Steroid Hormone Levels during Pregnancy and Incidence of Maternal Breast Cancer[1]

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Abstract

Previous studies evaluating pregnancy hormone levels and maternal breast cancer were limited to surrogate indicators of exposure. This study directly evaluates the association between measured serum steroid hormone levels during pregnancy and maternal risk of breast cancer. A nested case-control study was conducted to examine third-trimester serum levels of total unconjugated estradiol, estrone, estriol, and progesterone in women who were pregnant between 1959 and 1966. Cases ($n = 194$) were diagnosed with *in situ* or invasive breast cancer between 1969 and 1991. Controls ($n = 374$) were matched to cases by age at the time of index pregnancy, using randomized recruitment. Elevated progesterone levels were associated with a decreased incidence of breast cancer [odds ratio (OR) for progesterone ≥270 ng/ml, 0.49; 95% confidence interval (CI), 0.22–1.1] relative to those below the lowest decile. This association was stronger for cancers diagnosed at or before age 50 (OR for progesterone ≥270 ng/ml, 0.3; 95% CI, 0.1–0.9). Increased estrone levels were associated with an increased incidence overall (OR for estrone ≥18.7 ng/ml, 2.5; 95% CI, 1.0–6.2), whereas a positive association with estradiol was not observed. Too few cases occurred within 15 years of the index pregnancy to compare adequately the short- and long-term effects of pregnancy hormone exposure. When estrogen-to-progesterone ratios were evaluated, there was an indication of a modest increased incidence of breast cancer for those with high total estrogens and high estrone levels relative to progesterone. These findings suggest that pregnancy steroid hormone levels are risk factors for breast cancer.

Introduction

Full-term pregnancy has been associated with opposing influences on breast cancer risk, with adverse effects in the short term after childbirth and beneficial effects over the long term (1–3). However, at present the biological mechanisms underlying these associations are not understood. It is generally accepted that hormonal processes are linked to the etiological pathways of breast cancer (4). Pregnancy is characterized by elevated levels of circulating estrogens and progesterone, which increase with advancing gestational age (5–9). Thus, the breast is exposed to the highest concentrations of estrogens and progesterone during the third trimester of pregnancy.

Pregnancy hormone levels are hypothesized to influence maternal risk of breast cancer by virtue of their growth-promoting effects on breast cells. During pregnancy, estrogen and progesterone induce proliferative and differentiating effects on the ductal and lobular-alveolar epithelium (10–12). These hormone-induced changes are believed to reduce the susceptibility of breast tissue to malignant transformation in the long term (10). However, it is hypothesized that the growth-promoting effects of pregnancy hormones could also trigger the proliferation of existing tumor cells, leading to an increased risk of breast cancer shortly after pregnancy (13).

Endogenous steroid hormone exposure during pregnancy has received limited attention as a risk factor for maternal breast cancer. In the absence of available serum measurements, previous studies used pregnancy and fetal characteristics as surrogate measures of exposure to altered steroid hormone levels during pregnancy to indirectly estimate the effect of pregnancy hormones on maternal breast cancer risk. Studies evaluating twin pregnancies (14–23), pregnancy-induced hypertension (18, 19, 24), pregnancy nausea (18, 25), duration of gestation (18, 19), and fetal size (23, 26) have generally produced weak and inconsistent findings. The present study directly evaluates pregnancy hormones as risk factors for breast cancer in the mother by measuring third-trimester hormone levels in the blood of pregnant women who were subsequently followed for breast cancer occurrence.

Materials and Methods

This study is based on a case-control study initially conducted to examine the effects of third-trimester maternal serum α-fetoprotein levels on the incidence of breast cancer in the mother (27). The source population was a cohort of pregnant women who were members of the Kaiser Health Plan and

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enrolled in the CHDS[4] of the University of California at Berkeley. The CHDS recruited women from 1959 through 1966 to conduct multidisciplinary investigations of the health and development of children from pregnancy through childhood (28). Of the 15,528 pregnant women initially enrolled in the CHDS, in-depth information on socio-demographic data, reproductive history, and health-related behaviors was obtained from personal interviews and medical record abstraction for 12,552 women. Efforts were made to collect blood during each trimester of pregnancy. Samples were stored at −20°C.

