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ORIGINAL ARTICLE *Early pregnancy*

Concentrations of human chorionic gonadotrophin in very early pregnancy and subsequent pre-eclampsia: a cohort study

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STUDY QUESTION: Are low serum concentrations of human chorionic gonadotrophin (hCG) in very early pregnancy associated with pre-eclampsia risk?

SUMMARY ANSWER: Low hCG concentrations in very early pregnancy are associated with increased risk of severe pre-eclampsia.

WHAT IS KNOWN ALREADY: Low maternal serum concentrations of hCG early in pregnancy may indicate impaired proliferation or invasion of trophoblast cells, and thus low hCG concentrations may serve as a marker for impaired placental development. Impaired placental development is assumed to be a cause of pre-eclampsia, but there is little prospective evidence to support this hypothesis.

STUDY DESIGN, SIZE, DURATION: We performed a prospective cohort study of pregnancies after IVF at Oslo University Hospital 1996–2010 with linkage to the Medical Birth Registry of Norway to obtain information on pre-eclampsia development.

PARTICIPANTS/MATERIALS, SETTING, METHODS: We included 2405 consecutive singleton pregnancies and examined the association of maternal serum hCG concentrations (measured using Elecsys, Roche) on Day 12 after embryo transfer with the risk of any pre-eclampsia and of mild and severe pre-eclampsia.

MAIN RESULTS AND THE ROLE OF CHANCE: HCG concentrations were inversely associated with pre-eclampsia risk in a dose-dependent manner (P_{trend} 0.02). Compared with women with hCG ≥150 IU/l, women with hCG <50 IU/l were at 2-fold higher overall risk of pre-eclampsia [absolute risk 6.4 versus 2.8%; odds ratio (OR) 2.3, 95% confidence interval (CI) 1.2–4.7]. The inverse association was restricted to severe pre-eclampsia (P_{trend} 0.01), thus, women with hCG <50 IU/l were at 4-fold higher risk of severe pre-eclampsia than women with hCG ≥150 IU/l (absolute risk 3.6 versus 0.9%; OR 4.2, 95% CI 1.4–12.2). For mild pre-eclampsia, there was no corresponding association (P_{trend} 0.36).

LIMITATIONS, REASONS FOR CAUTION: Results for IVF pregnancies may not be generalizable to spontaneously conceived pregnancies.

WIDER IMPLICATIONS OF THE FINDINGS: Plausible causes of low maternal hCG concentrations very early in pregnancy include impaired placental development and delayed implantation. Thus, these results provide prospective evidence to support the hypothesis that impaired placental development may be associated with subsequent development of severe pre-eclampsia.

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Key words: placenta / chorionic gonadotrophin / pre-eclampsia / cohort study / epidemiology

Introduction

Impaired placental development is thought be an early event in pathophysiologic processes that lead to pre-eclampsia (Wang et al., 2009). In support of this hypothesis, reduced trophoblast invasion of the spiral arteries and other vascular changes have been observed in placental bed biopsies from women who present with pre-eclampsia (Starzyk et al., 1997; Zhou et al., 1997; Brosens et al., 2011). However, there is little prospective evidence to link early placental development with subsequent risk of pre-eclampsia. Human chorionic gonadotrophin (hCG) is synthesized by placental trophoblast cells (Muyan and Boime 1997; Kovalevskaya et al., 2002; Handschuh et al., 2007; Guibourdenche et al., 2010), and hCG stimulates trophoblast proliferation and invasion, and thereby placental development (Zygmunt et al., 2003; Handschuh et al., 2007; Herr et al., 2007; Licht et al., 2007; Cole, 2010). Therefore, low maternal concentrations of hCG early in pregnancy may indicate impaired proliferation or function of trophoblast cells, suggesting that low hCG concentrations may serve as a marker for impaired placental development. In this study of Norwegian women with pregnancies following IVF, we examined maternal serum concentrations of hCG on Day 12 after embryo transfer and risk of pre-eclampsia later in pregnancy.

