

# Role of early serum beta human chorionic gonadotropin measurement in predicting multiple pregnancy and pregnancy wastage in an *in vitro* ET fertilization cycle

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Submission: 21.03.2013  
Review completed: 09.06.2013  
Accepted: 03.09.2013

## ABSTRACT

**OBJECTIVE:** This study was performed to assess the prognostic value of serum beta human chorionic gonadotropin ( $\beta$ hCG), measured on day 14 post embryo transfer (ET) for predicting multiple gestation and pregnancy wastage in women undergoing *in vitro* fertilization ET (IVF-ET). **MATERIALS AND METHODS:** This retrospective study was performed between May 2009 and November 2012. Out of the 181 women who conceived, 168 were included and the remaining 13 were excluded as their pregnancy was biochemical. Serum  $\beta$ hCG was measured using a chemiluminescent enzyme immunometric assay. The predictive values of serum  $\beta$ hCG for establishing multiple pregnancy and pregnancy wastages were calculated by receiver operating characteristic (ROC) curve analysis. Median values of serum  $\beta$ hCG and outcome of all pregnancies were compared. **RESULTS:** Out of the 168 patients who conceived after IVF treatment, 114 (68%) were viable pregnancies (delivered/ongoing). Among the viable pregnancies, 97 (85%) had a successful pregnancy outcome and the remaining 17 patients are ongoing pregnancies. Median values of  $\beta$ hCG (625 IU/L) among viable pregnancies was significantly ( $P < 0.05$ ) higher than that of nonviable pregnancies (174 IU/L). The median values of  $\beta$ hCG for singleton (502 IU/L), twins (1093 IU/L), and triplets (2160 IU/L) was statistically significant ( $P < 0.05$ ). Using ROC curve it was predicted that for a value of  $\beta$ hCG at 375 IU/L, the sensitivity of viable pregnancy was 65% and specificity of viable pregnancy was also 65%, with positive and negative predictive values of 65 and 68%, respectively. Similarly for multiple pregnancy and pregnancy wastage the predictive values of  $\beta$ hCG were 808 and 375 IU/L, respectively; while the sensitivity and specificity is more than 65% each. **CONCLUSION:**  $\beta$ hCG cutoff values determined on day 14 post ET by ROC curve analysis are useful in discriminating between multiple pregnancy and pregnancy losses. The cutoff value might aid in the prognosis, clinical management, and counseling of the IVF patients.

**KEY WORDS:** Beta human chorionic gonadotropin, *in vitro* fertilization ET, multiple pregnancy, pregnancy wastage

## INTRODUCTION

Pregnancies achieved by *in vitro* fertilization ET (IVF-ET) are at an increased risk of adverse outcomes compared with natural conceptions.<sup>[1-3]</sup> Pregnancy losses such as preclinical (biochemical), spontaneous abortions, heterotopic pregnancies, ectopic pregnancies (two-five fold more), and multiple gestations (20-25%) are common; making the majority of patients anxious about the outcome of treatment and the viability of

their pregnancies.<sup>[2-6]</sup> Thus, the prediction of pregnancy outcome particularly at the time of pregnancy testing is of interest to many clinicians and infertile couples undergoing IVF-ET. Thus, there is a need for markers that can accurately detect the implantation and predict pregnancy outcome as early as possible after embryo transfer (ET). Earlier diagnosis of adverse pregnancy outcomes in patients who underwent IVF using  $\beta$ hCG aided the clinicians in counseling and management.<sup>[4,6-10]</sup>

