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ARTICLE

High concentration of first-measured HCG after embryo transfer is associated with subsequent development of pre-eclampsia

BIOGRAPHY

Beata Seeber, MD, MSCE, is an Associate Professor in the Department of Gynecological Endocrinology and Reproductive Medicine at the Medical University of Innsbruck. She completed her residency and fellowship training in reproductive endocrinology and infertility at the University of Pennsylvania, Philadelphia, USA. Her clinical expertise and research interests include infertility treatment, early pregnancy diagnosis and endometriosis.

Anna Aulitzky[1], Julia Lanbach[1,#], Sophie Falkensteiner[1,#], Sarah Maier[2], Hanno Ulmer[2], Bettina Toth[1], Beata Seeber[1,*]

KEY MESSAGE

First-measured HCG following single embryo transfer appears to serve as an early marker of poor placentation. Specifically, extremely high values of HCG were associated with later development of pre-eclampsia, even after controlling for age, BMI, parity and fresh versus frozen embryo transfer. Corroboration of these findings in larger populations is needed.

ABSTRACT

Research question: Are outlier high values of first-measured human chorionic gonadotrophin (HCG) following embryo transfer related to pregnancy complications, specifically pre-eclampsia?

Design: This retrospective cohort study screened 3448 women aged 18–45 years who underwent IVF between 2014 and 2019 and evaluated 614 women who had an intrauterine pregnancy following single embryo transfer (SET), 423 of whom had a live birth. Pregnancy and birth outcome information was available for final analysis in 280 cases. The setting was a university-based IVF centre. HCG was measured at a standardized time after the embryo transfer and the values correlated with adverse pregnancy outcomes associated with poor placentation.

Results: Women with first-measured HCG in the highest quintile had a higher incidence of pre-eclampsia than those with lower HCG concentrations (odds ratio [OR] 4.08, 95% confidence interval [CI] 1.41–11.82) even after controlling for age, body mass index, parity and type of embryo transfer. Additionally controlling for embryo stage at embryo transfer did not change the results (OR 3.97, 95% CI 1.37–11.46). No differences were found in the incidence of fetal growth restriction.

Conclusions: This is the first known report that links high first-measured HCG after SET to an adverse pregnancy outcome. If confirmed by future studies, initiation of preventive interventions at a very early stage of pregnancy merits further evaluation in this cohort of patients.

[1] Department of Gynecological Endocrinology and Reproductive Medicine, Medical University of Innsbruck, Austria (present address: Next Fertility IVF Bregenz, Bregenz, Austria)
[2] Department of Medical Statistics, Informatics and Health Economics, Medical University of Innsbruck, Innsbruck, Austria
[#] These authors contributed equally to this work.

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*Corresponding author. E-mail addresses: beata.seeber@i-med.ac.at, beata.seeber@tirol-kliniken.at (B. Seeber). https://doi.org/10.1016/j.rbmo.2022.10.007 1472-6483/© 2022 The Author(s). Published by Elsevier Ltd on behalf of Reproductive Healthcare Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/)
Declaration: The authors report no financial or commercial conflicts of interest.

KEYWORDS

HCG
IVF
Pre-eclampsia
Pregnancy outcome

INTRODUCTION

Human chorionic gonadotrophin (HCG) is a pregnancy-specific hormone produced primarily by trophoblastic cells. The presence of HCG can be detected in the maternal serum at the earliest 7 days after fertilization and serves as an important diagnostic tool to confirm an early pregnancy, while the measurement of HCG serially is the standardized approach to evaluating its course (*Seeber, 2012*). Women who conceive spontaneously often perform a urine pregnancy test at home after missing a period, obtaining a non-quantitative confirmation of the production of HCG. Following IVF and intrauterine embryo transfer, it is customary to test for the success of implantation by measuring very early concentrations of HCG in maternal serum, often only 10–14 days after embryo transfer. Unlike naturally-conceived pregnancies, for which the exact time of fertilization and implantation is not certain, the date and time of embryo transfer is routinely documented and thus the precise time until the first measurement of HCG can be calculated. Interestingly, it has been observed clinically that the first-measured serum HCG in successful pregnancies has a very wide range of values, from below 50 IU/l to over 2000 IU/l, despite otherwise identical conditions. To our knowledge, the reasons for and the potential significance of this large difference has not been thoroughly evaluated.

