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HYPERGLYCOSYLATED HUMAN CHORIONIC GONADOTROPIN AS AN EARLY PREDICTOR OF PREGNANCY OUTCOMES AFTER IN VITRO FERTILIZATION

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Abstract

Objective—To determine if hyperglycosylated hCG (hhCG), produced by invasive trophoblasts, measured as early as 9 days after egg retrieval can predict ongoing pregnancies (OP) after in vitro fertilization and fresh embryo transfer (IVF-ET).

Design—Cohort

Setting—Academic ART center

Patients—Consecutive patients undergoing IVF-ET

Interventions—Serum hhCG and hCG levels measured 9 (D9) and 16 (D16) days after egg retrieval

Outcome—Ongoing pregnancy (OP) beyond 9 weeks of gestation

Results—OP (62 of 112 participants) was associated with higher D9 levels of hhCG and hCG. However, hhCG was detectable in all D9 OP samples, while hCG was detectable in only 22%. D9 hhCG levels >110 pg/mL was 96% specific for OP, yielding a positive predictive value of 95%. Compared to D9 hCG levels, hhCG was more sensitive and had a larger area under the curve (0.87 vs. 0.67). Diagnostic test characteristics were similar between D16 hhCG and hCG levels.

Conclusions—In patients undergoing assisted reproduction, a test to detect pregnancy early and predict outcomes is highly desirable. HhCG is detectable in serum 9 days after egg retrieval IVF-

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ET cycles. At this early assessment, hhCG is superior to traditional hCG and highly predictive of ongoing pregnancies.

Keywords

Hyperglycosylated hCG; hCG; early pregnancy; in vitro fertilization

Introduction

Hyperglycosylated human chorionic gonadotropin (hhCG) is a variant of human chorionic gonadotropin (hCG). HCG is a molecule produced by placental trophoblast cells in pregnancy, in gestational trophoblastic disease, and by other tumors (1-3). The hCG glycoprotein consists of an alpha subunit, a beta subunit and variable carbohydrate content. Heterogeneity in either the protein or carbohydrate components distinguishes one form of hCG from another. The hyperglycosylated variant of hCG is structurally distinct because of four double size O-linked oligosaccharides and four larger N-linked sugar side chains (4). A monoclonal antibody highly specific for hhCG has been purified and has <10% cross-reactivity with regular hCG (5).

HhCG is a potential predictor of early pregnancy outcomes. Biologically, hhCG is produced by root and extravillous cytotrophoblast cells, where it appears to promote cell proliferation, invasion and implantation (6, 7). HhCG comprises the majority of total hCG produced in the early first trimester, dwindling to less than 1% of total hCG by early second trimester (8). Levels of hhCG are measurable in serum, and several studies have reported that absolute hhCG levels or the ratio of hhCG to total hCG can predict clinical pregnancy after in vitro fertilization (IVF) (9) or discriminate between early pregnancy loss and pregnancies progressing beyond 12 weeks in unassisted conceptions (10-12). However, these studies are limited in sample size, particularly in the peri-implantation time frame. Moreover, none have adequately examined the diagnostic test characteristics of hhCG in an unselected infertility population. In patients undergoing assisted reproduction, it is especially desirable to have a test to detect pregnancy early and predict outcomes. Currently, most IVF patients do not undergo serum hCG tests until 12-16 days after egg retrieval because of limited sensitivity of hCG assays and potential false positive testing from residual hCG injections used to induce ovulation. Studies on hCG to predict pregnancy outcomes in IVF have measured levels at these later time points (13-15).

The objectives of this study were to determine if hhCG can be detected in serum as early as 9 days after egg retrieval and to test if early hhCG levels can predict ongoing pregnancies in women undergoing in vitro fertilization and fresh embryo transfer. We hypothesized that hhCG levels on day 9 after egg retrieval would be highly predictive of ongoing pregnancies in this population.