Methods

Study population

We studied consecutive pregnancies following IVF at the Section for Reproductive Medicine, Rikshospitalet, Oslo University Hospital, Norway, between February 1996 and February 2010. We included pregnancies after transfer of fresh embryos from IVF with or without ICSI, or after transfer of frozen/thawed embryos. All oocytes used for treatment were autologous. Among 3422 pregnancies that lasted 20 weeks or more, we excluded 429 (12.5%) pregnancies without hCG measurement on Day 12 after embryo transfer and 588 (17.2%) multiple pregnancies, leaving 2405 pregnancies for analysis.

HCG measurements

HCG concentrations were measured in fresh maternal serum samples drawn on the morning of Day 12 after transfer of fresh or frozen/thawed cleavage stage embryos (2- to 4-cell stages corresponding to Day 2 after oocyte retrieval, or occasionally 6- to 8-cell stages corresponding to Day 3). The measurements were performed at the Department of Medical Biochemistry, Oslo University Hospital, using an electrochemiluminescence immunoassay (Elecsys, Roche) that measures intact hCG and free β-hCG chains. Control analyses at the hospital have shown a low within-series variation (coefficient of variation $<4\%$) and low variation over time (coefficient of variation $<5\%$) (Eskild et al., 2012).

Data collection

Information on type of IVF treatment, cause of infertility and maternal body mass index was recorded at the hospital prior to pregnancy.

We obtained information on pregnancy characteristics by linkage to the Medical Birth Registry of Norway [www.fhi.no/mfr/ (29 March 2014, date last accessed)] using the 11-digit unique identity numbers that are given to all Norwegian citizens. The Medical Birth Registry has prospectively recorded information on all births in Norway since 1967, and information on each pregnancy is obtained by compulsory notification on standardized forms by midwives or obstetricians. The recorded information includes gestational age at delivery, birthweight, maternal age and parity, pregnancy complications and, since December 1998, maternal smoking habits.

The diagnosis of pre-eclampsia in the Medical Birth Registry is based on development of hypertension (blood pressure $\geq$140/90 mmHg) and proteinuria (protein dip stick $\geq$1+ or $\geq$0.3 g/24 h) after 20 weeks of pregnancy. Since December 1998, clinical severity of pre-eclampsia is recorded, and pre-eclampsia is classified as clinically severe if blood pressure is $\geq$160/110 mmHg and proteinuria is $\geq$2+ on dip stick. In this study, we defined pre-eclampsia as severe if it was accompanied by any of the following conditions: (i) blood pressure $\geq$160/110 mmHg and proteinuria $\geq$2+ on dip stick, (ii) preterm delivery ($<$37 weeks of pregnancy) or (iii) delivery of a small-for-gestational-age (SGA) infant (birthweight $<$10th percentile, adjusted for gestational age and sex; Skjaerven et al., 2000). Other pre-eclampsia cases were classified as mild. The validity of the pre-eclampsia diagnoses in the Medical Birth Registry of Norway has been studied using hospital records as the gold standard, and 88% of pregnancies registered with pre-eclampsia were recorded with hypertension and proteinuria in the hospital records (Thomsen et al., 2013).

Statistical analyses

We grouped the women into four categories of hCG concentration ($<$50, 50–99, 100–149 and $\geq$150 IU/l). Logistic regression analysis was used to estimate odds ratios [OR, with 95% confidence intervals (CI)] for pre-eclampsia by hCG categories, using the highest category as the reference. We separately studied the associations with any pre-eclampsia and with pre-eclampsia by severity, classified as severe or mild. To test for trend across hCG categories, we assigned each woman the median hCG value within her category and used these medians as a continuous variable in the logistic regression model.

Information on clinical severity of pre-eclampsia and maternal smoking habits was available from December 1998 onwards. Therefore, we repeated the analyses among 2078 pregnancies with delivery after December 1998 and complete covariate information (86.4% of the total study population). In this sample, we examined whether the results changed after statistical adjustment for characteristics that may be associated with both hCG concentrations and pre-eclampsia risk, and we therefore adjusted for maternal age (Jukic et al., 2011), parity (Mooney et al., 1995), pre-pregnancy body mass index (Eskild et al., 2012), daily smoking at the beginning of pregnancy (Palomaki et al., 1993), number of transferred embryos (Delbaere et al., 2008), type of IVF treatment (Hui et al., 2003), year of embryo transfer and offspring sex (Illescas et al., 2013).

