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Maternal concentrations of human chorionic gonadotrophin in very early IVF pregnancies and duration of pregnancy: a follow-up study

Tom G Tanbo [a,b,*], *Manuela Zucknick* [c], *Anne Eskild* [b,d]

[a] *Department of Reproductive Medicine, Division of Gynaecology and Obstetrics, Oslo University Hospital, 0424 Oslo, Norway*

[b] *Institute of Clinical Medicine, University of Oslo, 0318 Oslo, Norway*

[c] *Oslo Centre for Biostatistics and Epidemiology, Institute of Basic Medical Sciences, University of Oslo, 0317 Oslo, Norway*

[d] *Department of Obstetrics and Gynecology, Akershus University Hospital, 1478 Lørenskog, Norway*

Anne Eskild is leader of the research section in the Department of Obstetrics and Gynecology at Akershus University Hospital, and Professor at the Institute of Clinical Medicine, University of Oslo, Norway. Her research interest is reproductive health in women.

KEY MESSAGE

In singleton pregnancies, the concentration of HCG at a fixed time after embryo transfer varied substantially. Low HCG concentrations in very early pregnancy were associated with longer duration of pregnancy, significantly in pregnancies delivered at term only.

A B S T R A C T

Research question: Are maternal concentrations of human chorionic gonadotropin (HCG) on a fixed day after embryo transfer associated with duration of pregnancy?

Design: A follow-up study of 1917 singleton pregnancies after IVF was performed. Embryos were cultured for 2 days and maternal HCG concentration quantified on day 12 after embryo transfer. Duration of pregnancy was obtained from the Medical Birth Registry of Norway. Association of HCG concentration (log2-transformed) with duration of pregnancy was estimated as hazard ratios (HR) with 95% confidence intervals (CI) by applying Cox regression proportional hazard models, where time to delivery for pregnancies shortened because of planned Caesarean delivery or induction of labour was treated as censored.

Results: The estimated median duration of pregnancy from embryo transfer was 266 days (95% CI 266–267 days). Maternal concentration of HCG on day 12 after embryo transfer varied from 1 to 588 IU/l (median 117 IU/l). Duration of pregnancy decreased by increasing HCG concentration, significantly in pregnancies delivered at full term ([257–270 days after embryo transfer; HR 1.127, 95% CI 1.026–1.238, P = 0.012). For each doubling of HCG

* Corresponding author.
E-mail address: t.g.tanbo@medisin.uio.no (TG Tanbo).

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concentration on day 12 after embryo transfer, duration of pregnancy was shortened by 0.51 days. Adjustment for maternal age, prepregnancy body mass index, being a first-time mother and number of embryos transferred did not change the association.

Conclusion: High maternal HCG concentration on a fixed day after embryo transfer is likely to indicate early embryo implantation. After embryo transfer, pregnancies with early implantation are shorter than pregnancies with late implantation.

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Introduction

A normal human pregnancy lasts approximately 40 weeks from the first day of the last menstrual period. However, there is large inter-individual variation in duration of pregnancy, and durations of 37 to 42 weeks from last menstrual period are considered normal. The causes of the large variation in duration of human pregnancy remain poorly understood (Gjessing et al., 1999; Jukic et al., 2013; Lie et al., 2006).

It is conceivable that the timing of spontaneous labour in a normal pregnancy is related to the gestational age of the fetus. However, in women with the same date of last menstruation, the true gestational age of the fetus may not be the same. The interval from last menstrual period until implantation of the embryo into the endometrium may vary (Baird et al., 1995; Jukic et al., 2011, 2013; Wilcox et al., 1999). Also the interval from ovulation until embryo implantation varies, and this variation has been estimated from 6 to 12 days (Jukic et al., 2013; Wilcox et al., 1999, 2000). Thus, in pregnancies with the same number of days since the last menstrual period, the true age of the fetus may vary considerably.

In pregnancies after IVF, the time of conception and the time of embryo transfer into the uterus are known. The exact time of embryo implantation, however, is not known (Englert et al., 1984; Ertzeid et al., 2000; Richard et al., 2001). Human chorionic gonadotrophin (HCG) is synthesized in trophoblast cells, and the maternal concentrations of HCG increase rapidly during the first weeks of pregnancy with a doubling time of 1–2 days shortly after implantation (Ertzeid et al., 2000; McChesney et al., 2005). The serum concentrations of HCG in very early pregnancy are therefore likely to indicate time since embryo implantation.