Materials and Methods

Participants underwent fresh, autologous in vitro fertilization-embryo transfer (IVF-ET) cycles between December 2010 and December 2011. Egg donation, gestational surrogacy, frozen embryo transfer and cancelled IVF cycles were excluded. Only the first eligible IVF-ET cycle for each patient during this time period was included. Conducted at a single, university-affiliated ART center, the study was reviewed by the Institutional Review Board at the University of California, San Diego and approved for waiver of informed consent.

First, we undertook a pilot case-control study to test the association between hhCG and pregnancy outcomes. Cases were patients with ongoing pregnancies beyond 9 weeks of

gestation, confirmed by ultrasound between 8 and 9 weeks of gestation (n=26). Controls were women with negative serum hCG testing 16 days after egg retrieval or spontaneous abortion prior to 9 weeks of gestation (n=26). Consecutive cycles from December 2010 to March 2011 were reviewed until the desired numbers of cases and controls were achieved. Of 212 cycles that were reviewed, 160 were excluded for the following reasons: frozen cycle (n=75), donor oocyte cycle (n=30), cycle cancellation (n=26), biochemical pregnancy (n=15), spontaneous reduction (n=14).

Second, we performed a retrospective cohort study to validate the case-control study findings and determine clinically useful cut-points for hhCG levels for predicting ongoing pregnancy. The cohort included all patients undergoing autologous fresh IVF cycles from March 2011 to December 2011 with serum samples available from 9 and 16 days after egg retrieval (n=112). None of the samples utilized in the pilot case-control study were included in the retrospective cohort study.

All participants underwent one of two controlled ovarian stimulation protocols: luteal phase leuprolide suppression (Abbot Laboratories, Chicago, IL) or GnRH antagonist with ganirelix (Abbot Laboratories, Chicago, IL) or cetrorelix (EMD Serono, Rockland, MA). Cycles were monitored with daily estradiol measurements starting on treatment day 3 and ultrasounds starting on treatment days 5 or 6. GnRH antagonists were initiated when the lead follicle reached 14mm. Pregnyl 10,000 IU (Merck, Whitehouse Station, NJ) was administered to trigger ovulation when 2 lead follicles reached 18mm in diameter. Transvaginal aspiration of oocytes was performed 35.5 hours later. Embryos were cultured to day 3 and day 5 per clinical criteria. Luteal support with intramuscular progesterone (50 mg/day) was initiated the day after egg retrieval. Serum progesterone levels were measured on day 9 after egg retrieval, and serum hCG levels were measured on day 16 after egg retrieval to test for pregnancy per routine clinical care. Obstetrical ultrasounds were performed between 5 and 6 weeks of gestation and repeated between the 8th- 9th week to confirm ongoing pregnancy.

Clinical characteristics, IVF cycle parameters, ultrasound results and pregnancy outcomes were abstracted from electronic patient records. Gestational age was calculated based on date of egg retrieval and confirmed by ultrasound dating. Serum samples from 9 and 16 days after egg retrieval were frozen within 4 hours after blood draw, after the sample for clinical assays was removed, and remained at -20C until assay for hhCG. None of the samples underwent a prior thaw.

Serum samples were collected and shipped on dry ice to Quest Diagnostics (Quest Diagnostics, Nichols Institute, San Juan Capistrano, CA) for measurements of hhCG and hCG. The ultrasensitive hhCG assay was performed using a procedure described previously (16). Briefly, hhCG was measured using an electrochemiluminescence (ECL) technique in a 96 well plates from Mesoscale discovery (MSD, Gaithersburg, MD). This immunometric assay used the hhCG specific antibody (B 152) as the coating antibody and hCG beta specific antibody (B 207) as the labeled antibody with sulfotag (MSD). Light signal (Relative light unit, RLU) generated by the antibody – antigen and antibody reaction was measured by a luminometer (MSD). RLU is directly proportional to the concentration of hhCG. The assay is sensitive to 5 pg/ml and specific to hhCG with less than 1% cross reactivity with hCG, LH or FSH. Using four different controls of various concentrations of hhCG, the intra assay was less than 6.5% and inter assay variation was less than 7.7 %. Samples with high concentrations of hhCG were diluted and reported as absolute values, corrected for dilution. The hCG assay was performed in an automated immunoassay analyzer (Centaur, Siemens). The assay sensitivity was 2 mIU/ml and total variation was less than 5.1 %.

