# The level of 17-beta estradiol in follicular fluid for patients undergoes IVF as correlation with the pregnancy rate

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## ABSTRACT

Estradiol (E2) is a steroid hormone 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 is an essential biochemical tool for the investigation of fertility. So that this study was done to assess the follicular fluid E2 in early follicular phase as a predictor of pregnancy rate among females undergoing in vitro fertilization (IVF) in Iraqi patient. The study population consisted of women undergoing in vitro fertilization and aged between 18 - 45 years. The medical records of 56 infertile patients undergoing IVF in the program at center of Baghdad center in the period between February 2015 and July 2015.

This study resulted in a pregnant women were 11 (20%) while the non-pregnant women were 45 (80%). The mean levels of E2 fluctuated among different age groups showing significantly with age >35 as compared with another group. The mean levels of E2 in follicular fluid fluctuated among different BMI groups showing that mid 30 year women had more chance to have pregnancy through IVF, and there's no significant between pregnant and non-pregnant. When related to BMI, IVF result showed that the chance of IVF success increased with decreased BMI. This implies that slim women had more chance to have pregnancy through IVF. Furthermore, level of E2 in follicular fluid couldn't be used in IVF as a predictor success of pregnancy.

## مستوى هورمون 17 بيتا استراديول في السائل الجريبي للمريضات اللواتي خضعن لعمليات اطفال الانابيب و علاقته مع معدل الحمل

هرمون استراديول ينتج أساسا من المبيض والمشيمة و كميات صغيرة منه من الغدة الكظرية والخصيتين. وهو في حالة من التوازن مع إيسترن، التي يمكن تحويلها إلى استريول بالكبد والمشيمة. وهو مثل هورمونات-LH-FSH البروجسترون من حيث كون تركيز الاستراديول ضروري لتحقيق الخصوبة. في هذه الدراسة تم تقييم E2 في السائل الجريبي في مرحلة ما قبل التبويض كمنبئ لمعدل الحمل بين الإناث اللواتي تمر بالاخصاب في الأنابيب (اطفال الانابيب)لالمرضى العراقيين . هذه الدراسة اجريت على النساء اللواتي تمر بالاخصاب في الأنابيب، والذين تتراوح أعمارهم بين 18-45 سنة. تم عمل مع عدد من المريضات 56 يعانين من العقم وخضعن لعمليات اطفال الانابيب في مركز بغداد للعقم في الفترة ما بين شباط 2015 وتموز 2015.

وأسفرت هذه الدراسة ان النساء الحوامل يمثلن 11 (20 في المائة) بينما كانت النساء غير الحوامل 45 (80%). وان متوسط مستويات E2 تغيرت بين مختلف الفئات العمرية في السائل الجريبي إلى حد كبير مع التقدم في السن اكبرمن 35 سنة مقارنة بمجموعة أخرى.ولقد تذذبت مستويات ال E2 فيم بين مختلف فئات مؤشر كتلة الجسم تبين أن المرأة لها منتصف السنة 30 فرصة أكبر للحمل عن طريق التلقيح الاصطناعي وانه لا يوجد فرق ملحوظ بين النساء الحوامل وغير الحوامل. وعندما يقارن بمؤشر كتلة الجسم فقد أظهرت النتائج أن فرص نجاح عمليات اطفال الانابيب زاد مع انخفض مؤشر كتلة الجسم . وهذا يعني أن المرأة ذات الوزن الاقل لها فرصة أكبر للحمل عن طريق اطفال الانابيب. وعلاوة على ذلك، لا يمكن استخدام مستوى ال E2 في السائل الجريبي لعمليات اطفال الانابيب كعامل متنبئ بالحمل .

## Introduction:

IVF means 'fertilisation in glass' (or nowadays a plastic dish) outside the female body [1].

A stimulated IVF cycle begins with drug administration to suppress the woman's menstrual cycle, and injections to stimulate oocyte (egg) development and maturation. At ovulation a trans vaginal ultrasound is used to guide a needle to the ovary and collect the eggs, which are subsequently mixed with the man's sperm. In the UK after three to five days either two (women under 40) or three (over 40) developing embryos are put into the uterus, with the goal of implantation and ultimately birth [2].

