

ASSOCIATION OF 17 β ESTRADIOL LEVELS ON THE DAY OF hCG ADMINISTRATION WITH PREGNANCY RATE IN IVF (IN VITRO FERTILIZATION) PATIENTS

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ABSTRACT

In vitro fertilization (IVF) is a commonly performed assisted reproductive technology (ART) procedure which is being adopted by infertile couples across the world. Despite the proven benefits, IVF has a low success rate. Evaluation of different parameters at different stages of this treatment is helpful for the clinicians to identify patients with poor prognosis and to improve the overall success rate. The study was planned to systematically review the association between 17 β Estradiol (E₂) levels on the day of β hCG administration and pregnancy achievement in IVF patients and also to assess whether fall or rise in E₂ is detrimental to pregnancy outcome. The study included 120 infertile female patients in the age range 25 - 40 years. The patients selected were subjected to standardized initial screening and baseline study between day 3-5 of menstrual cycle. Ovulation induction was done and when atleast 5 follicles had reached >14 mm of development, hCG (10000 IU/IM) was administered. On the same day, serum estradiol levels were measured and on its basis patients were grouped as (<1000; 1000-2500 & >2500pg/ml). The various steps of IVF protocol were performed and finally serum hCG was evaluated for chemical conformation of pregnancy and further after 15 days Transvaginalsonography was done for clinical conformation of pregnancy. A significantly higher pregnancy rate was observed in patients with E₂ level 1000-2500 pg/ml as compared to other groups. A rise or fall beyond it is detrimental for the success of IVF. Hence a specific range of serum 1000-2500 pg/ml is favorable for higher pregnancy rate in IVF patients.

Keywords: Estradiol, In Vitro Fertilization, IVF, ART, hCG, Pregnancy, Ovulation

INTRODUCTION

Infertility is defined as one year of unprotected intercourse without pregnancy. IVF (In Vitro Fertilization) is a commonly used technique for the treatment of infertility. It is an ART (Assisted Reproductive Technology) procedure which involves removal of eggs from a woman's ovaries and their fertilization outside her body. The resulting embryos are transferred into the woman's uterus through cervix (Steptoe *et al.*, 1978). IVF involves several steps viz. ovulation induction, egg retrieval, sperm preparation & insemination/Intracytoplasmic Sperm Injection (ICSI), assessment of fertilization, embryo development, luteal phase support, and embryo transfer (Michael *et al.*, 2000).

Despite its proven benefits, IVF remains confined to a limited population because of the involved expenses, lengthy procedure as well as the physical and emotional stress that the patient has to undergo. Prior to the treatment, patient has to be counseled and educated about the various steps involved. One of the most challenging tasks is to identify patients with low prognosis i.e. low chances of achieving live birth. Clinicians are, therefore, constantly searching for measures to improve the success rate as well as to make this procedure more patient friendly (Templeton *et al.*, 1996).

Sex hormones like Follicle stimulating hormone (FSH), Leutinizing hormone (LH), Prolactin (PRL) and Progesterone (P₄) serve as major markers during the IVF protocols. Assessment of the role of these hormone levels for IVF outcome has been the focus of interest for many years (Lass *et al.*, 1997). One such hormone is Estradiol (E₂). During ovarian stimulation for the purpose of medically assisted procreation, the assay of E₂ enables follicular maturation to be monitored and assessed. Quantitative measurement of estradiol is useful for the evaluation and management of the number of sexual disorders

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like delayed and precocious puberty, menstrual cycle disorders, menopause, ovulation induction, and gynecomastia. Estradiol is secreted from ovarian follicles during the female menstrual cycle and the placenta during pregnancy. It is also secreted to a lesser degree by the adrenal glands, testes and the peripheral conversion of androgen. The synthesis and secretion of estradiol is regulated via hypothalamic-pituitary axis through Leutinizing Hormone Releasing Hormone (LHRH), LH and FSH. Evaluation of the estradiol concentration in conjugation with LH, FSH, and P_4 assay, is useful for evaluating whether the hypothalamic-pituitary-gonadal axis is functioning correctly and is intact (Corsan and Kemmann, 1991; Pittaway and Wentz, 1983).

