Original Article

Does First Serum Beta-Human Chorionic Gonadotropin Value Prognosticate the Early Pregnancy Outcome in an *In-Vitro* Fertilisation Cycle?

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Abstract

Background: Pregnancies achieved through *in-vitro* fertilisation (IVF) are associated with adverse first trimester outcomes in comparison to spontaneously achieved pregnancies. In view of this, it is imperative to predict the success as well as prognosticate the pregnancy outcome of an IVF cycle not only for the clinicians but also the couples undergoing IVF. Serum beta-human chorionic gonadotropin (β-hCG) value has, thus, been used as a biomarker for pregnancy outcome after IVF and also an aid in counselling and management of the patient. **Aim:** The main objective of this study was to compare the predictive value of the first serum β-hCG value and the pregnancy outcome after an IVF cycle (whether fresh or frozen embryo transfer) in the two subgroups of patients. **Settings and Design:** The study was conducted at Assisted Reproductive Technology Centre of a tertiary care hospital, and it was a retrospective cohort study. **Methods and Materials:** A retrospective study was performed for post-IVF pregnancies at a single IVF centre from March 2014 to February 2015 with serum β-hCG values less than or equal to 1000 mIU/ml. The initial serum values of β-hCG on the day 16 of embryo transfer were correlated with first trimester pregnancy outcome and ongoing pregnancy rate (>12 weeks gestation). **Results:** Of the 208 post-IVF pregnancies included in the study, the group with β-hCG more than 500 mIU/ml had statistically significant higher ongoing pregnancy rates and a lesser poor pregnancy outcome. **Conclusion:** The study concluded that an early serum β-hCG value can be used as a predictor of a successful or an adverse first trimester pregnancy outcome helping in better counselling and monitoring of the high-risk precious IVF pregnancies.

Keywords: *Adverse pregnancy outcome, IVF, β-hCG*

Introduction

Human chorionic gonadotropin (hCG), the pregnancy hormone produced by the syncytiotrophoblast, can be detected in the maternal serum as early as 8 days post-conception.[1,2] As its serum level represents the trophoblastic mass and function with levels increasing proportionately during early gestation, a single determination of serum beta-human chorionic gonadotropin (β-hCG) 12–18 days after embryo transfer (ET) has been established as a reliable early indicator of pregnancy outcome after assisted reproductive techniques (ART).[3-5]

Pregnancies conceived after *in-vitro* fertilisation and ET (IVF-ET) are at an increased risk for adverse outcomes such as ectopic gestation, biochemical pregnancy or spontaneous abortions in comparison with spontaneously conceived pregnancies.[6,7] Multiple gestations also occur more frequently after an IVF.[7,8] Therefore, it is prudent to provide early and confirmatory information, not only about the success after ET, but also about the

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prognosis of such a pregnancy. Thus, β-hCG levels in addition to allaying the anxiety and stress of the subfertile couple also aid the clinicians in predicting the outcome and, allows for the modification for monitoring, treatment and counselling. Transvaginal ultrasound (TVS) examination, albeit a great boon for follow-up of an IVF pregnancy, has its limitation in the early post-implantation period, as it makes a gestational sac reliably visible only 3 weeks after ET. Thus, early absolute β-hCG values having an edge over TVS can be used as a marker to predict implantation and help in the early detection and follow-up of adverse pregnancy outcomes in conjunction with ultrasound subsequently.[9-11]

Herein, we performed a study with an aim to assess the predictive value of single serum β-hCG level, measured 15 days after ET and its correlation with pregnancy wastage and/or ongoing pregnancies after IVF-ET or frozen ET (FET) cycle.

Materials and Methods

Patients and study design

This was a retrospective comparative study conducted over a period of 1 year (March 2014 to February 2015) at the Assisted Reproductive Technology Centre of a tertiary care hospital. Records of 655 women, who conceived after a fresh ET (IVF-ET) or FET during this, were reviewed and analysed. Of these 655 successful IVF pregnancies, 208 fulfilled our inclusion criteria, which were (i) initial serum β-hCG values ≤ 1000 mIU/ml, (ii) serum hCG had been assayed on the 16th day post-ET, and its concentration was ≥50 mIU/ml and (iii) serum hCG had been assayed from the same laboratory. The women were excluded from the study if they were lost to follow-up for the early pregnancy outcome till the end of the first trimester. They were also not a part of the analysis if β-hCG was performed any day before or after the 16th day or were detected to have multiple gestation. The 208 women who were finally analysed and included were further divided into two groups; group I included women whose β-hCG values were ≤500 mIU/ml, whereas patients whose serum β-hCG values were 501–1000 mIU/ml formed group II.