Statistical Analysis

The primary exposure of interest was hhCG levels measured 9 days after egg retrieval. In addition, we examined hhCG levels 16 days after egg retrieval and hCG levels 9 and 16 days after egg retrieval. We also calculated the absolute change in hhCG levels (Delta hhCG) between days 9 and 16, as well as percent change in hhCG levels between days 9 and 16. The primary outcome of interest was ongoing pregnancy, confirmed by ultrasound between 8 and 9 weeks of gestation. The secondary outcome of interest was pregnancy, defined as a positive serum hCG test 16 days after egg retrieval. Spontaneous abortions were defined as pregnancy losses after a positive serum hCG test 16 days after egg retrieval and prior to 9 weeks of gestation.

Stata software (release 12; Stata Corp., College Station, TX) was used for analysis. HhCG and hCG values below detection thresholds were given half of the threshold value in analysis. Graphic displays of continuous variables were explored to determine data distributions. Continuous variables were summarized by means (standard deviation [SD] or medians (interquartile range [IQR]) and compared by pregnancy outcome status using the Student's t-test or Wilcoxon ranksum test, as appropriate. The distribution of hhCG and hCG levels did not meet the assumptions of normality, even with logarithmic transformation. Therefore, hhCG and hCG levels were compared by pregnancy outcomes using the Wilcoxon ranksum test. Categorical variables were characterized as proportions and compared using Chi-square test or Fisher exact test where appropriate.

Diagnostic test characteristics of sensitivity, specificity, positive predictive value and negative predictive value were calculated. Cut points in hhCG levels were selected to optimize the positive predictive value (PPV) for ongoing pregnancy (the probability that the subject who has an hhCG level above the cutpoint truly will have an ongoing pregnancy). Receiver operating characteristic curves were generated for hhCG and hCG on days 9 and 16 to compare areas under the curve (AUCs) for these diagnostic measures using the Chi-square test.

A priori sample size calculations were performed based on the confidence interval around the PPV estimate of hhCG for ongoing pregnancy. The lower bound of the 95% confidence interval around the positive predictive value was set at 90%. Assuming 90% power, Type I error of 5% and 50% incidence of ongoing pregnancy, 99 participants would be required to determine a positive predictive value with a lower bound of the 95% CI exceeding 90%.

Results

For the pilot case-control study, there were 26 participants with ongoing pregnancies beyond 9 weeks of gestation, 20 participants with spontaneous abortions prior to 9 weeks of gestation and 6 participants with negative hCG tests. Mean age (SD) was 35.7 $\pm$ 5.7. On day 9 after egg retrieval, hhCG was detectable in all ongoing pregnancy and spontaneous abortion samples. HhCG was also detectable in 5 of 6 women with negative pregnancy tests as measured by serum hCG. In contrast, hCG was detectable in 11/20 (55%) of ongoing pregnancy, 5/20 (25%) of spontaneous abortion and 0/6 of negative pregnancy test samples. In day 9 samples, median hhCG levels (IQR) were 14 (12), 41 (57), 51 (96) pg/ml in negative pregnancy, spontaneous abortion and ongoing pregnancy samples, respectively. In day 16 samples, median hhCG levels (IQR) were <1 (11), 3606 (3574), 9135(8170) pg/ml for negative pregnancy, spontaneous abortion and ongoing pregnancy samples, respectively. Participants with ongoing pregnancies had significantly higher hhCG levels on day 9 ($p=0.02$) and day 16 ($p<0.001$) compared to participants without ongoing pregnancies.