If the true gestational age of the fetus since embryo implantation is related to duration of an IVF pregnancy, it is likely that pregnancies with delayed embryo implantation may last longer than pregnancies with implantation shortly after transfer. If this is true, low maternal HCG concentrations on a fixed day after embryo transfer may be associated with longer duration of pregnancy.

This study examined the associations of maternal HCG concentration on day 12 after embryo transfer with duration of pregnancy. In total, 1917 successful singleton IVF pregnancies were included in the study.

Materials and methods

The study sample was recruited from the Department of Reproductive Medicine, Oslo University Hospital, Rikshospitalet, Norway during the years 1999–2013 (**Figure 1**). During these years, there were a total of 4693 pregnancies after IVF with delivery after 16 weeks of pregnancy. In the study sample, IVF pregnancies after fertilization with and without intracytoplasmic sperm injection (ICSI) were included, and only embryos from fresh cycles were transferred. All oocytes used for treatment were autologous. Only 3277 singleton pregnancies were eligible for the study, since in twin pregnancies, the HCG concentrations are higher and duration of pregnancy is shorter than in singleton pregnancies (Bjercke et al., 1999; Bortolus et al., 1999). To avoid possible bias associated with the age of the transferred embryo, pregnancies after transfer of day 2 embryos only (n = 2120) were included. Of these, the 1919 pregnancies for which maternal HCG concentrations were measured on the morning of day 12 after embryo transfer were included. One pregnancy was excluded because of outlying maternal HCG concentration (>1000 international units per litre (IU/l)), and one pregnancy was excluded because of outlying duration of pregnancy (313 days since embryo transfer). Thus, the study sample included 1917 pregnancies with HCG concentrations measured 12 days after transfer of day 2 embryos.

The study outcome measure was duration of pregnancy (time to delivery) in days, from embryo transfer until delivery. Information on date of embryo transfer was obtained from the electronic patient record at the Department of Reproductive Medicine, Oslo University Hospital, Rikshospitalet. Information on date of delivery was obtained by individual linkage to the Medical Birth Registry of Norway (Irgens, 2000), by using the mothers' unique person identification number. The Medical Birth Registry of Norway holds information about all births after 16 weeks of gestation since 1967, and the notification of births is mandatory by law.

The main exposure measure in this study was HCG concentration (IU/l) in maternal serum sample drawn in the morning on day 12 after embryo transfer. Serum HCG concentrations were quantified at the Department of Medical Biochemistry, Oslo University Hospital, Rikshospitalet by using an electro-chemiluminescence immunoassay method (Elecesys; Roche, Basel, Switzerland), which measures intact HCG and free β-HCG chain with a detection limit of 0.5 IU/ml. 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%). This is in agreement with the corresponding figures given by the manufacturer (Eskild et al., 2012).

The data analyses included the following potentially confounding factors: maternal age and prepregnancy maternal body mass index (BMI; kg/m^2) as continuous variables (Bergsjø et al., 1990; Eskild et al., 2012; Haavaldsen et al., 2014). Also, parity (first time mother; yes versus no) and number of embryos transferred (1 versus 2) were included (Almog et al., 2010; Bergsjø et al., 1990; Haavaldsen et al., 2014; Pinborg et al., 2005).

Ethical approval

This study was approved by the Regional Committee for Ethics in Medical Research in Norway on 27 February 2017 (reference number 2011/2465).

Statistical analysis

The distribution of HCG concentration and duration of pregnancy are presented in figures: for all pregnancies, for pregnancies with

