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Maternal hormones during early pregnancy: a cross-sectional study

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Abstract

Background—Little is known about correlates of first trimester pregnancy hormones as in most studies maternal hormones have been measured later in gestation. We examined the associations of maternal characteristics and child sex with first trimester maternal concentrations of 4 hormones implicated in breast cancer: human chorionic gonadotropin (hCG), α-fetoprotein (AFP), insulin-like growth factor (IGF)-I and IGF-II.

Methods—338 serum samples donated to the Northern Sweden Maternity Cohort (NSMC), 1975–2001, during the first trimester of uncomplicated pregnancies were analyzed for the hormones of interest as a part of a case-control study. The associations between maternal characteristics and child sex with hormone concentrations were investigated by correlation, general linear regression, and multivariate regression models.

Results—In the first trimester, greater maternal age was inversely correlated with IGF-I and IGF-II. In comparison with women carrying their first child, already parous women had higher IGF-I but lower hCG. Greater maternal weight and smoking were inversely correlated with hCG. No differences in hormone levels by child sex were observed.

Conclusions—Our analyses indicated that potentially modifiable maternal characteristics (maternal weight and smoking) influence first trimester pregnancy maternal hormone concentrations.

Keywords

pregnancy; cross-sectional study; human chorionic gonadotropin (hCG); α-fetoprotein (AFP); insulin-like growth factor (IGF)-I; IGF-II

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Introduction

The hormonal milieu of pregnancy is believed to underlie the effect of childbearing on cancer occurrence [1,2]. Studies that directly relate endogenous hormones during pregnancy to cancer risk are rare because of the substantial logistic difficulties in their design and conduct [3–5]. Instead, most investigations rely on surrogate markers of the endocrine environment of pregnancy, such as pregnancy conditions (e.g. morning sickness, preeclampsia), child birth weight, maternal age, parity or smoking [6,7]. Currently, relatively little is known about correlates of first trimester pregnancy hormones as in most studies maternal hormones have been measured in mid- or late pregnancy [8].

Early pregnancy may be a relevant period for subsequent cancer occurrence in the mother and the fetus as during that time important changes take place in both. In the mother, initial proliferation of breast epithelium sets the stage for the full breast differentiation and maturation that will culminate during the post-partum lactation period [9] and which is believed to play an important role in maternal protection from breast cancer [10]. In the fetus, this is the time when organogenesis takes place and tissue patterns and organ systems are established [11]. It has been proposed that hormonal alterations during pregnancy, specifically early in the pregnancy, may be of particular etiological importance for the development of testicular cancer in male offsprings [12].

We used data acquired from control subjects in a case-control study nested within the Northern Sweden Maternity Cohort (NSMC) [5,13], to examine associations of maternal characteristics and child sex with first trimester maternal concentrations of human chorionic gonadotropin (hCG), α-fetoprotein (AFP) and insulin-like growth factor (IGF)-I and IGF-II. The studied hormones have diverse origin: the synthesis of hCG takes place mainly in the syncytiotrophoblast and is then secreted into the maternal circulation through the villous interface [14], AFP originates primarily from the fetal liver [14], while IGF-I and II, as in the non-pregnant state, are mostly produced by maternal liver [15]. All these hormones have been implicated in maternal breast cancer [5,13].

Methods

Study population

The NSMC, based at the University Hospital in Umeå (Sweden), was established in November 1975 with the purpose of preserving for research purposes serum samples from pregnant women after mandatory tests for systemic infections. Cohort members are residents of one of the four northernmost counties of Sweden (total population ~800,000) who have attended a maternity health care clinic in the region during pregnancy. Blood samples are drawn mostly during the final weeks of the first trimester of pregnancy, or the early weeks of the second (weeks 6–18), and are periodically shipped frozen to a central repository at Umeå University Hospital, where they are analyzed for systemic infections and the remaining biological material is stored at −20 °C. The NSMC contains over 110,000 first-trimester serum samples from approximately 83,000 women.

Study subjects were selected among the controls included in a nested case-control study on pregnancy hormones and breast cancer risk [5,13] from the NSMC, 1975–2001. Eligibility criteria included blood donation in gestational weeks 6 to 15 during a spontaneous, singleton pregnancy resulting in the delivery of one live or stillborn infant, no use of hormonal medication during pregnancy and no invasive cancer diagnosis before index pregnancy (except non-melanoma skin cancer). A total of 338 women were included.