In 2003 – 2008, 23,737 patients in the UK underwent IVF, IVF has a 20% live birth rate b with fresh embryos, and 12% with frozen embryos 2 . These success rates are dependent on the female's age (if she uses her own eggs), decreasing significantly from a woman's mid30s.It is estimated that after five IVF cycles, approximately half the women under 34 will have conceived, but only 30% of those aged [3].

It is not surprising that the peak age of IVF usage correlates with the age at which female fertility rapidly declines [4].

Sex steroid hormones are located at the end of the cholesterol steroidogenesis pathway. The major circulating form of steroidogenic cholesterol in humans is LDL [5]. Delivery of LDL to the ovary allows for synthesis of androgens by thecal cells and aromatization of androgens to estrogen via the aromatase enzyme.

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, analyse the predictive role of mid-luteal phase for CC and (iii) validate the role of luteal phase E2 as predictors of (ongoing) pregnancy.

The synthesis of E2 in growing follicles occurs via interactions between Granulosa cell GC and cells of the theca interna. Estradiol synthesis influenced by LH and FSH levels, the number of luteinizing hormone recomponante  LHr and follicle stimulating hormone recompanate FSHr and the availability of precursor substrates. Studies characterizing E2 synthesis in the ovary have been completed in the bovine model and are similar to human ovarian steroidogenesis[6, 7].

## 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 infertility undergoing IVF in the program at Baghdad 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 Gonadotorophin 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 inflamma-tory disease, endometriosis); No

history of medical disorders (e.g.: thyroid dysfunction, hypertension, liver disease, DM, renal disease); Nonsmoker or alcohol consumer and exclude azoospermia as a cause of male infertility.

## Ovulation induction and IVF procedure:

Ovulation induction (OI) and IVF procedure was performed according to the standard long protocol [8]. 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 concentration5110 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 was assessed with a scoring system by Steer et al. [9]. Preceding embryo transfer (ETs) on day 2 or 3 [10]. Pregnancy was detected by hCG measurement on embryo transfer day fourteen (ETd14), 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 was 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) is a number calculated from a person's weight and height. BMI is a screening method that may indicate underlying health issues [11]

## 1 Estradiol assay

   E2 level was determined according to Tietz (1995)[12] method using TECO Diagnostics ELISA kit for E2.

## Data analysis:

Statistical calculations were made using the Statistical Package for the Social Sciences (version 12.0, SPSSInc., Chicago, IL, USA). For continuous variables Student's t-test and for categorical variables Chi-squared test and Fisher's exact test were used . Results are expressed as means ±SD or percentages (counts) as appropriate. Statistical significance was defined as a value of P <0.05

### 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 Baghdad center. The pregnant women

were 11 (20%) while the non-pregnant women were 45 (80%) as shown in figure (1). 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).
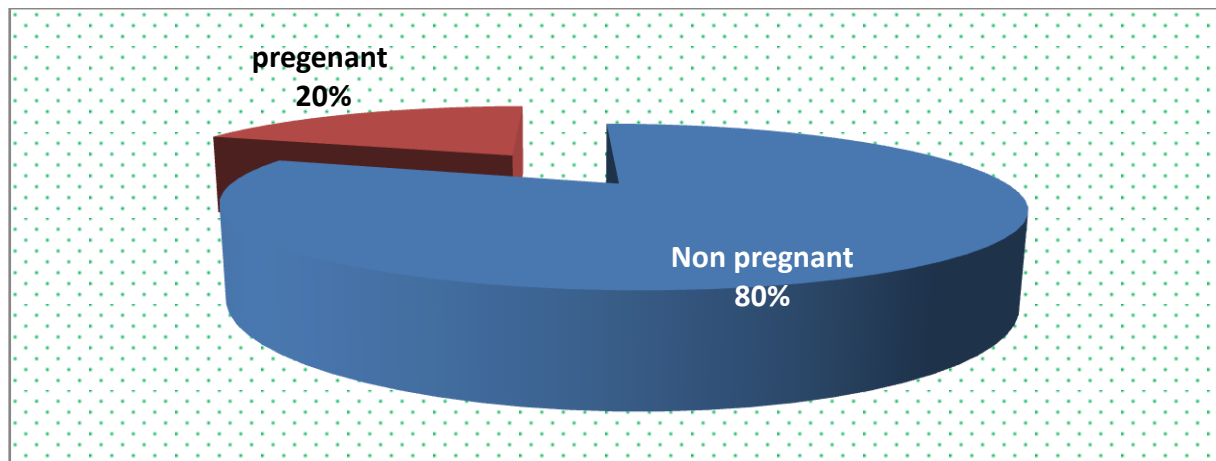