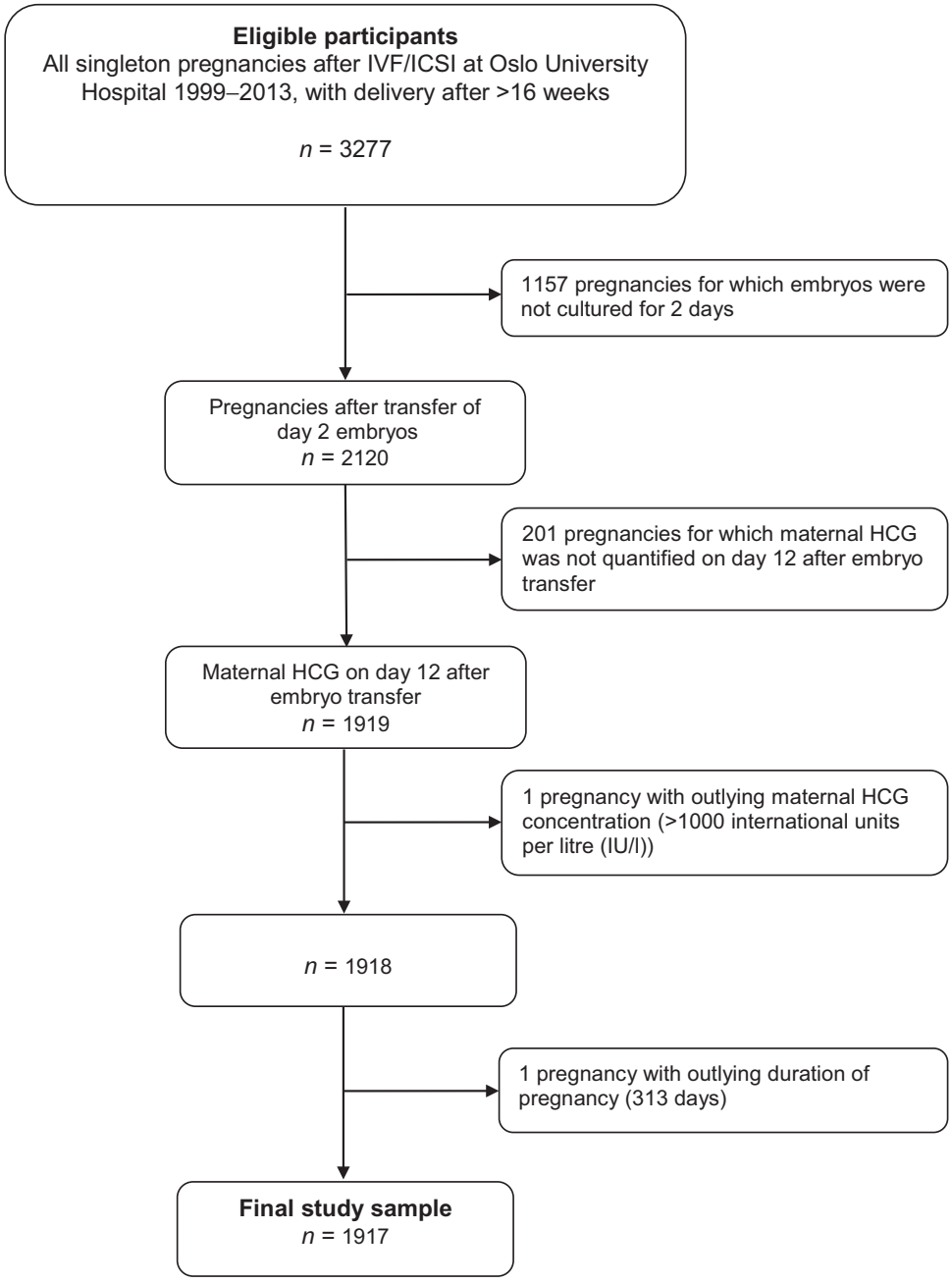

Figure 1 – Flow-chart. Selection of the study sample.

spontaneous onset of labour (including emergency Caesarean delivery), and for pregnancies that were shortened because of planned Caesarean delivery or induction of labour.

Within the pregnancy subtypes described above, means with standard deviation (SD) are presented for continuous study factors and percentages for categorical study factors for all pregnancies: for pregnancies with delivery at early term and pregnancies with delivery at full term. Early-term deliveries were pregnancies with a duration of 243–256 days (37.0–38.9 weeks) after embryo transfer, and full-term deliveries were pregnancies with a duration of 257–270 days (39.0–40.9 weeks) after embryo transfer. The corresponding duration of spontaneous pregnancies, would be 259–272 days (37–38 weeks) for early-term delivery, and 273–286 days (39–40 weeks) for full-term delivery. For this calculation, 2 days of embryo culture and 14 days from first day of last menstrual period until ovulation were added to the duration of the IVF pregnancies.