Oron et al. (2017) reported that initial serum HCG values are higher after the transfer of a single fresh blastocyst embryo compared with a single fresh cleavage-stage embryo, when standardized 16 days following oocyte collection (*Oron et al., 2015*). Slightly higher concentrations of HCG were observed in pregnancies following transfer of vitrified–warmed blastocysts versus fresh blastocysts (*Oron et al., 2017*). Studies have shown that lower serum HCG concentrations in very early pregnancy are associated with a higher maternal body mass index (BMI) (*Brady et al., 2018*; *Donovan et al., 2018*; *Mejia et al., 2018*), but additional maternal factors that may affect HCG have not been elucidated. The first serum concentration of HCG has likewise not been evaluated as a potential predictive factor for later pregnancy complications.

In the second trimester, a high HCG concentration for the corresponding gestational week of pregnancy has been shown to predict an increased risk of developing pregnancy-related complications, especially pre-eclampsia (*Liu et al., 2015*; *Panwar et al., 2019*; *Zheng et al., 2016*), a major cause of maternal and perinatal morbidity (*Rana et al., 2019*; *Tranquilli et al., 2014*). *Panwar et al. (2019)* showed a significant association between HCG concentrations in the second trimester (between 16 and 18 gestational weeks) and the development of pre-eclampsia. Furthermore, high maternal serum HCG concentrations at 15–20 gestational weeks served as an independent risk factor for pre-eclampsia (*Zheng et al., 2016*). A recent meta-analysis, which included 21 studies with over 28,000 subjects, confirmed that women who were diagnosed with pre-eclampsia were found to have higher early second-trimester concentrations of serum β-HCG multiple of the mean (MoM) compared with healthy controls, although first-trimester β-HCG concentrations were not significantly different (*Zhang et al., 2021*). Conversely, lower first-trimester β-HCG MoM (measured between 8+2 and 14+0 weeks of gestation) was found to be associated with subsequent development of pre-eclampsia in one small study, but these results have not been further corroborated (*Karahasanovic et al., 2014*).

It has been postulated that pre-eclampsia, as well as other pregnancy complications such as fetal growth restriction, including small for gestational age (SGA) and intrauterine growth restriction (IUGR), are the result of abnormal placentation stemming from an imbalance between pro- and anti-angiogenic factors (*Jardim et al., 2015*). Thus, the pathological mechanisms underlying the development of later pregnancy complications might be initiated in very early pregnancy by poor implantation and placentation (*Burton et al., 2009*), caused by endothelial dysfunction of the spiral arteries of the invading trophoblast (*Burton et al., 2009*; *Conde-Agudelo et al., 2004*; *Granger et al., 2001*; *Redman, 1991*; *Roberts and Redman, 1993*; *Steegers et al., 2010*).

In fact, the first-trimester screening test is not only a risk assessment for fetal genetic abnormalities but also aids in predicting the development of pre-eclampsia (*Levine et al., 2004*; *O'Gorman et al., 2016*; *Poon et al., 2012*; *Tayyar et al., 2015*, *2016*; *Tsiakkas et al., 2015*; *Wright et al., 2015*). In women who are deemed at high risk of developing pre-eclampsia based on this test, acetylsalicylic acid (aspirin) is recommended as a preventive measure. When started before 16 gestational weeks at a dose of at least 100 mg per day, aspirin reduces the risk of developing pre-eclampsia by up to 50% in a dose-dependent fashion (*Askie et al., 2007*; *Crandon and Isherwood, 1979*; *Roberge et al., 2013*).