Data about index pregnancy were obtained by linkages with the Swedish Birth Registry and review of the original medical records of the women, as described in detail previously [16]. The Swedish Birth Registry is based on data from standardized medical records used in all Swedish maternal care and delivery units since 1973, and more than 99% of all births in Sweden are registered [17]. In general, the quality of the information is considered high, although individual variables have variable completeness, as procedures to collect information have changed over the years. In addition, a full copy of the maternity and delivery records was requested from the hospital closest to the registered place of residence. If the medical record of a subject was not found in this hospital, requests were sent to one or two of the nearest hospitals. Data from the medical records were abstracted with duplicate entry for dates of sampling and last menstrual period. Gestational day at blood donation was calculated as the difference in days between dates at blood donation and last menstrual period. All maternal characteristics studied were measured at the time of the first visit to the maternity care unit, when a blood sample was collected.

Laboratory methods

IGF-I and IGF-II were quantified by immunoradiometric (IRMA) assays from Nichols Institute Diagnostics (San Clemente, CA) and Diagnostic System Laboratories (Webster, TX), respectively. The intra- and inter-batch coefficients of variation (CV) at 12.2 nmol/L IGF-I concentration were 6.3 % and 7.6 % and for an IGF-II concentration of 108 nmol/L were 9.0 % and 11.0%, respectively. AFP was quantified by enzyme-linked immunosorbent assay (Genentech, Inc., San Francisco, CA) with intra- and inter-assay CVs of 3.2% and 6.7%, estimated on measurements of Bio-Rad 17 μg/L AFP control samples. hCG was quantified with the Coat-a-Count IRMA assay (Diagnostic Products Corporation, Los Angeles, CA), which is highly specific for intact hCG, with low cross-reactivity to other glycoprotein hormones present in patient samples, including α and β hCG subunits. Intra- and inter-batch CVs were 2.8% and 3.8%, estimated on measurements of Bio-Rad 170 mIU/mL hCG control samples. In samples drawn in 1988 and thereafter, there was an evident reduction in measured hCG concentrations to levels incompatible with pregnancy, while no such effect on other hormone concentrations was observed. Therefore, all subjects whose baseline blood sample had been drawn after January 1, 1988, were excluded from analyses on hCG (n=56).

Statistical analyses

As expected, levels of hCG and AFP varied substantially according to gestational day at blood donation, while no such effect was observed for IGF-I and there was only a weak positive correlation of IGF-II with gestational day (Figure 1, the solid lines showing the progression of hormones during pregnancy, estimated by local linear regression [18]). To account for these variations, all statistical analyses for IGFs and AFP were adjusted for gestational day (linear term), while for hCG, a cubic term of gestational day was used because of the non-linear association of hCG with gestational day. Prior to analysis, original hormone levels were natural log-transformed to limit heteroscedasticity. Outliers were defined as log-hormone concentrations exceeding 3 times the interquartile range and such measurements were set to missing: 2 for IGF-I and 1 for IGF-II and AFP.

Association between continuous variables was evaluated by calculating Spearman partial correlations (adjusted for gestational day). Variables that showed significant correlations with hormone concentrations were investigated also in tertiles (e.g. maternal age and weight). General linear regression models were used to estimate geometric means of hormones levels and compare them across exposure categories of maternal age, weight, height, parity, smoking and child sex. For continuous variables (maternal age, weight and height), tests for trend were computed by treating continuous variables as ordered categorical variables. For dichotomous variables (parity, smoking and child sex), an F-test was used to assess the significance. To

explore the independent effect of maternal characteristics on hormones, multivariate regression models including maternal age, parity, weight, height and smoking were also evaluated. Estimates from models that reached either statistical significance ($p < 0.05$) or borderline significance ($p<0.10$) are presented. Addition of child sex to the adjusted model was also explored. Possible interactions between categories of some of the studied variables (e.g. maternal age and parity) were evaluated by inclusion of cross-product terms in the regression models that included the main effects for these variables, but none of them was found to be significant. SAS version 9.1 was used for all analyses.

The study was approved annually by the Regional Ethics Committees of the University of Umeå, Sweden.