The original case-control study was conducted among CHDS participants who (*a*) completed an interview questionnaire; (*b*) were at least 21 years of age or married at the time of enrollment; (*c*) delivered a liveborn or stillborn infant from the index pregnancy; and (*d*) had a blood sample taken and the serum frozen during the last trimester of the index pregnancy, which was the last pregnancy occurring during the CHDS enrollment period: June 1959 to September 1966. Of the 710 women (247 breast cancer cases and 463 controls) eligible for the original case-control study, 594 had a third-trimester serum sample remaining in storage at the time of the present study. Of these, 26 were excluded for reasons including fetal death, twin pregnancy, gestation >44 weeks, insufficient serum, missing date of blood draw, blood draw on same day as delivery, or blood draw >100 days before delivery. The final analysis was conducted with 194 cases and 374 controls.

All participants consented to participate in the CHDS at the time of enrollment. The data available for the present study contained no personal identifiers, nor could they be linked to the original data source by study investigators. This study was approved by the Texas A&M Institutional Review Board for Human Subjects in Research.

Outcome Assessment. Cases were identified as women who had histologically confirmed primary *in situ* or invasive breast carcinoma (based on International Classification of Diseases, 9th Ed., Code 174) identified in the California Cancer Registry between 1969 and December 1991. Linkage of the CHDS cohort to the California Cancer Registry was completed by probabilistic matching by name, date of birth, sex, and race. Addresses and patient record numbers were used to verify matches. Cancer deaths occurring since 1960 were identified from the death records of the state of California to ascertain breast cancer cases missed before cancer registry reporting became complete for the entire state in 1988. Controls were selected from the remaining members of the cohort who met the eligibility criteria and were not identified as breast cancer cases by the methods described above. Randomized recruitment (29) was used to match approximately two controls to every case by 5-year groupings of age at index pregnancy.

Exposure Assessment. Using frozen sera obtained during the third trimester of the last pregnancy in the CHDS enrollment period, the steroid hormone laboratory at Quest Diagnostics/Nichols Institute (San Juan Capistrano, CA) assayed for total unconjugated estradiol, estrone, estriol, and progesterone. Estradiol and estrone were measured by RIA preceded by organic extraction and Celite chromatography. Estriol and progesterone levels were measured by organic extraction and RIA. Free steroids (not bound to protein) were not measured separately in this analysis because they have been shown to increase with prolonged storage because of the dissociation of weak bonds (30).

Laboratory investigators were blinded to the case-control status of the specimens. All samples were assayed in duplicate in randomly sorted batches of ~60 samples/assay. Three quality-control pools (low, medium, and high) were included in the front, middle, and back of each assay batch to document the absence of significant laboratory drift. Seven-point standard curves were constructed at the beginning and end of all batches to identify and correct for any shifts in measurements. Assays were repeated if <50% of the sample was recovered at the end of the process or if the coefficient of variation was >20% for patient samples or for more than two-thirds of the control pools. If these quality-control specifications were met, the two results from the duplicate assays were averaged. The within-assay coefficients of variation were 14% for estradiol, 9% for estrone, 9% for estriol, and 12% for progesterone. Between-assay coefficients of variation were 10, 10, 9, and 11%, respectively.

Adjustment of Hormone Levels for Timing of Blood Draw. Only a single blood sample was available for each participant during the third trimester of pregnancy. The timing of the blood draw ranged from 26 to 42 (mean, 34.5) weeks of gestation and preceded delivery by an average of 36 days (range, 1–100). For data analysis, hormone measurements were adjusted for timing of blood draw to account for the increasing estrogen and progesterone levels that accompany advancing gestation. Mean log-transformed hormone levels were predicted for each day of blood draw by estimating generalized additive models (31) for each hormone in the control group. Two definitions of timing of blood draw were assessed: days preceding delivery and gestational age at blood draw. Model fit and proportion of variance explained were very similar for both variables, so timing of blood draw was defined in the final models as days preceding delivery. Linearity was assessed by χ^2 tests for differences between linear fits, and flexible trends were estimated using local regression smoothing. These tests yielded a low P (< 0.05) only for progesterone. A linear relationship was assumed for estradiol, estrone, and estriol.

Using the results of the linear and local regression models, we calculated predicted hormone levels for all cases and controls according to the timing of the woman's blood draw. To depict how far the observed hormone measurement was above or below the expected value at a given point during pregnancy, residual values were calculated by subtracting the predicted value from the actual hormone measurement observed on a given day of blood draw. The residuals were used as hormone levels adjusted for timing of blood draw (referred to below as "date-adjusted levels"). For ease of interpretation, all residual values were adjusted to the same point of reference by adding a constant equivalent to the predicted (log) hormone level occurring 35 days before parturition (*i.e.*, 2.5, 3.1, 2.1, and 5.2 for estriol, estradiol, estrone, and progesterone, respectively). Finally, the date-adjusted hormone levels were transformed back to their original units in ng/ml.