**Figure (1): Shows the pregnancy rate .**

The mean levels of E2 in relation to the age of the study population in blood serum 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 levels of 1875.55 ±1516.02 pg/ml for age ≤25 year and level 3516.95±2670.811 pg/ml at age 26-35 years at age (year) >35 year 2505.23±2421.656 (p=0.4, P=0.1and 0.02 respectively). Group C showed significant correlation between age>35 with S.E2 as compared with another group in table 1 and figure (2,3,4).

**Table 1 : Shows the mean levels of E2 among different age group .**

| Hormone level | Age (year) ≤25 | Age (year) 26-35 | Age (year) >35 |
|---|---|---|---|
| **E2 ( pg/ml)** | 1875.55 ±1516.02 | 3516.95±2670.811 | 2 505.23±2421.656 |
| **P value** | 0.4 | 0.1 | 0.02 |

All values are expressed as mean ±SD.

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

The mean levels of, E2 in relation to the BMI of the study population in blood serum are illustrated in Table 2. According to their BMI, the study population was divided into three groups: Group A (Normal) BMI ≤ 24.9 (Kg/m2) , Group B (Overweight) BMI 25– 29.9 (Kg/m2), Group C (Obese) BMI ≥ 30 (Kg/m2). The
mean levels of E2 in blood serum fluctuated among different BMI groups showing the 3028.18±2581.787 for Group A (Normal) BMI, the 3597.65±2688.991pg/ml for Group B (Overweight) and level 1455.84±1110.442 for Group C (Obese). There's no significantly in all Group related to group shown in table 2 and figures (5,6,7).
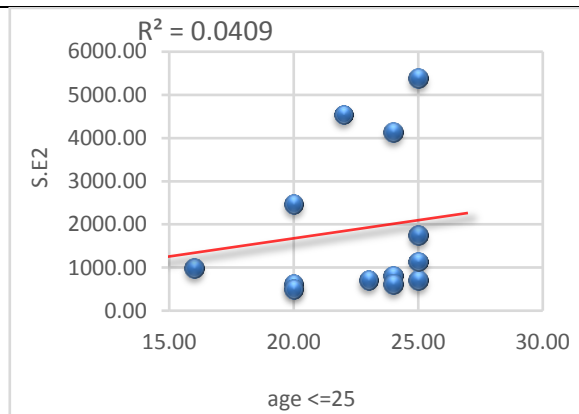
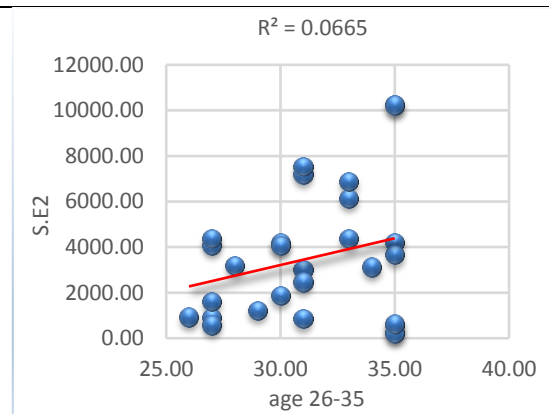Figure 2: Illustrate the correlation between S.E2 with age ≤ 25



Figure 3: Illustrate the correlation between S.E2 with age 26-35
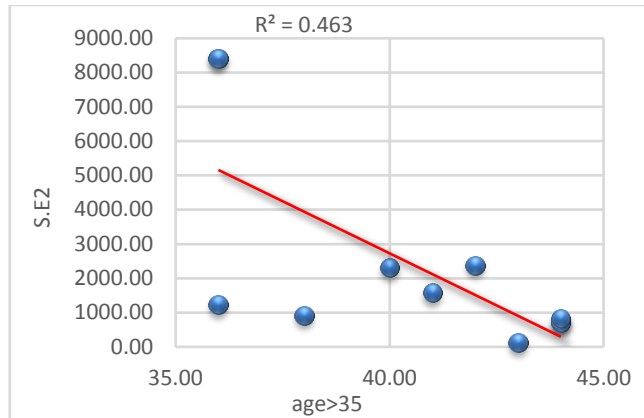