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10.4103/0974-1208.121425

Ultrasound examination is a part of the routine follow-up after IVF-ET, but the gestational sac is reliably visible only after the 3<sup>rd</sup> week post ET. Therefore, to confirm pregnancy and assess its outcome at an earlier stage attempts have been made to identify serum biomarkers. We are aware that human chorionic gonadotropin (hCG) can be detected in the maternal serum as early as 8 days post conception.<sup>[6,10]</sup> hCG is produced by syncytiotrophoblast, and its serum level represents the trophoblastic mass.<sup>[11-13]</sup> Human chorionic gonadotropin levels dynamically increase during early gestation and the levels are significantly greater in viable pregnancies than in ectopic gestation, biochemical pregnancy, or spontaneous abortions.<sup>[14-20]</sup> Similarly, the hCG concentrations are significantly higher in multiple pregnancy as compared with singleton. This observation has been used to predict the probability of a higher order birth.<sup>[13,17-19]</sup> The absolute values can therefore be used as markers to predict implantation and help in the early detection of multiple gestation and along with ultrasound assist in the early diagnosis of ectopic pregnancy and pregnancy wastage.<sup>[5,9,14-18,20,21,22]</sup>

The aim of this study was to assess the prognostic value of serum  $\beta$ hCG, measured on day 14 post ET for predicting pregnancy wastage and multiple gestations in women undergoing IVF-ET.

## MATERIALS AND METHODS

This retrospective study was conducted during May 2009 till November 2012. Records of 181 women who conceived during this period were reviewed. Out of the 181 women, 168 were included and the remaining 13 were excluded as their pregnancy was biochemical. The indications for IVF were tubal factor, male factor, endometriosis, anovulatory/polycystic ovary syndrome (PCOS), unexplained infertility, and diminished ovarian reserve.

All patients underwent IVF-ET according to the conventional protocols<sup>[23]</sup>. In the long or agonist protocol pituitary downregulation was done using a midluteal administration of a gonadotropin releasing hormone (GnRH) agonist (Leuprolide) 1 mg subcutaneously. In the antagonist protocol Cetorelix 0.25 mg (Serono, SA, the Netherlands) subcutaneous injections were started when the leading follicle reached 14 mm after ovarian stimulation. Ovarian stimulation was achieved using recombinant follicle stimulating hormone (FSH; Gonal F; Serono, SA, the Netherlands) and follicle monitoring was done by transvaginal ultrasonography from day 5 of stimulation. When at least three leading follicles reached the size of 18 mm, recombinant hCG 250  $\mu$ g (Ovitrelle; Serono, SA) subcutaneously was administered. Transvaginal ultrasound-guided aspiration

of follicles was performed 34-36 h later. Retrieved oocytes were fertilized by either conventional IVF or intracytoplasmic sperm injection. A maximum of two embryos were transferred and the rest were cryopreserved for future use. Micronized vaginal progesterone 600 mg or intramuscular progesterone 100 mg was given daily as luteal phase support until a pregnancy test was performed 2 weeks later.

Samples of venous blood were collected routinely on the 14<sup>th</sup> day after ET. Serum  $\beta$ hCG concentrations were measured using chemiluminescent microparticle enzyme immunoassay for the total  $\beta$ hCG molecule (Access total  $\beta$ hCG REF 33500, Beckman Coulter Inc, USA.) in the IVF laboratory of our hospital. The test has been standardized according to the Third International Standard for Chorionic Gonadotropin from the National Institute for Biological Standards and Control (75/537).

Pregnancy was confirmed when the  $\beta$ hCG level was  $\geq 50$  IU/L. Biochemical pregnancy was defined as pregnancy detected by  $\beta$ hCG measurement ( $< 50$  IU/L) and no gestational sac was seen on transvaginal ultrasound. Ongoing pregnancy was defined as a successful progression beyond 12 weeks gestation (singleton or multiple). Pregnancy wastage or nonviable pregnancy comprised of biochemical pregnancy, blighted ovum, first and second trimester abortions, and ectopic pregnancies. Viable pregnancies comprised of successful ongoing pregnancies, which continued beyond 12 weeks of gestation.

Confirmation of clinical pregnancy was done by a transvaginal ultrasound examination 1 week post  $\beta$ hCG test. The scans were performed on Siemens Acuson Antares, 2007 using EV9F4 (transvaginal). Conversion of crown-rump length from measurement in millimeters to the compatible gestational age was done using the Robinson's table.