Statistical Analysis. Date-adjusted third-trimester levels of total unconjugated estradiol, estriol, estrone, and progesterone and estrogen-to-progesterone ratios were evaluated as risk factors for maternal breast cancer. Geometric mean hormone levels and 95% CIs were calculated for cases and controls. Unconditional logistic regression was used to estimate ORs and 95% CIs. Logistic regression models were estimated using the GENMOD procedure in SAS (32), which allows the specification of offsets to incorporate the control sampling probabilities

[4] The abbreviations used are: CHDS, Child Health and Development Studies; CI, confidence interval; OR, odds ratio.

used in randomized recruitment (29). ORs and CIs are rounded to two significant digits.

In the absence of a biological basis for predicting risk variation by level of pregnancy hormone exposure, quadratic spline regression models were estimated to evaluate the shape of the association. The selection of category boundaries was guided by the nonmonotonic shape of the spline curves. To confirm that the shape of the association was not being distorted by the choice of category cutpoints, the odds of being a case were graphed for the mean of each defined category and compared with the spline curves. Four hormone exposure categories were defined by equally spaced boundaries on the date-adjusted hormone levels scale, with 10% of the subjects remaining in each extreme (*i.e.*, the 10th percentile, the 90th percentile, and their mid-range), using the distributions among the controls. This method of categorization is reported to have improved statistical efficiency over equally spaced percentile cutpoints (*e.g.*, tertiles or quartiles; Ref. 33). Tests for trend in the category-specific effect estimates were calculated by entering an ordered four-level exposure variable into the model that assigned the category mean hormone level to each person.

Covariates evaluated as potential confounders included race, age at first full-term pregnancy, age at menarche, number of previous full-term pregnancies, smoking, alcohol and caffeine consumption, prepregnancy body mass index, and third-trimester α-fetoprotein levels. Confounders were defined as covariates that changed the estimated exposure ORs for the hormone levels by >10% or changed their estimated SEs by >20% upon removal from the model. Missing covariates were evaluated using the complete-subjects analysis approach (34). Only number of previous full-term pregnancies and age at first full-term pregnancy met our criteria for confounding. Thus, the final models were adjusted for number of previous full-term pregnancies (0, 1, 2, ≥3), age at first full-term pregnancy (<21, 21–25, 26–30, ≥31 years), and the matching factor, age at index pregnancy (continuous).

We evaluated all covariates as potential modifiers of the effect estimates by comparing stratum-specific ORs and tests for homogeneity. There was no persuasive evidence of effect measure modification of the association between pregnancy hormones and incidence of breast cancer in these data.

The effect estimates for pregnancy hormones on breast cancer incidence were also stratified by age at diagnosis (≤50 *versus* >50 years) and time elapsed between index pregnancy and breast cancer diagnosis (<15 years *versus* ≥15 years). In each model, the subset of cases was compared with all 374 controls. The controls could not be stratified across the specified subgroups because the strata definitions (*i.e.*, characteristics of diagnosis) are unique to breast cancer cases and the controls were not pair-matched to cases.

Table 1 Characteristics of breast cancer cases and controls at index pregnancy

	Cases (%) (n = 194)	Controls (%) (n = 374)	OR[a] (95% CI)
Age (yrs)			
≤20	6.2	5.4	1.0
21–25	15.5	17.4	0.81 (0.35–1.9)
26–30	24.7	23.5	1.5 (0.65–3.2)
31–35	28.4	27.5	2.6 (1.2–5.8)
≥36	25.3	26.2	3.2 (1.5–7.1)
Race			
White	60.8	66.3	1.0
Black	28.4	23.0	1.3 (0.89–2.0)
Other	10.8	10.7	1.1 (0.63–2.0)
Age at first pregnancy (yrs)			
≤20	19.1	26.7	1.0
21–25	39.2	41.2	1.3 (0.81–2.1)
26–30	28.4	21.9	1.9 (1.1–3.2)
≥31	13.4	10.2	2.1 (1.1–4.1)
Previous pregnancies			
0	21.1	19.8	1.0
1	26.3	23.5	1.0 (0.60–1.7)
2	27.3	21.7	1.1 (0.65–2.0)
≥3	25.3	35.0	0.57 (0.32–1.0)
Age at menarche (yrs)			
≤11	16.5	13.6	1.0
12	22.7	21.7	0.85 (0.48–1.5)
13	22.2	17.9	0.99 (0.55–1.8)
≥14	13.9	17.1	0.70 (0.37–1.3)
Missing	24.7	29.7	
Prepregnancy body mass index (kg/m^2)			
<20	18.6	18.5	1.0
20–24	47.4	47.9	1.0 (0.64–1.7)
25–29	11.3	12.3	0.95 (0.49–1.8)
≥30	4.1	3.2	1.2 (0.46–3.4)
Missing	18.6	18.2	—
Smoking (cigarettes/day)			
Nonsmokers/past smokers	68.0	68.5	1.0
1–9	12.4	9.6	1.3 (0.78–2.4)
10–19	7.7	6.7	1.2 (0.59–2.3)
≥20	11.9	14.2	0.82 (0.48–1.4)
Missing	0.0	1.1	
Alcohol (drinks/wk)			
Nondrinkers	42.3	48.9	1.0
1–3.5	25.8	21.7	1.4 (0.93–2.2)
≥4	16.0	15.0	1.2 (0.72–2.0)
Missing	16.0	14.4	
Caffeine (cups/day)			
<1	15.5	15.8	1.0
1–3	37.6	35.8	1.1 (0.63–1.8)
4–6	16.5	22.7	0.80 (0.43–1.5)
>6	16.0	12.8	1.3 (0.68–2.6)
Missing	14.4	12.8	

