Human chorionic gonadotropin levels are equally predictive for pregnancy outcome after fresh and vitrified-warmed blastocyst transfer

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Abstract

Purpose The aim of the present study was to evaluate if the live birth predictive values of β -hCG levels differ in fresh and vitrified-warmed blastocyst transfer cycles.

Methods In the retrospectively designed study, 775 cycles with positive β -hCG values 13 days after fresh blastocyst transfer (fresh ET; $n=568$) or vitrified-warmed blastocyst transfer (FET; $n=207$) were selected for analysis. Average β -hCG levels stratified according to pregnancy outcome (biochemical pregnancy, spontaneous abortion, ectopic pregnancy, and singleton or twin birth) were compared between fresh ET and FET cycles. To determine the optimal sensitivity and specificity of β -hCG levels for live birth prediction, a ROC curve was constructed. Fisher's exact test was used to compare the positive predictive values (PPV).

Results Average β -hCG levels stratified according to pregnancy outcome were not statistically different between fresh ET and FET cycles. In fresh ET and in FET group, the β -hCG levels were significantly higher in pregnancies resulting in live birth compared to non-viable pregnancies (1,035 vs. 462 IU/L, $p<0.001$ and 968 vs. 411 IU/L, $p<0.001$). Optimal cut-off level for live birth prediction was 495 IU/L (sensitivity 83.0 %, specificity 71.8 %) after ET and 527 IU/L (sensitivity 80.0 % and specificity 76.6 %) after FET. The PPV for live birth rate in the groups after ET and FET were 90.6 % and 84.9 % respectively, without statistically significant difference ($p>0.05$).

Conclusion Beta-hCG levels after fresh and vitrified-warmed blastocyst transfer are equally predictive for pregnancy

outcome. Clinicians can be encouraged to interpret β -hCG results in the same manner.

Keywords Beta-hCG levels · Predicting pregnancy outcome · Blastocyst transfer · Frozen embryo transfer

Introduction

A single determination of serum beta human chorionic gonadotropin (β -hCG) 12–18 days after embryo transfer (ET) is an established, reliable early indicator of pregnancy after medically assisted reproduction (MAR). However, as many as 22 % of these pregnancies are non-viable and result in a biochemical pregnancy, spontaneous abortion or ectopic pregnancy [1]. Considering this, it is very important to provide early and reliable information, not only regarding conception after ET, but also about the prognosis of such pregnancy. This is especially important in the light of increased levels of stress associated to infertility treatment [2, 3].

Several studies have investigated the value of β -hCG in the prediction of viable pregnancy after MAR and found correlation between β -hCG levels and pregnancy outcome [1, 4–11]. The threshold levels of β -hCG with best prediction as well as the reliability of prediction, however, vary between studies. Differences could be the result of disparities in study designs, in the time period of blood sampling, and in analytical methods used to measure β -hCG levels. What is more, some studies have shown that many other factors could have influence on β -hCG level dynamics; such as day of ET, culture media, aetiology of infertility and MAR technique [7–9, 12, 13].

Recent data also suggest that the rate of β -hCG rise is higher following ET of cryopreserved embryos (FET) [14]. However, only a few published studies included the analysis of β -hCG levels after FET and they present with inconclusive

Capsule Beta-hCG levels 13 days after fresh and vitrified-warmed blastocyst transfer are equally predictive for pregnancy outcome.

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results [1, 6, 10]. Furthermore, there is no data specifically considering differences in β -hCG levels after transfer of vitrified-warmed embryos at blastocyst stage compared to fresh blastocyst transfer.

The aim of our study was to evaluate if the levels and live birth rate predictive values of β -hCG differ in fresh and vitrified-warmed blastocyst transfer cycles.

