Article

Serum oestradiol and β-HCG measurements after day 3 or 5 embryo transfers in interpreting pregnancy outcome

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Abstract

The aim of this study was to assess the clinical value of serum oestradiol concentration 8 days after embryo transfer (D8E2) and β-human chorionic gonadotrophin (HCG-β) concentration 12 days after embryo transfer (D12HCG-β) in the prediction of pregnancy and the outcome of pregnancy following assisted reproduction, taking into account the day of transfer, which was either day 3 (D3) or day 5 (D5). The objective was to improve patient counselling by giving quantitative and reliable predictive information instead of non-specific uncertainties. A total of 2035 embryo transfer cycles performed between January 2003 and June 2005 were analysed retrospectively. Biochemical pregnancy, ectopic pregnancy and first-trimester abortions were classified as non-viable pregnancies; pregnancies beyond 12 weeks gestation were classified as ongoing pregnancies (OP). Significantly higher D8E2 and D12HCG-β were obtained in D5 transfers compared with D3 transfers with regard to pregnancy and OP ($P \leq 0.001$). For D3 embryo transfers, the cut-off value of D8E2 in predicting OP was 130 pg/ml (sensitivity 80%, specificity 72%), compared with 98 mIU/ml (sensitivity 89%, specificity 69%) for D12HCG-β. For D5 embryo transfers, the values were 179 pg/ml (sensitivity 79%, specificity 84%) and 257 mIU/ml (sensitivity 78%, specificity 81%) respectively. It appears that serum post-embryo transfer D8E2 and D12HCG-β concentrations provided clear information regarding pregnancy and the outcome of pregnancy following IVF–embryo transfer.

Keywords: *β-human chorionic gonadotrophin, embryo transfer, IVF, oestradiol, pregnancy outcome*

Introduction

Patients undergoing assisted reproduction treatments are under a lot of stress, and anxious about the treatment outcome. Moreover, assisted reproduction pregnancies are associated with a higher incidence of ectopic pregnancies and early pregnancy losses than in spontaneous pregnancies (Ben-Rafael *et al.*, 1988; Strandell *et al.*, 1999). Therefore, it is important to be prepared for an adverse pregnancy outcome and to counsel the couple accordingly. The early prediction of pregnancy outcome therefore has great importance for both couples and clinicians in the IVF team.

A test with a high predictive value in the early days of pregnancy, before a demonstrable gestational sac on transvaginal ultrasound, may help to reduce the anxiety of patients and assist the clinician in early pregnancy monitoring and management. Reliable serum markers to discriminate normal and adverse pregnancy outcome in early pregnancy after assisted conception cycles are greatly needed and are being investigated extensively. Serum oestradiol, progesterone and β-human chorionic gonadotrophin (HCG-β) measurements have been investigated as determinants of pregnancy viability (Hutchinson-Williams *et al.*, 1989; Yamashita *et al.*, 1989; Bjercke *et al.*, 1999; Homan *et al.*, 2000; Sugantha *et al.*,

2000; Poikkeus *et al.*, 2002; Urbancsek *et al.*, 2002; Carmona *et al.*, 2003; Anckaert *et al.*, 2005; Ioannidis *et al.*, 2005).

A single measurement of serum HCG-β concentration, as early as 11–12 days after embryo transfer, is not only diagnostic but also has good predictive value for pregnancy outcome (Schmidt *et al.*, 1994; Glatstein *et al.*, 1995; Qasim *et al.*, 1996; Bjercke *et al.*, 1999; Sugantha *et al.*, 2000; Carmona *et al.*, 2003), and is therefore being used as part of the routine follow-up after IVF/intracytoplasmic sperm injection (ICSI).

Serum oestradiol concentration after embryo transfer has also been investigated as a predictor of pregnancy outcome. Significantly higher oestradiol concentrations were observed in conception cycles 8 days after HCG administration (Hutchinson-Williams *et al.*, 1989). This raised early luteal oestradiol secretion in conception cycles of the corpus luteum is suggested to be stimulated by the early embryo (Baird *et al.*, 1997). However, serum oestradiol measurement is not routine in IVF pregnancy follow-up, unlike HCG-β.