[a] ORs for all variables except age were adjusted for the matching factor, age at index pregnancy.

Results

Cases and controls were similarly distributed across all categories of age at index pregnancy, race, body mass index, smoking, and caffeine consumption (Table 1). Cases had first full-term pregnancies at later ages, reported slightly fewer previous pregnancies, experienced menarche at somewhat earlier ages than controls, and were somewhat more likely to consume alcohol. Missing information on age at menarche, body mass index, and alcohol and caffeine intake was distributed similarly across cases and controls.

On the basis of the date of last menstrual period, gestational age at delivery ranged from 34 to 44 weeks (mean, 39.6 weeks; median, 40.0 weeks). Mean age at index pregnancy was 31 years for both cases and controls (range, 17–44 years). Mean age at breast cancer diagnosis was 50.9 years (range, 31–69 years), and mean time elapsed between index pregnancy and breast cancer diagnosis was 20.5 years (range, 4–32 years). Geometric mean estrogen levels adjusted for timing of blood draw were similar for cases and controls, but there was some suggestion of higher mean progesterone levels among controls (Table 2). All estrogen and progesterone levels were positively correlated with one another (Table 3), with estradiol and estrone demonstrating the strongest association.

A pattern of decreasing incidence of breast cancer was

Table 2 Geometric mean hormone levels[a] (ng/ml) for breast cancer cases and controls

	Cases (n = 194)		Controls (n = 374)	
	Mean	95% CI	Mean	95% CI
Estone	12.5	12.0–13.1	12.4	11.9–12.9
Estradiol	22.8	21.5–24.1	22.8	21.8–23.9
Estriol	8.8	8.0–9.6	8.3	7.7–8.9
Progesterone	175.4	167.4–183.8	180.4	174.9–186.1

[a] Hormone levels adjusted for timing of blood draw

Table 3 Pearson correlations for $\log_e$-transformed third-trimester estrone, estradiol, estriol, and progesterone[a]

	Estrone	Estradiol	Estriol	Progesterone
Estrone	1.00			
Estradiol	0.76	1.00		
Estriol	0.39	0.54	1.00	
Progesterone	0.22	0.40	0.46	1.00

[a] Hormone levels were adjusted for timing of blood draw. $P < 0.0001$ for all correlations (n = 568).

found for increasing levels of progesterone, with incidence reduced by 40–50% for those with progesterone levels >197.11 ng/ml (Table 4). Those with the highest estrone levels had an incidence of breast cancer that was more than two times greater than those with the lowest levels. For estriol levels, there was an indication of an inverse U-shaped effect in which the two middle categories (7.39 to <13.52 and 13.52 to <19.65 ng/ml) had modestly elevated ORs, and the upper extreme category was indistinguishable from the reference group. No association with breast cancer incidence was evident for estradiol. Estrogen-to-progesterone ratios were weakly associated with breast cancer incidence, with a modest dose-response gradient for estrone-to-progesterone and combined estrogen-to-progesterone ratios (Table 5).

Information on cancer histology was available from the CHDS on 139 of the 194 breast cancers, 11% of which were *in situ*. When the analyses were repeated for invasive cases only, the results were largely unchanged. All 194 cases were included in the remaining analyses.