The aim of the present study was to evaluate whether very early HCG in maternal serum, measured at a standardized time following SET, was associated with later complications of pregnancy, specifically pre-eclampsia and fetal growth abnormalities. It was postulated that a very high maternal first-measured HCG concentration may be a sign of aberrant placentation, which will manifest later as adverse pregnancy outcome. Women undergoing IVF are an important study group because single gestations following IVF have higher risks of pregnancy complications compared with spontaneously conceived pregnancies (*Shah et al., 2021*). As such, first-measured HCG could be more than just a marker confirming implantation of an embryo; it could also be a very early biomarker of later pregnancy complications.

MATERIALS AND METHODS

This was a retrospective cohort study. Data were abstracted from electronic medical records and written informed consent was obtained from patients to access their outside records in cases of incomplete or missing data. The study was approved by the Ethics Committee of the Medical University of Innsbruck (MUI), reference 1145/2020, in May 2020.

All women who underwent IVF at the study academic IVF centre between 2014 and 2019 were screened. Inclusion criteria were: age 18–45 years at time of treatment, single intrauterine gestation following a SET, first HCG measured in the study central laboratory between 9 and 15 days following blastocyst embryo transfer, and delivery of an infant ≥24 weeks. Women who did not measure the first HCG in the study laboratory or with first HCG values measured before

day 9 or after day 15 following embryo transfer were excluded. Also excluded were twin gestations, vanishing twin and extrauterine gestations, and gestations ending in miscarriage <24 weeks. Ultimately, only those women who had a singleton live birth ≥24 weeks following SET and for whom delivery information and pregnancy outcomes and complications were known were included in the analyses. Luteal support following fresh embryo transfer and natural cycle–frozen embryo transfer was with micronized vaginal progesterone 600 mg/day. Frozen embryo transfer (using vitrified–warmed embryos) following a programmed cycle were supported with oral oestradiol 6 mg/day in addition to the same dose of progesterone and was continued in all cases until HCG measurement and then until at least weeks 8–10 of gestation.

The analysis of serum HCG concentrations was performed at the central laboratory of the University Hospital of Innsbruck, using an electrochemiluminescence immunoassay (Elecsys, Roche, Germany) that measures intact HCG and free β-HCG chains (functional sensitivity <0.6 mIU/ml). HCG was customarily measured in the early morning 11 days following the transfer of a single day 5 blastocyst (range 9–15 days), corresponding to 16 days following oocyte retrieval in fresh cycles. In the rare cases of transfer of cleavage-stage embryos (day 2 or 3), the day of HCG measurement was adjusted accordingly (day 14 or 13 post-transfer, respectively) to standardize the same number of days from oocyte retrieval.

To account for variations in post-embryo transfer day of HCG measurement due to weekends, holidays or patient availability, all values of HCG were standardized to day 11 as it was the most frequent measurement day. Based on the known standard exponential rise in HCG, the values were extrapolated to day 11 for all patients by using the formula: HCG (day 11) = $\frac{\mathrm{HCG}(t)}{\sqrt{2}^{(d-11)}}$. Sensitivity analyses were subsequently performed by varying the presumed slope of the rise in HCG from, 180 to 220 percent over 48 hours in an attempt to account for known variations in the clinical setting.

Demographic and historical data including age, BMI, medical and obstetrics history were collected from electronic medical records. IVF data collected included cleavage-stage versus blastocyst embryo, fresh versus frozen (vitrified) embryo transfer, and for the fresh cycles: type of protocol (agonist versus antagonist), type of trigger (HCG versus gonadotrophin-releasing hormone [GnRH] agonist), and development of ovarian hyperstimulation syndrome (OHSS) following fresh embryo transfer.

Patient characteristics were subsequently evaluated by quintiles of HCG (1st to 4th quintile HCG [HCG Q1–Q4] versus 5th quintile HCG [HCG Q5]) using mean and SD. Differences between groups were analysed using *t*-test, Mann–Whitney test or chi-squared test, as appropriate.