In separate analyses restricted to pregnancies without pre-eclampsia, we also examined whether hCG concentrations could be associated with the risk of gestational hypertension (development of sustained hypertension after 20 weeks of pregnancy without concomitant proteinuria), preterm delivery ($<$37 weeks of pregnancy) or SGA delivery (birthweight $<$10th percentile, adjusted for gestational age and sex).

We performed several supplementary analyses. First, we examined the associations of hCG concentrations with the risk of preterm (delivery $<$37 weeks) and term pre-eclampsia (delivery $\geq$37 weeks of pregnancy). Secondly, we examined the association of hCG concentrations with pre-eclampsia risk by type of IVF treatment, cause of infertility and number of transferred embryos. Thirdly, we repeated the main analyses using hCG categories defined by multiples of the median (MoM) hCG concentration. Fourthly, among pregnancies with delivery after December 1998 and complete covariate information, we evaluated whether hCG measurements may have changed during the study period by estimating geometric mean hCG concentration for each year of embryo transfer. In that analysis, we used linear regression to adjust for maternal age, parity, pre-pregnancy body mass index, daily smoking at the beginning of pregnancy, type of IVF, number of transferred embryos and offspring sex.

All analyses were performed using Stata version 12.1 (Stata Corporation, College Station, TX, USA).

Ethical approval

The study was approved by the Regional Committee for Medical Research Ethics and the Advisory Board of the Medical Birth Registry of Norway, and the use of clinical data was approved by the data inspection officer at Oslo University Hospital.

Results

Characteristics of the study participants are given in Table I. Among 2405 pregnancies, 105 (4.4%) were complicated by pre-eclampsia, 48 (2.0%) by severe pre-eclampsia and 57 (2.4%) by mild pre-eclampsia. Among the 48 pre-eclampsia pregnancies classified as severe, 26 were clinically severe, 17 had delivery before 37 weeks of pregnancy and 22 resulted in delivery of an SGA infant.

HCG and pre-eclampsia risk

Concentrations of hCG on Day 12 after embryo transfer were inversely associated with pre-eclampsia risk in a dose-dependent manner (P_{trend} 0.02). Compared with pregnancies with hCG concentrations in the highest category (≥150 IU/l), pregnancies with hCG in the lowest (<50 IU/l) were at 2-fold higher overall risk of pre-eclampsia (OR 2.3, 95% CI 1.2–4.7). The inverse association was, however, restricted to severe pre-eclampsia (P_{trend} 0.01), and women in the lowest category (hCG <50 IU/l) were at 4-fold higher risk of severe pre-eclampsia (OR 4.2, 95% CI 1.4–12.2) compared with women in the highest (hCG

Table I Characteristics of the 2405 pregnancies, overall and by pre-eclampsia status.[a]

	Total study population (n = 2405)	Severe pre-eclampsia[b] (n = 48)	Mild pre-eclampsia[b] (n = 57)	No pre-eclampsia (n = 2300)
Serum hCG on Day 12 after embryo transfer (IU/l), median (IQR)	111 (76–156)	93* (61–125)	113 (72–145)	112 (77–157)
Maternal age at delivery (years), mean (SD)	33.2 (3.5)	32.7 (4.1)	32.7 (3.9)	33.3 (3.5)
Parity (no. of previous births), %				
0	71.3	85.4	80.7	70.7
1	25.2	12.5	17.5	25.6
≥2	3.6	2.1	1.8	3.7
Maternal pre-pregnancy body mass index (kg/m^2), median (IQR) (n = 2340)	22.7 (20.9–25.3)	25.6* (23.1–29.3)	25.0* (21.8–28.6)	22.7 (20.8–25.1)
Maternal daily smoking at the beginning of pregnancy, % (n = 2116)				
No	80.8	82.2	89.6	80.5
Yes	7.5	4.4	4.2	7.6
Did not consent to record information	11.8	13.3	6.3	11.9
Male cause of infertility,[c] %	34.5	35.4	36.8	34.4
Type of IVF treatment, %				
Fresh embryo without ICSI	55.1	43.8	54.4	55.3
Fresh embryo with ICSI	38.0	41.7	38.6	38.0
Frozen/thawed embryo	6.9	14.6	7.0	6.7
Number of transferred embryos, %			*	
1	29.4	35.4	19.3	29.5
2	68.9	64.6	75.4	68.8
3	1.7	0.0	5.3	1.7
Gestational age at delivery (weeks), n (%) (n = 2319)				
<34	101 (4.4)	9 (18.8)	0 (0.0)	92 (4.2)
34–36	86 (3.7)	8 (16.7)	0 (0.0)	78 (3.5)
≥37	2132 (91.9)	31 (64.6)	55 (100.0)	2046 (92.3)
Offspring birthweight z score,[d] median (IQR) (n = 2309)	−0.1 (1.0)	−0.8 (1.1)	0.1 (1.0)	−0.1 (1.0)
Male offspring, % (n = 2397)	53.1	35.4*	40.4*	53.8