Progesterone was strongly and inversely associated with breast cancer diagnosed at or before age 50, but not for cancer diagnosed after age 50 (Table 6). No distinct associations emerged between cases diagnosed at or before age 50 and any of the estrogenic compounds. For cases diagnosed after 50 years of age, the suggestion of an inverted U-shaped effect surfaced for each estrogen, but the point estimates were imprecise.

When the cases were stratified by time elapsed between index pregnancy and breast cancer diagnosis (Table 6), few cases (n = 40) were diagnosed within 15 years after the index pregnancy. Thus, lack of precision made any suggested associations in this subgroup difficult to evaluate.

Discussion

The present study evaluated the association between serum hormone levels during pregnancy and incidence of maternal breast cancer in the CHDS cohort of pregnant women who were subsequently followed for breast cancer. Increasing progesterone levels were associated with a lower incidence of maternal breast cancer, and this relationship was stronger for breast cancers diagnosed at or before age 50. Those with the lowest estrone and estriol levels tended to have a reduced risk, especially among cases diagnosed after age 50. A similar association was not observed for estradiol levels. The discrepant results among the estrogens could be attributable to differing bioavailable fractions, which could not be evaluated in these data. Higher concentrations of total estrogens relative to progesterone were associated with increased incidence of breast cancer, and this association appeared to be driven primarily by the estrone:progesterone ratio.

Because of the limited sample size, it was not possible to separate the effects by age at diagnosis from those specific to the time elapsed since pregnancy. Age at diagnosis is correlated with time since pregnancy (r = 0.64) because cancers detected at younger ages are more likely to occur during or shortly after the childbearing years. In these data, the two factors were interrelated, with 90% of cases diagnosed within 15 years of the index pregnancy occurring by age 50, and 64% of cases diagnosed after 15 years of pregnancy occurring after age 50. Although the results appeared to vary by age at diagnosis, too few cases occurred within 15 years of the index pregnancy to reliably detect differences by time elapsed since pregnancy.

Pregnancy estrogens and progesterone increase with advancing gestational age (5, 35, 36). However, more sharply increasing estriol levels and decreasing progesterone levels have been reported with approaching parturition (6–8). Therefore, when we adjusted the hormone measurements for timing of blood draw, two definitions were considered: day of gestation at blood draw and number of days preceding delivery. Although the two measures were similar predictors of steroid hormone measurements, gestational age estimated from day of last menstrual period is potentially less accurate than the calculated interval between recorded dates of blood draw and delivery. Thus, we proceeded to adjust the steroid hormone measurements for number of days before delivery. The final analyses were repeated after hormone levels were adjusted for day of gestation, and the patterns of association remained largely the same. Furthermore, eliminating blood draws within 20 days of delivery had no impact on the overall results.

To ensure comparable cancer surveillance among cases and controls, it would have been preferable to restrict the control population to verified California residents, as recommended by present CHDS guidelines for nested case-control cancer studies. Residence, however, was not an applied inclusion criterion when the cases and controls where selected for the original case-control study. Thus, unidentified breast cancers could have occurred among controls who moved out of state and were lost to follow-up. Residence has remained largely stable among CHDS cohort members who completed the first 5 years of active follow-up. Among cohort members who completed interviews before pregnancy, had third-trimester blood samples, and completed the initial 5-year observation period, 8% were lost to follow-up by 1992. Of the controls selected for the present study, 24 deaths were recorded as of 1991. Of the remaining controls believed to be alive, 66% were confirmed residents of California as of 1987 and 62% as of 1991.

Bias from loss to follow-up or incomplete case ascertainment is improbable because neither in-state nor out-of-state migration patterns are likely to be associated with hormone levels during a prior pregnancy. In support of this conclusion, no differences in mean (log-transformed) hormone levels were observed overall or among the control group when we compared those with and without verified California residence as of 1991. Undetected breast cancers among the control group

Table 4 ORs and 95% CIs for incidence of breast cancer associated with total unconjugated progesterone, estrone, estradiol, and estrone levels (ng/ml) in the third trimester of pregnancy

