Serum HCG 12 days after embryo transfer in predicting pregnancy outcome

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BACKGROUND: Assisted reproduction treatment (ART) entails a risk of ectopic pregnancy and early pregnancy loss. Serum HCG has been found to be predictive of pregnancy outcome. Our aim was to assess the clinical value of a single early HCG assay in ART pregnancies taking into account the aetiology and treatment of infertility.

METHODS: During 1994–1999, we studied 774 embryo transfer cycles resulting in pregnancy defined as a serum HCG concentration of $\geq 5$ IU/l on day 12 following embryo transfer. The treatment included IVF in 518, ICSI in 119, and frozen embryo transfer in 137 cycles. Serum HCG concentrations were measured by fluoroimmunometric assay. Pregnancies were classified as viable (live fetus at $\geq 22$ weeks gestation) or non-viable (biochemical pregnancy, miscarriage, ectopic pregnancy and molar pregnancy). Data on the outcomes were retrospectively retrieved from the records.

RESULTS: The median HCG concentration was 126 IU/l in viable pregnancies and 31 IU/l in non-viable pregnancies ($P < 0.0001$). The median HCG concentration was 115 IU/l in singleton pregnancies and 201 IU/l in multiple pregnancies ($P < 0.0001$). Male factor infertility was associated with viable pregnancies ($P = 0.004$) and tubal factor with non-viable pregnancies ($P = 0.003$); the lowest HCG level (88 IU/l) was observed in subjects with both male factor infertility and ICSI treatment ($P = 0.001$). An HCG value of 76 IU/l emerged as the most suitable cut-off point to predict viable pregnancy. Probabilities of each type of outcome related to the HCG level are given.

CONCLUSIONS: A single HCG reading on day 12 after embryo transfer helps to plan the subsequent follow-up. Male factor infertility and ICSI are associated with relatively low HCG values in viable pregnancies.

Key words: assisted reproduction treatment/ectopic pregnancy/HCG/infertility/pregnancy outcome

Introduction

Early prediction of outcome is important in pregnancies following assisted reproduction treatment (ART). In these pregnancies, the incidence of ectopic pregnancies (EP) varies from double to nearly 5-fold (Dubuisson \textit{et al.}, 1991; Strandell \textit{et al.}, 1999) compared with that in spontaneous pregnancies. In particular, patients with tubal factor infertility are at an increased risk of EP and should therefore receive special attention to avoid further impairment of fertility. The rate of multiple gestation is also high (20–25\%) (Bergh \textit{et al.}, 1999) and early pregnancy loss is common, which causes anxiety in the couples involved.

Markers have been sought to distinguish between viable and non-viable pregnancies before verification of live intrauterine pregnancy by transvaginal sonography (TVS) is possible. A single determination of serum HCG concentration has been found to be predictive of pregnancy outcome (Schmidt \textit{et al.}, 1994; Glatstein \textit{et al.}, 1995; Sugantha \textit{et al.}, 2000), even as early as 11–12 days after embryo transfer (Qasim \textit{et al.}, 1996; Bjercke \textit{et al.}, 1999).

In only two studies (Gold \textit{et al.}, 2000; Homan \textit{et al.}, 2000) have different treatments or causes of infertility been compared in relation to early HCG concentration and pregnancy outcome. In subjects with unexplained infertility, ICSI may result in lower than expected HCG levels (Gold \textit{et al.}, 2000).

The objective of our study was to use day 12 post-embryo transfer HCG concentrations to predict outcome of pregnancies following ART, taking into account the aetiology and treatment of infertility.

Materials and methods

\textit{Subjects}

During 1994–1999, a total of 1888 embryo transfers were carried out at the Infertility Clinic of Helsinki University Central Hospital, Helsinki, Finland. Records of the subjects who conceived following ART during that period were analysed. Of these, 774 embryo transfer cycles fulfilled our inclusion criteria, which were (i) serum HCG had been assayed on day 12 post embryo transfer and its concentration was $\geq 5$ IU/l, (ii) the data on the outcome was available, and (iii) there were no known chromosomal anomalies or signs of ‘vanishing twin’ syndrome in subsequent ultrasonography.
The number of women was 650. Their mean age at treatment was 32 years (range 21–41). The main cause of infertility was anovulation in 47 (6%) cycles, endometriosis in 105 (14%), male factor in 128 (17%), tubal factor in 235 (30%), unexplained in 203 (26%), and combined in 56 (7%).

Treatment protocols
The women underwent previously described treatment protocols (Simberg et al., 1998; Tiitinen et al., 2001). Briefly, in IVF and ICSI cycles, long luteal phase pituitary down-regulation with nafarelin or buserelin was followed by ovarian stimulation with either urinary or recombinant FSH. When two or more follicles reached a diameter of >17 mm (in TVS), 10 000 IU of HCG was administered. The half-life of the HCG used was 33 h. Transvaginal follicle aspiration was performed 36 h after HCG administration, and embryo transfer took place 48 h later. Micronized vaginal progesterone at a daily dose of 600 mg was used for 2 weeks for luteal support.