[a]For variables with missing data, the no. of pregnancies with available data is given in parentheses.
[b]Differences from pregnancies without pre-eclampsia were tested using χ^2 test or t-test, after log-transformation where appropriate, and P-values <0.05 are marked with an asterisk. We did not test differences in gestational age at delivery and offspring birthweight between the groups, as these variables were used to categorize severe and mild pre-eclampsia.
[c]Male but no known female cause of infertility.
[d]Adjusted for gestational age and offspring sex.

≥150 IU/l). For mild pre-eclampsia, there was no corresponding association (P_{trend} 0.36) (Table II).

The observed associations did not substantially change when we restricted the analysis to pregnancies with delivery after December 1998 and complete covariate information, and the estimates remained essentially unchanged after statistical adjustment for potentially confounding factors (Table II).

HCG and risk of gestational hypertension, preterm delivery or SGA delivery

In separate analyses of pregnancies without pre-eclampsia, we examined whether hCG concentrations were associated with gestational hypertension, preterm delivery or SGA delivery, but found no evidence for any association with these pregnancy outcomes (Table III).

Supplementary analyses

We separately analyzed the risk for preterm and term pre-eclampsia (Supplementary data, Table SI). Women with hCG <50 IU/l were at strongly increased risk for preterm pre-eclampsia (OR 5.4, 95% CI 1.3–22.7) compared with women with hCG ≥150 IU/l, although there was no significant trend (P_{trend} 0.17). For term pre-eclampsia, there was significant trend indicating inverse association of hCG concentration with pre-eclampsia risk (P_{trend} 0.02), and women with hCG <50 IU/l appeared to be at ~2-fold higher risk for term pre-eclampsia (OR 1.9, 95% CI 0.8–4.5) compared with women with hCG ≥150 IU/l, although the difference was not statistically significant.

The association of hCG concentrations with pre-eclampsia risk did not substantially differ by type of IVF treatment (Supplementary data, Table SII), cause of infertility (Supplementary data, Table SIII) or number of transferred embryos (Supplementary data, Table SIV).

We repeated the main analyses using hCG categories defined by MoM hCG concentrations, and this yielded similar results as the original analyses (Supplementary data, Tables SV and SVI).

There was no convincing association of year of embryo transfer with mean hCG concentration (Supplementary data, Fig. S1), suggesting that hCG measurements did not substantially change during the study period.

Discussion

In this prospective follow-up of pregnancies conceived after IVF, maternal concentrations of hCG on Day 12 after embryo transfer were inversely associated with the risk for severe pre-eclampsia in a dose-dependent manner.

To our knowledge, this is the first study to provide evidence that low hCG concentrations very early in pregnancy may be associated with

Table II Odds ratios [OR, with 95% confidence intervals (CI)] of pre-eclampsia by categories of maternal serum hCG concentrations on Day 12 after embryo transfer.