So far, the role of E_2 level on day of hCG administration in pregnancy achievement has only been evaluated retrospectively. Moreover, these studies have suggested contradictory results (Chen *et al.*, 2003; Chenette *et al.*, 1990; Joo *et al.*, 2010; Kyrou *et al.*, 2009; Mettler *et al.*, 1989; Ng *et al.*, 2000; Simon *et al.*, 1995; Valbuena *et al.*, 2001). The aim of the present study was to assess the association of Estradiol (E_2) level on day of hCG administration on the pregnancy rate in IVF patients.

MATERIALS AND METHODS

The study was conducted on the infertile female patients visiting the infertility clinic “Jaipur Fertility Centre (JFC) of Mahatma Gandhi Medical College and Hospital, Jaipur”. Of the total 268 patients who visited the clinic within a period of 8 months, 120 females were included in the current study. Inclusion criteria were infertile female patients, age 25 to 40 years and first IVF attempt. Embryo transfer was performed on day 5. Of the total 268 patients, 07 due to a lack of response, 33 due to age, 20 due to risk of Ovarian Hyper Stimulation Syndrome (OHSS), 39 due to poor embryo quality, 38 due to insufficient number of embryos, and 11 due to some other unexplained reasons were excluded and finally, 120 patients were selected for further analysis.

The patients selected were subjected to standardize initial screening to outline the general cause of infertility. A complete medical, surgical, and gynecologic history of the female patient was obtained by the physician. Thorough physical examination of the females was performed including height, weight, body habitus, hair distribution, thyroid glands, and pelvic examination. Blood sample was collected and subjected to routine haematological, biochemical and serological analysis. Hormones viz. Serum Thyroid Stimulating Hormone (TSH) & Prolactin (PRL) were also analyzed. Ultrasonography (uterus adenexa) and Video-Hystero-laprosopy (VHL) were conducted to check the endometrial thickness and to assess of the external architecture of the tubes and, visualization of the fimbria. Following initial screening the patient was asked to skip one cycle and come between day 3rd to 5th of the next cycle. In this visit, the patient was to baseline study which included transvaginal sonography (TVS) of uterus adenexa and hormonal assay viz. Serum Leutinizing Hormone (LH), Follicle Stimulating Hormone (FSH) and 17 β estradiol (E_2). Based on the observations of the baseline study a suitable ovulation protocol was chosen. Ovulation induction was done by FSH/re-combinent FSH (Sitrodine, Gonal-F, Newmone-R, Folligraph, Fostine, etc.) or human Menopausal Gonadotrophin (Menagon, Menapure, Persinal). When the ovarian follicles (05 or more) had reached (>14 mm) of development, human Chorionic Gonadotropin (hCG) injection 10000 IU/IM was administered followed by oocyte retrieval 33 to 36 hours after the hCG injection. Serum E_2 level was measured again approximately 12 hours before hCG administration. All the hormone assays in the present study were by enzyme linked fluorescent assay (ELFA) on VIDAS using Biomerieux kits. All patients were divided into three groups based on the serum E_2 levels on the day of hCG administration: (i) < 1000 pg/ml (n = 42); (ii) 1000 – 2500 pg/ml (n = 40) and (iii) >2500 pg/ml (n = 38).

Along with oocyte retrieval, simultaneous sperm preparation was performed. This was followed by insemination or ICSI. Fertilization was assessed 12 to 16 hours after insemination or ICSI and a close monitoring of the embryo development was observed from day 2 to day 5. Embryo transfer was performed on day 5 after insemination. After 15 days of embryo transfer serum β hCG (on VIDAS instrument and kits) was evaluated to confirm chemical pregnancy. Clinical pregnancy was confirmed by TVS 15 days after the confirmation of chemical pregnancy. The pregnancy rate by (both chemical and

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clinical) thus obtained, was correlated with the serum E₂ levels on the day of β hCG administration. The results were statistically analyzed by applying Chi-square test. The P- value ≤ 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

The present study was conducted on 120 infertile female patients in the age range of 25 to 40 years (30.6 $\pm$ 3.8). The study was planned to systematically review the association between serum E₂ levels on the day of hCG administration and pregnancy achievement in IVF and also to access whether fall or rise in E₂ is detrimental to pregnancy outcome.