Materials and methods

Single-centre retrospective analysis of 4,373 IVF/ICSI cycles in the time period from 2008 to 2010 was conducted. All couples included in IVF/ICSI ovarian stimulation programme undergoing fresh or vitrified-warmed blastocyst transfer were included regardless of age. Exclusion criteria were cycle cancellation, ET cancellation, transfer of cleavage stage embryos and negative β -hCG result after ET. Only cycles with available data on pregnancy outcome and measurement of β -hCG level in our laboratory on day 13 after ET were included in further analysis. The study was approved by the local institutional review

The indication for IVF/ICSI was female infertility in 31.4 %, male infertility in 32.7 %, female and male infertility in 21.0 % and unexplained infertility in 14.9 % of couples. Patients underwent ovarian stimulation using protocols with combination of GnRH agonist/GnRH antagonist and recombinant FSH (Gonal-F, Serono International SA, Geneva, Switzerland)/HMG (Menopur, Ferring Pharmaceuticals Inc., Saint-Prex, Switzerland) that were previously described in detail [15]. In the study population, IVF was performed in 35.9 % and ICSI in 64.1 % of cycles. After oocyte fertilization using, embryos were cultured in the Blast Assist extended culture media (Origio, Denmark). Embryo quality was assessed by an experienced embryologist at day two and three after oocyte fertilization. Day five blastocyst transfer was performed if more than three optimal embryos were available on day three according to our standard policies. After consultation with the patients, time of embryo transfer was adjusted to day 3 or day 5 according to doctor-patient agreement. Blastocysts were graded according to our established grading system 5 days after oocyte fertilization [16, 17]. Surplus blastocysts, not selected for transfer were cryopreserved using embryo vitrification procedure [17]. Vitrification was performed by using the combination of dimethyl sulfoxide (DMSO) and ethylene glycol cryoprotectants. Blastocysts were first exposed to equilibration media (7.5 % DMSO/ethylene glycol) for 10 min and later to vitrification solution (15 % DMSO/ethylene glycol) for 1 min (Irvine Scientific, Santa Ana, CA). Before vitrification, blastocysts were placed in closed straws (High security vitrification kit, Cryo Bio System, Paris, France). For thawing, the embryo carrier was expelled from the straw and quickly plunged into the thawing

medium (1 M sucrose) for 1 min. Blastocysts were then transferred in dilution solution (0.5 M sucrose) 3 times for 3 min. They were cultured in recovery medium (Blast Assist System, Origio, Denmark) for at least 4 h before they were transferred into the uterus.

After ET, patients received luteal supplementation using 600 mg/day of vaginal progesterone (Utrogestan, Ferring Pharmaceuticals Inc., Saint-Prex, Switzerland). Transfer of vitrified-warmed blastocyst stage embryos was performed in natural menstrual cycles monitored by ultrasound and urinary LH surge tests in patients without ovulation disorders. In the presence of ovulation disorder, transfer was performed in cycles prepared by oestrogen/progesterone.

Serum β -hCG level measurement was scheduled 13 days after blastocyst ET. All measurements were performed using hemiluminescent microparticle immunoassay (CMIA) method (Abbot Laboratories, Ireland) in the same laboratory. The assay has a detection range from 1.2 to 15,000 IU/ml. All patients underwent transvaginal ultrasound examination at 6 weeks of gestation to determine the pregnancy status. To obtain the data about pregnancy outcome, patients completed a questionnaire.

Cycles were classified according to pregnancy outcome. Pregnancy was defined as a rise in β -hCG above 20 IU/L. Biochemical pregnancy was diagnosed if no sign of pregnancy was evident on ultrasound examination. Spontaneous abortion was defined as pregnancy loss after ultrasonic demonstration of fetal gestational sac. Ectopic pregnancies were diagnosed by transvaginal ultrasound and/or laparoscopy. Non-viable pregnancies included biochemical pregnancies, spontaneous abortions and ectopic pregnancies. Live birth was recorded where one or more babies were born.