Serum hCG (IU/l)	No. of cases/pregnancies	Risk (%)	Total study population		Pregnancies with delivery ≥ December 1998 and complete covariate information[a]			
			Unadjusted		Unadjusted		Adjusted[b]	
			OR	95% CI	OR	95% CI	OR	95% CI
Any pre-eclampsia								
<50	14/220	6.4	2.3	1.2–4.7	2.8	1.3–6.0	2.2	1.0–4.9
50–99	39/810	4.8	1.7	1.0–3.0	2.1	1.2–3.9	1.8	1.0–3.3
100–149	33/702	4.7	1.7	1.0–3.0	1.8	0.9–3.3	1.8	0.9–3.3
≥150	19/673	2.8	1.0	Reference	1.0	Reference	1.0	Reference
P_{trend}			0.02		0.004		0.03	
Severe pre-eclampsia								
<50	8/220	3.6	4.2	1.4–12.2	4.2	1.4–12.8	3.7	1.2–11.6
50–99	18/810	2.2	2.5	1.0–6.4	2.6	1.0–6.6	2.5	1.0–6.6
100–149	16/702	2.3	2.6	1.0–6.7	2.5	1.0–6.5	2.7	1.0–7.2
≥150	6/673	0.9	1.0	Reference	1.0	Reference	1.0	Reference
P_{trend}			0.01		0.01		0.02	
Mild pre-eclampsia								
<50	6/220	2.7	1.4	0.5–3.8	1.8	0.6–5.3	1.3	0.4–4.0
50–99	21/810	2.6	1.4	0.7–2.7	1.8	0.8–3.9	1.3	0.6–2.9
100–149	17/702	2.4	1.3	0.6–2.6	1.3	0.6–3.0	1.1	0.5–2.6
≥150	13/673	1.9	1.0	Reference	1.0	Reference	1.0	Reference
P_{trend}			0.36		0.12		0.49	

[a]N = 2078, 86.4% of the total study population.

[b]Adjusted for maternal age at delivery (<30, 30–34 or ≥35 years), parity (0 or ≥1 previous births), maternal pre-pregnancy body mass index (<25, 25–29, 30–34 or ≥35 kg/m^2), maternal daily smoking at the beginning of pregnancy (yes, no or did not consent to record information), type of IVF (fresh embryo without ICSI, fresh embryo with ICSI or frozen/thawed embryo), number of transferred embryos (1 or ≥2), year of embryo transfer (1998–2002, 2003–2006 or 2007–2010) and offspring sex.

Table III Odds ratios [OR, with 95% confidence intervals (CI)] of gestational hypertension, preterm delivery and small-for-gestational age (SGA) delivery by categories of maternal serum hCG concentrations on Day 12 after embryo transfer among 2300 pregnancies without pre-eclampsia.

Serum hCG (IU/l)	No. of cases/pregnancies	Risk (%)	Total study population		Pregnancies with delivery ≥December 1998 and complete covariate information[a]			
			Unadjusted		Unadjusted		Adjusted[b]	
			OR	95% CI	OR	95% CI	OR	95% CI
Gestational hypertension								
<50	5/206	2.4	1.5	0.5–4.2	1.7	0.6–5.0	1.7	0.6–5.0
50–99	17/771	2.2	1.3	0.6–2.8	1.4	0.6–2.9	1.2	0.5–2.7
100–149	14/669	2.1	1.2	0.6–2.8	1.2	0.5–2.7	1.3	0.6–3.0
≥150	11/654	1.7	1.0	Reference	1.0	Reference	1.0	Reference
P_{trend}			0.41		0.31		0.45	
Preterm delivery[c]								
<50	13/194	6.7	0.8	0.4–1.6	1.0	0.5–1.9	1.0	0.5–1.9
50–99	57/732	7.8	1.0	0.7–1.4	1.0	0.6–1.5	0.9	0.6–1.4
100–149	49/649	7.6	0.9	0.6–1.4	1.0	0.6–1.5	1.0	0.6–1.5
≥150	51/641	8.0	1.0	Reference	1.0	Reference	1.0	Reference
P_{trend}			0.71		0.89		0.73	
SGA delivery[d]								
<50	17/193	8.8	0.9	0.5–1.6	1.0	0.5–1.8	1.0	0.5–1.8
50–99	68/729	9.3	1.0	0.7–1.4	1.0	0.7–1.5	1.0	0.7–1.5
100–149	74/648	11.4	1.2	0.8–1.7	1.1	0.8–1.6	1.1	0.7–1.6
≥150	61/636	9.6	1.0	Reference	1.0	Reference	1.0	Reference
P_{trend}			0.76		0.91		0.98	