Advances in assisted reproduction techniques might have possible effects on serum HCG-β concentrations. For example, the medium used to culture embryos from days 1 to 3 was shown to affect the ability of the embryo to secrete HCG after implantation (Orasanu *et al.*, 2006). Embryos obtained from assisted conception cycles are transferred to the uterus at different developmental stages. Therefore, in counselling patients about pregnancy and the outcome of pregnancy following IVF–embryo transfer, serum oestradiol and HCG-β values might be displaying different outcomes depending on the transfer day. Delay of embryo transfer by 2 days might result in different interpretations of serum oestradiol and HCG-β concentrations. Zhang and colleagues (2003) concluded that HCG-β concentrations in pregnancies resulting from day 5 transfers were lower than those from day 3 transfers when assessed at equivalent intervals from fertilization.

The objective of the current study was to assess the clinical value of serum oestradiol concentrations 8 days after embryo transfer and HCG-β concentrations 12 days after embryo transfer in the prediction of pregnancy and the outcome of pregnancy following assisted reproduction, taking into account the day of transfer. The probabilities of pregnancy outcomes were further evaluated for each HCG-β concentration and compared according to embryo transfer day. Thus, it is hoped to improve patient counselling by giving quantitative and reliable predictive information instead of non-specific, uncertain words.

Materials and methods

Patients and treatment protocols

The files of patients treated at Istanbul Memorial Hospital ART and Genetics Centre during the period January 2003 to June 2005 were retrospectively reviewed. Embryo transfer cycles with available data on concentrations of serum post-embryo transfer day 8 oestradiol (D8E2) and day 12 HCG-β (D12HCG-β) and pregnancy outcome were included and grouped according to the day of embryo transfer. A total of 2035 embryo transfers performed either on day 3 (D3) or day 5 (D5) after oocyte retrieval were analysed (1422 on day 3 and 613 on day 5).

Ovarian stimulation was performed with either mid-luteal gonadotrophin-releasing hormone (GnRH) agonist, long or GnRH agonist flare-up or GnRH antagonist protocol. Gonadotrophin doses used were 225–450 IU daily. When the leading follicle reached a diameter of 20 mm, 10,000 IU of HCG was administered and oocytes were collected 35–36 h later under transvaginal ultrasound guidance. IVF or ICSI procedures were performed as described previously (Kahraman *et al.*, 1999). On day 3 after oocyte retrieval, two to five embryos with the best morphological grade were selected for transfer. If there were more than five embryos with 8 cells and minimal or no fragmentation, the transfer was planned to be done on day 5. Delaying embryo transfer was suggested to allow selection of the most vital embryos for transfer (Borini *et al.*, 2005). On day 5, two to five embryos with the best morphological scores were transferred. The number of embryos transferred depended mainly on the patient's age, the number of previous attempts and embryo quality. Luteal phase support was provided with i.m. progesterone 75 mg daily, starting on the day after oocyte retrieval, and in case of pregnancy, intravaginal progesterone (Progestan; Kocak, Istanbul, Turkey) 600 mg daily was prescribed until 12 weeks gestational age. In cases of D12HCG-β concentrations >10 mIU/ml, which indicated conception, the test was repeated after 2 days. If the second HCG-β value increased two-fold, transvaginal ultrasound was performed 21 days later to visualize the fetal heartbeat.

Pregnancy outcome

Three groups of women were defined according to pregnancy outcome: non-pregnant, women with non-viable pregnancy and women with ongoing pregnancy.

Non-viable pregnancies included ectopic pregnancies, biochemical pregnancies (an initial rise of serum HCG-β concentrations without a progressing pregnancy), and first-trimester abortions (eventual arrest of development of intrauterine gestational sac/embryo seen on transvaginal ultrasound). Ongoing pregnancy was defined as one that proceeded beyond the first trimester at the time the data collection was ended (September 2005).

Differentiation between ongoing pregnancies versus non-viable ones and multiple pregnancies versus singleton ones were analysed according to D8E2 and D12HCG-β values, taking into account the day of transfer.