In natural frozen embryo transfer cycles, embryo transfer took place 3 days after the LH surge, as measured by using a home test kit. Micronized vaginal progesterone at a dose of 200 mg daily was administered for 2 weeks after embryo transfer. In substituted frozen embryo transfer cycles, hormone replacement with estradiol valerate given orally (4–6 mg daily) was used until endometrial thickness reached ≥7 mm per two layers. Micronized vaginal progesterone (600 mg/day) was then started, followed by embryo transfer after 3 to 4 days. Estrogen and progesterone treatment was continued until the end of the first trimester in viable pregnancies.

There were 518 (67%) IVF cycles, 119 (15%) ICSI cycles and 137 (18%) frozen embryo transfer cycles. Of the frozen embryo transfer cycles, 96 (70%) were substituted and 41 (30%) were natural. The mean number of embryos transferred in the studied cycles was 1.8. The last transfers of three embryos were carried out in 1994. In 1999, the number of embryos transferred per cycle was 1.7.

Pregnancy outcome
Viable pregnancy was defined as one resulting in delivery of at least one live fetus at ≥22 weeks gestation, which conforms to the current World Health Organization’s (WHO) definition of childbirth. Non-viable pregnancies constituted biochemical pregnancies (a temporary rise of serum HCG without signs of intrauterine pregnancy in TVS), ectopic pregnancies (EPs; diagnosed in TVS or laparoscopy), miscarriages (cessation of development of intrauterine pregnancy seen in TVS), and pregnancies with hydatidiform mole (a pathologic-anatomic diagnosis).

Assay of HCG
At day 12 after embryo transfer, each patient had her HCG level assessed. Serum concentrations of intact HCG (with both α and β subunits) were measured by solid phase, two-site fluorometric immunoassay (AutoDELFIA®; Wallac, Turku, Finland). The intra-assay variation was 2.7–5.1% and inter-assay variation 1.6–3.9% at the studied HCG levels. The standards had been calibrated against the WHO Third International Standard of HCG for immunoassay (code 75/537).

Statistical analysis
The measured HCG values were non-normally distributed. In viable pregnancies, an exponential transformation gave a good fit to normal distribution allowing use of Student’s t-test and one-way analysis of variance (ANOVA). However, the HCG values in non-viable pregnancies remained non-normally distributed even after logarithmic or exponential transformations. Analyses involving non-viable pregnancies were therefore carried out by the non-parametric Mann–Whitney U-test or Kruskal–Wallis analysis of variance.

The χ²-test was used for comparison of proportions. A level of P < 0.05 was regarded as statistically significant. A receiver operating characteristic (ROC) curve was used to determine the HCG cut-off level that best discriminated between viable and non-viable pregnancies.

The 774 measurements available of day 12 HCG were divided in 10 deciles, each containing 77 or 78 cases, and the proportions of each outcome type within each decile were calculated. Statistical analysis was performed using NCSS 2000 software.

Results
The median HCG concentration was 126 IU/l in viable pregnancies and 31 IU/l in non-viable pregnancies (P < 0.0001) (Table I). The median HCG concentration in twin pregnancies was almost double that in singleton pregnancies (201 IU/l versus 115 IU/l, P < 0.0001).

When calculating the results for the 290 fresh versus the 72 frozen embryo cycles resulting in a viable singleton pregnancy, no difference in the median HCG values was observed [114 versus 115 IU/l respectively; not significant (NS)]. Neither was there a difference between the medians of the 18 natural and the 54 substituted cycles (111 versus 123 IU/l; NS). Median HCG concentrations in singleton live pregnancies following transfer of one versus two embryos were 104 IU/l and 119 IU/l respectively (NS).

The ROC curve (Figure 1) suggests that an HCG level of 76 IU/l is a suitable cut-off point for predicting viable pregnancy with 80% sensitivity and 82% specificity. At HCG levels of 76 IU/l, the positive predictive value (PPV) for viable pregnancy was 87% and the negative predictive value (NPV) was 74%.