After detecting a significant association between hhCG and ongoing pregnancy, we conducted a retrospective cohort study to determine diagnostic test characteristics of hhCG measurements. Of 126 potential patients, 112 women had both day 9 and day 16 serum samples available and were included in the study. Baseline characteristics of the 14 women who were excluded did not differ from the remaining cohort (data not shown). Table 1 depicts baseline characteristics of the population. Fifty-five percent of participants (n=62) had an ongoing pregnancy beyond 9 weeks of gestation, and 66% (n=74) had a positive pregnancy test by serum hCG on day 16 after egg retrieval. There were no ectopic pregnancies during this recruitment interval. Participants with ongoing pregnancies were younger and had lower day 3 FSH levels and higher numbers of oocytes retrieved and fertilized embryos than participants without ongoing pregnancies. Participants with positive pregnancy test by serum hCG on day 16 were younger and had higher numbers of oocytes retrieved and fertilized embryos than participants who were not pregnant.

Figure 1 summarizes hhCG, delta hhCG, hCG and delta hCG levels by sample day and pregnancy outcome. Percent changes in hhCG and hCG had similar results to delta hhCG and hCG, respectively (data not shown). Ongoing pregnancy or positive pregnancy samples were associated with higher day 9 and 16 measurements of both hhCG and hCG. However, day 9 hCG levels were detectable in only 11/62 (22%) of ongoing pregnancy, 1/12 (8%) of spontaneous abortion and 1/37 (3%) of negative pregnancy samples. In contrast, all day 9 samples from ongoing pregnancies and spontaneous abortions had detectable hhCG, while 35/38 (92%) of samples from participants who were not pregnant also demonstrated presence of hhCG. However, the hhCG level on day 9 was significantly lower in the non-pregnant patients, 35 vs. 91 pg/ml ($P=0.001$)

Table 2 summarizes diagnostic test characteristics of hhCG and hCG on days 9 and 16 for pregnancy outcomes. For ongoing pregnancies, day 9 hhCG levels greater than 110 pg/mL was 96% specific for OP, yielding a positive predictive value of 94%. This finding did not differ between day 3 and day 5 embryo transfers. Compared to day 9 hCG measurements, hhCG had higher sensitivity (47% versus 18%). In receiver operating characteristic curve analysis, day 9 hhCG had a higher area under the curve than hCG (AUC 0.80 versus 0.67, $p=0.002$). Diagnostic test characteristics were similar between day 16 hhCG, day 16 hCG and delta hhCG measurements.

To predict a positive pregnancy as measured by hCG on day 16, day 9 hhCG levels greater than 81 pg/mL was 95% specific, yielding a positive predictive value of 95%. Day 9 hCG levels greater than 4 mIU/mL had similar specificity and positive predictive value, but sensitivity was significantly lower (24% versus 54%). On day 16, hhCG levels greater than 242 pg/mL, delta hhCG levels greater than 226 pg/mL and percent change hhCG greater than 4 diagnosed all positive pregnancies identified by elevated hCG on day 16. There were 23 twin pregnancies (37%) among 62 ongoing pregnancies. Day 9 hhCG levels were not significantly different between twin and singleton ongoing pregnancies (median 99 [IQR 83] versus 77 [214] pg/mL, $p=0.88$). By 16 days after egg retrieval, hhCG levels were significantly higher with twin ongoing pregnancies than singleton (median 20,150 [IQR 15,610] versus 11,470 [8775] pg/mL, $p=0.002$).