	Category mean	Cases (n = 194)	Controls (n = 374)	OR[a]	95% CI	P for trend	OR[b]	95% CI	P for trend
Progesterone									
<124.25	106.4	26	37	1.0	Referent		1.0	Referent	
124.25 to <197.11	163.5	106	203	0.69	0.39–1.2		0.66	0.38–1.2	
197.11 to <269.97	226.3	46	97	0.63	0.34–1.2		0.57	0.30–1.1	
≥269.97	321.4	16	37	0.56	0.26–1.2		0.49	0.22–1.1	
						0.16			0.08
Estrone									
<3.36	2.6	10	38	1.0	Referent		1.0	Referent	
3.36 to <11.03	7.0	113	219	2.0	0.94–4.1		1.8	0.87–3.9	
11.03 to <18.70	14.7	45	80	2.1	0.96–4.8		2.0	0.88–4.5	
≥18.70	25.9	26	37	2.7	1.1–6.5		2.5	1.0–6.1	
						0.09			0.12
Estradiol									
<13.39	11.0	19	38	1.0	Referent		1.0	Referent	
13.39 to <26.66	19.9	103	200	1.0	0.56–1.9		1.0	0.55–1.9	
26.66 to <39.92	31.6	57	98	1.2	0.61–2.4		1.1	0.58–2.3	
≥39.92	49.4	15	38	0.77	0.33–1.8		0.71	0.30–1.7	
						0.68			0.48
Estriol									
<7.39	6.0	11	37	1.0	Referent		1.0	Referent	
7.39 to <13.52	10.6	96	172	1.9	0.91–3.9		1.9	0.91–4.0	
13.52 to <19.65	15.9	73	128	1.8	0.88–3.9		1.9	0.88–3.9	
≥19.65	24.8	14	37	1.2	0.49–3.1		1.3	0.50–3.2	
						0.84			0.90

[a] OR controlling for age at index pregnancy (n = 568).
[b] OR controlling for age at index pregnancy, number of previous full-term pregnancies, and age at first full-term pregnancy (n = 568).

Table 5 ORs and 95% CIs for incidence of breast cancer associated with estrogen-to-progesterone ratios (ng/ml)

	Category mean	Cases (n = 194)	Controls (n = 374)	OR[a]	95% CI	P for trend	OR[b]	95% CI	P for trend
Estrone:Progesterone									
<0.019	0.015	13	37	1.0	Referent		1.0	Referent	
0.019 to <0.060	0.038	99	207	1.4	0.70–2.7		1.3	0.67–2.7	
0.060 to <0.101	0.078	57	92	1.8	0.86–3.7		1.8	0.84–3.7	
≥0.101	0.149	25	38	2.1	0.90–4.8		2.0	0.85–4.6	
						0.06			0.08
Estradiol:Progesterone									
<0.071	0.060	14	37	1.0	Referent		1.0	Referent	
0.071 to <0.141	0.108	99	186	1.4	0.74–2.8		1.4	0.70–2.7	
0.141 to <0.210	0.170	56	113	1.4	0.67–2.8		1.3	0.64–2.7	
≥0.210	0.260	25	38	1.9	0.83–4.4		1.7	0.74–4.0	
						0.24			0.33
Estriol:Progesterone									
<0.041	0.034	15	37	1.0	Referent		1.0	Referent	
0.041 to <0.074	0.059	78	172	1.2	0.61–2.3		1.2	0.62–2.4	
0.074 to <0.108	0.088	82	127	1.7	0.87–3.3		1.9	0.95–3.7	
≥0.108	0.124	19	38	1.3	0.55–2.9		1.4	0.62–3.3	
						0.16			0.07
$E_1 + E_2 + E_3$:Progesterone[c]									
<0.147	0.125	13	37	1.0	Referent		1.0	Referent	
0.147 to <0.273	0.216	93	192	1.4	0.70–2.8		1.3	0.64–2.6	
0.273 to <0.399	0.327	61	107	1.7	0.82–3.6		1.6	0.76–3.3	
≥0.399	0.489	27	38	2.2	0.96–5.1		2.0	0.87–4.7	
						0.04			0.06

[a] OR controlling for age at index pregnancy.
[b] OR controlling for age at index pregnancy, number of previous full-term pregnancies, and age at first full-term pregnancy.
[c] E_1, estrone; E_2, 17β-estradiol; E_3, estriol.

would be expected to lead to conservative estimates of the true associations. As a quality-control check, case-control status was subsequently verified for this study population by cancer linkage data through 1996. The review identified three controls diagnosed with breast cancer as of 1991, five controls diagnosed with breast cancer after 1991, and three cases confirmed to be invalid matches with the cancer registry. These data were analyzed according to breast cancer status at the conclusion of

Table 6 ORs[a] and 95% CIs for incidence of breast cancer associated with third-trimester steroid hormone levels (ng/ml) by age at diagnosis and time since index pregnancy

Three cases excluded because of missing date of diagnosis.