## Statistical analysis

Data was analyzed using Statistical Package for Social Sciences (SPSS) version 16.0 software. Pregnancy rates were compared across different categories using Chi-square test. Serum  $\beta$ hCG levels were found to be non-normal even after logarithmic transformation. Therefore, median values of  $\beta$ hCG were computed and compared using nonparametric tests (Man-Whitney U test for two categories and Kruskal-Wallis for more than two categories). Nonparametric receiver operating characteristics (ROC) curve was drawn to assess the cutoff value of  $\beta$ hCG for predicting viable pregnancy, multiple pregnancies, and pregnancy wastages with adequate sensitivity and specificity. For all the statistical tests, the level of significance was taken as  $P < 0.05$ .

## RESULTS

Baseline characteristics of the patients have been described in Table 1. The minimum and maximum age of the patients was 22 and 42 years, respectively with mean age  $\pm$  standard deviation (SD) of  $31.1 \pm 3.6$  years. Almost 81% of patients were within 35 years of age. The main indicators for assisted reproductive technology (ART) were tubal factor (42%) followed by male (16%) and other factors. In more than 80% of patients agonist protocol was used and a maximum of three embryos were transferred depending on the age of the woman.

Among the 168 patients who successfully implanted following IVF treatment, 114 (68%) were viable pregnancy (delivered/ongoing). Similarly, the viable pregnancy rate amongst agonist and antagonist protocol patients was 67% (91/135) and 70% (23/33), respectively and there was no statistical significance between these two rates ( $P > 0.05$ ). Age group analysis of viable pregnancy rate varied between 63% (31–35 years) and 71% (<35 years) and no significant ( $P > 0.05$ ) difference was noted between these age groups. Among the viable pregnancies, 97 (85%) turned out to be successful deliveries and the remaining 17 patients crossed 12 weeks of gestation period.

Serum  $\beta$ hCG level assessment by pregnancy outcome is given in Table 2. Median values of  $\beta$ hCG among viable pregnancies was 625 IU/L and it was significantly ( $P < 0.05$ ) higher than that of nonviable pregnancies (174 IU/L). Among the viable pregnancies, the median value of  $\beta$ hCG

was higher (1007 IU/L) in women with endometriosis followed by unexplained infertility (962 IU/L). However, the median values of  $\beta$ hCG did not vary significantly ( $P > 0.05$ ) across different IVF factors [Figure 1].

The median value (502 IU/L) for  $\beta$ hCG in singleton viable pregnancies was significantly ( $P < 0.05$ ) less than that of multiple viable pregnancies (1,280 IU/L)<sup>[24]</sup>. The median values of  $\beta$ hCG for singleton, twins, and triplets of viable pregnancies were 502, 1,093, and 2160 IU/L, respectively and there was a significant ( $P < 0.05$ ) variation between these categories [Figure 2]. Among nonviable pregnancies the median values of  $\beta$ hCG varied between 39.5 IU/L in biochemical pregnancy wastage and 2,152 IU/L in aborted cases due to twin pregnancy [Figure 2] 14 days post ET.

### Predictive value of serum human chorionic gonadotropin level for viable pregnancy

Nonparametric ROC curve was drawn by taking serum  $\beta$ hCG (logarithmic values) as predictor variable and successful deliveries as event variable. The area under ROC curve and its level of significance is presented in Figure 3.

The area under ROC curve was 0.73 (0.05) and it was statistically significant ( $P < 0.05$ , 95% CI 0.64-0.83). Using the coordinates of ROC it was predicted that for a value of  $\beta$ hCG at 375 IU/L, the sensitivity and specificity of viable pregnancy is 65% with positive and negative predictive values of 65 and 68%, respectively.

### Prediction of serum beta human chorionic gonadotropin level for multiple pregnancy and pregnancy wastage

Nonparametric ROC curve was drawn by taking serum  $\beta$ hCG values as predictor variable for both multiple pregnancies and pregnancy wastages and depicted in Figure 4.

