Correlation of Serum Hormone Concentrations in Maternal and Umbilical Cord Samples[1]

Rebecca Troisi,[2] Nancy Potischman, James M. Roberts, Gail Harger, Nina Markovic, Bernard Cole, David Lykins, Pentti Siiteri, and Robert N. Hoover

Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland 20892 [R. T., R. N. H.], Dartmouth Medical School, Hanover, New Hampshire 03755 [R. T., B. C.]; Division of Cancer Control and Population Sciences, National Cancer Institute, Bethesda, Maryland 20892 [N. P.]; Magee Women's Hospital, University of Pittsburgh, Pittsburgh, Pennsylvania 15238 [J. M. R., G. H., N. M., D. L.]; and University of California San Francisco School of Medicine, San Francisco, California 94122 [P. S.]

Abstract

Evidence suggests that adult cancer risk of hormonally related tumors may be influenced by the *in utero* environment, and most speculation on the biological mechanism has focused on the hormonal component. Epidemiological studies investigating the biological nature of pregnancy and maternal factors associated with offspring's cancer risk have relied on maternal hormone measurements. The degree to which maternal hormone levels represent the fetal environment, however, is not widely known. Pregnancy estrogen, androstenedione, testosterone, dehydroepiandrosterone (DHEA), and DHEA-sulfate concentrations were measured in maternal and mixed umbilical cord sera from 86 singleton pregnancies. Spearman correlations between maternal and cord hormone levels generally ranged between 0.2 and 0.3. The correlation was 0.26 for estriol, the estrogen of highest concentration in pregnancy, and 0.27 for estradiol, the most biologically active estrogen. The correlations between mother and offspring for the estrogens and DHEA appeared similar for males and females, whereas there was a suggestion that the maternal-umbilical cord correlations for other androgens varied in magnitude by fetal sex, and all correlations appeared higher in pregnancies lasting <38 weeks compared with longer gestational lengths, although these stratified findings may have been attributable to chance. These data show a moderate degree of correlation in hormone concentrations between the maternal and fetal circulation. Studies using maternal hormone concentrations as a proxy for the fetal environment should consider the misclassification resulting with the use of this marker.

Introduction

Epidemiological studies provide some evidence of altered cancer risks in offspring associated with several maternal, perinatal, and pregnancy characteristics, most notably for breast cancer, a decreased risk with preeclampsia (1, 2), and an increased risk with birth weight (1, 3–12). Investigations have been pursued to understand the underlying biological nature of the effects by identifying hormonal alterations associated with these factors (13–17). Differences in maternal hormone concentrations by pregnancy and perinatal factors have been noted, although data for most risk factors are sparse. These studies have focused primarily on hormones in the maternal circulation (13–17). Consequently, the interpretation of findings related to reasons for subsequent cancer risk in the offspring relies on the assumption that maternal levels are reflective of the fetal circulation or environment.

Several studies have reported correlations between hormone concentrations in the maternal and fetal circulations, (18–23), although most have been small ($n < 43$; Refs. 18–20 and 23) and focused primarily on estrogens (18–21). In this study, we examined correlations between maternal and mixed cord serum concentrations of androstenedione, testosterone, DHEA,[3] DHEAS, estradiol, estriol, and estrone.

Materials and Methods

Study Population. Subjects were a sample from an ongoing study of pre-eclamptic pregnancies being conducted at the Magee Women's Hospital, University of Pittsburgh. In the parent study, all women with pre-eclampsia who delivered at Magee Women's Hospital from February, 1994 through May, 1998 and were ≥14 years of age were invited to participate. All women attending the Magee Women's Hospital's obstetric practice during the same time period who were ≥14 years of age were invited to participate in the study as controls; 52% agreed to participate. For every case, we attempted to choose a control that matched the case on parity, length of pregnancy at delivery, type of delivery, and maternal age (±5 years). We included the first 100 cases that agreed to participate. After excluding pregnancies involving multiple fetuses, and cases without blood sampled during the appropriate time period or who could not be matched to a control, there were 86 pre-eclamptic cases and 86 controls for study. This control group was used in the present study to assess the relationship between maternal and cord hormone concentrations in women with normal pregnancies. Informed consent for the questionnaire, interview, and blood collection were obtained from all study participants.

Hormone Assays. Maternal sera were collected at admission for labor and delivery, and mixed venous and arterial cord sera

Received 8/9/02; revised 12/18/02; accepted 1/31/03.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported in part by NIH Grant 2 PO1 HD30367.