Estradiol levels fall rapidly in the luteal phase of ART procedure. Ovarian stimulation is necessary for multiple follicular developments and is accompanied by serum 17 β estradiol (E₂) levels. Following ovulation induction, E₂ level can be increased more than 10 fold compared with those found during spontaneous cycle. On the basis of the fact that cyclic changes in the endometrium are regulated by ovarian steroid hormones, the increased E₂ level due to controlled ovarian hyper-stimulation may compromise endometrial receptivity for embryo implantation (Garcia *et al.*, 1984; Hadi *et al.*, 1994; Pittaway and Wentz, 1983). So far, the effect of this estradiol decline on pregnancy outcome has remained controversial.

A negative association between the probability of pregnancy and E₂ level on the day of hCG administration has been reported. High E₂ levels are responsible for cases of impaired endometrial receptivity without affecting embryo quality. Moreover high E₂ level has been shown to adversely affect the embryonic implantation (Ng *et al.*, 2000; Simon *et al.*, 1995; Valbuena *et al.*, 2001). On the other hand several studies have suggested that the pregnancy achievement is dependent on E₂ level on the day of hCG administration or there is a positive association between them (Chenette *et al.*, 1990; Joo *et al.*, 2000). Some studies suggested that E₂ level does not affect the pregnancy rate in IVF cycles (Chen *et al.*, 2003; Kyrou *et al.*, 2009; Mettler *et al.*, 1989).

According to the serum E₂ concentration on the day of hCG administration, all patients were divided in to three groups viz. <1000pg/ml (n = 42), 1000 – 2500 pg/ml (n = 40) and >2500 pg/ml (n=38). The association between serum E₂ levels on the day of hCG administration and pregnancy rate, both chemical and clinical was reviewed. Chemical pregnancy is confirmed positive for serum β hCG levels of > 50.0 U/L. The present study reported 42.5 % positive chemical pregnancies (Table 3). Serum quantitative β hCG levels, lower than 50 IU/L are associated with a 35% chance of an ongoing intrauterine pregnancy; levels greater than 500 IU/L are predictive of successful outcome in more than 95% of cases (Homan *et al.*, 2000). However, clinical pregnancy was confirmed by TVS, 15 days after the confirmation of chemical pregnancy.

Table 1: Positive chemical & clinical pregnancy cases in the groups based on S Estradiol levels on the day of hCG administration

E ₂ levels	No. of Patients (n)	S. β hCG Positive	% Positive	Clinical pregnancy	% Positive
<1000 pg/ml	42	12	28.6	9	21.4
1000-2500 pg/ml	40	28	70.0	23	57.5
>2500 pg/ml	38	11	28.9	10	26.3
Total	120	51	42.5	42	35.0
Chi-square (x²)			45.18		32.62
P-value			0.000		0.000

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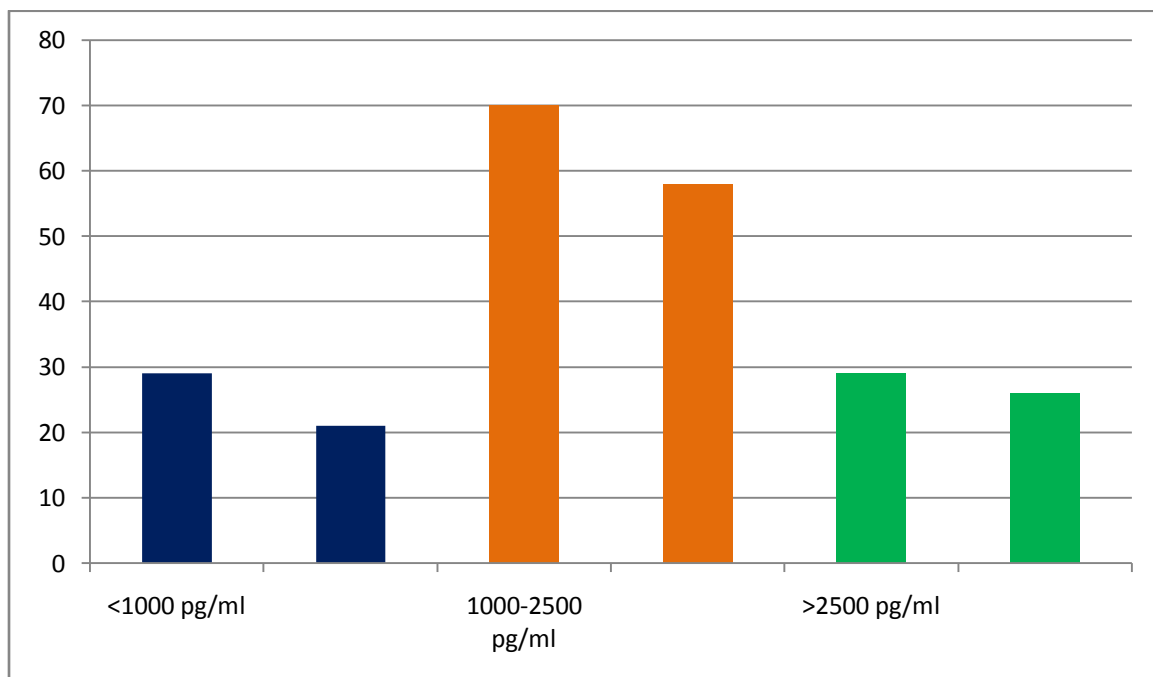