Figure 2 shows the probabilities (%) of the different outcomes within each of the 10 deciles of the 774 day 12 post-embryo transfer HCG measurements. All biochemical pregnancies were found at HCG levels <100 IU/l. Within the 3rd decile (HCG range 29–45 IU/l), the probabilities of biochemical pregnancy, miscarriage, and normal pregnancy were similar, about 31% each. Within the uppermost decile (median 263, range 221–683 IU/l), normal singleton pregnancy

| Table I. HCG concentrations (IU/l) in viable and non-viable pregnancies |
|-----------------------------|------------------|-----------------|-----------------|-----------------|
| HCG                         | n    | %     | Median | Range | IQ range |
| Viable pregnancies          |      |       |       |       |          |
| Singletons                  | 459  | 59.1  | 126   | 5–683 | 88–188   |
| Twins                       | 362  | 47    | 115   | 5–397 | 80–169   |
| Triplets                    | 96   | 12    | 201   | 48–683| 148–260  |
| Non-viable pregnancies      | 315  | 40.9  | 31    | 5–268 | 15–64    |
| Biochemical                 | 154  | 20    | 18    | 5–81  | 11–29    |
| Ectopic                     | 20   | 3     | 35    | 5–144 | 15–61    |
| Miscarriages, all           | 138  | 17.6  | 60    | 5–268 | 32–89    |
| Miscarriages, late          | 5    | 0.6   | 96    | 74–116| 80–113   |
| Hydatidiform mole           | 2    | 0.3   | 99    | 39–160| 39–160   |
| Total                       | 774  | 100   |       |       |          |

IQ range = interquartile range (i.e. 25–75 centile range).
Figure 1. ROC curve for predicting viable pregnancy by serum HCG concentration at 12 days post embryo transfer. The ROC curve was constructed by plotting the true positive rate (sensitivity) on the y-axis and the false positive rate (1-specificity) on the x-axis for a given HCG concentration.

Figure 2. Probability (%) of each pregnancy outcome within each of the 10 centiles of serum HCG concentration at 12 days post embryo transfer. Open (white) symbols denote viable pregnancies and closed (black) symbols non-viable pregnancies. The two cases of hydatidiform mole are not depicted.

Discussion

The previous studies on ART cycles relating early HCG determination with pregnancy outcome consisted of 77–662 cycles and have been reviewed (Sugantha et al., 2000). Our study with 774 cycles is thus the largest of its kind. Our time point for HCG sampling was the earliest suggested as being useful (Legro et al., 1995; Qasim et al., 1996; Bjercke et al., 1999).

In our series, the median HCG concentrations in viable pregnancies were about 4-fold compared with those in non-viable ones, which is in agreement with the results reported before (Qasim et al., 1996; Bjercke et al., 1999). Low maternal \(\beta\)HCG is also associated with non-viable pregnancy later in the first trimester (Ong et al., 2000).

and twin pregnancy showed similar probabilities of \(\sim 50\%\). The EP represented \(5 \text{–} 9\%\) at HCG ranges up to \(\sim 80\) IU/l, and only one subject with EP had a level higher than this (144 IU/l).

In the subjects with tubal factor infertility, the overall rate of EP was 14/235 (6%). The rate decreased from 20% within the 1st decile (HCG 5–14 IU/l) to 10% within the 2nd–4th deciles (HCG 15–66 IU/l) and to 0.8% for HCG values of \(\geq 67\) IU/l.

Among viable singleton pregnancies, when male factor was present, the median HCG concentration was low compared with the remainder (100 IU/l, \(P = 0.006\)). When ICSI was present the median HCG concentration was also low (93 IU/l, \(P = 0.004\)). The lowest HCG concentrations (median 88 IU/l, \(P = 0.001\)) were observed in the cases with both male factor infertility and ICSI treatment. These results remained essentially the same when multiple pregnancies were included. Male factor infertility was present in 45 of 72 (63%) ICSI cycles; the other aetiological factors were anovulation in one, combined in 13, endometriosis in three, tubal factor in two and unexplained in eight ICSI cycles. In non-viable pregnancies, no significant differences in HCG concentrations were detected either in the aetiological subgroups or in the different treatment groups.

The outcome of pregnancies classified according to the treatment type is shown in Table II. Table III shows the aetiology of infertility in relation to the outcome. The proportion of viable pregnancies associated with male factor infertility was relatively high (\(P = 0.004\)) and it was relatively low as regards tubal infertility (\(P = 0.03\)). The rate of viable pregnancies was 71, 61, 53 and 48% in women aged up to 29, 30–34, 35–39 and \(\geq 40\) years respectively (\(P = 0.002\)). No correlation between age and HCG level was observed in viable pregnancies (\(P = \text{NS}\)).
doubled HCG level in twin pregnancies is in accordance with the results of previous studies (Schimdt et al., 1994; Glatstein et al., 1995; Bjercke et al., 1999).

Our cut-off level for predicting a viable pregnancy (76 IU/l) was slightly higher than those reported for similar (~80%) sensitivity: 42 mIU/ml (Qasim et al., 1996); 55 IU/l (Bjercke et al., 1999); and 50 IU/l (Sugantha et al., 2000).