[2] To whom requests for reprints should be addressed, at Epidemiology and Biostatistics Program, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6120 Executive Boulevard, Bethesda, MD 20892-7246. E-mail: troisir@mail.nih.gov.

[3] The abbreviations used are: DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone-sulfate; RIA, radioimmunoassay.

Table 1 Maternal and umbilical cord serum hormone concentrations in 86 pregnancies by sex of the offspring

	Maternal levels			Cord levels		
	Mean (SD)	Median	Range	Mean (SD)	Median	Range
DHEA (ng/dl)	644 (537)	432	(57–2,478)	548 (230)	531	(103–1,470)
Males	678 (581)	420	(57–2,383)	526 (203)	509	(103–1,161)
Females	600 (475)	456	(103–2,478)	577 (261)	534	(126–1,470)
DHEAS (μg/dl)	120 (90)	94	(11–461)	211 (126)	187	(20–885)
Males	126 (91)	102	(11–461)	221 (143)	214	(20–885)
Females	112 (90)	90	(14–405)	197 (98)	160	(71–528)
Androstenedione (ng/dl)	413 (264)	356	(77–1,719)	362 (152)	344	(91–996)
Males	454 (308)	358	(77–1,719)	368 (159)	352	(128–996)
Females	359 (183)	340	(97–919)	354 (144)	337	(91–700)
Testosterone (ng/dl)	184 (149)	150	(38–921)	26 (21)	21	(2–166)
Males	212 (184)	152	(43–921)	29 (24)	22	(5–166)
Females	147 (70)	148	(38–393)	22 (17)	16	(2–65)
Estradiol (pg/ml)	24,146 (10,744)	22,848	(815–75,137)	12,420 (12,226)	9,510	(402–81,083)
Males	23,735 (12,044)	22,528	(815–75,137)	12,782 (13,074)	9,606	(2,331–81,083)
Females	24,691 (8,866)	22,967	(5,599–53,000)	11,941 (11,164)	8,879	(402–48,333)
Estrone (pg/ml)	8,772 (7,908)	7,682	(635–60,948)	39,644 (29,042)	30,815	(2,753–147,865)
Males	8,151 (8,930)	5,252	(1,004–60,948)	37,305 (26,493)	28,609	(6,608–142,154)
Females	9,595 (6,333)	8,577	(635–35,200)	42,742 (32,223)	39,241	(2,753–147,865)
Estriol (ng/ml)	18 (7.5)	18	(1.1–34)	249 (146)	223	(45–768)
Males	17 (6.3)	18	(1.1–31)	210 (126)	192	(48–768)
Females	20 (6.4)	19	(7.4–34)	300 (156)	279	(45–620)

were collected at delivery. The samples were allowed to clot at room temperature, centrifuged, and stored at −80°. Blood samples were analyzed by Quest Diagnostics (San Juan Capistrano, CA). Levels of unconjugated estrone, estradiol, and androstenedione were measured by an in-house method of RIA after extraction with organic solvent and purification by celite chromatography (24, 25). Unconjugated testosterone and estriol were measured by extraction and RIA and DHEAS by dilution and RIA. Blinded aliquots of pooled sera from normal and pregnant women and normal babies constituted 10% of each batch of study samples. The coefficients of variation (representing total inter- and intra-assay error) for the maternal hormones were 18.6% for DHEA, 8.5% for DHEAS, 10.2% for androstenedione, 9.6% for testosterone, 13.7% for estradiol, 10.3% for estrone, and 6.8% for estriol and 8.1, 6.6, 8.5, 15.2, 10.9, 16.7, and 9.2%, respectively, for the same hormones in cord blood.

Statistical Analysis. Means and SDs for maternal and cord hormone concentrations overall and by offspring's sex were calculated. The correlations between maternal and cord hormones were calculated overall and by offspring's sex, gestational length, and race. Correlations among the hormones within the maternal and cord samples separately were also calculated. Spearman correlations were calculated because the hormone values were not normally distributed.

We present a hypothetical example of the degree of attenuation in the true risk estimate from a study of daughter's subsequent breast cancer risk from fetal estrogen exposure that would result from using maternal levels as a proxy for fetal hormone exposure. This was estimated using the concordance between maternal and cord hormone values that we observed in this study and an approach for illustrating attenuation in the relative risk calculated as an odds ratio from a 2 × 2 table, as described in Willett (26).