Figure 4: Illustrate the correlation between S.E2 with age>35

**Table 2: Shows the mean levels of serum E2 in relation to the BMI of the study groups.**

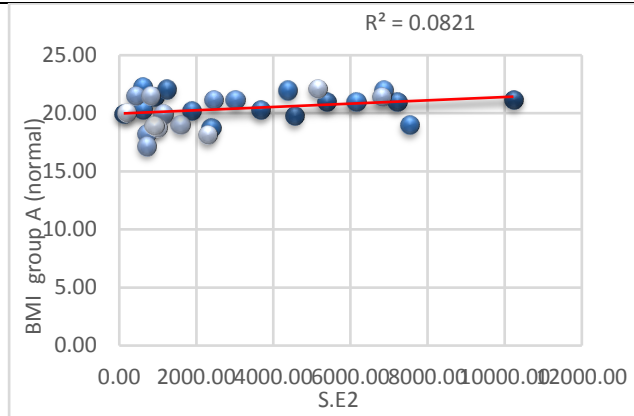| | Blood serum | | |
|---|---|---|---|
| | Group A (Normal) BMI ≤ 24.9 (Kg/m2) | Group B (Overweight) BMI 25– 29.9 (Kg/m2) | Group C (Obese) BMI ≥30 (Kg/m2) |
| E2 ( pg/ml) | 3028.18±2581.78 | 3597.65±2688.991 | 1455.84±1110.442 |
| P value | 0.12 | 0.053 | 0.059 |

Figure 5: Illustrate the correlation between BMI and S.E2 in group A (normal)
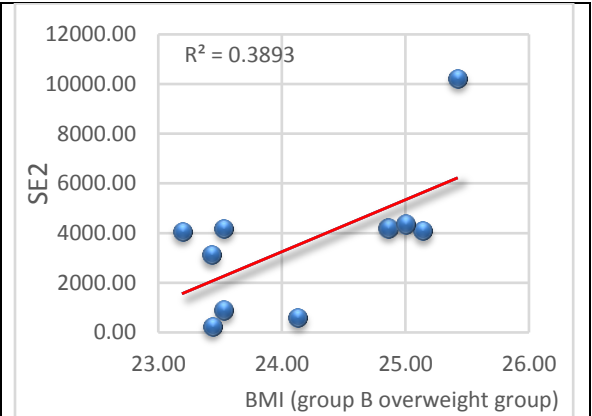


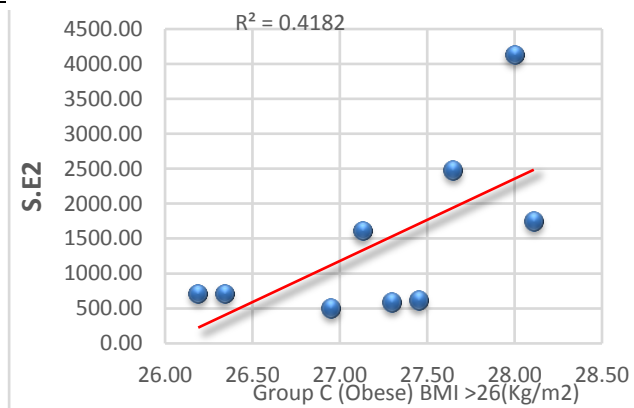Figure 6: Illustrate the correlation between BMI and S.E2 in group B (overweight)



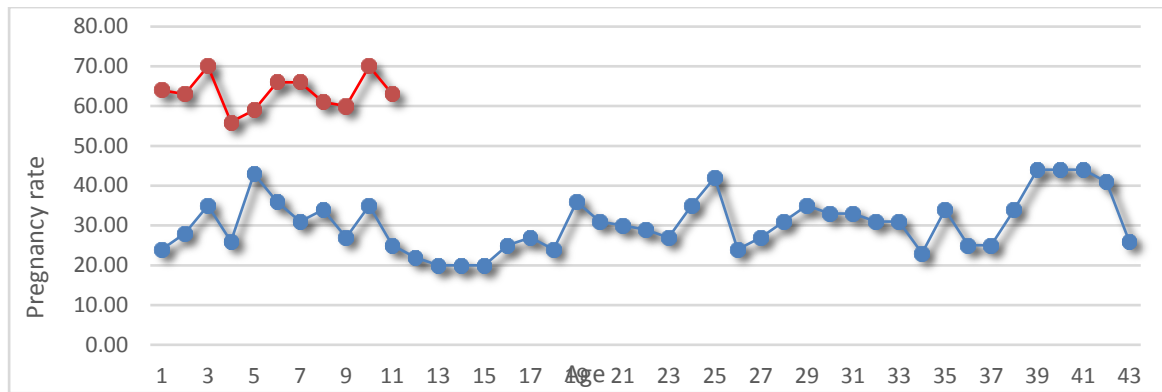Figure 7: Illustrate the correlation between BMI and S.E2 in Group C (obese)