The area under ROC curve for multiple pregnancy was 0.74 (0.05) (95% CI 0.64-0.85) and at  $\beta$ hCG level 808 the sensitivity and specificity was 72%. The area under ROC curve for pregnancy wastage was 0.74 (0.05) (95% CI 0.64-0.83) and at  $\beta$ hCG level 375 the sensitivity and specificity was 65% respectively.

## DISCUSSION

Pregnancy survival depends upon successfully completing

**Table 1: Baseline characteristics of study population (n=168)**

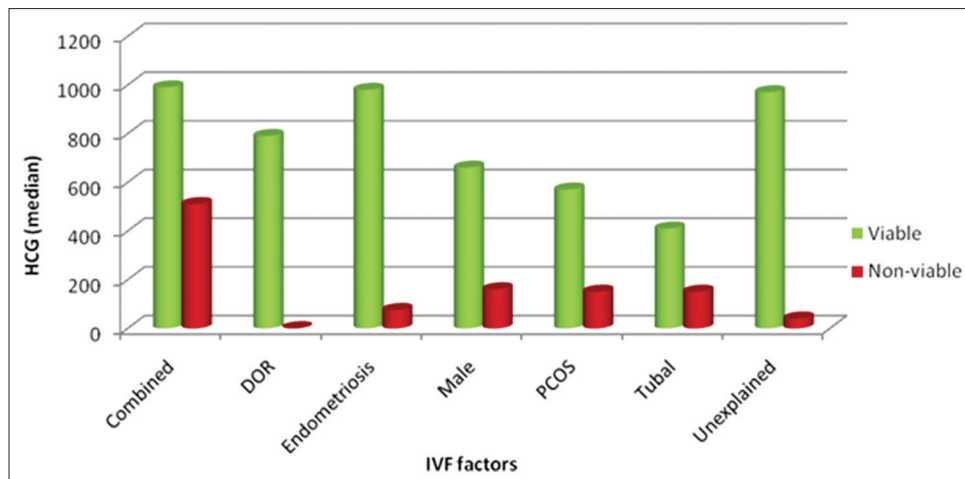
Characteristics	Numbers	Percentage
Age groups (years)		
≤30	80	47.6
31-35	68	40.5
≥35	20	11.9
IVF factors		
Combined	19	11.3
Endometriosis	11	6.5
Male	27	16.1
PCOS	16	9.5
Tubal	70	41.7
Unexplained	20	11.9
Diminished ovarian reserve	5	3.0
Protocol used		
Agonist	135	80.4
Antagonist	33	19.6
Number of embryo transferred		
One	3	1.8
Two	25	14.9
Three	140	83.3

IVF=*In vitro* fertilization; PCOS=Polycystic ovary syndrome

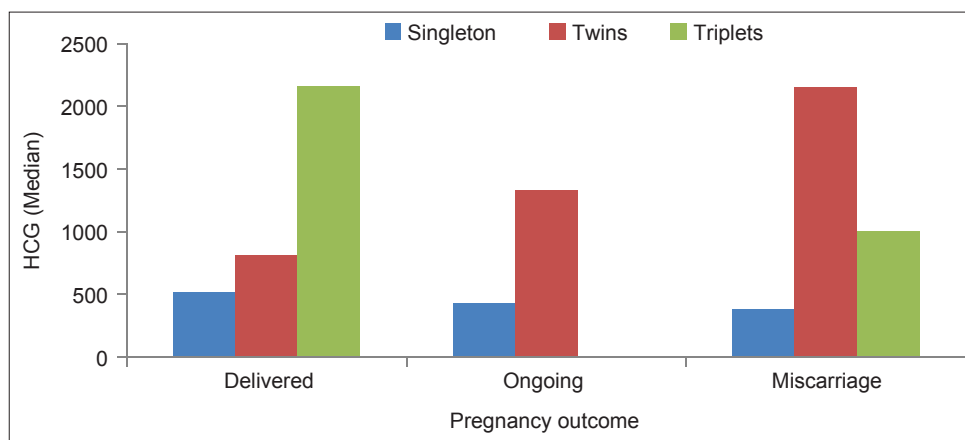
**Table 2: Serum  $\beta$ hCG (IU/L) levels among pregnancy types**