Discussion

IVF is a challenging process through which patients are wrought with anxiety regarding treatment outcome. A test that can predict pregnancy outcomes would be particularly desirable for this population. This study demonstrated that hhCG measurements 9 days after egg retrieval, 7 days earlier than current standard hCG tests in our center, is highly predictive of ongoing pregnancies beyond 9 weeks of gestation in an unselected cohort of

infertile women undergoing IVF and fresh embryo transfer. By 16 days after egg retrieval, hhCG measurements (absolute or change in hhCG levels) were not superior to standard hCG levels in predicting the probability of ongoing pregnancy. These findings suggest that the clinical advantage of assessing hhCG in the IVF setting is in earlier pregnancy detection and prediction.

These results contribute significantly to limited data on hhCG measurements in assisted reproduction. To our knowledge, only three smaller studies to date have examined hhCG in IVF (9, 11, 17). Kovalevskaya, et al. measured daily urinary hhCG in 22 donor egg recipients who were not exposed to exogenous hCG injections (11). Of 12 pregnant participants, detection of hhCG in urine was reported to be as early as 5 days after a day 3 embryo transfer (mean 6.75 days, range 5-10 days) (11). This is on the same chronologic day as our detection of serum hhCG on day 9 after egg retrieval. Early serum samples were not available in the Kovalevskaya study, and the number of participants was too small to determine clinical cutpoint for urine hhCG. A second study of 87 women who became pregnant after IVF measured hhCG between days 14 and 20 after embryo transfer, significantly later than the current study (17).

A recent study sought to identify cut-off values for hhCG to predict clinical pregnancy after IVF (9). Strom et al. recruited a cohort of 56 patients from four fertility centers and measured serum and urine hhCG levels 4, 6, 8 and 12 days after embryo transfer regardless of embryo stage. HhCG measurements in the Strom study on day 4 for blastocysts would correspond to day 9 levels in our study. Among 32 blastocyst transfers, hhCG levels > 25 pg/mL 9 days after egg retrieval were 82% sensitive, 87% specific for clinical pregnancy. This is a lower cut-off value than the 110 pg/L threshold found in the current study. There are two potential explanations for the discrepancy. First, their outcome of clinical pregnancy was based on ultrasound identification of gestational sac or gestational sac with heart beat. These assessments occur as early as 5-6 weeks of gestation, significantly earlier than the 9+ weeks of follow up in the current study. Thus, the clinical pregnancy group included early pregnancy losses prior to 9 weeks, and as hhCG levels in spontaneous abortions tend to be lower, the resultant cut-off values would be expected to be lower. Second, the small sample size and patient selection may have resulted in a population different than in this study. Advancing the findings of these prior studies, the current, well-powered study was able to identify a highly specific threshold value to predict ongoing pregnancies at 9 weeks, a longer-term, clinically important outcome for IVF patients.

In evaluating hhCG as a diagnostic test for early pregnancy outcomes, both cutpoint and participant selection are important. A diagnostic test has multiple test characteristics, including sensitivity, specificity, PPV and negative predictive value, which change depending on the cutpoint used. An ideal diagnostic test would have both high sensitivity and specificity. Unfortunately, like most clinically used biomarkers, hhCG does not encompass both of these attributes in the prediction of early pregnancy outcomes. In considering cutpoint selection, a highly specific threshold value of hhCG would minimize false positives, or falsely predicting ongoing pregnancies in patients who will not be pregnant or suffer an earlier pregnancy loss. In contrast, a highly sensitive threshold value would minimize false negatives but increase the number of patients with false positive results. Because the existing hCG test is already highly sensitive and may be used in conjunction with hhCG, we reasoned that the benefit of an additional test for IVF patients would be derived from a highly specific test that could reassure patients who test positive that they had a high probability of a successful pregnancy beyond 9 weeks of gestation.

In clinical care, it is important to have knowledge of the predictive value of hhCG testing. As predictive values are determined not only by sensitivity and specificity, but also by

prevalence of ongoing pregnancies in the population, participant selection is key to the ability to generalize the findings of this study. In prior pregnancy outcome studies on hhCG, sample sizes were limited and there are no data on how participants who enrolled differed from the source population. A strength of this study was the inclusion of consecutive IVF patients undergoing fresh transfers in this study. The ongoing pregnancy rate of 45% is consistent with our clinic-based data over the past five years.