Statistical analyses were performed using Statsoft Inc. Statistica 8.0 data analysis software system. Mean and standard deviation for each continuous variable were calculated. Beta-hCG levels and other variables between fresh ET and FET group were compared. Student's *t*-test or Mann–Whitney *U* test was used to assess parametric variables, depending on the data distribution. Chi-squared test was used to compare differences in frequencies. Statistical significance was set at $p < 0.05$. Receiver operator characteristic curves (ROC) were constructed for each group to identify sensitivity and specificity for live birth prediction at specific β -hCG levels. Area under the ROC curve measures the diagnostic accuracy of the test, with a greater area corresponding to a greater ability to discriminate between patients with viable and non-viable pregnancy. An area of 1.0 under the curve denotes a perfect test, whereas an area of 0.5 is not better than chance. Fisher's exact test was used to compare the positive predictive values (PPV).

Table 1 Comparison of pregnancy outcome after fresh and vitrified-warmed blastocyst transfer

	Fresh ET (<i>N</i> =568)	FET (<i>N</i> =207)	<i>p</i> -value
Biochemical pregnancy <i>N</i> (%)	51 (9.0)	36 (17.4)	NS
Spontaneous abortion <i>N</i> (%)	63 (11.1)	35 (16.9)	NS
Ectopic pregnancy <i>N</i> (%)	5 (0.9)	6 (2.9)	NS
Non-viable pregnancy <i>N</i> (%)	119 (21.0)	77 (37.2)	<0.05
Singleton live birth <i>N</i> (%)	386 (68.0)	120 (58.0)	<0.05
<i>ET</i> blastocyst transfer			
Twin live birth <i>N</i> (%)	63 (11.1)	10 (4.8)	NS
<i>FET</i> vitrified-warmed blastocyst transfer			
Total live birth <i>N</i> (%)	449 (79.1)	130 (62.8)	<0.01

Results

Fresh blastocyst transfer (fresh ET) was performed in 2,573 stimulated cycles and vitrified-warmed blastocyst transfer (FET) in 1,064 cycles. There were 1,044 (40.6 %) pregnancies after fresh ET and 282 (26.5 %) after FET cycles. Ultimately, 775 (51.5 %) cycles were selected for detailed analysis; 568 after fresh ET and 207 after FET.

Women undergoing fresh ET were on average statistically significant younger than women undergoing FET (31.81 ± 4.03 and 32.56 ± 3.96 years respectively, $p < 0.05$) Number of transferred embryos was significantly higher after FET cycles compared to fresh ET cycles (1.64 ± 0.52 vs. 1.40 ± 0.50 , $p < 0.001$).

Biochemical pregnancy, spontaneous abortion and ectopic pregnancy rates were comparable in both groups. Live birth rate was significantly higher after fresh ET (Table 1).

The mean value of β -hCG level was statistically significantly higher in the group after fresh ET compared to the group after FET (937 ± 637 vs. 761 ± 646 IU/L, $p < 0.001$). However, if cycles were stratified according to pregnancy outcome, the mean β -hCG levels between these two groups were not statistically significantly different if pregnancy ended with biochemical pregnancy, spontaneous abortion, ectopic pregnancy, singleton or twin birth (Table 2).

Furthermore, cycles were classified according to viable (singleton or twin live birth) or non-viable pregnancy (biochemical pregnancy, spontaneous abortion, ectopic pregnancy). In this analysis, β -hCG levels were significantly higher in viable pregnancies compared to non-viable pregnancies ($1,035 \pm 631$ vs. 462 ± 467 IU/L, $p < 0.001$). The same was true for the group after fresh ET ($1,054 \pm 615$ vs. 496 ± 514 IU/L, $p < 0.001$) and after FET (968 ± 682 vs. 411 ± 383 IU/L, $p < 0.001$).

To determine the optimal sensitivity and specificity for the β -hCG values with regard to live birth, a ROC curve was used in both groups (Fig. 1). The area under the curve for group after fresh ET and after FET were 0.796 (95 % confidence interval: 0.743–0.848) and 0.807 (95 % confidence interval: 0.743–0.871), respectively.