[a]$N = 1985$, 86.3% of pregnancies without pre-eclampsia.
[b]Adjusted for maternal age at delivery (<30, 30–34 or ≥35 years), parity (0 or ≥1 previous births), maternal pre-pregnancy body mass index (<25, 25–29, 30–34 or ≥35 kg/m^2), maternal daily smoking at the beginning of pregnancy (yes, no or did not consent to record information), type of IVF (fresh embryo without ICSI, fresh embryo with ICSI or frozen/thawed embryo), number of transferred embryos (1 or ≥2), year of embryo transfer (1998–2002, 2003–2006 or 2007–2010) and offspring sex.
[c]A total of 84 (3.7%) of 2300 pregnancies were excluded from this analysis due to lack of information on gestational age at delivery.
[d]A total of 94 (4.1%) of 2300 pregnancies were excluded from this analysis due to lack of information on adjusted birthweight.

subsequent risk of pre-eclampsia. Previously, the results of some studies have suggested that low hCG concentrations in late first trimester may be associated with increased pre-eclampsia risk (Ong *et al.*, 2000; Canini *et al.*, 2008; Keikkala *et al.*, 2013), but those studies could not assess hCG concentrations during the initial phase of placental development. In the study by Keikkala *et al.* (2013), increased pre-eclampsia risk was related to low first trimester concentrations of hyperglycosylated hCG, which is assumed to stimulate endometrial trophoblast invasion (Handschuh *et al.*, 2007; Cole, 2010; Guibourdenche *et al.*, 2010). In the second and third trimester, high hCG concentrations have been associated with increased pre-eclampsia risk (Said *et al.*, 1984; Chandra *et al.*, 2003; Dugoff *et al.*, 2005; Morris *et al.*, 2008; Olsen *et al.*, 2012), and it has been suggested that high hCG concentrations in the second and third trimester of pregnancy may reflect a compensatory angiogenic response to fetoplacental hypoxia (Crosignani *et al.*, 1974).

The standardized hCG measurements on Day 12 after embryo transfer is an important feature of our study, because of the steep increase in hCG concentrations at the initial stage of pregnancy with a mean doubling time of 1–2 days (Ertzeid *et al.*, 2000; McChesney *et al.*, 2005). Another strong feature is the use of a single laboratory with little variation in hCG measurements during the inclusion period. The immunoassay used in this study detects most hCG forms, including hyperglycosylated hCG (Cole, 2012), which is the dominant form of hCG in maternal serum in very early pregnancy (Cole, 2010). We obtained complete follow-up information on individual pregnancy outcomes by linkage to the Medical Birth Registry of Norway. This information could not be biased, because obstetricians who diagnosed pre-eclampsia were not aware of the hCG measurements early in pregnancy, and therefore, the diagnoses could not be influenced by these measurements. We had information on maternal pre-pregnancy characteristics that may influence both hCG increase and the risk for pre-eclampsia, but statistical adjustment for these potentially confounding factors did not change the main findings.

Ovulation was induced in all women with fresh embryo transfers by injecting 6500–10 000 international units of hCG [Profasi or Ovitrelle (Serono) and Pregnyl (Organon)] 34–36 h before oocyte retrieval and the induction took place 16 days prior to the sampling of serum for hCG measurement. The exogenous hCG is, however, not detectable in serum after 14 days following injection (Damewood *et al.*, 1989), and therefore, the ovulation induction is unlikely to bias our results.

We cannot exclude the possibility that the inverse association with severe pre-eclampsia that we detected in IVF pregnancies may not be present in spontaneously conceived pregnancies. However, in

spontaneous pregnancies, it will be very difficult to achieve standardized measurements of hCG on a specified day shortly after conception in a large number of women. Prospective studies of IVF pregnancies may therefore provide the most reliable evidence related to early hCG concentrations and later risk of pre-eclampsia. The absolute risk for pre-eclampsia may be higher in pregnancies following IVF compared with spontaneously conceived pregnancies (Jackson *et al.*, 2004), but the overall risk of pre-eclampsia in our study population (4.4%) did not substantially differ from the overall risk of pre-eclampsia in Norway (~4%) during the same time period (Klungsoyr *et al.*, 2012).