The result of IVF (positive pregnancy, negative pregnancy) in relation to age of cases is 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 shown in table 3.

**Table 3: Shows the difference between the pregnant and non-pregnant out-come with their age.**

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

All values are expressed as mean ±SD.

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

**Figure (8): Shows the correlation between the out- come of IVF with age.**

The result of IVF (positive pregnancy, negative pregnancy) in relation to BMI of cases is provided in Table 4. 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 shown in table 4 and figure(9).
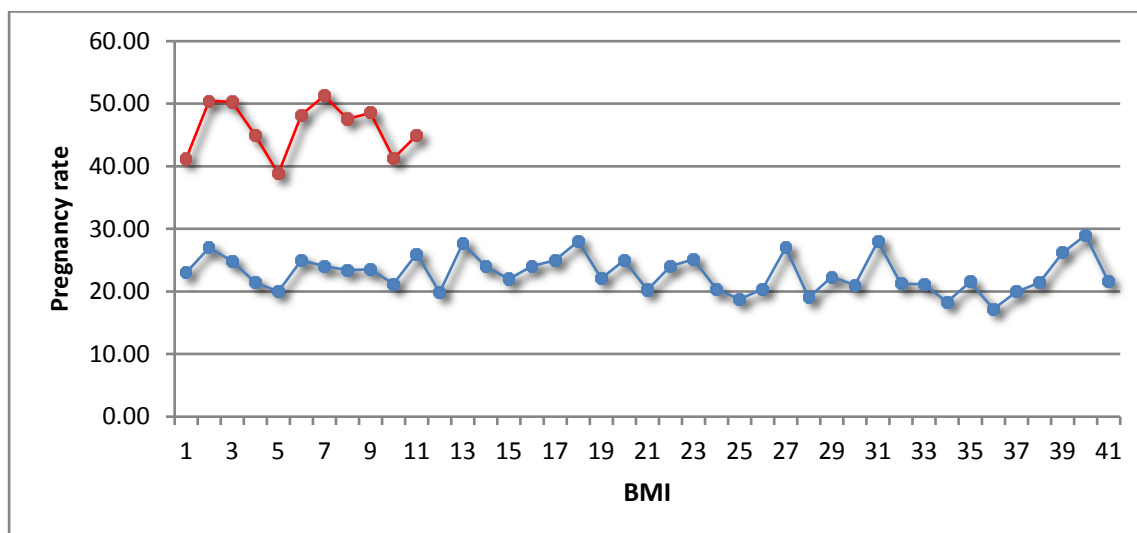
**Table 4 : Shows the difference between the out-come of IVF with their BMI.**

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

All values are expressed as mean ±SD. * Positive: pregnancy occurred.

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



**Figure (9): Showed the correlation between BMI and IVF outcome (pregnant and non-pregnant women).**

Table 5. Summarizes the outcome of IVF (positive pregnancy, negative pregnancy ) in relation to the mean levels of E2 in blood serum . The mean level of E2 in serum was observed in negative pregnancy 2924.05±2570.09, followed by positive pregnancy 2604.80±3014. The difference in E2 levels among these classes was no significant difference (p>0.05) as showed in table 5 and figure (10).