For the fresh ET group, the β -hCG threshold level of 496 IU/L was selected as optimal for predicting pregnancy

resulting in live birth, with sensitivity of 83.0 %, specificity of 71.8 % and positive predictive value of 90.6 %. For the FET group, β -hCG value of 527 IU/L yielded optimal sensitivity (80.0 %) and specificity (76.6 %) and had 84.9 % positive predictive value for live birth. The difference in PPV between groups was not statistically significant.

Discussion

Replacement of vitrified-warmed embryos currently represents a significant part of infertility treatment and is becoming increasingly important and successful, especially with recent advances in embryo cryopreservation methods. Although there are reports of lower pregnancy and higher miscarriage rates after FET compared to fresh ET [18], others report of similar success rates after both approaches [19]. Therefore, early prediction of pregnancy outcome after FET is particularly important.

It is well documented in the literature that levels of β -hCG in early pregnancy are highly predictive for pregnancy outcome after fresh ET, however prognostic values and β -hCG thresholds are not consistent [1, 4–11]. The most important factor for inconsistent results is different timing of the β -hCG sampling. However, several other parameters that have impact on embryo growth can also influence β -hCG levels. For example, it was shown that culture media can influence the growth rate and implantation of human embryos [13]. In a study comparing two embryo culture media, the one promoting

Table 2 Comparison of β -hCG levels (IU/L) after fresh and frozen blastocyst transfer in regard to pregnancy outcome

β -hCG levels (IU/L)	Fresh ET (<i>N</i> =568)	FET (<i>N</i> =207)	<i>p</i> -value
Biochemical pregnancy	206±303	256±288	NS
Spontaneous abortion	699±495	540±390	NS
Ectopic pregnancy	912±957	587±533	NS
Non-viable pregnancy	496±514	411±383	NS
Singleton live birth	989±553	920±598	NS
Twin live birth	1,456±804	1,534±1,250	NS
Total live birth	1,054±615	968±682	NS

Fig. 1 Receiver operator characteristic (ROC) curve for β -hCG values in predicting live birth 13 days after fresh (ET—red line) and vitrified-warmed blastocyst transfer (FET—blue line). The y-axis represents sensitivity and x-axis 1-specificity. The value of 496 IU/L for the ET group and value 527 IU/ml for the FET group is circled. The two circles represent the optimal sensitivity and specificity for the assay

faster growth resulted in pregnancies where β -hCG increased earlier and to higher levels compared with the other medium [20]. Different conditions and duration of embryo cultivation may explain the observations that values of early β -hCG measurements are different following cleavage or blastocyst stage ET. However, the results are conflicting; Zhang et al. [12] and Papageorgiou et al. [8] have demonstrated lower values after blastocyst cultivation of embryos compared to cleavage stage ET. But subsequently published data on β -hCG levels after cleavage and blastocyst stage ET have shown higher or comparable β -hCG levels after blastocyst transfer compared to cleavage stage ET [7, 8]. One of the explanations could be that significant development of embryo culture media in the last decade and improved embryo development environment exerted less negative influence on trophoblast production in cultivated embryos in these studies.

However, studies investigating β -hCG levels after fresh ET present with inconsistent results so it is impossible to use these findings in predicting pregnancy outcome after FET. There is a possibility that freezing and thawing may damage the trophoblast and thereby negatively influence implantation, embryo growth and β -hCG production. In contrast to this, some recent data suggest that the rate of β -hCG increase is higher following FET [14]. There is no data specifically considering differences in β -hCG levels after FET compared to fresh ET. Only a few published studies included analysis of β -hCG levels after FET with very limited data on this topic [1, 6, 10]. These studies have shown no differences in β -hCG levels after fresh ET and FET. However, to our knowledge, there are no studies comparing β -hCG dynamics and pregnancy prognostic value of β -hCG in cycles after fresh and cryopreserved blastocyst transfer. Thus, the aim of our study was to evaluate β -hCG levels 13 days after fresh or vitrified-warmed blastocyst transfer.