Results

Maternal characteristics at enrolment in the study, child sex and hormone concentrations in the study population are presented in Table 1. Blood samples were collected during the first trimester of pregnancy (6–15 gestational weeks), 10 gestational weeks being the median time of blood draw. Maternal age ranged from 18.8 to 44.4 years (median =31.5 years) and about half of the women (52 %) donated a blood sample during their first pregnancy (primiparous). A slightly greater percentage of mothers were carrying a boy (53 vs. 47% mothers of girls). Of the women who provided information, 23% were smoking, but no detail of number of cigarettes smoked per day was available.

Hormone concentrations were in the range expected for that period of pregnancy and their concentrations changed with increasing gestational day for AFP, hCG and slightly for IGF-II, but were stable for IGF-I, as anticipated (Figure 1, Table 2) [14,19–21]. As expected, the strongest correlation between any two hormones was between IGF-I and IGF-II ($r = 0.47$). AFP was moderately directly correlated with IGF-II ($r = 0.23$) and IGF-I ($r = 0.14$). In general, continuous maternal characteristics were either weakly or not correlated with hormone concentrations (Table 2). Maternal age was inversely associated with IGF-I and IGF-II. In analyses across tertiles of maternal age, all investigated hormones showed a tendency to decrease with increasing maternal age, but significant trend was observed only for levels of IGF-II (Table 3). Maternal weight was only associated (inversely) with hCG, while maternal height was not significantly correlated with any of the hormones (Table 2).

In comparison with primiparous women, women who had already delivered a child had lower concentrations of IGF-II and hCG, while their levels of IGF-I and AFP were slightly higher, but the difference was significant only for IGF-II and hCG (Table 3). Mothers who smoked had significantly lower hCG concentrations than non-smoking mothers. There were no differences in hormone levels by child sex.

Multivariate regression models including baseline maternal characteristics (age, parity, weight, height and smoking) showed that age was independently associated with lower IGFs, and that multiparous women had higher IGF-I but lower hCG than primiparous women (Table 4). Maternal weight was associated inversely with hCG and smoking women had significantly lower hCG (Table 4). Addition of child sex to any of these models had only minor, negligible effect on the estimates. There was no indication of interaction between maternal age and parity for any of the 4 hormones. Analyses using local linear regression to account for variation of hormone levels with gestational age yielded almost identical results.

Discussion

To date, few studies have investigated in detail correlates of IGF-I, IGF-II, AFP and hCG during the first trimester of pregnancy. Our analyses indicated that several maternal

characteristics, including age, parity, weight, and smoking are associated with hormone concentrations.

Maternal age

Greater maternal age was inversely correlated with both IGF-I and II, and the association remained significant after adjustments for other baseline maternal characteristics. The observed relationship between age and IGF-I is consistent with the well-established effect of age on hormone concentrations staring from puberty until very old ages [22]. In contrast, no association of IGF-II with age has been reported in non-pregnant women or in men [22]. Little is known about IGF-II regulation both during and out of pregnancy and these results warrant further investigation. No effect of maternal age on hCG and AFP concentrations was observed, in line with observations for these hormones during the second trimester of pregnancy in previous studies [23].

Parity

In comparison with women carrying their first child, multiparous women had significantly lower levels of hCG, consistent with observations about the effect of parity on hCG concentrations during the second trimester of pregnancy in previous studies [23–25]. The reason for this decline, particularly for a hormone of placental origin is unknown [24]. Parous women had higher concentrations of IGF-I, which could be an effect observed only during pregnancy as IGF-I does not differ by parity in non-pregnant women [26–28]. For IGF-II, after the effect of age was taken into account, multiparous women had hormone concentrations similar to those of primiparous women. No effect of parity on AFP was observed, consistent with the observations in the second trimester in previous studies [24,29]. To our knowledge only one study has reported first trimester AFP concentrations according to parity, which appeared to be slightly reduced only in women with more than 2 previous pregnancies, as compared to primiparous [30].

Maternal weight

Maternal weight is associated with decreased maternal concentrations of hormones of placental and fetal origin, as the amount of hormone secreted is more diluted in the larger blood volume of heavy-set women [30]. A wealth of cross-sectional studies has shown an inverse association of maternal weight with second trimester hCG and AFP [24]. Similar association have been reported for first trimester AFP and free β-hCG (the β-subunit of hCG, moderately correlated with hCG [30,31]. In our data, we observed the expected inverse association between maternal weight at blood donation and hCG, but not with AFP. No association of maternal weight with IGF-I or IGF-II was observed, consistent with observations in non-pregnant women [32–35]. No association of pre-pregnancy BMI with second / third trimester IGF-I concentrations was reported in one other study [36].