Hormone assays

Serum oestradiol and HCG-β concentrations were measured

by the ECLIA electrochemiluminescence immunoassay (Roche E170/ Elecsys 1010/2010, Roche Diagnostics, USA). This method has been standardized against the 4th International Standard for Chorionic Gonadotrophin (code 75/589). Intra- and inter-assay co-efficients of variations were <20% for both oestradiol and HCG-β and the assay sensitivities were 0.1 mIU/ml for HCG-β and 5 pg/ml for oestradiol.

Statistical analysis

Student's *t*-test or Mann–Whitney *U*-test was used for the comparison of parametric variables depending on data distribution, and chi-squared test was used to assess the differences in frequencies. $P < 0.05$ was considered statistically significant.

The discriminative value of serum D8E2 and D12HCG-β concentrations between two compared groups (ongoing versus non-viable pregnancies, and multiple pregnancies versus singletons) according to transfer day was determined using receiver operator characteristic (ROC) curve analysis.

Data were analysed with the Statistical Package for Social Sciences for Windows, version 12.0 (SPSS Inc., USA) and MedCalc (MedCalc software, Ghent, Belgium).

Results

The mean ages of women in D3 and D5 transfer cycles were 31.1 ± 4.9 and 30.0 ± 4.9 years respectively, the difference being significant ($P < 0.0001$). The mean numbers of embryos transferred were significantly higher in D3 embryo transfer cycles compared with D5 embryo transfer cycles (3.7 ± 1.2 and 3.5 ± 1.1 respectively; $P < 0.0001$). However, the pregnancy rates were found to be similar (53 and 56% in D3 and D5 cases respectively). **Table 1** shows pregnancy outcome according to the day of transfer.

Serum D8E2 and D12HCG-β concentrations according to pregnancy outcome and the transfer day are summarized in **Table 2**. Serum D8E2 and D12HCG-β values were found to be significantly higher both in D3 and D5 transfer cases when pregnancy versus non-pregnancy, ongoing versus non-viable pregnancy and multiple versus singleton pregnancy values were compared (for results and *P*-values, see **Table 2**).

When serum D8E2 concentrations were analysed according to transfer day, significantly higher values (nearly two-fold) were observed in day 5 transfers compared with day 3 ones with regard to pregnancy ($P = 0.001$), ongoing pregnancy ($P = 0.001$) and multiple pregnancy ($P = 0.002$) (**Table 2**). On the other hand, statistically similar values were found with regard to non-viable pregnancy and singleton pregnancy.

When serum D12HCG-β measurements were compared between day 3 and day 5 transfers, significantly higher concentrations (almost two-fold) were observed in day 5 cases with regard to pregnancy ($P < 0.0001$), non-viable pregnancy ($P = 0.001$), ongoing pregnancy ($P < 0.0001$), singleton pregnancy ($P < 0.0001$) and multiple pregnancy ($P < 0.0001$) (**Table 2**).

ROC curve analysis results of D8E2 and D12HCG-β concentrations in differentiating between ongoing versus non-viable and multiple versus singleton pregnancies according to transfer day are demonstrated in **Table 3**.

Probabilities of non-viable or ongoing pregnancy for each D12HCG-β concentration according to transfer day were also investigated, and are shown in **Figure 1**. HCG-β concentrations of <50 mIU/ml were associated with a low probability of ongoing pregnancy (29% in D3 and 7% in D5 transfers), whereas concentrations above 200 mIU/ml were associated with an ongoing pregnancy chance of 92% in day 3 and 80% in day 5 transfers (**Figure 1**).

Table 1. Pregnancy outcome according to the day of embryo transfer.

	Day 3 transfer cycles (n = *1422*)	%	*Day 5 embryo embryo transfer cycles* (n = *613*)	%
Non-pregnant	671	47	271	44
Non-viable pregnancy	192	14	103	17
Ongoing pregnancy	559	39	239	39
Singleton pregnancy	317	22	125	20
Multiple pregnancy	242	17	114	19

There were no statistically significant differences between the two groups.

Table 2. Serum post-embryo transfer day 8 oestradiol and day 12 human chorionic gonadotrophin-β concentrations according to transfer day and pregnancy outcome.