Treatment protocol

All the patients underwent IVF-ET according to the conventional protocols. In the long or agonist protocol, pituitary downregulation was performed using gonadotropin-releasing hormone agonist (leuprolide acetate) 0.5 mg subcutaneously from the 21st day of the menstrual cycle. In the antagonist protocol, Cetrorelix 0.25 mg (Serono, SA, The Netherlands) subcutaneous injection was started when the leading follicle reached 14 mm after ovarian stimulation. Ovarian stimulation was achieved using recombinant follicle-stimulating hormone (Gonal F; Serono, SA, The Netherlands), and follicular monitoring was performed by transvaginal ultrasonography from the day 5 of stimulation. When at least three leading follicles reached the size of 18 mm, recombinant hCG 250 μg (Ovitrelle; Serono, SA, The Netherlands) was subcutaneously administered. TVS-guided aspiration of the follicles was performed 34–36 h later. The retrieved oocytes were fertilised by either conventional IVF or intracytoplasmic sperm injection depending on the patient's history. A maximum of two to three grade I embryos at 8-cell stage were transferred on the day 3, and the supernumerary embryos were cryopreserved. For the luteal phase support, intramuscular progesterone 100 mg was given daily along with tablet dydrogesterone 10 mg twice a day (Duphaston 10 mg) until a serum β-hCG assay was performed 15 days after the ET.

For the FET cycles, endometrial preparation was performed using hormone replacement with estradiol valerate given orally (4–6 mg daily; Tab Progynova; Bayer Zydus) from the 2nd day of the menstrual cycle, until the endometrial thickness reached ≥7 mm per two layers. Intramuscular progesterone 100 mg was then started, followed by 8-cell ET after 3 days. Oestrogen and progesterone treatment was continued till serum β-hCG levels were measured on the 16th day and continued until the end of the first trimester in viable pregnancies.

Beta-human chorionic gonadotropin

As a protocol, we performed serum β-hCG assay routinely 15 days after ET. Serum β-hCG concentrations were measured in the laboratory of our hospital using chemiluminescent microparticle enzyme immunometric assay for the total β-hCG molecule. The test had been standardised according to the Third International Standard for Chorionic Gonadotropin from the National Institute for Biological Standards and Control (75/537).

Pregnancy outcome

Pregnancy or positive IVF status was said to have occurred when the β-hCG level was ≥50 IU/L. If the day 16 β-hCG test was positive, but less than 200 mIU/ml, a second β-hCG concentration was performed 48 h later as a protocol with an aim to assist in predicting a viable intrauterine pregnancy or to assist in evaluating a possible ectopic pregnancy. However, the first β-hCG value was considered for the statistical analysis. Thereafter, the confirmation of clinical pregnancy was made by a TVS 3 weeks post-ET. Our primary outcome measure was to study the pregnancy outcome till the end of the first trimester and the secondary outcome measure to ascertain pregnancy wastage or nonviable pregnancy.

Nonviable pregnancies were the ones with biochemical pregnancy, falling β-hCG concentrations, which ultimately became negative, blighted ovum (an empty gestational sac on ultrasound), first trimester abortions and ectopic pregnancies. Biochemical pregnancy was defined as pregnancy detected by β-hCG measurement (>50 IU/L) and no gestational sac visible on serial TVS. Ongoing pregnancy was defined as a successful progression beyond 12 weeks gestation (singleton or multiple). Multiple gestations were defined by having more than one embryo with cardiac activity; however, they were not a part of our analysis.

Statistical analysis

Data were analysed using the Statistical Package for the Social Sciences version 16.0 software (SPSS Inc., Chicago, IL, United States). Group comparisons were made using Chi-square (χ^2) test (for categorical variables) or Student's *t*-test (for scalar variables). Pregnancy rates were compared across different categories using χ^2 test. For all the statistical tests, the level of significance was taken as $P < 0.05$.

Results

Of the 208 women who formed the study group, 106 women belonged to the group I with β-hCG levels less than or equal to 500 mIU/ml, whereas 102 patients had levels 501–1000 mIU/ml. Baseline characteristics of the patients of both the groups have been depicted in Table 1, and they were comparable. The contributory cause for subfertility and the reason for undergoing ART were tubal factor, ovarian causes (endometriosis and anovulation), male factor and other factors as depicted in Figures 1 and 2. The cycle characteristics of both the study group patients are shown in Table 2. In more than 80% of the patients, agonist protocol was used, and a maximum of three embryos were transferred depending on the age of the woman. In group I, 11 women underwent a FET after an IVF, whereas nine patients had an FET in the second group.

It was observed that among the 106 positive IVF pregnancies in group I (β-hCG ≤ 500), pregnancy wastage or poor pregnancy outcome was more in comparison to the women in group II. The difference in the number of ectopic pregnancies and first trimester abortions was also statistically significant [Table 3], and although the number of biochemical pregnancies was greater in group I, it was not significant. In fact, we observed in our study that if the β-hCG levels were greater than 500 mIU/ml, there was no biochemical pregnancy [Figure 3]. Eighty-nine patients (87.25%) in the second group as against 65 patients (61.32%) in group I had ongoing pregnancies, that is, they had crossed the first trimester This viable pregnancy rate between the two group is also significant statistically ($P < 0.05$).