Smoking

Mothers who reported smoking at enrollment had lower hCG concentrations than non-smoking mothers, consistent with data indicative of decreased hCG or free β-hCG both during the first [37,38] and second trimester in smoking mothers [24,29,39,40]. Interestingly, no correlation between hCG and number of cigarettes smoked per day has been observed [24,39]. It has been proposed that morphological changes of the villus barrier and the trophoblasts in the placenta of women who smoke may account for the decrease in hCG concentrations [29,30]. The absence of an effect of smoking on first trimester AFP is consistent with previous studies [30]. Only a negligible increase in second trimester AFP associated with smoking has been reported [24]. Similarly, the absence of an effect of smoking on first trimester IGFs is in line with observations obtained soon after childbirth [41] or outside pregnancy [36,28,42].

Child sex

No differences in hormone levels by child sex were observed, although women carrying a female fetus had slightly higher first trimester IGF-I and hCG (Table 3). Mothers of girls have consistently been reported to have higher hCG during the first [30,43] and the second trimester [44] than mothers of boys. Differences in hCG by fetal sex have been observed even in very early pregnancy (3 week) [43] and might result from differential expression of genes (e.g., inactivation of X-linked genes in the placenta) that play a role in hCG metabolism [43]. The absence of an effect of child sex on first trimester AFP has been reported also by others [30, 45]. It should be noted, however, that most studies show differences during the second trimester, with mothers of girls having lower concentrations than mothers of boys [46,47]. No differences in maternal IGF-I by fetal sex have been reported previously [48], despite that cord blood IGF-I has been found to be higher in girls than in boys [49–51]. However, IGF-I does not cross the placenta into fetal circulation [52].

The effect of pregnancy on maternal breast cancer risk is likely to be the result of the cumulative hormonal exposure throughout its duration [8,53]. Early hormonal events would establish the basis for the more profound changes that occur later in gestation. Furthermore some degrees of correlation (usually about 0.40 or higher) between hormone concentrations during the first and second or third trimester of the same pregnancy [54] or during the first trimester of successive pregnancies have been reported by others [55,56]. For hCG in particular, concentrations during the mid and latter parts of the first trimester maybe the most etiologically relevant in terms of breast cancer risk, as during this period of gestation they peak and the proportion of the intact, biologically active form of the hormone is highest [57]. It has been proposed that hCG plays an important role in breast differentiation during pregnancy which will ultimately result in reduced risk of maternal breast cancer [58]. Support for this hypothesis comes from animal experiments showing that short term treatment of young virgin animals with recombinant hCG induces protection similar to that conferred by pregnancy [59] and from epidemiological observations that women who took hCG as part of a weight loss program were at decreased risk of breast cancer [60] and that elevated hCG concentrations during first trimester pregnancy could be inversely related to risk of breast cancer [5]. The lower hCG concentrations in women with greater weight fit epidemiological observations of increased postmenopausal breast cancer risk associated with pregnancy weight gain [61]. The high hCG concentrations in primiparous women are in line with the important effect of the first full-term pregnancy, particularly at an early age, on maternal risk of breast cancer [62]. Most studies have not shown an association of smoking with breast cancer [63,64], but some have indicated that smoking particularly at early age may be associated with increased risk [65,66]. One might speculate that women who smoke at very young age (as teenagers) are more likely to continue to smoke also during pregnancy and thus experience lower hCG concentrations and less breast differentiation.

Studies within the NSMC have indicated that women with elevated IGF-I during the first trimester of pregnancy are at increased risk of breast cancer [13,16]. IGF-I levels were higher in younger women and multiparous women, but the relevance of these findings to the pregnancy association with maternal breast cancer risk is unclear. The observation of inverse association between maternal age and IGF-II during pregnancy is novel, as very little is known about the regulation of this hormone, but awaits confirmation from further studies.

Strengths of our study are its relatively large sample size and the availability of measurements of several hormones implicated in breast cancer pathogenesis. Direct abstracting of pregnancy data from medical records ensured high quality of the information at hand. Recently we reported excellent correspondence between smoking reported in the medical record and as assessed by cotinine measurements in maternal serum (Kappa statistic of 0.84) [16]. Weaknesses include the lack of detail about smoking which precluded more in-depth dose-

response analyses. Furthermore, there were no data on pregnancy conditions, such as diabetes and pre-eclampsia, which may influence maternal hormones. The long-term storage of the serum specimens at relatively high temperature (−20°C) could have caused some analyte deterioration. However, it was reassuring that hormonal variations with gestational day closely followed what has been reported in the literature [14,19–21] and with the exception of hCG degradation in samples collected after 1988, there was no indication that duration of storage influenced hormone concentrations.