	D8E2			*D12HCG-β*		
	D3	*D5*	P-*value*	*D3*	*D5*	P-*value*
Non-pregnant	50 ± 2	28 ± 2	<0.0001	0	0	
Pregnant	384 ± 20	527 ± 36	0.001	249 ± 8	466 ± 27	<0.0001
P-value	<0.0001	<0.0001				
Non-viable pregnancy	184 ± 20	306 ± 63	NS	98 ± 8	246 ± 24	0.001
Ongoing pregnancy	454 ± 25	622 ± 42	0.001	301 ± 10	589 ± 35	<0.0001
P-value	<0.0001	<0.0001		<0.0001	<0.0001	
Singleton pregnancy	291 ± 23	344 ± 35	NS	185 ± 8	360 ± 25	<0.0001
Multiple pregnancy	603 ± 38	838 ± 63	0.002	404 ± 14	730 ± 52	<0.0001
P-value	<0.0001	<0.0001		<0.0001	<0.0001	

Values are means ± SEM; D8E2 = serum post-embryo transfer day 8 oestradiol concentration (pg/ml); D12HCG-β = serum post-embryo transfer day 12 human chorionic gonadotrophin-β concentration (mIU/ml); D3 = day 3 embryo transfer cycles; D5 = day 5 embryo transfer cycles.

Table 3. Receiver operating characteristic curve analysis results showing optimal cut-off values of serum day 8 oestradiol and day 12 human chorionic gonadotrophin-β concentrations for the prediction of ongoing and multiple pregnancies according to transfer day.

	D8E2		*D12HCG-β*	
	D3	*D5*	*D3*	*D5*
Cut-off for OP versus NVP	130	179	98	257
Sensitivity (%)	80	79	89	78
Specificity (%)	72	84	69	81
Cut-off for MP versus SP	191	308	249	551
Sensitivity (%)	72	76	70	58
Specificity (%)	63	68	80	86

D8E2 = serum post-embryo transfer day 8 oestradiol concentration (pg/ml); D12HCG-β = serum post-embryo transfer day 12 human chorionic gonadotrophin-β concentration (mIU/ml); D3 = day 3 embryo transfer cycles; D5 = day 5 embryo transfer cycles; NVP = non-viable pregnancy; MP = multiple pregnancy; OP = ongoing pregnancy; SP = singleton pregnancy.

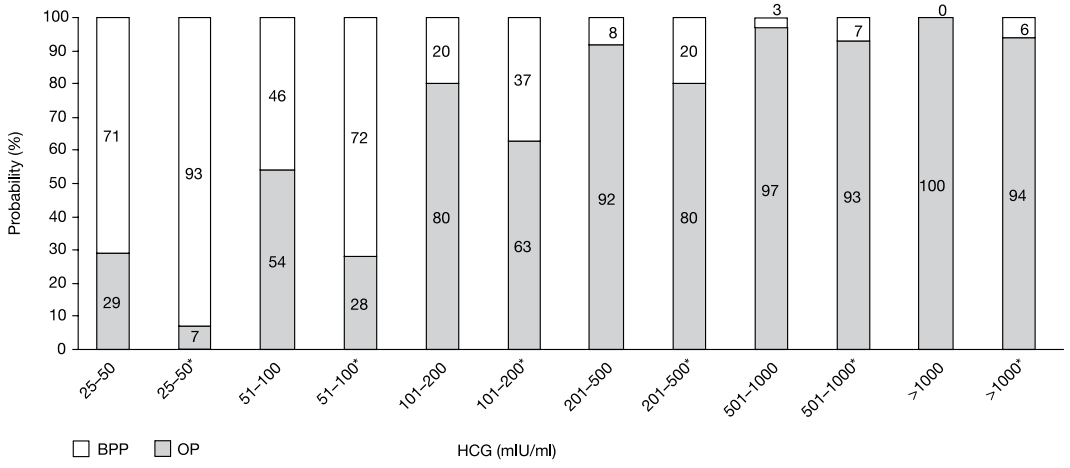

Figure 1. Probabilities (%) of non-viable and ongoing pregnancy for a given day 12 human chorionic gonadotrophin-β (HCG-β) concentration according to transfer day. HCG-β ranges with asterisk designate day 5 embryo transfer cases and without asterisk designate day 3 embryo transfer cases. NVP, non-viable pregnancy; OP, ongoing pregnancy.