Figure 1: Comparison of Positive chemical & clinical pregnancy rate in the groups based on S Estradiol levels on the day of hCG administration

The synchronized effect of E_2 followed by P_4 produces a receptive endometrium that is essential for blastocyst implantation (Narvekar *et al.*, 2010).

A negative association between the probability of pregnancy and E_2 level on the day of hCG has been reported. Simon *et al.*, 1995 have suggested that high E_2 level is responsible for cases of impaired endometrial receptivity without affecting embryo quality. Moreover high E_2 level has been shown to adversely affect the embryonic implantation (Simon *et al.*, 1995; Valbuena *et al.*, 2001). On the contrary, several studies have suggested that the pregnancy achievement is dependent on E_2 level on the day of hCG administration. In other words, they suggest a positive association between the two (Chenette *et al.*, 1990; Gelety and Bualos, 1995; Joo *et al.*, 2010). Some recent studies have, however, suggested that E_2 level does not affect the pregnancy rate in IVF cycles (Chen *et al.*, 2003; Kosmas *et al.*, 2004; Kyrrou *et al.*, 2009; Mettler *et al.*, 1989; Sharara and Mc Clamrock, 1999).

The study suggests a positive association between serum E_2 levels 1000 – 2500 pg/ml on day of hCG administration and the chemical pregnancy rate (S. β hCG positive) as compared to levels below and higher than this range (Table 1). In the follicular phase, E_2 causes proliferation of surface epithelium, glands, stroma and blood vessels in endometrium and regulation of P_4 receptors. The synchronized effect of E_2 followed by P_4 produces a receptive endometrium that is essential for blastocyst implantation. The luteal phase in ART procedure has been noted to be truncated with the duration of ovarian steroid production usually shorter due to an abrupt decline in serum E_2 and P_4 levels. It is well established that the iatrogenic corpus luteal deficiency necessitates P_4 supplementation in the luteal phase. However, there is no agreement on whether the accompanying E_2 fall is detrimental to pregnancy outcome, and if so whether luteal E_2 supplementation would improve the outcome (Narvekar *et al.*, 2010).

The chemical pregnancy rate is significantly high ($p < 0.05$) in the subgroup with serum E_2 levels 1000 – 2500 pg/ml on the day of hCG administration (Figure 1). A rise or fall beyond this range is detrimental for the success of IVF. The overall success rate (clinical pregnancy) in the present study was 35 %. The success rate of IVF is still very low. Since its introduction almost 33 years ago, attempts to improve the IVF success rate are going on. Any factor that can increase the pregnancy rate is therefore looked forward to. Our study recommends a specific range of serum E_2 (1000 – 2500 pg/ml) as favorable for a higher

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pregnancy rate. The above findings can be confirmed further by conducting similar studies on larger cohorts. The study also proposes the combination of current finding with other probabilities or variations in the IVF protocol to improve the success rate further.

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