Pregnancy status	Numbers	Mean ( $\pm$ SD)	Median (range)
Delivered	97	934.47 (1188.53)	572 (128-9322)
Ongoing	17	1065.41 (926.21)	977 (234-3899)
Pregnancy wastage	54	520 (863.07)	174 (10-5028)

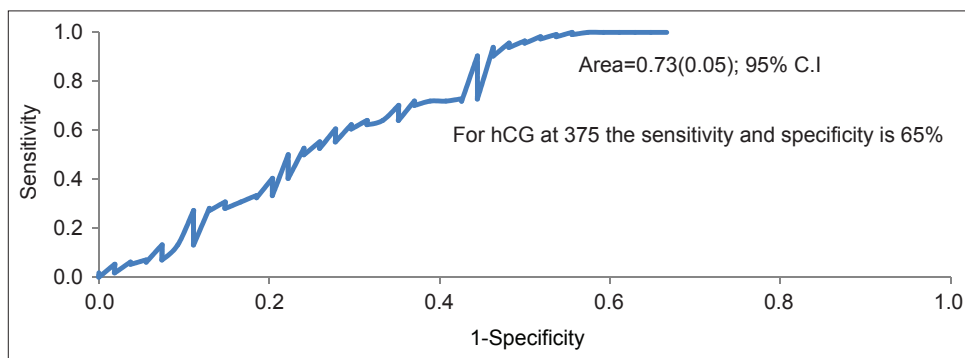
SD=Standard deviation;  $\beta$ hCG=Beta human chorionic gonadotro



**Figure 1:** Median human chorionic gonadotropin values for viable and non-viable pregnancies and factors contributing to IVF



**Figure 2:** Median HCG for pregnancy outcome by pregnancy type

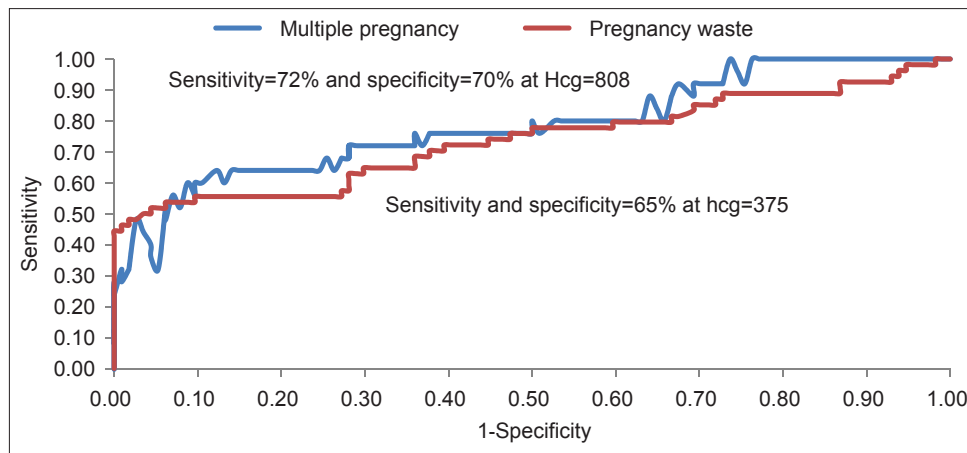


**Figure 3:** ROC for predicting viable pregnancy by serum HCG concentration at 14 days of post embryo transfer

each step of embryonic development in a strict destined script. Serum  $\beta$ hCG levels represent trophoblastic mass and function. Its concentration keeps on multiplying with a relatively narrow range of values at the early stages of pregnancy and every divergence from this predefined incremental curve raises a suspicion of abnormality. It has been suggested that  $\beta$ hCG has more predictive value than other biochemical markers for predicting and assessing pregnancy outcome.<sup>[1,5,11]</sup>