We found a higher pregnancy as well as live birth rate after fresh ET compared to FET. Besides lower implantation rate

after FET, higher biochemical pregnancy and abortion rates were observed. This is the reason that the mean value of β -hCG level was higher in the group after fresh ET compared to the group after FET. If β -hCG levels were compared between these two groups stratified according to pregnancy outcome, differences were not noticed. Our study has not shown any statistically significant difference in β -hCG levels when transferring fresh or vitrified-warmed blastocysts, which is in accordance with the data published by Lawler et al. [1], who studied β -hCG levels after transfer of fresh and cryopreserved cleavage stage embryos. Method of freezing, however, was not defined in the mentioned study and it is known that embryo freezing protocols could also produce differential effects on β -hCG levels. It has been demonstrated that vitrification of embryos results in significantly lower damage to developing embryos compared to slow freezing method, which also translates to higher pregnancy rates [21]. Due to lower trophoblast damage, this could relate to higher β -hCG levels early after transfer compared to transfer of embryos cryopreserved by slow freezing. Additionally, differences in treatment protocols before embryo transfer could also influence β -hCG values due to differing impact on endometrial maturation [9]. Beta-hCG values after fresh ET and FET cycles could also differ due to differing exogenous gonadotropin action on the endometrium. However, the effects are not likely to be significant once pregnancy is achieved as our results do not demonstrate any differences in average β -hCG levels between the studied groups. Direct comparison of β -hCG levels after transfer of vitrified embryos with previously reported values in the literature is not possible due to different study protocols and timing of β -hCG analysis. According to our data, it can be presumed that comparable β -hCG levels are achieved after transfer of fresh and of vitrified-warmed blastocysts.

Our results have confirmed that β -hCG levels are significantly higher in viable than in non-viable pregnancies

regardless of the mode of ET. Thus, after fresh ET or FET, the levels do not differ in each of the outcomes studied. Higher β -hCG levels in viable pregnancies have been demonstrated by many investigators [1, 4–11]. Our study has shown that 13 days after ET, average β -hCG levels in viable pregnancies are twice as high compared to non-viable pregnancies. The optimal threshold levels with acceptable sensitivity and specificity for predicting live birth after ET in the present study were 496 IU/L for ET and 527 IU/L for FET group. Furthermore, sensitivity and specificity for predicting pregnancy outcome in each of the studied groups was comparable and PPV was not statistically different. Considering variations in timing of β -hCG sampling and in methods used for β -hCG measurement in different studies, these numbers are difficult to evaluate against other findings in the literature. Nonetheless, sensitivity and specificity for predicting live birth in the present study was comparable with findings of other authors [1, 7, 8].

Our study is limited by its retrospective design and possible selection bias. 1,510 cycles with positive β -hCG result following fresh ET and FET were evaluated, but only a little more than half of them were included in further analysis. The main reason for drop-out is the fact that significant part of our patients comes from abroad. In these patients, pregnancy test is usually performed in their own country with a different method. To avoid differences in the types of assay used, only measurements performed in our laboratory were considered. Another important drop-out reason is that we do not schedule β -hCG measurements during weekends and that only cycles with β -hCG sampled on day 13 after ET were included. With these limitations in mind, we strongly believe that included participants represent a general patient population, since their characteristics are similar to those reported in other studies. Nonetheless, these results should be validated by future, properly designed prospective studies.

In conclusion, our study suggests that β -hCG levels 13 days after ET are a useful tool in predicting the course of the confirmed pregnancy regardless of fresh or vitrified-warmed blastocyst transfer. Therefore, clinicians counselling couples after vitrified-warmed blastocyst transfer can be encouraged to interpret β -hCG levels in the same manner as in the setting of fresh blastocyst transfer.

Conflict of interest The authors declare that they have no conflict of interest.

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