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A Prospective study: Correlation between Serum Estradiol levels and Fertilization Rates in Assisted Reproductive Technology Outcomes

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Abstract

Background and Objective: Advancements in reproductive procedures require an understanding of factors influencing in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) outcomes. The study objective is to evaluate the impact of serum estradiol (E2) levels and fertilization rates on the success of IVF/ICSI in infertile women. **Materials and Methods:** The study comprised one hundred and twenty infertile women undergoing IVF/ICSI treatment. Hormone levels were measured using enzyme-linked immunosorbent assays (ELISA). The fertilization rate (FR) was estimated by dividing the fertilized oocytes by the total number of microinjection oocytes. Based on the IVF/ICSI outcomes, participants were categorized into pregnant and non-pregnant groups. **Results:** The pregnant group had a significantly higher mean E2 level (1480.84 ± 564.29) than the non-pregnant group (1147.84 ± 440.56) ($p < 0.001$). There were significant correlations between baseline E2 levels and IVF/ICSI outcomes ($r = -0.317$, $p < 0.001$). Furthermore, the pregnant group had a significantly higher mean of FR (72.22 ± 9.56) than the non-pregnant group (44.88 ± 37.17) ($p < 0.001$). Additionally, significant correlations were found between FR and IVF/ICSI outcomes ($r = -0.420$, $p < 0.001$).

Conclusion: Higher E2 levels and higher FR significantly affect the probability of success in IVF/ICSI treatments. More research is needed to substantiate these findings and clarify the underlying processes at work.

Keywords: Estradiol (E2); Fertilization Rate; ICSI; IVF.

Introduction:

Assisted reproductive technology (ART), notably IVF, has transformed the field of reproductive medicine, providing couples battling infertility with hope and a road to fatherhood (1). As the demand for ART continues to rise, there is a constant challenge to improve treatment results by effectively addressing the factors that influence them. The

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predictors of IVF results are still unknown, and the total rate of successful IVF pregnancies is around 30% (2).

Achieving effective IVF outcomes is a complex process driven by several interconnected factors, including age, embryo quality, endometrial receptivity, ovarian response to stimulation, and reasons for infertility (3,4,5,6). Multivariate analysis provides a thorough method for simultaneously evaluating several factors of IVF outcomes. Although prior research has identified the fertilization rate (FR) as a significant parameter associated with clinical outcomes, the precise degree of its influence remains uncertain (7). Further research has consistently reported that the chance of achieving a successful pregnancy through IVF techniques is directly linked to a higher FR. Additionally, the FR can be used as a valuable biological indicator to evaluate the implantation rate in IVF procedures, leading to more favorable reproductive outcomes(8).

Serum estradiol is crucial for endometrial receptivity, myometrium spiral artery remodeling, and placental development (9). However, despite advancements in understanding the detrimental effects of elevated estradiol levels, the relationship between high E2 levels and clinical IVF outcomes remains equivocal (10). Previous research has linked higher serum E2 levels to decreased endometrial receptivity and lower rates of clinical pregnancy (11). Another study reported that higher E2 levels during the proliferative phase were possibly associated with an unfavorable uterine environment during peri-implantation (12,13). Furthermore, it has been observed that lower rates of implantation are associated with elevated serum E2 levels before embryo transfer in fresh IVF cycles (14). New research reported that maintaining optimal E2 levels can improve IVF outcomes (15).

According to the obtained data, it is evident that the elevated E2 levels and high FR in IVF/ICSI outcomes are still a matter of debate and controversy. Consequently, our research aims to explore the association between serum E2 levels and FR and their impact on the overall success rate of IVF/ICSI.

Materials and Methods

A prospective study involved 120 infertile patients who underwent IVF/ICSI treatment at Kamal Al-Samarra'ay Specialized Hospital between November 2021 and October 2022. The study adhered to ethical standards and was approved by the hospital's ethics committee. All participants provided signed informed consent before their inclusion in the study.

Demographic data and pregnancy outcomes were obtained for analysis in this study. According to IVF/ICSI outcomes, the participants were categorized into patients who achieved pregnancy and those who failed to achieve pregnancy.