	OR (95% CI)			
	Age at Dx[b] (yrs)		Time to Dx (yrs)	
	≤50 (90 cases; 374 controls)	>50 (101 cases; 374 controls)	<15 (40 cases; 374 controls)	≥15 (151 cases; 374 controls)
Progesterone				
<124.25	1.0	1.0	1.0	1.0
124.25 to <197.11	0.54 (0.27–1.1)	0.86 (0.40–1.9)	1.0 (0.34–3.2)	0.58 (0.32–1.1)
197.11 to <269.97	0.41 (0.18–0.92)	0.79 (0.34–1.8)	0.72 (0.20–2.6)	0.52 (0.26–1.0)
≥269.97	0.30 (0.10–0.90)	0.87 (0.31–2.5)	0.21 (0.02–2.1)	0.55 (0.24–1.3)
P for trend	0.04	0.86	0.13	0.33
Estrone				
<3.36	1.0	1.0	1.0	1.0
3.36 to <11.03	0.85 (0.33–2.2)	3.5 (1.2–11)	5.3 (0.68–42)	1.5 (0.67–3.2)
11.03 to <18.70	1.0 (0.37–2.9)	3.8 (1.2–12)	6.1 (0.72–51)	1.6 (0.69–3.8)
≥18.70	1.5 (0.52–4.6)	2.6 (0.67–10)	2.6 (0.21–31)	2.2 (0.87–5.6)
P for trend	0.08	0.52	0.98	0.07
Estradiol				
<13.39	1.0	1.0	1.0	1.0
13.39 to <26.66	0.49 (0.20–1.2)	1.9 (0.83–4.4)	0.68 (0.25–1.8)	1.3 (0.61–2.7)
26.66 to <39.92	0.63 (0.25–1.6)	1.8 (0.73–4.6)	0.78 (0.26–2.4)	1.4 (0.61–3.1)
≥39.92	0.42 (0.14–1.3)	0.82 (0.24–2.8)	0.16 (0.02–1.4)	0.94 (0.36–2.5)
P for trend	0.69	0.66	0.22	0.95
Estriol				
<7.39	1.0	1.0	1.0	1.0
7.39 to <13.52	1.3 (0.52–3.2)	2.7 (0.87–8.1)	2.2 (0.48–10)	1.7 (0.78–3.8)
13.52 to <19.65	1.3 (0.54–3.4)	2.5 (0.81–7.8)	1.9 (0.41–9.1)	1.8 (0.79–4.0)
≥19.65	0.81 (0.24–2.8)	1.9 (0.51–7.2)	1.5 (0.23–9.5)	1.2 (0.43–3.3)
P for trend	0.60	0.90	0.82	0.94

[a] Controlling for age at index pregnancy, number of previous full-term pregnancies, and age at first full-term pregnancy.
[b] Dx, diagnosis.

1991, which is consistent with the original date of diagnosis criterion for case identification (*i.e.*, controls diagnosed with breast cancer before 1991 were treated as cases, invalid cases were included as controls). Removing all controls who were subsequently diagnosed with breast cancer had no meaningful impact on the results.

The serum hormone measurements in this study were subject to the potential effects of long-term freezer storage and within-person variation. The measures for the archived specimens were largely (91–96%) within the reported reference ranges for third-trimester levels (5, 6, 8, 38). Furthermore, the stability and individual rankings of serum steroid hormone concentrations have been shown to be essentially unaffected after up to 8 years of low-temperature freezer storage (39–42). Given similar storage intervals and thawing episodes for case and control specimens, differential effects of storage would not be expected. Although single serum samples may not capture diurnal variations, limited day-to-day variation has been reported for pregnancy estrogens (43, 44), although progesterone levels may be somewhat more variable (45). Nevertheless, exposure measurement error attributable to biological variation is unlikely to be differential with respect to cases and controls and would most likely lead to an underestimation of the effect estimates (46–48).

The data in this study were obtained from the last pregnancy recorded in the CHDS, which was either a first or subsequent pregnancy for each woman. On evaluating primiparous women separately from multiparous women, we found no evidence of effect estimate modification between these two groups. Previous reports of highly correlated total estradiol levels in successive pregnancies (49) gives support to the similarity of hormonal effects between first and subsequent pregnancies in our analysis.