Discussion

The present study has investigated the predictive value of serum D8E2 and D12HCG-β concentrations on pregnancy outcome after day 3 or day 5 transfers in women undergoing assisted reproduction. About two-fold higher D8E2 and D12HCG-β values were found in D5 transfers compared with D3 ones regarding pregnancy, which is logical when embryo transfer day is considered as the time point from which the measurements were performed.

Serum HCG, progesterone and oestradiol determinations in early pregnancy were shown to be reliable predictors of pregnancy outcome after assisted reproduction, not only at week 2, but up to week 4 (Yamashita *et al.*, 1989) or 6 (Anckaert *et al.*, 2005) after embryo transfer.

There are many studies in the literature about serum oestradiol concentration on the day of HCG administration and subsequent IVF outcome, although the results are controversial. In one review, it has been suggested that ovarian stimulation and the associated hyperoestrogenism are unlikely to be responsible for recurrent implantation failure (Urman *et al.*, 2005). As far as luteal serum oestradiol concentrations are concerned, serum oestradiol concentrations were shown to be statistically higher in conception IVF cycles compared with non-conception ones even as early as 4 days post-embryo transfer (Greb *et al.*, 2004). Greb *et al.* (2004) evaluated luteal oestradiol concentrations at different time points either before or after embryo transfer and observed comparable oestradiol patterns before embryo transfer between conception and non-conception cycles, which indicated that higher early luteal oestradiol concentrations in conception cycles could be attributed to events after embryo transfer. In another study, although in a small group of 22 IVF patients, conception cycles could be differentiated from non-conception ones by serum post-HCG day 8 oestradiol concentrations (Hutchinson-Williams *et al.*, 1989). In the present study, significantly higher post-embryo transfer day 8 serum oestradiol concentrations were observed in conception versus non-conception cycles, and also in ongoing versus non-viable pregnancies both in day 3 and day 5 transfer cases (for *P*-values, see **Table 2**). When analysed according to the day of transfer, 1.5–2 times higher serum post-embryo transfer day 8 oestradiol values were found in D5 transfers compared with D3 ones, which reached significance in conception, ongoing pregnancy and multiple pregnancy (for *P*-values, see **Table 2**). However, a significant difference was not found with regard to non-viable pregnancy and singleton pregnancy, which might have a clinical value but could not be explained.

HCG-β was reported to be the most reliable predictive test among biochemical markers in the assessment of pregnancy outcome (Homan *et al.*, 2000). HCG production by the blastocyst *in vitro* starts at approximately 160 h post-insemination and rises exponentially with maximal HCG-β production around day 10 (Woodward *et al.*, 1993). It was also postulated that HCG variants were produced by secretory endometrium even before embryo implantation, and this may be the main source of the earliest rise in plasma HCG during early implantation (Alexander *et al.*, 1998).

In previous studies related to the predictive value of HCG-β, timing of the HCG-β measurement was different; some authors evaluated according to ovulatory (HCG) day, some according to oocyte retrieval day, some according to fertilization, and some according to transfer day. In the present study, transfer day was considered as the point and serum oestradiol and HCG-β measurements were taken at 8 and 12 days after transfer day respectively.

Zhang *et al.* (2003) observed 50% higher HCG-β values in pregnancies resulting from day 3 transfers compared with those following day 5 ones, measured 13 days after oocyte aspiration, regardless of transfer day. In the current study HCG-β was measured 12 days after embryo transfer. The mentioned study found lower HCG-β values in pregnancies following day 5 transfers, but if data were analysed according to transfer day, higher HCG-β values would be obtained in pregnancies resulting from day 5 transfers, similar to the present findings.

The results of this study show that, in patients who were given embryos on day 5, D12HCG-β concentrations were significantly higher than those of patients who were given embryos on day 3 regarding all kinds of pregnancy outcome (for *P*-values see **Table 2**). It is logical that a blastocyst transferred on day 5 can produce higher HCG-β values compared with a cleavage stage embryo given on day 3, when measurements were made at a fixed time after transfer day. The actual amount of HCG is thought to reflect the number of viable trophectoderm cells producing the hormone (Woodward *et al.*, 1993).