All participants underwent controlled ovarian stimulation with gonadotropins, followed by an ovulation induction protocol tailored to their follicle maturity. Venous blood samples were collected on the second or third day of the menstrual cycle to measure hormone levels, such as follicle-stimulating hormone (FSH), luteinizing hormone (LH), estrogen (E2), progesterone (P), testosterone (T), prolactin (PRL), and anti-Mullerian hormone (AMH).

After 36 hours, the retrieved oocytes were stripped of their outer layer and rinsed with a culture medium to evaluate their level of maturity. The fertilization process, either through IVF or ICSI, was carried out and the presence of two pronuclei was observed 16-20 hours later to confirm fertilization. The FR was calculated by dividing the number of fertilized oocytes by the total number of microinjection oocytes (16). The quality of the embryo was assessed based on various factors such as morphology, cell division patterns, and the presence of fragmentation (17). Embryos are transferred on either day 2 or day 3 after fertilization, with the number of embryos determined by the patient's age and embryo quality.

The beta hCG levels were evaluated along with transvaginal ultrasound examinations to establish clinical pregnancy. Patients with beta hCG levels greater than 25 units and demonstrated cardiac activity on transvaginal ultrasound were considered to be clinically pregnant. (18).

Statistical analysis:

The IBM SPSS Statistics 27 was used for data analysis. The quantitative data are expressed as Mean and standard deviation (SD) for normal distribution. One-way analysis of variance (ANOVA) was used to compare study groups. Chi-square tests were used to compare categorical variables. Correlation analysis was used to assess the associations between variables. Pearson's correlation coefficient (r) was used for assessing the strength and trend of the correlations. The statistical significance of the observed association was assessed using a significance level of $p \leq 0.05$.

Results:

Table 1 revealed that out of 120 subjects, 49 (40.83%) became pregnant, while 71 (59.17%) did not. There was a significant difference in basal E2 levels between the pregnant (1480.84 ± 564.29 pg/ml) and non-pregnant (1147.84 ± 440.56 pg/ml, $p < 0.001$) groups. Furthermore, the pregnant participants had a significantly higher mean FR (72.22 ± 9.56) compared to the non-pregnant subjects (44.88 ± 37.17) ($p < 0.001$).

Moreover, pregnant subjects exhibited higher levels of progesterone and AMH, along with increased numbers of retrieved and mature oocytes and embryos. However, these findings lack statistical significance.

Table 2 displays the results of the correlation analysis conducted between infertility parameters and IVF/ICSI outcomes in the study participants. The analysis revealed significant negative correlations among various parameters. Specifically, a notable inverse correlation was identified between the E2 levels of pregnant and non-pregnant subjects ($r = -0.317$, $p < 0.001$). Additionally, a significant negative correlation was found between the FR in both groups ($r = -0.420$, $p < 0.001$). These findings indicate that there is a negative relationship between these parameters and the IVF/ICSI outcomes in non-pregnant subjects.

Table 1- Demographic and Clinical Characteristics Analysis Based on IVF/ICSI Outcomes of the Studied Groups.

Variables	**PREGNANT (n=49) 40.83%**	**NON PREGNANT (n=71) 59.17%**	**P value**
Age	31.34 ± 5.28	32.50 ± 5.41	0.246
Duration of infertility	6.14 ± 3.60	5.38 ±3.67	0.263
BMI	26.49±4.28	27.51 ± 4.37	0.209
Estradiol (pg/ml)	**1480.84 ±564.29**	**1147.84 ± 440.56**	**0.000**
FSH (IU/L)	4.74 ± 1.16	5.08 ± 1.66	0.213
LH (IU/L)	2.95 ± 1.18	3.30 ± 1.76	0.216
FSH/LH Ratio	0.64 ± 0.32	0.67 ± 0.35	0.65
Progesterone (ng/L)	0.39 ± 0.11	0.37 0.09	0.44
Testosterone (ng/L)	0.40 ± 0.13	0.46 ± 0.18	0.056
Prolactin	16.95 ± 4.74	18.53 ± 6.90	0.166
AMH (ng/L)	1.71± 0.93	1.66 ± 0.86	0.782
Oocyte number (mean ±SD)	9.02± 1.87	8.84 ±3.14	0.728
Mature oocyte	6.73 ± 1.65	6.42± 2.91	0.401
No of embryo	5.83 ± 1.55	5.53 ± 1.72	0.329
Embryo Transfers	3.22 ± 1.17	3.49±1.06	0.197
Fertilization Rate	**72.22 ± 9.56**	**44.88±37.17**	**0.000**

Table 2- The correlation between IVF/ICSI outcomes with other infertility parameters.