Table 1: Demographic profile of the two study groups

Parameter	Group I (n = 106)	Group II (n = 102)	P value
Average age	26 years	26.4 years	NS
Average duration of infertility	6.7 years	6.9 years	NS
Cause of infertility			
Tubal factor (%)	24	21	NS
Ovarian factor (%)	25	23	NS
Male factor (%)	20	26	NS
Unexplained (%)	29	25	NS
Uterine factor (%)	8	7	NS

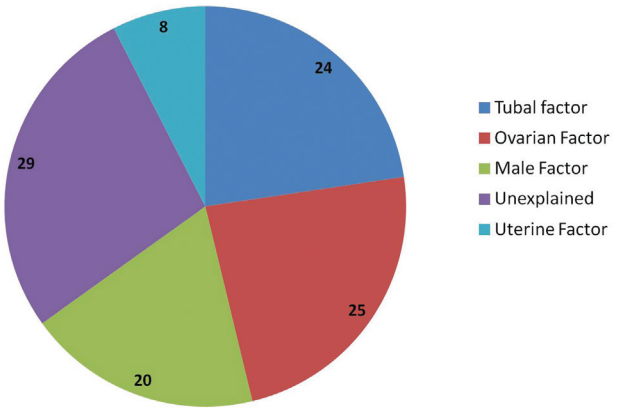

Figure 1: Causes for subfertility in group I (n = 106; β-hCG ≤ 500)

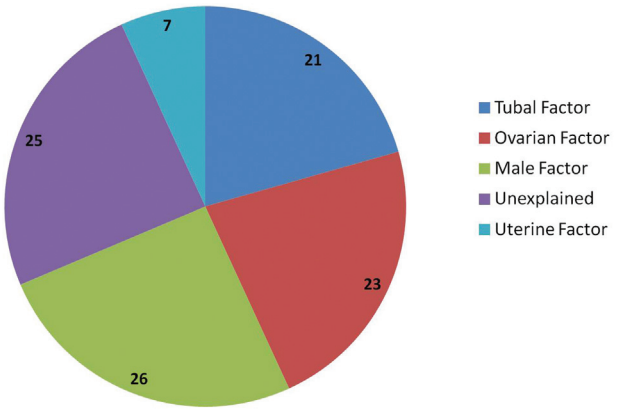

Figure 2: Causes for subfertility in group II (n = 102; β-hCG 501–1000)

The respective mean ages of the patients in the various categories of the pregnancy outcomes of the whole study group (group I and group II) such as ectopic pregnancy, missed abortion or ongoing pregnancy were also comparable. The mean β-hCG value of the women who had an ongoing pregnancy irrespective of which group they belonged was significantly higher as compared to poor pregnancy outcome [Table 4].

Discussion

β-hCG or β human chorionic gonadotropin, the first trophoblastic signal of the ensuing pregnancy, can be detected in the maternal serum as early as 8–9 days after fertilisation or 2–3 days after implantation. Thus, β-hCG level has been used as a yardstick to ascertain the outcome of an IVF cycle to allay the anxiety of the couple and to guide the clinicians for monitoring these high-risk pregnancies, which are associated with early pregnancy complications namely ectopic pregnancy, blighted ovum, missed abortion, etc.[6,7]

Quantitative serum β-hCG assessment for predicting pregnancy outcome has been in vogue since the 1960s. Zegers-Hochschild *et al.*[12] compared early hCG levels in patients who conceived naturally or through assisted reproduction and noted that the former had significantly higher hCG levels. Confino *et al.*[3] also concluded that the patients with poor outcomes had statistically lower hCG levels than the normal pregnancies. Many workers, thereafter, have published their data on serum hCG as a predictor of pregnancy outcome from the western countries.[1,13] In the Indian setting, Singh *et al.*,[14] in their study, correlated the age of the patient and the initial serum values of β-hCG on the day 14 of ET with ongoing pregnancy (>12 weeks gestation). Differing from their

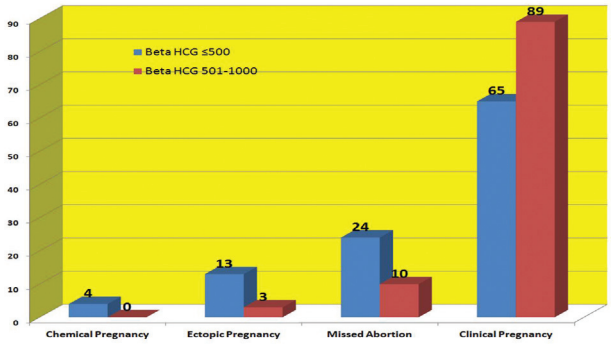

Figure 3: Comparison of pregnancy outcome in the two study groups

Table 2: Cycle characteristics of the study group

Cycle characteristics	Group I (n = 106)	Group II (n = 102)	P value
Fresh IVF cycle	95	93	NS
FET cycle	11	9	NS
IVF protocol: LP	103	100	NS
IVF protocol: AP	3	2	NS
Average number of embryo transferred per cycle	2	2	NS

IVF = *in vitro* fertilisation, FET = frozen embryo transfer, LP = long protocol, AP = antagonist protocol.