In addition, logistic regression modelling was used to analyse the relationship of elevated HCG Q5 with the occurrence of pre-eclampsia (defined according to national guidelines (*Guideline of the German Society of Gynecology and Obstetrics, 2019*) as elevated blood pressure >140/90 mmHg, proteinuria >300 mg/day and/or signs of end-organ dysfunction not otherwise explained) or abnormal fetal growth defined according to national guidelines (*Guideline of the German Society of Gynecology and Obstetrics, 2017*) as SGA or IUGR. In the first model the unadjusted crude odds ratio (OR) and its 95% confidence interval (CI) were estimated. In the second model OR was adjusted for age, BMI and parity while a third model additionally adjusted for type of embryo transfer (fresh versus frozen), and a fourth model included embryo stage at transfer (cleavage versus blastocyst). Variable selection for the adjusted models was based on clinical relevance for the outcomes.

Due to the explorative nature of the study, an a priori sample size calculation was not performed. A post-hoc power calculation based on the per-group sample size, the proportion of PEL seen per group, and setting $\alpha = 0.05$, a power of $\beta = 0.79$ was obtained. Statistical analyses were performed using SPSS Statistics for Windows, Version 27 (IBM Corp., Armonk, NY, USA).

RESULTS

A total of 3448 women were identified who underwent IVF and embryo transfer between 2014 and 2019. Of these women, 2834 were excluded for the following reasons: negative pregnancy test, double embryo transfer, multiple gestation or vanishing twin gestation, ectopic pregnancy or pregnancy of unknown location, no HCG value available from the central laboratory following embryo transfer or value outside the pre-set time range. This left 614 women for further consideration, of which 423 (69%) delivered a live infant while 191 (31%) experienced a miscarriage. Sufficient pregnancy and neonatal data were available for analysis for 280 women, as depicted in FIGURE 1.

The mean age of the patients was 32.91 ± 3.84 years and the mean BMI was 24.36 ± 4.97 kg/m^2, with approximately two-thirds of the women being primipara, as shown in TABLE 1. Ninety-six (34.3%) of the women had undergone a fresh embryo transfer while the remaining 184 (65.7%) had a frozen embryo transfer. Only 3 (1.1%) of the women had pre-existing hypertension prior to pregnancy. The mean HCG concentration measured on day 11, or standardized to that day, was 368 ± 230 IU/l, with a wide range of values from 18 to 2036 IU/l.

Although there was not sufficient pregnancy data for the 143 women excluded from further analyses, these women had a similar age (32.92 ± 4.68 years old, $P = 0.93$), BMI (23.83 ± 4.05 years old, $P = 0.74$) and mean HCG concentration (336.32 ± 185.68 IU/l, $P = 0.24$) as the 280 women analysed.

According to the value of the first-measured serum HCG, patients were separated into a high HCG group, defined as the top 5th quintile ($n = 56$) and corresponding to a mean HCG of 712 ± 233 IU/l, and a normal-range HCG group representing the 1st to 4th quintiles with a mean of 282 ± 126 IU/l ($n = 224$), as shown in TABLE 1. The HCG groups were compared according to baseline characteristics and no differences were seen in age, gravidity or parity, pre-existing hypertension, or pregnancy achieved with fresh versus frozen embryo transfer. The women in the high HCG group had a slightly lower BMI of 23.14 ± 4.36 versus 24.66 ± 5.10 kg/m^2 for those in the normal-range HCG group. There was likewise no difference between the two HCG groups in the type of controlled ovarian stimulation (agonist or antagonist protocol), type of trigger (HCG versus GnRH agonist), or development of OHSS