Results

The women were in their mid-20s, on average ($\mu = 26.2$ years), and ~60% were Caucasian and 40% African-American. The average length of pregnancy was ~38 weeks (because in the initial study, controls were matched on gestational length to preeclampsia cases, who tend to deliver slightly early), although the median gestation was closer to 39 weeks (the range was 26–41 weeks, and the 25th and 75th percentile cut points were 37 and 39 weeks, respectively). Means for maternal and cord hormone levels are presented in Table 1. Distributions were skewed with lower medians than means for all hormones. Except for DHEAS, androgens were higher in maternal than in cord serum, with the greatest difference demonstrated for testosterone. Estradiol concentrations were twice as great in maternal than in cord serum, whereas estrone and estriol concentrations were lower. Pregnancies with male fetuses tended to have slightly higher androgen concentrations in the maternal and cord circulations, whereas those involving female fetuses tended to have slightly higher estrogen concentrations.

Spearman correlations between maternal and cord hormones overall were generally in the range of 0.2–0.3 (Table 2). Although the correlations between mother and offspring for the estrogens and DHEA were similar for males and females, correlations with some of the androgens appeared higher with male offspring, whereas the correlation for DHEAS appeared higher with female offspring. In general, correlations between the maternal and fetal hormone concentrations appeared higher in pregnancies with shorter than longer gestational lengths. Sample size was reduced in these analyses, and the results may be attributable to chance. Results were similar when the batches with DHEA values for quality control samples >2 SD from the batch mean were excluded (data not shown).

When maternal and cord serum hormone concentrations were categorized into quartiles based on their own distributions, there was between 30 and 50% agreement for the lowest and highest quartiles, *e.g.*, 33% of the lowest quartile of cord estriol measures was categorized into the lowest quartile of maternal estriol, whereas 14% was categorized into the highest quartile of maternal estriol, and the remainder (53%) was categorized into the middle quartiles. Likewise, 36% of the highest quartile of cord estriol was categorized into the highest quartile of

Table 2 Spearman correlation coefficients between maternal and cord serum hormone levels by sex of the offspring, gestational length, and race

	DHEA	DHEAS	Androstenedione	Testosterone	Estradiol	Estrone	Estriol
Overall (n = 86)	0.27[a]	0.15	0.24[a]	0.23[a]	0.27[a]	0.41[b]	0.26[a]
Male (n = 49)	0.33[a]	0.05	0.41[b]	0.34[a]	0.28[a]	0.41[b]	0.28
Female (n = 37)	0.24	0.36[a]	0.01	0.07	0.27	0.40[a]	0.21
<38 weeks (n = 24)	0.43[a]	0.39[a]	0.42[a]	0.33	0.34	0.50[a]	0.41[a]
38+ weeks (n = 62)	0.20	0.07	0.17	0.21	0.24	0.37[b]	0.15
Caucasian (n = 50)	0.21	0.13	0.21	0.04	0.35[a]	0.41[b]	0.13
African-American (n = 34)	0.31	0.18	0.31	0.49[b]	0.14	0.43[a]	0.40[a]

[a] $P < 0.05$.
[b] $P < 0.01$.

Table 3 Spearman correlation coefficients among serum hormone concentrations, within maternal and umbilical cord samples

	DHEA	DHEAS	Androstenedione	Testosterone	Estradiol	Estrone	Estriol
DHEA							
Maternal	1.0	0.73[a]	0.39[a]	0.32[a]	0.54[a]	0.45[a]	−0.08
Cord	1.0	0.45[a]	0.51[a]	0.43[a]	0.45[a]	0.47[a]	0.47[a]
DHEAS							
Maternal		1.0	0.43[a]	0.39[a]	0.53[a]	0.50[a]	−0.20
Cord		1.0	0.28[a]	0.39[a]	0.22[b]	0.27[b]	−0.009
Androstenedione							
Maternal			1.0	0.89[a]	0.38[a]	0.17	0.009
Cord			1.0	0.70[a]	0.72[a]	0.82[a]	0.52[a]
Testosterone							
Maternal				1.0	0.34[a]	0.12	0.03
Cord				1.0	0.74[a]	0.50[a]	0.24[b]
Estradiol							
Maternal					1.0	0.62[a]	0.39[a]
Cord					1.0	0.74[a]	0.40[a]
Estrone							
Maternal						1.0	0.17
Cord						1.0	0.49[a]
Estriol							
Maternal							1.0
Cord							1.0

[a] $P < 0.01$.
[b] $P < 0.05$.

maternal estriol, whereas 14% was categorized into the lowest quartile.