The investigation of maternal pregnancy hormone levels as a risk factor for breast cancer originates from evidence of short-term increases and long-term decreases in risk after full-term pregnancy (1–3). No previous studies have directly evaluated the effect of serum hormones during pregnancy on the incidence of maternal breast cancer. Pregnancy hormone levels were recently evaluated for differences between two populations with (Boston, MA) and without (Shanghai, China) a high incidence of breast cancer (50). Median levels of estradiol, estriol, and progesterone at 16 weeks of gestation were reported to be substantially higher among the Chinese women, who are known to have a lower incidence of breast cancer. At 27 weeks of gestation, estradiol and estriol levels remained higher for the Chinese women, but median progesterone levels were lower than levels reported for the American women. Therefore, high third-trimester progesterone levels were not associated with reduced risk in this ecological study.

The lack of agreement between our findings and those reported by Lipworth *et al.* (50) is not surprising given that associations on the aggregate level do not necessarily reflect associations at the individual level. Furthermore, because the average week of gestation at blood collection in our study was 39 weeks, compared with 27 weeks reported by Lipworth *et al.* (50), the hormone concentrations reported in both studies may characterize different stages of pregnancy. We believe our data more closely approximates maximum hormone level exposure because concentrations progressively increase with duration of

gestation. The third-trimester blood draw in the study by Lipworth *et al.* was taken ~1 week earlier for Chinese women than for those from Boston, which could explain lower average progesterone levels among the Chinese women but does not account for unaffected estrogen levels. There were too few Asians ($n = 20$) in our study population to analyze breast cancer incidence separately for this subgroup.

In the absence of available serum hormone measurements during pregnancy, several studies have used pregnancy and fetal characteristics as surrogates of exposure to altered pregnancy hormones to address this question. Findings from studies assessing surrogates of elevated estrogen levels, such as twin pregnancies (14–23), pregnancy nausea (18, 25), and large fetal size (23, 26), as predictors of maternal breast cancer have been largely mixed and inconclusive. Furthermore, one study's evaluation of Rh factor as a proxy for high progesterone and estrogen levels (51–53) showed no association with breast cancer (19). Alternatively, studies evaluating conditions characterized by lower estrogen levels or reduced duration of exposure, such as pregnancy-induced hypertension (18, 19, 24) and extreme preterm birth (<30 weeks of gestation; Ref. 19), have shown consistent inverse associations with breast cancer in the mother.

The inconsistency of previous surrogate studies could result from the poor quality of these surrogates as indicators of pregnancy hormone levels. Although correlations and patterns of association between pregnancy hormone levels and the commonly used proxies have been observed (51–62), the strength of the association and the accuracy of the substitute indicators have gone largely unstudied. A recent evaluation of the association between birth weight and estriol levels reported a positive dose-response for mean estriol levels across birth weight and birth weight for gestational age categories (63). Although the specificity of the authors' measure of high birth weight as a predictor of high estriol exposure was good (0.93), the sensitivity was very poor (0.28). Thus, the use of birth weight, or other untested surrogate indicators in use today, is likely to lead to pronounced bias in the estimates of hormonal effects.

During pregnancy, high concentrations of progesterone induce lobular-alveolar development and differentiation in preparation for lactation (64), whereas estrogens stimulate ductal growth (11, 12). Although there is some evidence that estrogen metabolites can bind directly to DNA (65), the cancer-promoting effects of estrogens are believed to act through their increase in breast cell division rates (66). Through this mechanism, the carcinogenic effects are dependent on the rate and duration of cell proliferation, which reflect the dose and duration of endogenous hormones (66). An inverse association between breast cancer incidence and high concentrations of progesterone during pregnancy is biologically plausible given recent evidence that high-dose progesterone elicits a growth-inhibiting response from normal and cancerous breast cells (64, 67, 68). Although the initial response to progesterone is transiently growth stimulatory, sustained exposure to high progesterone levels, as in pregnancy, has been shown to inhibit proliferation in normal human breast epithelial cells *in vivo* (67) and in human breast cancer cell lines *in vitro* (68) through cross-talk between progesterone and growth factor/cytokine-mediated pathways (64).

In summary, this study is the first to directly evaluate serum steroid hormone levels during pregnancy as a risk factor for breast cancer in the mother. Our results indicate that higher concentrations of progesterone are inversely related to breast cancer incidence relative to those with the lowest levels. This association is most pronounced among cancers diagnosed at or before age 50. The suggestion of reduced breast cancer risk for those with low estrone and estriol levels was more prominent among cases diagnosed after age 50. Furthermore, higher concentrations of estrone and total estrogens relative to progesterone were also associated with an elevated incidence of breast cancer. The mechanism by which pregnancy affects maternal breast cancer incidence is not fully understood, but these findings indicate that normal variations in steroid hormone levels during pregnancy could influence subsequent breast cancer incidence.

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