The observed duration of pregnancy was calculated as number of days from date of embryo transfer until date of delivery. In addition, the median duration of pregnancy was estimated with 95% confidence interval by applying the Kaplan-Meier method. In such analyses, the fact that pregnancies with induction of labour or planned Caesarean section would have lasted longer if such interventions were not performed is accounted for. These pregnancies were censored at the date of delivery (survival analyses).

The associations between HCG concentration and duration of pregnancy were estimated by using survival analyses. Thus, the associations with time to delivery were estimated as crude hazard ratios (HR) with 95% confidence intervals (CI) by fitting univariable Cox proportional

hazards models. In these analyses, the HCG concentrations were log2 transformed to achieve normal distribution of HCG concentrations. All pregnancies were studied, and separate analyses were performed for pregnancies with early-term delivery and pregnancies with full-term delivery. Adjustments were also made for the study factors described above by including them as co-variables in multivariable Cox models. For 12 women, information about BMI was missing, and BMI values were imputed by random draws from the distribution. The Cox model assumptions of linearity and proportional hazards were assessed by Martingale and scaled Schoenfeld residuals.

Finally, for pregnancies with delivery at full term, the fitted Cox model was used to estimate the change in duration of pregnancy (in days) for each doubling of maternal HCG concentration at day 12 after embryo transfer. As a baseline for these calculations, the mean log2 HCG concentrations estimated at the median duration of pregnancy were used.

All statistical tests were performed as two-sided tests, and *P*-values <0.05 were considered statistically significant. The analyses were performed with the statistical computing environment R version 3.2.0 and R packages Hmisc (v3.17) and Survival (v2.38), R Foundation for Statistical Computing, Vienna, Austria.

Results

The mean age of the women was 33.4 years, and 69% were first-time mothers (**Table 1**).

In the sample as a whole, the median observed duration of pregnancy was 264 days from embryo transfer (ranges 97 to 288, mean = 260.4, SD = 16.9 days; **Figure 2a**) corresponding to 280 days in spontaneous births (**Figure 2b**). For pregnancies with planned Caesarean delivery (**Figure 2c**), as well as for pregnancies with labour induction (**Figure 2d**), the duration of pregnancy would have been longer if such intervention had not been performed. Therefore, when we treated the observed duration of these pregnancies as censored by using the Kaplan-Meier method, median pregnancy duration was estimated to 266 days (95% CI 266–267 days) corresponding to 282 days in spontaneous pregnancies (**Table 2**).

HCG concentrations on day 12 after embryo transfer varied from 1 to 588 IU/l, median 117 IU/l. The HCG concentrations displayed a skewed distribution (**Figure 3**). In all sub-samples of pregnancies, a positive association of HCG concentration on day 12 after embryo transfer (log2 transformed) with risk of delivery (HR>1.0) was estimated; however, a statistically significant association was only found in women with delivery at full-term (HR 1.127, 95% CI 1.026–1.238, *P* = 0.012) (**Table 2**). Thus, women with a high HCG concentration had a shorter duration of pregnancy (higher HR of delivery) than women with a low HCG concentration. **Figure 4** illustrates the results in **Table 2** for full-term pregnancies. For each doubling of HCG concentration on day 12 after embryo transfer, the duration of pregnancy decreased by 0.51 days. Adjustment for maternal age, prepregnancy BMI, being a first-time mother (yes/no), and number of embryos transferred did not change any of the above associations notably (**Table 2**).

Discussion

The main finding of this study was that low maternal HCG concentration on day 12 after embryo transfer is associated with longer duration of pregnancy, significantly in pregnancies with delivery at full term only. To the authors' knowledge, the association of maternal HCG concentration on a fixed day in early IVF pregnancies with duration of pregnancy has not previously been reported.

Almost all successful singleton pregnancies after transfer of day 2 embryos at the largest IVF clinic in Norway during the years 1999–2013 were included. By linkage to the Medical Birth Registry of Norway, the date of delivery for all pregnancies with duration beyond 16 weeks was obtained. Thus, there was complete follow-up and it was possible to calculate the exact duration of pregnancy. Survival analyses were used to account for the shortening of pregnancy duration caused by planned Caesarean section or induction of labour. Although the study sample included a large number of pregnancies that were followed until delivery, the number of pregnancies delivered at early term were limited. Type two errors may therefore have occurred. Adjustment for maternal age, prepregnancy BMI, being a first-time mother and number of embryos transferred did not alter the associations notably.