Correlations among the serum hormone concentrations within the maternal and umbilical cord samples are presented separately in Table 3. The hormones were positively correlated with each other in both the maternal and cord samples with a few exceptions (estriol with DHEA and DHEAS in maternal serum and estriol with DHEAS in cord serum). Correlations among hormones within the maternal and cord samples tended to be higher than correlations between the two samples. In general, the highest correlations were demonstrated between hormones that are proximal in the metabolic pathway, *e.g.*, between androstenedione and testosterone (0.89 and 0.70 in maternal and cord serum, respectively) and between estradiol and estrone (0.62 and 0.74, respectively). Correlations among the maternal hormones generally were similar when stratified by fetal sex, and although there was some variation, there were no consistent patterns.

The attenuation in the true risk estimate for daughter's subsequent breast cancer risk from fetal estrogen exposure that would result from using maternal levels as a proxy can be estimated using the concordance between maternal and cord hormone values that we observed in this study (26). Assuming a hypothetical, true risk estimate for a daughter's breast cancer of 5.4 comparing the highest to lowest quartiles of estriol exposure, and that misclassification is nondifferential, the observed risk estimate in a study using maternal instead of cord concentrations would be 2. Likewise, if the true estimate was 2.25, the attenuated estimate using maternal hormone concentrations would be 1.4.

Discussion

Studies have reported correlations in the range of 0.3–0.5 between estrogen concentrations in the maternal and fetal circulations (18–23). Our results for estrogens are generally of similar magnitude. There was a suggestion that the correlations vary for certain hormones by fetal sex and gestational length, but the sample sizes were too small to evaluate this interaction. Studies on the maternal-fetal correlation for androgen concentrations are sparse. One study, however, found no correlation for testosterone between mothers and either sex (20), and another found no correlation for DHEAS (22). These findings suggest caution is warranted in interpreting circulating maternal androgen levels as they relate to cancer risk factors.

Mixed cord blood includes blood leaving the placenta via the umbilical vein and returning from the fetus to the placenta via the umbilical artery. Which provides a better representation of average fetal hormonal exposure is unclear. However, for estrogens, this may not be important, because of the high degree of correlation between venous and arterial concentrations [$r = 0.65$ for estradiol (21) and $r = 0.71$ (21) and $r = 0.7$ (22) for estriol]. In the case of androgens, which are actively extracted by the placenta from the blood delivered to it by the fetal artery, the data are not sufficient to draw conclusions. Because only small amounts of androstenedione and testosterone escape aromatization in the placenta where they are metabolized to estrogens, androgen concentrations in the umbilical cord vein in normal pregnancy should be quite low. Correlations between umbilical cord arterial and venous androstenedione and testosterone concentrations have not been reported, although the correlation for DHEAS in one study was high ($r = 0.9$; Ref. 22). Although stronger correlations with maternal levels have been observed in some studies in which the umbilical cord vein and artery were sampled separately, the data are sparse, and results are not consistent across studies or consistently stronger by source (vein or artery; Refs. 19 and 21).

We measured maternal and fetal hormone levels late in pregnancy, but the critical time period during which fetal exposure to variations in hormone levels as they relate to later cancer risk is not known. An effect on the breast later in pregnancy, however, is suggested by the association of preeclampsia with daughter's breast cancer risk (1, 2), because this condition commonly occurs in late pregnancy. Even if hormone levels earlier in pregnancy are more relevant, maternal values are only a proxy for the fetal circulation and, ultimately, for which the fetal breast is exposed. Although the possibility exists that hormone levels late in pregnancy do not reflect earlier levels, sampling fetal blood at any time before delivery is not feasible. Another limitation of having to sample fetal blood at delivery is the possibility that the stress of labor and delivery affects hormone levels. Regarding the maternal-fetal correlations, this stress likely also affects the maternal hormones, possibly to the same degree and likely in the same direction. We cannot, however, reject the possibility that labor and delivery affected the correlations we observed.

Our data showing higher testosterone concentrations in males than in females are consistent with some previous studies that have reported higher testosterone concentrations in the cord serum of male babies (27–30). Cord estrogens, in contrast, have generally not been found to differ by sex (28, 30–34). The explanation for the difference we observed in the magnitude of maternal-cord correlations for some hormones by fetal sex is unclear.

Random error in the hormone measurements undoubtedly resulted in an attenuation of the observed correlations between maternal and cord levels. The combined inter- and intra-assay laboratory errors, calculated using blinded replicates, were $>$10% in several cases. The coefficient of variation was particularly high for DHEA, although the correlations with DHEA did not change materially with exclusion of the batches with quality control sample means indicating the largest degree of error. Stratification by fetal sex, gestational length, and race reduced the sample sizes and could explain some of the variation in the estimates of correlation that was observed in these subgroups. In contrast, the many comparisons may have resulted in some statistically significant results entirely by chance.