The differences could be partly due to the different HCG assays used, and partly due to our decision, unlike the others, to regard the five late miscarriages (which have relatively high initial HCG) as non-viable pregnancies. A single cut-off level is always a matter of choice. Had we chosen a cut-off level of 41 IU/l, the sensitivity would have risen to 97%, but the specificity would have decreased to 62%.

We provide probabilities for each type of outcome for a given HCG level to help clinicians to counsel the patient and to plan the next step in her follow-up. Each HCG level has given HCG level to help clinicians to counsel the patient and to plan the next step in her follow-up. Each HCG level has given HCG level to help clinicians to counsel the patient and to plan the next step in her follow-up. Each HCG level has given HCG level to help clinicians to counsel the patient and to plan the next step in her follow-up. Each HCG level has given HCG level to help clinicians to counsel the patient and to plan the next step in her follow-up. Each HCG level has given HCG level to help clinicians to counsel the patient and to plan the next step in her follow-up. Each HCG level has given HCG level to help clinicians to counsel the patient and to plan the next step in her follow-up. Each HCG level has given HCG level to help clinicians to counsel the patient and to plan the next step in her follow-up. Each HCG level has given HCG level to help clinicians to counsel the patient and to plan the next step in her follow-up. Each HCG level has given HCG level to help clinicians to counsel the patient and to plan the next step in her follow-up. Each HCG level has given HCG level to help clinicians to counsel the patient and to plan the next step in her follow-up. Each HCG level has given HCG level to help clinicians to counsel the patient and to plan the next step in her follow-up. Each HCG level has given HCG level to help clinicians to counsel the patient and to plan the next step in her follow-up.

The treatment protocols in fresh and frozen embryo transfers differ, which may have an impact on endometrial maturation and on the HCG levels. Endometrial maturation and early HCG have usually been compared either within fresh embryo cycle subgroups or between fresh embryo transfers and spontaneous pregnancies (Tulchinsky et al., 1996; Hsu et al., 1998; Ertzeid et al., 2000). Our study did not include comparison with spontaneous pregnancies, but others have reported that early HCG in spontaneous pregnancies is higher than that in the IVF pregnancies (Tulchinsky et al., 1996; Ertzeid et al., 2000). We found similar HCG levels in fresh and frozen embryo transfer cycles as well as within the frozen embryo transfer group divided into natural and substituted cycles. No differences have been found in the early HCG in fresh embryo transfer pregnancies related to ovarian stimulation (Hsu et al., 1998; Ertzeid et al., 2000). Our results suggest that the HCG concentrations are similar even when no ovarian stimulation is used. This is in accordance with previous findings (Homan et al., 2000; Sugantha et al., 2000).

We observed similar HCG concentrations in singleton pregnancies regardless of the number of embryos transferred. Thus our results are equally applicable to double and single embryo transfers. This also implies that the ‘vanishing twin’ syndrome (early cessation of development of one twin) is rare.

In viable pregnancies, the HCG concentrations in ICSI treatment cycles were a little lower than in other treatment groups. The same was true for the subgroup of male factor infertility. The presence of both factors together resulted in the lowest HCG concentrations among viable pregnancies. A previous study (Gold et al., 2000), reported lower HCG concentrations 16 days after embryo transfer in ICSI compared with IVF in the subgroup of unexplained infertility. The explanation for this was not clear. Although the early embryo cleavage is delayed in ICSI-derived embryos and the fragmentation of embryos is increased, the implantation potential is comparable with IVF-derived embryos (Hsu et al., 1999; Frattarelli et al., 2000).

In previous series, no differences in the proportions of viable and non-viable pregnancies among the various aetiopathological subgroups were reported. The aetiology of infertility played some role in our study. Male factor infertility was associated with viable pregnancy, probably reflecting normal female fertility combined with suboptimal male fertility. In contrast, tubal factor infertility was associated with non-viable pregnancies, at least partly due to the increased incidence of EP (6% with tubal factor infertility compared with 0.8% without). Rates of ectopic pregnancy as high as 11% have been reported elsewhere (Dubuisson et al., 1991).

Although the EP in our series were clearly associated with low HCG values, they could not be distinguished from the much larger group of other pregnancies with similar levels of HCG. A low HCG concentration alone is therefore not a suitable marker for EP. Among the women with tubal factor infertility, we found EP in 10–20% at the HCG values of 5–66 IU/l. Thus, a history of tubal infertility together with a low HCG calls for a second HCG and early localization of the pregnancy by transvaginal ultrasonography.

It has been suggested that HCG has more predictive value than other biochemical markers for assessing pregnancy outcome (Homan et al., 2000). We therefore continue to include the assay of HCG 12 days after embryo transfer in our IVF protocol.

References
Serum HCG 12 days after embryo transfer


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