Therefore, contradictory results obtained in the present study compared with that performed by Zhang *et al.* (2003) might be the consequence of HCG-β measurement time, which was according to embryo transfer day in this study and oocyte aspiration day in the mentioned study. HCG-β measurements after IVF treatments should be considered and standardized according to the day of transfer, which will eradicate the confusion in the literature.

Furthermore, the results of the present study would be of help in clinical practice in interpreting the possible differences in HCG-β measurements in different embryo transfer groups. Transfer day should be taken into account when counselling patients since, as seen in the study, 2 days' delay in embryo transfer results in different interpretations of oestradiol and HCG-β. One might misinterpret the outcome of day 5 embryo transfer pregnancy if using the cut-off values of day 3 embryo transfers.

Some investigators have suggested that early HCG-β concentrations were predictive of implantation outcome, not the final pregnancy outcome, i.e. live birth (Glatstein *et al.*, 1995). However, cut-off concentrations for HCG-β were calculated in predicting ongoing pregnancy. Bjercke *et al.* (1999) found a single HCG-β measurement of 55 IU/l on day 12 after embryo transfer to be reliable in predicting the occurrence of early pregnancy loss. In another study, 143 IU/l was found as the optimal cut-off value for HCG-β at day 11 post-embryo transfer in differentiating between viable and non-viable pregnancies after assisted reproduction, excluding biochemical pregnancies (Anckaert *et al.*, 2005). In another study, 50 IU/l cut-off value for HCG-β was found on day 14 after embryo transfer in prediction of viable pregnancies (Sugantha *et al.*, 2000). In the present study, HCG-β measurement 12 days after transfer of 98 mIU/ml in day 3 transfer cycles, and 257 mIU/ml in day 5

ones were found as the values most reliably predicting ongoing pregnancy. ROC curve analysis revealed an HCG-β value of 98 mIU/ml as the cut-off point for predicting ongoing pregnancy after day 3 transfer, which is similar to the value of 76 IU/l after day 2 transfer found by Poikkeus *et al.* (2002). Serum HCG-β concentrations roughly double every 2 days in early pregnancy, corresponding to a 40% rise per day. Due to various findings in the literature, even though not in a wide range, it was suggested that each centre should validate its own threshold values for serum HCG-β measurement based on their own experience (Glatstein *et al.*, 1995). Transfer day also should be taken into account while interpreting HCG-β values and counselling patients about pregnancy outcome, although existence of overlapping values might be difficult to explain for the clinician.

In a study performed by Homan *et al.* (2000), HCG-β results 16 days after ovulation were analysed in predicting pregnancy outcome according to patient age, since it is known that women aged >40 have a greater risk of spontaneous abortion (Hansen 1986). It was demonstrated that at an HCG-β concentration of 200 IU/l, the probability of an ongoing pregnancy is 80% in patients <40 years while it is 50% in those older than 40 years. A test describing probability of ongoing pregnancy may be clinically beneficial to both physician and patient. If HCG-β concentration indicates a non-viable pregnancy, it would be more reliable to talk in terms of probabilities than uncertainties, which would provide better preparation of patients emotionally. If the probability of non-viable pregnancy is high and the patient is informed accordingly, disappointment and anxiety during the waiting period will be lower. Patients prefer to receive clear accurate information about their care. In this study, it was found that for post-embryo transfer day 12 HCG-β concentrations <50 mIU/ml, the probability of an ongoing pregnancy is 29% in day 3 transfer cycles, and 7% in day 5 ones. When the value is above 200 mIU/ml the probability of ongoing pregnancy is 92% in day 3 transfers, and 80% in day 5 ones. The findings of such studies might be included in a patient information booklet or sheet to inform about the probabilities of outcome at different HCG-β concentrations.

In conclusion, from the present data, it appears that serum post-embryo transfer day 8 oestradiol and day 12 HCG-β concentrations provided clear information regarding pregnancy and the outcome of pregnancy following assisted reproduction and day 5 transfers yielded higher values compared with day 3 transfers, as expected.

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