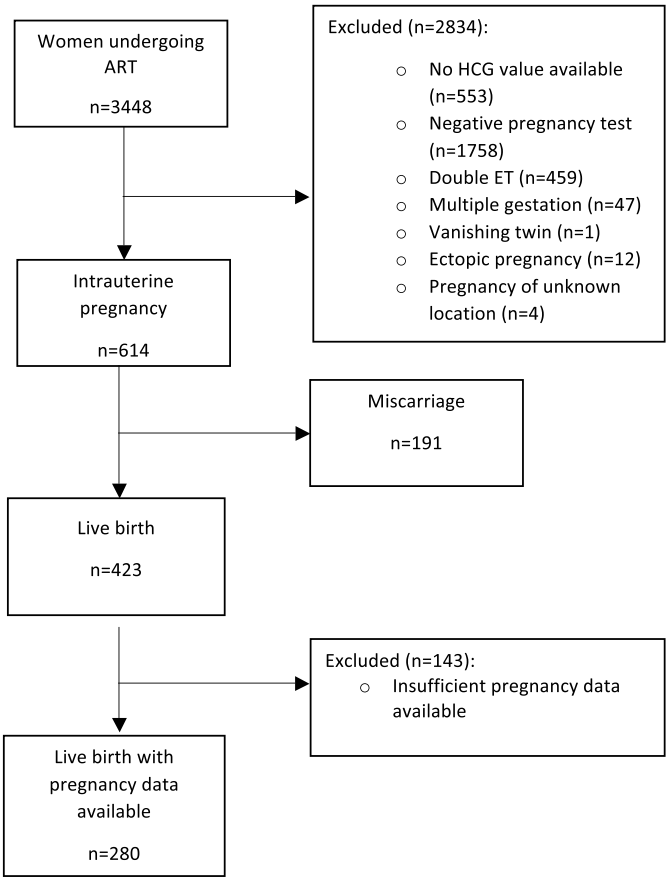

FIGURE 1 Flow chart showing patients screened and ultimately included in the study.

following fresh embryo transfer for the pregnancies achieved with fresh embryo transfer.

The incidence of pregnancy complications between women in the high HCG and normal-range HCG groups is shown in TABLE 2. Women in the high HCG group had a markedly higher incidence of pre-eclampsia, namely 8 of 56 (14.3%) versus 8 of 224 (3.6%) in the normal-range HCG group, respectively (P = 0.005). Of the 16 women with PEL, four had early-onset manifestation requiring preterm delivery before 34 weeks and 12 had late-onset. All four early-onset PEL cases were in the 1st to 4th HCG quintiles group (normal-range HCG). A high HCG in the top 5th quintile remained a significant independent predictor of pre-eclampsia with an OR of 4.76 (95% CI 1.61–12.59) after adjusting for age, BMI and parity. Additionally adjusting for type of embryo transfer reduced the OR slightly to 4.08 (95% CI 1.41–11.82), while considering embryo stage gave a very similar outcome (OR 3.97; 95% CI 1.37–11.46), as shown in TABLE 2.

The sensitivity analysis using a conservative 180% rise in calculated HCG over 2 days showed that the highest quintile of HCG predicted pre-eclampsia with an OR of 4.11, P = 0.010, while controlling for the confounding variables. Similarly, after controlling for confounders, a rapid rise of 220% in HCG over 2 days predicted pre-eclampsia with an OR of 5.13, P = 0.003. Neither the incidence of IUGR nor SGA showed a significant difference between HCG groups.

DISCUSSION

This retrospective cohort study confirmed that first-measured HCG following embryo transfer has a wide range of values, as previously seen in other studies (*Oron et al., 2015*, *2017*). When standardized to 11 days post-blastocyst embryo transfer (16 days post-oocyte retrieval), it was found that the mean HCG value was 368 IU/l in women who went on to have a live birth. In isolated cases, women whose HCG was very low on the first measurement (18 IU/l being the lowest limit) nonetheless delivered a live-born infant.