In conclusion, we observed that maternal age, parity and weight are associated with hCG or IGFs concentrations during early pregnancy. Among these, of particular importance are the effects of smoking and maternal weight as they are potentially modifiable.

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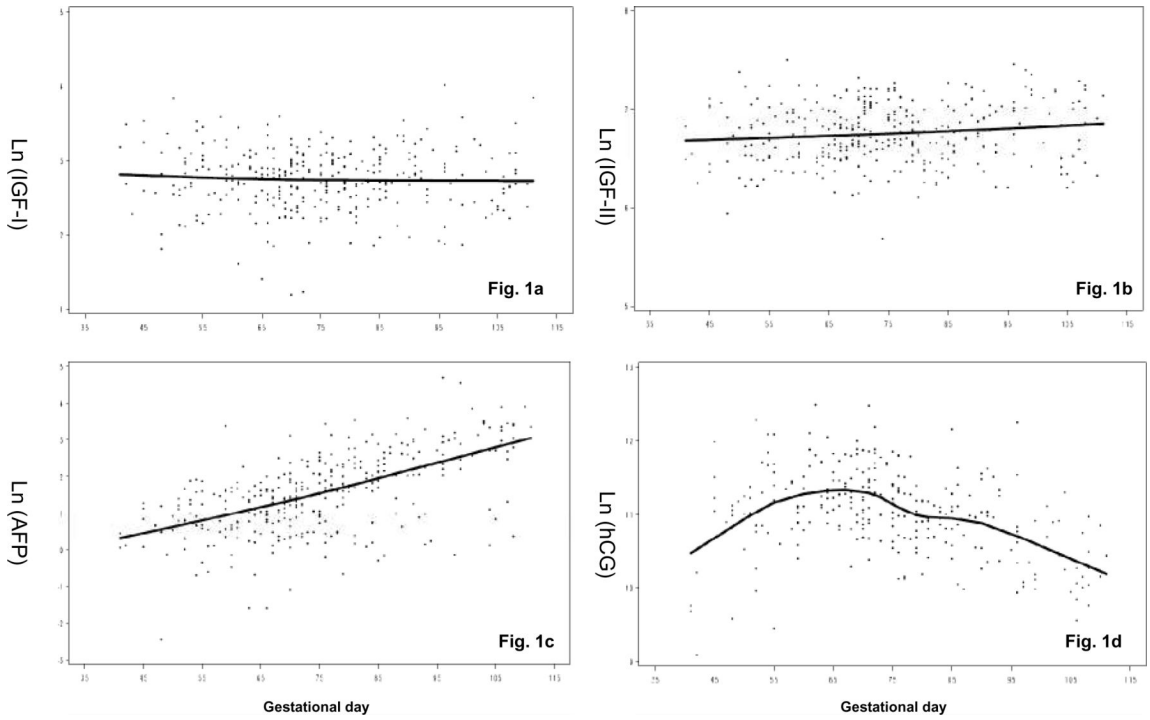

Figure 1.

Scatterplot of log-scale IGF-I (Fig.1a), IGF-II (Fig.1b), AFP (Fig.1c), and hCG (Fig.1d) by gestational day in 338 uncomplicated pregnancies from the Northern Sweden Maternity Cohort, 1975–2001. The solid line shows the progression of hormones during pregnancy, estimated by local linear regression [18].

Table 1

Maternal characteristics at enrollment and child sex for index birth and hormone concentrations in 338 uncomplicated pregnancies

Characteristics* / hormone	n	Median(10th, 90th percentile) or percentage
Gestational week (weeks)	338	10 (8–14)
Maternal age (years)	338	31.5 (25.3–37.6)
Parity		
Primiparous	175	52%
Multiparous	163	48%
Maternal weight (kg)	338	60.9 (52.2–74.0)
Maternal height (cm)	330	165 (157–172)
Maternal smoking		
Yes	78	23%
No	255	77%
Child sex		
Boy	177	53%
Girl	160	47%
Hormone concentrations **		
IGF-I (nmol/L)	336	15.7 (9.4–25.4)
IGF-II (nmol/L)	337	860 (580–1,260)
AFP (μg/L)	337	4.6 (1.3–19.6)
hCG (mlU/mL)***	280	61,828 (27,734–131,098)