In this dataset, 35 of 38 participants with negative serum hCG test on day 16 had detectable hhCG levels in their day 9 samples. Because of the high proportion, this finding most likely resulted from residual medication used to induce ovulation and less likely from an early pregnancy that did not progress between days 9 and 16. As well, longitudinal data from the Strom study suggest greater residual hCG drug effect if serum is measured as early as day 5 after egg retrieval (9). Therefore, similar to traditional hCG assays, detectability alone is not predictive of pregnancy outcomes; rather the value of hhCG measurement rests on the identification of a threshold hhCG level which optimize specificity for prediction of pregnancy outcomes. One future direction of this study is to assess early hhCG levels in frozen embryo transfer cycles or donor recipient cycles in which no exogenous hCG is used. It would also be interesting to study early hhCG levels in fresh cycles where lower doses of purified hCG, recombinant hCG or only GnRH agonist are used to trigger ovulation. The majority of samples where a pregnancy occurred had undetectable hCG levels on day 9. This indicates that the traditional hCG assay used in our study (Siemens Centaur) was not as sensitive on day 9 as hhCG and that this particular assay did not fully recognize hhCG.

Several limitations to this study ought to be considered. The study had limited power to examine the ability of hhCG to discriminate between singleton and twin gestation, or ongoing pregnancy versus pregnancy loss prior to 9 weeks. While levels of hhCG on day 9 differed between ongoing pregnancy and early pregnancy losses in the case-control pilot, and levels on day 16 differed between singleton and twins in the cohort study, larger datasets will be required to examine diagnostic test characteristics for these outcomes. While day 9 hhCG appeared higher in twin compared to singleton gestations, this did not reach statistical significance and was likely due to a sample size as overall day 9 hhCG levels were low and variability was moderate. Second, we were unable to test hhcg/hCG ratios on day 9 samples because the majority of samples did not yield detectable hCG levels at this early date. Third, we had two time points measured one week apart and are unable to test if changes in hhCG levels over closer intervals would improve prediction. However, neither absolute nor relative change in hhCG over one week was a better predictor than absolute levels on day 9 alone. Finally, we limited this study to early pregnancy outcomes. There are some data that support an association between lower mid-trimester hhCG levels and higher risk of pre-eclampsia, a disorder associated with abnormal placentation (18,19). As ART has been linked to disorders of placentation (21, 22,23) and hCG receptors have been identified in the endometrium (20), further studies are needed to examine the association between hhCG levels and perinatal complications.

In summary, we have conducted a cohort study on early measurement of hyperglycosylated hCG to predict pregnancy outcomes in an unselected in vitro fertilization population. The results of this study support the ability of single measurement of this biomarker to predict ongoing clinical pregnancy beyond 9 weeks of gestation after fresh in vitro fertilization cycles as early as 9 days after egg retrieval.

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Figure 1.

Comparisons of hhCG and hCG levels by pregnancy outcomes. By ongoing pregnancy outcomes, A) Day 9 levels, B) Day 16 levels, and C) Delta hhCG levels. By pregnancy outcomes, D) Day 9 levels, E) Day 16 levels, and F) Delta hhCG levels. Data are depicted as medians with 25th to 75th percentile values. Absolute levels are reported immediately below the graph. *depicts $p < 0.001$ compared to positive pregnancy outcome.