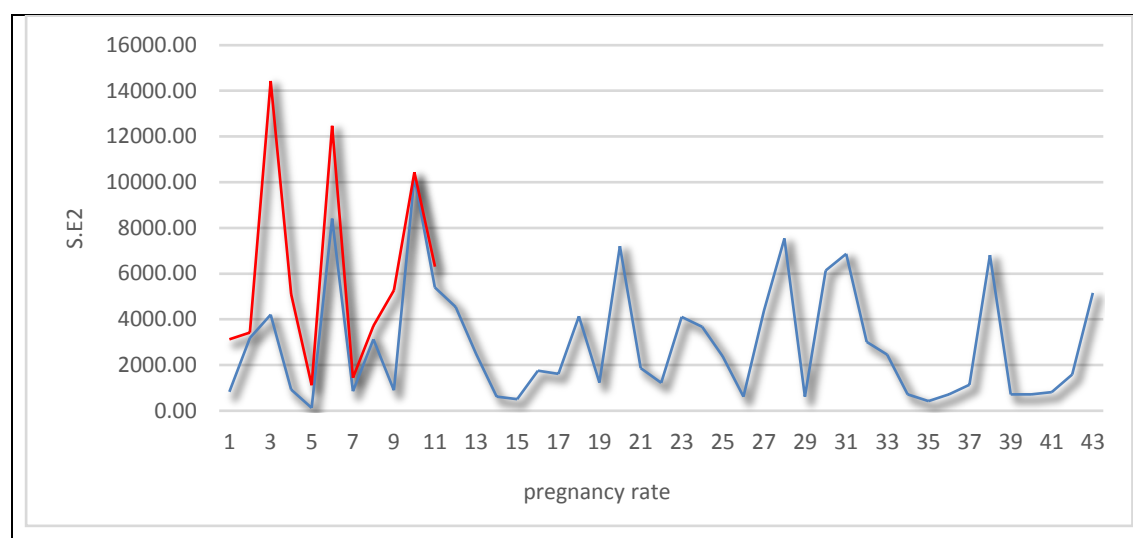**Table 5 : Showed the difference in E2 and IVF out come .**

| Blood serum | | | |
|---|---|---|---|
| **Pregnany** | **positive** | **negative** | **p-value** |
| **E2 ( pg/ml)** | **2604.80±3014** | **2924.05±2570.09** | 0.121 |

All values are expressed as mean ±SD.

Positive: pregnancy occurred.

Negative: no pregnancy

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



**Figure (10): Showed the correlation between S.E2 with IVF outcome (pregnant and non-pregnant women).**

**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[13]; Dehghani-Fieouzabadi et al., 2008[14] , Mohammed M. Laqqan;2010[15] .but was close to that reported from the Netherlands (33.8 years) and UK studies (30.36year) [3]HFEA's IVF National data Statistics 1/04/2000 – 31/03/2001, (van Rooij et al., 2002 [16]).

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 study the number of patients, their age different with another studies 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) (*Hala Abd El-Fttah Ali, *et al* ;2013[17]). In previous study BMI (p value) was 0.25, pregnant group mean = 25.1, non-pregnant group mean = 25.0, (Tiffany et al 2005)[18] and not similar with our studies. In another study morbidly obese women had significantly lower clinical pregnancy rates after IVF.

Data presented in this study showed that the mean levels of E2 in patient undergoes IVF was fluctuated among BMI groups, the maximum level of E2 was in BMI 23– 25 (Kg/m2) . The detrimental effect of BMI on delay in conception is attributed to its effects on the ovary as well as endometrium. Maximum number of study group comprising of overweight women with BMI ≥26. This result is supported by Esinler et al who correlated multiple endocrine and metabolic alterations in obese women giving rise to infertility [19,20]. Our study is not Conformity in the same linear with (*Hala Abd El-Fttah Ali, *et al* ;2013)[17] 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 .

In vitro fertilization results presented in this study showed that the chance of IVF success not related 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 ((Advanced Fertility Center, 2010)[21] and (van Rooij *et al*., 2002)[16]. Previous study is 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 Gnoth *et al*. (2008)[21] 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)[22]. The mean levels of E2, was slightly varied in different classes of IVF outcome with no significant differences between the classes. mean level of E2 in serum was observed in negative pregnancy followed by positive pregnancy. Similar results were exhibited by Smeenk et al. (2007)[22].

In the follicular phase, as a result of growth of follicles serum 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. The association between E2 level and IVF outcome was evaluated by many studies. Therefore our studies different with (Aktan E, Bozkurt K; 2004)[24] and (Phophong P;2000)[25]. In our study a lower pregnancy rate was achieved in women who had a low E2. Many other studies suggested that with the higher E2 levels, higher pregnancy rates were achieved. theres no significant between S.E2 in pregnant and non-pregnant women.

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