Our data also shows that there were significant differences in the value of  $\beta$ hCG levels studied 14 days post ET in women with viable versus nonviable pregnancies. The median value was higher in successfully delivered (572 IU/L) and ongoing (977 IU/L) pregnancies compared to nonviable pregnancies (174 IU/L) which was similar to the results reported earlier.<sup>[1,3-5,15-18,21,25,26-28]</sup> The levels of day 14  $\beta$ hCG was found to be significantly higher in women





**Figure 4:** Receiver operating characteristic for predicting human chorionic gonadotropin level for multiple pregnancy and pregnancy wastage

with higher order pregnancies compared to those with singletons ( $P < 0.01$ ), which is similar to studies reported in literature.<sup>[1,2,4,7-9,14,16,19,29]</sup> The present study is unique as it defines the cutoff value of  $\beta$ hCG as early as day 14 post ET. The testing of  $\beta$ hCG was performed in a single laboratory, minimizing intraassay and interassay variability which is similar to previous studies.<sup>[9,11,14,16,18,28,29]</sup>

It is well-documented that age is a contributing factor to pregnancy loss and higher pregnancy wastage occurs in women older than 35 years. In our study though 71.5% pregnancy was viable in  $\leq 30$  years patient and it was not statistically significant ( $P < 0.568$ ) between the different age groups. Similarly, 67.6% women successfully delivered in the age group of  $\leq 30$  years. Present study did not find any significance of initial  $\beta$ hCG in relation with age which is similar to the study by McCoy, *et al.*<sup>[2]</sup>

Luteal support, protocol used, and duration of hormonal stimulation were included in the analysis; but the results demonstrated that they had no predictive value.

Median concentration of hCG in tubal factor infertility was considerably low, which is similar to other studies.<sup>[4,18]</sup> Present study showed the median concentration of  $\beta$ hCG (1,007 IU/L) in endometriosis was far greater than other factors in viable and successfully delivered pregnancies.

At  $\beta$ hCG level of 375 IU/L the sensitivity and specificity of viable pregnancy was 65% with positive and negative predictive values as 65 and 68%, respectively. It is discriminated between pregnancy wastages and viable pregnancies; the serum level is higher than other studies because we have included second trimester abortions in this group. We sought a cutoff value to predict multiple gestations with the greatest certainty. The cutoff level that had optimal sensitivity (72%) and specificity (70%) was 808 IU/L.

By choosing day 14 as the test for  $\beta$ hCG, unnecessary delay of diagnosis could be avoided, which is crucial both from a psychological and a medical point of view. Relief of tension might not only be important for the patients' well-being, but also for the outcome of pregnancy. As all IVF patients use progesterone as luteal phase support, an early and reliable test for pregnancy is desirable in order to terminate luteal support in patients not achieving pregnancy. We therefore continue to include the assay of  $\beta$ HCG 14 days after ET in our IVF protocol.

## CONCLUSION

The results show that  $\beta$ hCG cutoff values determined on day 14 after ET by ROC curve analysis are useful in discriminating between multiple pregnancy and pregnancy losses. The cutoff value might aid in the prognosis, clinical management, and counseling of the IVF patients. Pregnant patients with  $\beta$ hCG values greater than the cutoff value can be followed-up clinically and with transvaginal ultrasound at variable intervals.

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**How to cite this article:** Singh N, Begum AA, Malhotra N, Bahadur A, Vanamail P. Role of early serum beta human chorionic gonadotropin measurement in predicting multiple pregnancy and pregnancy wastage in an *in vitro* ET fertilization cycle. *J Hum Reprod Sci* 2013;6:213-8.  
**Source of Support:** Nil, **Conflict of Interest:** None declared.

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