Variables	IVF/ ICSI outcome	
	r	**P-value**
Age	0.107	0.246
Duration of infertility	-0.103	0.263
BMI	0.116	0.209
Estradiol	**-0.317****	**0.000**
FSH (IU/L)	0.114	0.213
LH (IU/L)	0.114	0.216
FSH/LH Ratio	0.042	0.650
Progesterone (µg/L)	-0.07	0.445
Testosterone (µg/L)	0.175	0.056
Prolactin	0.127	0.166
AMH	-0.026	0.782
Oocyte number	-0.032	0.728
Mature oocyte	-0.077	0.401
No of embryo	-0.090	0.329
Embryo Transfers	0.119	0.197
Fertilization Rate	**-0.420****	**0.000**

Discussion:

The present study demonstrated clear differences in E2 levels between pregnant and non-pregnant women. The pregnant women had significantly higher levels of E2 than the non-pregnant subjects. Though there were variations in the serum E2 levels associated with the highest pregnancy rates compared to previous research, these differences may be due to variances in hormonal analysis methods and other contributing factors. Our findings support previous research indicating that higher levels of serum E2 are linked to increased rates of implantation and clinical pregnancy (10)(15). A retrospective study reported that higher E2 levels were correlated with higher pregnancy rates, with no significant association between elevated levels of E2 and negative perinatal consequences (19).

The current study demonstrated a significant negative correlation between the outcome of IVF/ICSI and the E2 levels, suggesting that higher levels of E2 may reduce the chances of achieving pregnancy. In contrast, a prospective study reported that elevated E2 levels had negative effects on both the development of embryos and the endometrium (20). Following previous research, it has been reported that the use of gonadotropins for ovarian stimulation could potentially reduce endometrial receptivity, which in turn lowers the chances of successful embryo implantation and subsequent pregnancy (21). Moreover, increased E2 levels during ovarian stimulation in IVF compromise trophoblast invasion and placentation, raising the risk of pregnancy complications (22).

It is worth noting that the hormone parameters - P, T, PRL, and AMH - did not show any significant differences between the pregnant and non-pregnant groups. However, it is worth mentioning that there was a trend towards significance for T levels (p=0.056). Our findings support recent research that indicates that higher levels of androgens can disrupt the ovulation process, leading to infertility (23). However, our results are in contrast to recent clinical evidence that reported that higher levels of androgen are associated with better ovarian response, which in turn leads to improved IVF outcomes (24)(25).

Furthermore, the current study revealed a substantial difference in FR between pregnant and non-pregnant women. The results additionally revealed that higher FR levels increase the likelihood of successful embryo development and favorable IVF/ICSI outcomes. These findings are consistent with recent evidence that indicates a favorable correlation between FR and ICSI outcomes (26). Moreover, another study emphasized the significance of FR as a valid indicator of implantation outcomes (8).

Conclusion:

The chance of successful conception in IVF/ICSI is enhanced with higher E2 levels and FR. However, more research is needed to confirm these conclusions and gain a better understanding of the fundamental processes involved.

ETHICAL DECLARATIONS

Ethics Approval and Consent to Participate

Written approval was obtained from the Ethics Committee of the College of Medicine, Al-Iraqia University, Baghdad, Iraq (CM/SA/296). The study data were used purely for research purposes, and all participants provided informed consent before their inclusion in the study.

Consent for Publication

Not applicable (no individual personal data included).

Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that there is no conflict of interest.

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Authors' Contributions

All the authors listed have made a significant, direct, and intellectual contribution to the work and approved it for publication.

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