Table 1

Baseline characteristics by pregnancy outcome

	All participants (n=112)	Ongoing Pregnancy		Pregnancy	
		Positive (n=62)	Negative (n=50)	Positive (n=74)	Negative (n=38)
					p-value
Age, Mean (SD)	36.4 (4.2)	35.4 (3.8)	37.7 (4.3)	35.9 (3.9)	37.5 (4.5)
					0.06 ^a
Years of infertility, Mean (SD)	2.4 (2.1)	2.3 (2.0)	2.6 (2.2)	2.5 (2.2)	2.3 (2.0)
					0.55 ^a
Diagnosis, n (%)					
Decreased ovarian reserve	36 (32.1)	11 (17.7)	25 (50.0)	19 (25.7)	17 (44.7)
Male	34 (30.4)	27 (43.5)	7 (14.0)	27 (36.5)	7 (18.6)
Tubal	12 (10.7)	7 (11.3)	5 (10.0)	8 (10.8)	4 (10.5)
Ovulatory	11 (9.8)	6 (9.7)	5 (10.0)	8 (10.8)	3 (7.9)
Unexplained	8 (7.1)	5 (8.1)	3 (6.0)	5 (6.8)	3 (7.9)
Other	11 (9.8)	6 (9.7)	5 (10.0)	7 (9.5)	4 (10.8)
					0.32 ^b
Body Mass Index, Mean (SD)	23.7 (4.5)	23.3 (4.0)	24.3 (5.1)	23.6 (4.2)	24.1 (5.2)
					0.63 ^a
Day 3 FSH (mIU/ml), Mean (SD)	8.3 (4.0)	7.6 (2.6)	9.2 (5.0)	7.8 (3)	9.1 (5.2)
					0.16 ^a
Antral follicle count, Mean (SD)	17.3 (12.1)	19.1 (12)	15 (11.9)	18.4 (11.9)	15 (12.2)
					0.16 ^a
Gravidity, n (%)					
0	60 (53.6)	32 (51.6)	28 (56.0)	38 (51.4)	22 (57.9)
1-2	43 (38.4)	24 (38.7)	19 (38.0)	30 (40.1)	13 (34.2)
>2	9 (8.0)	6 (9.6)	3 (6.0)	6 (8.1)	3 (7.9)
					0.79 ^b
Day 3 Transfer, n (%)	44 (39.3)	27 (43.5)	17 (34.0)	23 (31.1)	21 (55.3)
					0.01 ^c
Retrieved oocytes, Mean (SD)	11.2 (6.6)	12.8 (7.1)	9.3 (5.4)	12.2 (6.9)	9.3 (5.7)
					0.03 ^a
% fertilization, Mean (SD)	69.9 (20.3)	70.1 (17.8)	69.5 (23.2)	71.1 (19.0)	66.6 (23.0)
					0.30 ^a
Embryos transferred, n (%) ^b					

	All participants (n=112)	Ongoing Pregnancy		Pregnancy	
		Positive (n=62)	Negative (n=50)	Positive (n=74)	Negative (n=38)
1	28 (25.0)	14 (22.6)	14 (28.0)	18 (24.3)	10 (26.3)
2	59 (52.7)	37 (59.7)	22 (44.0)	45 (60.8)	19 (50.0)
>2	25 (22.3)	11 (17.7)	14 (28.0)	11 (14.9)	9 (23.7)
					0.44 ^b

^a Student's t-test,

^b Fisher's exact test,

^c Chi-square

Diagnostic test characteristics for prediction of pregnancy and ongoing pregnancy beyond 9 weeks of gestation

Table 2

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Ongoing Pregnancy				
Day 9 hhCG > 110 pg/ml	47	96	94	59
Day 9 hCG > 5 mIU/ml	18	96	85	48
Day 16 hhCG > 8165 pg/ml	77	96	96	77
Day 16 hCG > 237 mIU/ml	79	96	96	79
Delta hhCG >8150 pg/ml	77	96	96	77
Pregnancy				
Day 9 hhCG > 81 pg/ml	54	95	95	51
Day 9 hCG > 4 mIU/ml	24	97	95	40
Day 16 hhCG > 242 pg/ml	100	100	100	100
Delta hhCG >226 pg/ml	100	100	100	100