At the opposite extreme, it was noted that some first-measured HCG concentrations were extremely high. The pregnancy complications in women who made up this group of outliers, defined as having an HCG value in the 5th quintile, were evaluated. A markedly high incidence of pre-eclampsia was found in 14%, suggesting that markedly high HCG could be an early marker for later development of pre-eclampsia. In fact, high HCG remained an independent predictive factor for pre-eclampsia, even after controlling for known possible confounders, including BMI, stage and type of embryo transfer.

The aetiology of pre-eclampsia is not completely understood but there is evidence that abnormal placentation due to poor trophoblast invasion serves as one risk factor. Shallow implantation induces a hypoxic state in the developing placenta, increasing proinflammatory cytokines and the inflammatory response, and further releasing free radicals – all contributing to a state of oxidative stress. Through these processes, endothelial injury may occur and set the stage for the clinical entity of pre-eclampsia.

IVF pregnancies are known to have a higher risk of placental-related disorders, especially hypertensive disorders, HELLP (haemolysis, elevated liver enzymes and low platelets) syndrome, pre-eclampsia and related adverse pregnancy outcomes such as preterm birth, low birthweight or SGA, need for Caesarean section, among others (*Chih et al., 2021*; *Gui et al., 2020*; *Qin et al., 2017*). To our knowledge, these pregnancy outcomes have never been studied in relation to first-measured HCG concentrations.

The current findings are consistent with those of other studies, although these analysed HCG values much later in pregnancy, at 16–20 gestational weeks (*Kaur et al., 2012*; *Liu et al., 2015*; *Panwar et al., 2019*; *Zheng et al., 2016*). These likewise showed that higher concentrations of HCG are related to an increased severity of pre-eclampsia (*Kaur et al., 2012*; *Panwar et al., 2019*). Furthermore, transformation of undifferentiated cytotrophoblastic

TABLE 1 PATIENT CHARACTERISTICS ACCORDING TO FIRST-MEASURED HCG CONCENTRATION

Characteristic	All patients	1st to 4th quintiles HCG	5th quintile HCG	*P*-value
Number of patients	280	224 (80)	56 (20)	
Day of HCG measurement post-ET				0.110
Mean (SD)	11.36 ± 1.16	11.30 ± 1.14	11.59 ± 1.22	
Minimum day	9	9	10	
Maximum day	15	15	15	
Median	11	11	11	
HCG (11) (IU/l):				<0.001
Mean (SD)	368 ± 230	282 ± 126	712 ± 233	
Minimum	18	18	507	
Maximum	2036	504	2036	
Median	337	282	663	
Age (years)	32.91 ± 3.84	32.85 ± 4.00	33.14 ± 3.23	0.576
BMI (kg/m^2)	24.36 ± 4.97	24.66 ± 5.10	23.14 ± 4.36	0.026
Parity				0.849
Primipara	188 (67.1)	151 (67.4)	37 (66.1)	
Multipara	92 (32.9)	73 (32.6)	19 (33.9)	
Pre-existing hypertension	3 (1.1)	3 (1.3)	0 (0)	0.384
Type of transfer				0.023
Frozen	184 (65.7)	140 (62.5)	44 (78.6)	
Fresh	96 (34.3)	84 (37.5)	12 (21.4)	
Embryo stage at transfer				0.029
Cleavage	18 (6.4)	18 (8)	0 (0)	
Blastocyst	262 (93.6)	206 (92)	56 (100)	

Data are given as *n* (%) or mean ± SD.

BMI = body mass index; ET = embryo transfer; HCG = human chorionic gonadotrophin.

into syncytiotrophoblastic cells results in a hypersecretory state of HCG in pre-eclampsia (*Redman, 1991*; *Redman and Staff, 2015*). It is speculated that this pathophysiology may explain the connection between elevated serum HCG and the development of pre-eclampsia. HCG acts in conjunction with vascular endothelial growth factor (VEGF) to regulate angiogenesis and vasculogenesis during placental development. In case of a dysfunctional placental endothelium, HCG, along with other placental factors, might be released in higher concentrations into the maternal circulation. Particularly in preterm pre-eclampsia, the invasion of the trophoblast does not reach the myometrial segments and the spiral arteries are poorly dilated, resulting in reduced placental blood supply (*Brosens et al., 2011*).