* Data missing: 8 women for maternal height, 5 women (1%) for smoking, and 1 woman for child sex;

** IGF-I, IGF-II, AFP and hCG concentrations were geometric means;

*** n=280 as samples drawn in 1988 and thereafter were excluded.

Table 2

Spearman partial correlation coefficients between hormone levels (log-scale) and maternal characteristics at enrollment in 338 uncomplicated pregnancies from the Northern Sweden Maternity Cohort, 1975–2001[&]

Characteristics	IGF-I	IGF-II	AFP	hCG
Gestational day	−0.02	**0.12** *	**0.60** **	**– 0.34** **
Maternal age (years)	**– 0.12** *	**– 0.33** **	−0.03	−0.09
Maternal weight (kg)	0.06	0.01	−0.04	**– 0.12** *
Maternal height (cm)	0.04	−0.01	0.00	−0.01
IGF-I (nmol/L)				
IGF-II (nmol/L)	**0.47** **			
AFP (μg/L)	**0.14** *	**0.23** **		
hCG (mlU/mL)	0.07	**0.16** *	0.00	

* $p < 0.05$

** $p < 0.001$

[&] partial correlations for IGF-I, IGF-II, and AFP were adjusted for a linear term of gestational day; for hCG for a cubic term of gestational day.

Table 3

Geometric mean (gestational day adjusted)* of maternal IGF-I, IGF-II, AFP and hCG levels by maternal characteristics at enrollment and child sex in 338 uncomplicated pregnancies from the Northern Sweden Maternity Cohort, 1975–2001

Characteristic	N	IGF-I nmol/L	IGF-II nmol/L	AFP ug/L	hCG mlU/mL
Maternal age (years)					
1st tertile (<28.6)	112	16.6	955	5.3	62,454
2nd tertile (28.6–33.8)	114	15.3	872	4.3	65,674
3rd tertile (≥33.8)	112	15.3	763	4.4	57,514
p-trend		**0.13**	**<0.0001**	**0.13**	**0.30**
Parity					
Primiparous	175	15.3	923	4.6	66,434
Multiparous	163	16.2	797	4.7	57,936
p-value		**0.23**	**<0.0001**	**0.70**	**0.03**
Maternal weight (kg)					
1st tertile (<58.0)	111	15.2	871	4.7	62,615
2nd tertile (58.0–65.3)	114	16.0	841	4.6	66,019
3rd tertile (≥65.3)	113	16.0	867	4.7	57,094
p-trend		**0.37**	**0.93**	**0.96**	**0.25**
Maternal height (cm)					
1st tertile (<163)	117	15.4	860	4.7	62,141
2nd tertile (163–168)	106	15.2	846	4.2	62,042
3rd tertile (≥168)	107	16.4	875	4.9	61,485
p-trend		**0.27**	**0.68**	**0.80**	**0.90**
Maternal smoking					
Yes	78	16.7	865	5.1	54,298
No	255	15.4	861	4.5	64,584
p-value		**0.13**	**0.91**	**0.27**	**0.02**
Child sex					
Boy	177	15.3	855	4.8	61,096
Girl	160	16.3	866	4.6	62,747
p-value		**0.14**	**0.69**	**0.69**	**0.68**

* for IGF-I, IGF-II, and AFP, geometric mean adjusted for a linear term of gestational day; for hCG, geometric mean adjusted for a cubic term of gestational day.

Table 4

Percent change for hormone concentrations per unit increase in the independent variables (p values) from multivariate linear regression models with adjustments for maternal age, parity, weight, height, and smoking at enrollment in 338 uncomplicated pregnancies from the Northern Sweden Maternity Cohort, 1975–2001*

Independent variable	Dependent variable (hormones)			
	IGF-I	IGF-II	AFP	hCG
Maternal age (years)	−0.014 (0.01)	−0.017 (<0.0001)		
Multiparous vs. primiparous	0.108 (0.04)			−0.150 (0.05)
Maternal weight (kg)				−0.010 (0.01)
Maternal smoking (yes vs. no)				−0.166 (0.03)

* for IGF-I, IGF-II, and AFP, models were adjusted for a linear term of gestational day; for hCG, models were adjusted for a cubic term of gestational day.