Low hCG concentrations in very early pregnancy may indicate impaired placental development because hCG is synthesized by placental trophoblast cells (Muyan and Boime, 1997; Kovalevskaya *et al.*, 2002; Handschuh *et al.*, 2007; Guibourdenche *et al.*, 2010) and hCG stimulates trophoblast invasion and placental development (Zygmunt *et al.*, 2003; Handschuh *et al.*, 2007; Herr *et al.*, 2007; Licht *et al.*, 2007; Cole, 2010). Therefore, it seems plausible that hCG concentrations very early in pregnancy are linked to the number and function of trophoblast cells. Delayed implantation may be another cause of low hCG concentrations in very early pregnancy (Wilcox *et al.*, 1999; Jukic *et al.*, 2011), but it is not known whether delayed implantation is associated with later pre-eclampsia development. Previously, it has been suggested that low hCG concentrations in very early pregnancy may be associated with subsequent pregnancy loss (Qasim *et al.*, 1996; Bjercke *et al.*, 1999; Homan *et al.*, 2000; Poikkeus *et al.*, 2002). In these pregnancies, low hCG concentrations may indicate impaired placental development (Hustin *et al.*, 1990; Romero *et al.*, 2011) or delayed implantation (Wilcox *et al.*, 1999), or low hCG could be an early consequence of a pregnancy loss in progress.

The clinical presentation of pre-eclampsia ranges from mild disease diagnosed at term to clinically severe and early-onset pre-eclampsia combined with fetal growth restriction. Pre-eclampsia may originate from a multitude of causes, but impaired placental development may be more important for the severe forms of pre-eclampsia than for mild pre-eclampsia (Vatten and Skjaerven, 2004), and our findings strongly support this hypothesis. Nonetheless, we cannot exclude that the validity of the pre-eclampsia diagnoses in the Medical Birth Registry may be lower for mild than for severe pre-eclampsia, and this could have contributed to the different associations for severe and mild pre-eclampsia that we observed.

Although pre-eclampsia may originate from impaired early placental development, the clinical condition does not present itself until the second half of pregnancy. The chain of events from placental development to onset of pre-eclampsia is incompletely understood, but one promising hypothesis is that impaired placental development leads to fetoplacental hypoxia as fetal demands increase during the second and third trimesters. Hypoxia may initiate an angiogenic response that leads to high maternal serum concentrations of hCG, sFlt-1, s-endoglin and other angiogenic markers, some of which may be causes of the maternal hypertension and proteinuria that characterize pre-eclampsia (Crosignani *et al.*, 1974; Levine *et al.*, 2006; Maynard *et al.*, 2008; Smith and Wear, 2009; Vatten *et al.*, 2012).

It has recently been suggested that low concentrations of hyperglycosylated hCG in maternal serum between 8 and 13 weeks of pregnancy may be a useful predictor for early-onset pre-eclampsia (Keikkala *et al.*, 2013). In our study, however, there was a large overlap in hCG concentrations between women who subsequently developed pre-eclampsia and women who remained normotensive. Also, the steep increase in hCG concentration in very early pregnancy suggests that detailed information about the time from conception to serum sampling is essential for the evaluation of hCG concentrations in very early pregnancy and later development of pre-eclampsia. Therefore, a single measurement of total hCG concentration in very early pregnancy may not be a useful tool for individual prediction of pre-eclampsia risk.

In summary, maternal hCG concentrations very early in pregnancy were inversely associated with the risk for severe pre-eclampsia in a dose-dependent manner. Our findings support the hypothesis that impaired placental development very early in pregnancy may be an underlying cause of severe pre-eclampsia.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

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Authors' roles

All authors contributed to study design, analysis and interpretation of data. T.G.T. and A.E. collected the data. B.O.Å. analyzed the data and drafted the manuscript together with L.J.V. and T.G.T. and A.E. critically revised the manuscript. All authors approved the version to be published.

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Conflict of interest

None declared.

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