In spontaneous pregnancies, there is variation in time from ovulation to embryo implantation, and delayed embryo implantation has

Table 1 – Distributions of study factors.

Parameter	*n*	Age, mean (SD)	Body mass index, mean (SD)	Log2(HCG), mean (SD)	HCG, mean (SD)	First-time mother, *n* (%)	Single embryo transfer, *n* (%)
All births	1917	33.48 (3.65)	23.81 (7.08)	6.83 (0.79)	130.50 (69.18)	1323 (69.01)	1061 (55.35)
Early-term only	343	33.92 (3.56)	24.03 (4.47)	6.79 (0.83)	127.48 (68.48)	219 (63.85)	184 (53.64)
Full-term only	984	33.29 (3.79)	23.45 (3.81)	6.87 (0.75)	133.09 (69.78)	658 (66.87)	546 (55.49)
Spontaneous births	1441	33.28 (3.62)	23.68 (7.81)	6.86 (0.77)	132.13 (68.34)	985 (68.36)	839 (58.22)
Early-term only	209	33.86 (3.47)	23.59 (4.43)	6.88 (0.75)	133.74 (68.66)	133 (63.64)	121 (57.89)
Full-term only	803	33.17 (3.77)	23.30 (3.66)	6.89 (0.75)	134.26 (69.86)	534 (66.50)	464 (57.78)
Planned Caesarean births	129	34.57 (3.70)	24.25 (4.32)	6.77 (0.97)	128.26 (69.25)	79 (61.24)	47 (36.43)
Early-term only	58	34.69 (3.70)	24.47 (4.14)	6.63 (1.15)	119.78 (67.27)	32 (55.17)	19 (32.76)
Full-term only	59	34.56 (3.86)	24.08 (4.44)	6.96 (0.74)	140.92 (71.20)	37 (62.71)	22 (37.29)
Induced births	347	33.90 (3.66)	24.15 (4.08)	6.74 (0.81)	124.57 (72.40)	259 (74.64)	175 (50.43)
Early-term only	76	33.47 (3.65)	24.91 (4.71)	6.68 (0.70)	116.16 (67.73)	54 (71.05)	44 (57.89)
Full-term only	122	33.43 (3.81)	24.11 (4.29)	6.71 (0.80)	121.59 (67.91)	87 (71.31)	60 (49.18)

age = maternal age; body mass index = prepregnancy maternal body mass index; HCG/log2(HCG) = HCG (in UI/l) and log2 concentrations of HCG (in UI/l) at day 12 after embryo transfer.

