no significant between age and F.E2 and can be shown in these study agreement with (Volpe A, Coukos G, D'Ambrogio G, et al 1999)⁽²⁴⁾ because Estradiol is the most important type of estrogen found in a woman's body and it is responsible for keeping the eggs healthy as well as facilitating pregnancy. If the test shows a high Estradiol count then there is a problem with egg numbers and/or quality. Our study is different with (Nasrin Sheikh1, IrajAmiri, et.al; 2011)⁽²⁵⁾ because the E2 have positive correlation with the number and size of follicular fluid and therefore the follicle is decrease with increase the age and normally E2 is decline.

Data presented in this study showed that the mean levels of E2 in patient undergoes IVF generally showed fluctuations in different BMI groups, maximum level of E2 in follicular fluid level of estradiol are highly increasing in group B and group C more than group A and this study disagreement with (RehanaRehman, ZahirHussain*, NaveedFaraz; 2012)⁽²⁶⁾. In Rehana study the Estradiol level became lower in overweight than in normal-weight women and to be inversely correlated with BMI because the follicular phase, as a result of growth of follicles E2 concentration progressively increase which causes endometrium hyperplasia of both glandular and stromal components. It also induces the production of specific proteins, growth factors and the receptors of estrogen and progesterone. This study was in Simi same line with (Tiffany et al 2005)⁽²³⁾. Our patient have ovarian stimulation by (GnRH) agonist when a patient is aging and obesity lead to decreases the level of E2 and require to increase the ovarian stimulation and all these lead to increase the follicle size despite to mature or not, therefore at last that causes increase in E2 level in overweight (BMI 23-26).

In vitro fertilization results presented in this study showed that the chance of IVF success increased with decreased age in relation to age. Out of the 56 women enrolled in the IVF programs, 11 (20. %) had pregnancy, 45 (80%) had no pregnancy. Our result is congruent with that and (van Rooij et al., 2002)⁽²¹⁾. Previous study implies that younger women had a better chance to have a successful pregnancy. This is supported by previous result that ovarian reserve and response increased with decreasing age making more chance for pregnancy to occur. In addition, our result is different with that of (Gnothet al. 2008)⁽²⁷⁾ who found that pregnancy outcome of IVF program was significantly higher in younger women than that in the older ones. Similar result was also reported by (Smeenk et al. 2007)⁽²⁸⁾, but the difference in the pregnancy outcome of IVF program was not significant. The difference in our result with another previous study we taken 56 patient and already the pregnancy rate is low as comparison with world studies especially UK (2511 patient) and US study (800-900 patient) and different in study age group.

In vitro fertilization results presented in this study showed that the chance of IVF success increased with decreased BMI. Out of the 56 women enrolled in the IVF programs, 11 (20. %) had pregnancy, 45 (80%) had no pregnancy. Many stimulation cycles in assisted reproduction are associated with failed pregnancy despite the transfer of apparently healthy and morphologically normal embryos.

Most studies to date report decreased success in obese patients treated with in vitro fertilization (IVF). Obese patients are more likely to have polycystic ovaries, which places them at greater risk of hyperstimulation and the need for cancellation. Monitoring obese patients during stimulations may be more difficult due to the ovaries being located outside the normal sonographic focal plane. Due to body habitus, oocyte retrieval and embryo transfer might also be more difficult. While much data has been collected and analyzed regarding patients with elevated BMI and reproductive outcome, we chose to evaluate details surrounding the embryo transfer as an independent variable in assessing the question.

The detrimental effect of BMI on delay in conception is attributed to its effects on the ovary as well as endometrium. The fact is revealed in our study with maximum number of study group comprising of obese women with BMI 22-25. This result is supported by (Esinler et al) who correlated multiple endocrine and metabolic alterations in obese women giving rise to infertility. Overweight and obesity through certain pathological mechanisms affect the endocrine status of the patient. Our studies different with, (*HalaAbd El-Fttah Ali, *Ola Mohamed Ebraheem, **SaharNagueb Mohamed ;2013)⁽²²⁾ because Estradiol showed a tendency to be lower in overweight than in normal-weight women and to be inversely correlated with BMI in the whole study population and a donor oocyte recipient model has clearly demonstrated that endometrial receptivity is not affected by elevated BMI. The study divided 536 women undergoing their first donor oocyte IVF cycle into four BMI groups: underweight, normal, overweight, and obese. They found no statistically significant differences in implantation rate, ongoing pregnancy rate or spontaneous loss rate between groups. In follicular fluid the maximum mean level of E2 was observed in positive pregnancy. Followed by negative and there's no significant ($p>0.005$). Three studies suggested a detrimental role of high E2 levels on pregnancy achievement. Estradiol level in follicular fluid found as abundant amount and responsible for ovarian function where an important predictor for ovarian response but increased above normal value can lead to E2 may cause endometrial damage and disrupt the implantation and this property may be responsible for the negative effect of E2 on IVF outcome. This study agreement with (Valbuena D,2001)⁽³⁰⁾ and (Ng EHY;2000)⁽³¹⁾ this might also be due to additional confounding factors such as the small number of patients included in some studies or patients contributed more

than one cycle for analysis. The restricted number of patients in our study may be criticized. But one should realize that this study reflects the results of a patients group whose members are inhabitant of low socio-economic level with low financial support to complete a successful treatment program.

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