Table 3: Comparison of pregnancy outcome in the two study groups

β-hCG group	Chemical pregnancies	Ectopic pregnancies	Missed abortion/an embryonic pregnancies	Clinical pregnancies >12 weeks	Total
	n (%, 95% confidence interval)	n (%, 95% confidence interval)	n (%, 95% confidence interval)	n (%, 95% confidence interval)	
Group I (≤500 mIU/ml)	4 (3.77, 1.21–8.85)	13 (12.26, 6.99–19.57)	24 (22.64, 15.43–31.32)	65 (61.32, 51.80–70.22)	106
Group II (500–1000 mIU/ml)	0 (0, 0.00–2.89)	3 (2.94, 0.75–7.79)	10 (9.80, 5.08–16.77)	89 (87.25, 79.69–92.72)	102
P value	0.13 (not significant)	0.01 (significant)	0.012 (significant)	0.000 (significant)	

Table 4: Comparison of mean β-hCG values of various pregnancy outcomes in the study population (combined group I and group II)

	Mean age (years)	Mean β-hCG (mIU/ml)
Chemical pregnancies	29.25 ± 5.31	163.820
Abortions	29.67 ± 3.95	403.654
Ectopic pregnancies	28.8125 ± 3.600	224.906
Ongoing pregnancy	29.37 ± 3.891	565.824

study, we divided our patients into two subgroups of patients with two different ranges of β-hCG values and analysed the outcome. In our study, it was observed that the higher values of initial serum β-hCG levels were associated with good pregnancy outcome, and more of these pregnancies were likely to continue beyond 12 weeks. In them, 89 of the 102 women had an ongoing pregnancy if the initial β-hCG level was greater than 500 mIU/ml, whereas only 65 of the 106 patients had an ongoing pregnancy in group I, and this difference was statistically significant. Our finding is also in congruence to other studies.[15,16]

The hCG concentrations have also been elucidated to be significantly higher in multiple pregnancy as compared with singleton, and this observation has been used to predict the probability of a higher order birth.[17,18] Singh *et al.*[17-19] also documented that the levels of the day 14 β-hCG in their study was significantly higher in women with higher order pregnancies compared to those with singletons, similar to studies reported in literature. However, we did not include multiple pregnancies in our analysis and excluded them, as our aim was to predict the outcome only in positive β-hCG values less than 1000 mIU/ml, which would not be predictive of all higher order pregnancies.

It has been postulated that many factors influence the β-hCG level dynamics such as day of ET, type of culture media, aetiology of infertility and the type of ART utilised.[1,20,21] Recent studies also suggest that the rate of β-hCG rise is higher following ET of cryopreserved embryos (FET).[22] Relji *et al.*[22] found that the average β-hCG levels were different as per the pregnancy outcome but were not statistically different between fresh ET and FET cycles. In our study, we did not stratify the pregnancy outcome as per the type of transfer; however, both of our study groups included embryos transferred either in the fresh cycle or frozen cycle.

Thus, our study has reiterated that an early or first β-hCG value does prognosticate the pregnancy outcome after an IVF cycle. Considering a cut-off of 1000 mIU/ml and below, and further subdividing them into subgroups have both given a uniqueness to our study as well as a message that, with β-hCG values less than 500 mIU/ml, the clinicians have to be wary of the outcome and monitor them more closely as they are at a higher risk for ectopic gestation and pregnancy failure. These early values would further help us in guiding about the termination of luteal support and counselling of the patients as well.

Conclusion

While the quantitative measurement of serum β-hCG after ET gives the information about the success of an IVF cycle, but its absolute value is also indicative of the pregnancy outcome if the results are favourable. Initial low β-hCG values will prevent the unnecessary delay of diagnosis, especially in the cases of adverse outcomes such as an ectopic or a biochemical pregnancy. Thus, the β-hCG levels can be used to assist and guide the clinician in better management and monitoring of post-IVF pregnancies in conjunction with a transvaginal sonography. Larger multicentre studies can further stratify the cut-off levels for poor pregnancy outcomes and can be used universally.

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Conflicts of interest

There are no conflicts of interest.

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