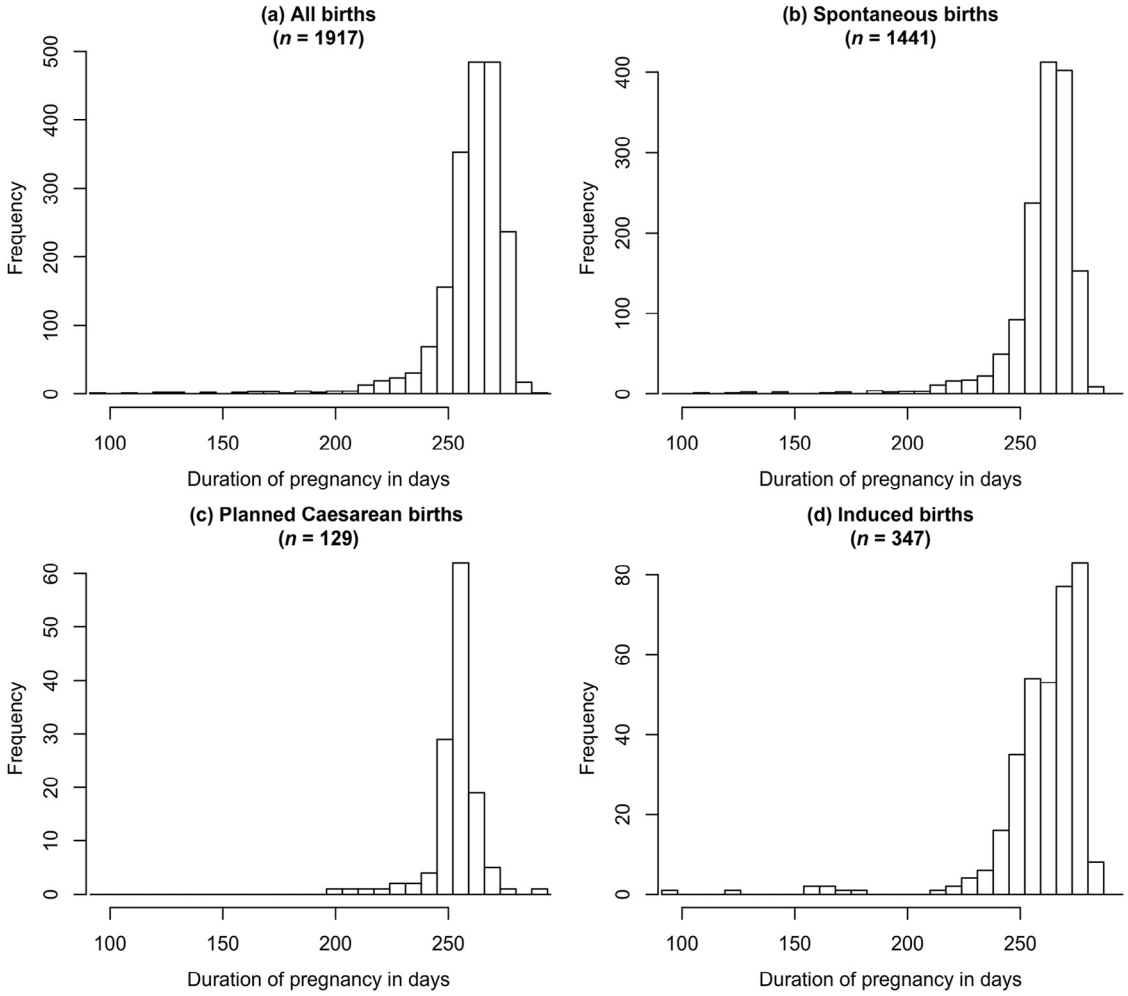

Figure 2 – Distributions of duration of pregnancy (in days).

been associated with longer duration of pregnancy (Wilcox et al., 1999); Jukic *et al.*,2013). Preimplantation embryos synthesise HCG, but to detect HCG in the maternal circulation, the trophoblastic cells have to invade the spiral arteries. Thus, the time elapsed since embryo implantation is likely to be an important determinant of maternal HCG concentration in very early pregnancy (Ertzeid et al., 2000).

In the present study, maternal HCG concentrations varied between 1 and 588 IU/l at day 12 after embryo transfer. With an HCG doubling time of 1.5 days, the implantation window for cleavage-stage embryos may therefore be up to 8 days (Ertzeid et al., 2000). Thus, the variation in the implantation window in IVF pregnancies may be similar to the variation from ovulation to implantation in spontaneous pregnancies (Wilcox et al., 1999).

Furthermore, the association of low HCG concentration with longer pregnancy duration in the present study suggests that the timing of embryo implantation after transfer may explain some of the normal variation in duration of IVF pregnancies. The risk of preterm delivery was not associated with HCG concentration in this study. Thus,

Table 2 – Associations[a] of HCG concentration (log2 concentrations of HCG in IU/l) on day 12 after embryo transfer with duration of pregnancy (in days).

Births	*n*	Median duration 95% CI	Crude HR 95% CI *P*-value	Adjusted HR 95% CI *P*-value
All births	1917	266 (266, 267)	1.053 (0.986, 1.125) NS	1.046 (0.978, 1.118) NS
Early-term only	343	254 (253, 255)	1.141 (0.955, 1.364) NS	1.124 (0.935, 1.350) NS
Full-term only	984	265 (265, 265)	1.127 (1.026, 1.238) 0.012	1.100 (1.000, 1.210) NS

HR = Hazard ratio; median duration = median duration of pregnancy (in days); *n* = number of pregnancies; 95% CI = 95% Confidence interval; NS = not statistically significant.

Adjustments are made for maternal age, prepregnancy body mass index (kg/m^2), first-time mother (yes/no) and single embryo transfer (yes/no).