The strengths of this study are that all pregnancies occurred after IVF at a single university-based centre with standardized stimulation and IVF protocols. In the sub-cohort of women studied, it was possible to access the medical records for the entirety of pregnancy. The study excluded women with double embryo transfer and twin pregnancies, thus precluding the possibility that the high HCG concentrations were due to the implantation of multiple embryos. Nonetheless, the very low possibility that very early vanishing twin pregnancies were unknowingly included cannot be ruled out.

The limitations of the study are the retrospective study design and a limited sample size to evaluate the rare outcomes of interest. In addition, the HCG values obtained outside of day 11 were mathematically extrapolated, possibly with an unknown margin of error. In an attempt to account for this potential error, a sensitivity analysis was performed with various slopes of HCG increase over 48 h (180–220%) and found the number of pre-eclampsia cases per group and thus the study outcomes did not differ.

Future studies should evaluate whether first-measured HCG values are associated with pre-eclampsia and the more severe HELLP syndrome in larger patient cohorts. In addition, it would be interesting to evaluate these outcomes in naturally-conceived pregnancies with high initial HCG values, although the feasibility of such a study is unlikely due to the difficulty in assessing the exact time of fertilization and implantation in spontaneously conceived pregnancies.

In summary, although preliminary, these findings suggest that high first-measured HCG after embryo transfer might help identify women with risk of developing hypertensive disorders, especially pre-eclampsia, at a very early stage of pregnancy. If these findings are confirmed by future larger studies, then interventions that are currently employed for pre-eclampsia prevention, such as acetylsalicylic acid, might be evaluated as a preventive measure in this group of patients.

TABLE 2 INCIDENCE OF PREGNANCY COMPLICATIONS ACCORDING TO FIRST-MEASURED HCG CONCENTRATION

	Total patients	1st to 4th quintiles HCG	5th quintile HCG
Number of patients	280 (100)	224 (80)	56 (20)
Pregnancy complications	41 (14.6)	28 (12.5)	13 (23.2)
Pre-eclampsia	16 (5.7)	8 (3.6)	8 (14.3)
IUGR	10 (3.6)	9 (4.0)	1 (1.8)
SGA	15 (5.4)	11 (4.9)	4 (7.1)

		Pre-eclampsia incidence	OR	95% CI	*P*-value
Model 1[a]	HCG Q1–Q4	8/224 (3.6)	Ref.		
	HCG Q5	8/56 (14.3)	4.50	1.61–12.59	0.004
Model 2[b]	HCG Q1–Q4	8/224 (3.6)	Ref.		
	HCG Q5	8/56 (14.3)	4.76	1.66–13.64	0.004
Model 3[c]	HCG Q1–Q4	8/224 (3.6)	Ref.		
	HCG Q5	8/56 (14.3)	4.08	1.41–11.82	0.010
Model 4[d]	HCG Q1–Q4	8/224 (3.6)	Ref.		
	HCG Q5	8/56 (14.3)	3.97	1.37–11.46	0.011

Data are presented as *n* (%) unless otherwise stated.

BMI = body mass index; CI = confidence interval; HCG = human chorionic gonadotrophin; IUGR = intrauterine growth restriction; OR = odds ratio; SGA = small for gestational age.

[a] Model 1 univariate.

[b] Model 2 adj. for age, BMI and parity.

[c] Model 3 adj. for age, BMI, parity and type of transfer.

[d] Model 4 adj. for age, BMI, parity, type of transfer and embryo stage.

DATA AVAILABILITY STATEMENT

The data underlying this article will be shared on reasonable request to the corresponding author.

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Received 30 May 2022; received in revised form 14 September 2022; accepted 13 October 2022.