[a] For this analysis, we estimated (expected) duration of pregnancy by applying Cox regression analyses. Births which were not spontaneous (i.e. planned Caesarean sections and induced births) were treated as censored cases.

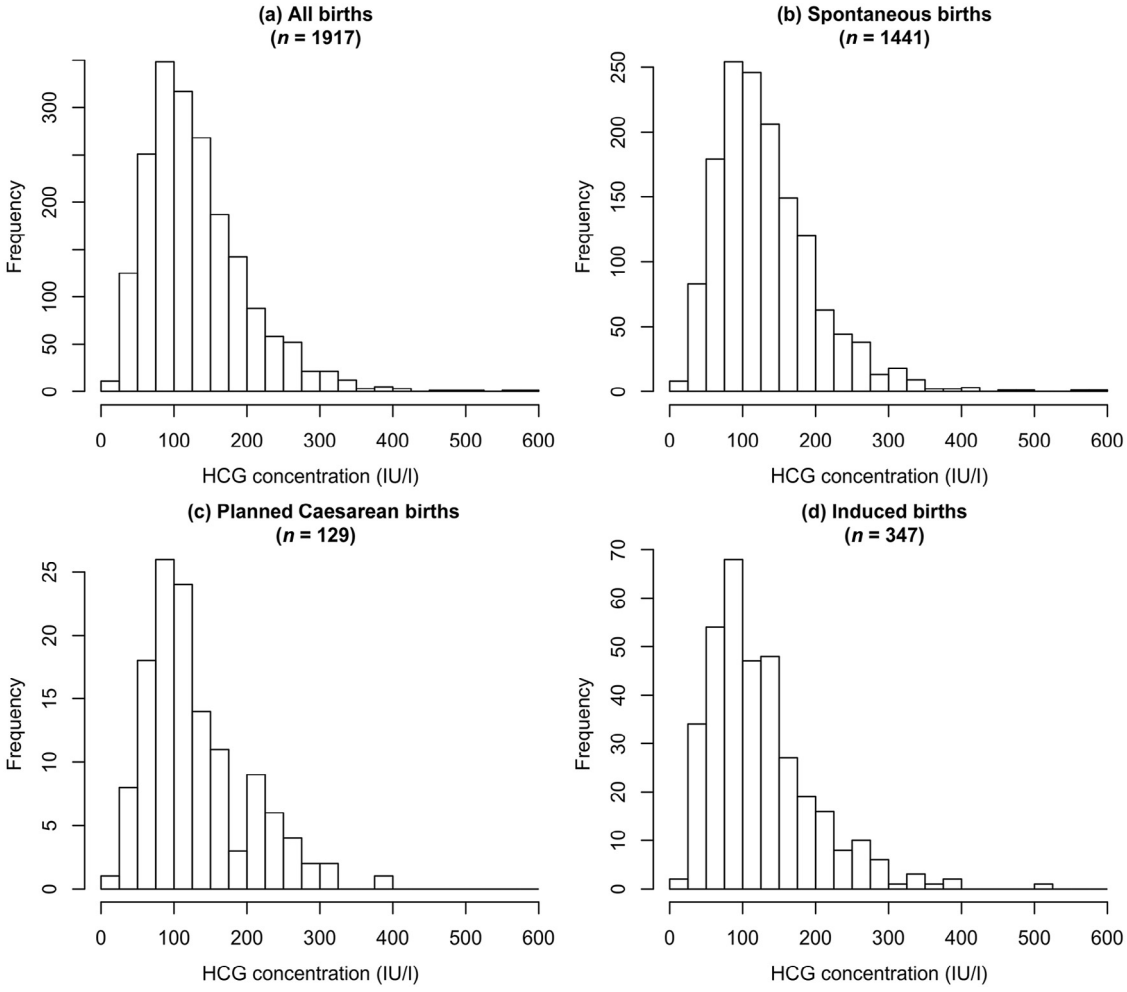

Figure 3 – Distributions of HCG concentration (IU/l) at day 12 after embryo transfer.

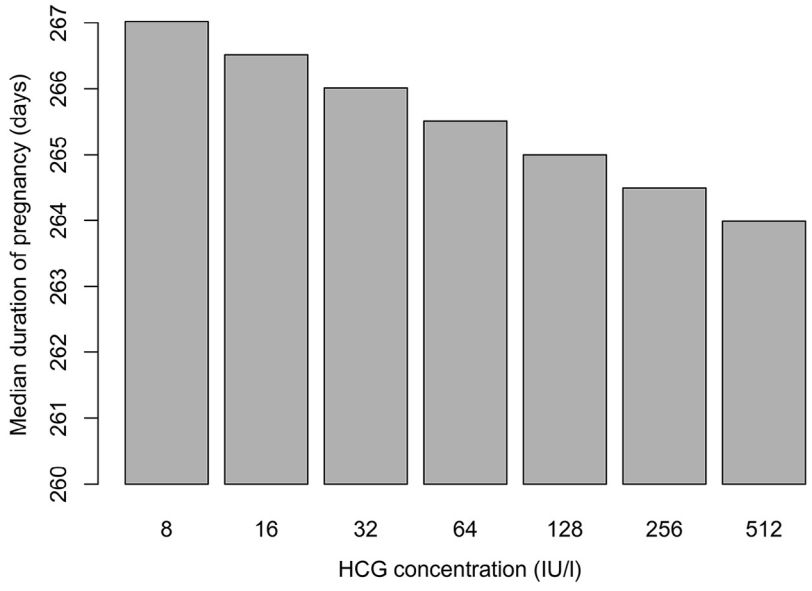

Figure 4 – Estimated median duration of pregnancy (in days) for each doubling of HCG concentration (IU/l) at day 12 after embryo transfer, in pregnancies with delivery at full term.

the risk of preterm delivery may not be related to the timing of embryo implantation, and HCG concentration in early pregnancy may not be predictive of preterm delivery.

Only hatched blastocysts can implant into the endometrium. The mean time from fertilization of the oocyte to development of a blastocyst is 4–5 days, assuming a cell-doubling time of approximately 16 h. However, this time interval may vary considerably (Edwards et al., 1981). Synchronous timing of the maturation of the endometrium with the hatching stage of the blastocyst is a prerequisite for implantation. Thus, any factor that delays the maturation of the endometrium or cell division in the preimplantation stage embryo may also delay implantation (Fréour et al., 2013; Horcajadas et al., 2008; Roque et al., 2013; Stewart et al., 1992; Tanbo and Eskild, 2015).

It is conceivable that slow proliferation of trophoblastic cells or impaired trophoblast invasion into the endometrium could cause low HCG concentration. Low HCG concentration in very early pregnancy has been associated with development of preeclampsia, preterm preeclampsia in particular (Asvold et al., 2014a, 2014b). Preeclampsia is assumed to be caused by impaired early placental development (Wang et al., 2009). These previous findings support the idea that low HCG concentration in very early pregnancy may by an indicator of slow trophoblast proliferation or impaired early placentation.

Pregnancies with slow trophobast proliferation may possibly need longer time to mature, and thereby last longer. It has been reported that women with advanced age or high weight have longer duration of pregnancy (Jukic et al., 2013; Roque et al., 2013), and that HCG concentrations are lower in these women (Eskild et al., 2012; Haavaldsen et al., 2014).

The duration of human pregnancies varies considerably (Bergsjø et al., 1990). Similar variations have been found in chimpanzee pregnancies (Wildman et al., 2011). In the sample of pregnancies after IVF in the present study, the observed median pregnancy duration was 264 days after embryo transfer, and that corresponds to 280 days from start of the last menstruation in spontaneous pregnancies (Jukic et al., 2013). When estimating pregnancy duration, taking into account that pregnancies with planned Caesarean section or induction of labour would have lasted longer, the median duration of pregnancy was 2 days longer.

The present findings may have clinical implications for IVF pregnancies. Maternal HCG concentration at a fixed day after embryo implantation could provide additional information about expected date of delivery. Thus, HCG concentration, in addition to date of embryo transfer, could improve the estimation of the true gestational age of the fetus, and thereby improve the timing of clinical interventions in pre-and post-term IVF pregnancies.

In conclusion, this study found that among 1917 pregnancies after IVF, low maternal HCG concentration on day 12 after embryo transfer was associated with longer duration of pregnancy. This association was significant in pregnancies with delivery at full term only.

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