In summary, moderate correlations were demonstrated overall between maternal and umbilical cord hormone concentrations. There was some suggestion that the correlations varied by certain characteristics of the mother and offspring, but these findings should be replicated in a larger study. Studies using maternal hormone concentrations as a proxy for the fetal environment should consider the misclassification resulting from its use.

References

1. Ekbom, A., Trichopoulos, D., Adami, H-O., Hsieh, C-C., and Lan, S-J. Evidence of prenatal influences on breast cancer risk. Lancet, *340:* 1015–1018, 1992.

2. Ekbom, A., Chung-cheng, H., Lipworth, L., Adami H-O., and Trichopoulos, D. Intrauterine environment and breast cancer risk in women: a population-based study. J. Natl. Cancer Inst. (Bethesda), *89:* 71–76, 1997.

3. Andersson, S. W., Bengtsson, C., Hallberg, L., Lapidus, L., Niklasson, A., Wallgren, A., and Hulthen, L. Cancer risk in Swedish women: the relation to size at birth. Br. J. Cancer, *84:* 1193–1198, 2001.

4. Hilakivi-Clarke, L., Forsen, T., Eriksson, J. G., Luoto, R., Tuomilehto, J., Osmond, C., and Barker, D. J. Tallness and overweight during childhood have opposing effects on breast cancer risk. Br. J. Cancer, *85:* 1680–1684, 2001.

5. Vatten, L. J., Mæhle, B. O., Lund Nilsen, T. I., Tretli, S., Hseih, C-C., Trichopoulos, D., and Stuver, S. O. Birth weight as a predictor of breast cancer: a case-control study in Norway. Br. J. Cancer, *86:* 89–91, 2002.

6. Michels, K. B., Trichopoulos, D., Robins, J. M., Rosner, B. A., Manson, J. E., Hunter, D. J., Colditz, G. A., Hankinson, S. E., Speizer, F. E., and Willett, W. C. Birthweight as a risk factor for breast cancer. Lancet, *348:* 1542–1546, 1996.

7. Sanderson, M., Williams, M. A., Malone, K. E., Stanford, J. L., Emanuel, I., White, E., and Daling, J. R. Perinatal factors and risk of breast cancer. Epidemiology, *7:* 34–37, 1996.

8. Sanderson, M., Williams, M. A., Daling, J. R., Holt, V. L., Malone, K. E., Self, S. G., and Moore, D. E. Maternal factors and breast cancer risk among young women. on breast cancer risk in the offspring of preeclamptic pregnancies. Paediatr. Perinat. Epidemiol., *12:* 397–407, 1998.

9. Kaijser, M., Lichtenstein, P., Granath, F., Erlandsson, G., Cnattingius, S., and Ekbom, A. In utero exposures and breast cancer: a study of opposite-sexed twins. J. Natl. Cancer Inst. (Bethesda), *93:* 60–62, 2001.

10. Hubinette, A., Lichtenstein, P., Ekbom, A., and Cnattingius, S. Birth characteristics and breast cancer risk: a study among like-sexed twins. Int. J. Cancer, *91:* 248–251, 2001.

11. DeStavola, B. L., Hardy, R., Kuh, D., dos Santos Silva, I., Wadsworth, M., and Swerdlow, A. J. Birthweight, childhood growth and risk of breast cancer in a British cohort. Br. J. Cancer, *83:* 964–968, 2000.

12. Innes, K., Byers, T., and Schymura, M. Birth characteristics and subsequent risk for breast cancer in very young women. Am. J. Epidemiol., *152:* 1121–1128, 2000.

13. Petridou, E., Panagiotopoulou, K., Katsouyanni, K., Spanos, E., and Trichopoulos, D. Tobacco smoking, pregnancy estrogens and birth weight. Epidemiology, *1:* 247–250, 1990.

14. Petridou, E., Katsouyanni, K., Spanos, E., Skalkidis, Y., Panagiotopoulou, K., and Trichopoulos, D. Pregnancy estrogens in relation to coffee and alcohol intake. Ann. Epidemiol., *2:* 241–247, 1992.

15. Petridou, E., Katsouyanni, K., Hsieh, C. C., Antsaklis, A., and Trichopoulos, D. Diet, pregnancy estrogens and their possible relevance to cancer risk in the offspring. Oncology, *49:* 127–132, 1992.

16. Panagiotopoulou, K., Katsouyanni, K., Petridou, E., Garas, Y., Tzonou, A., and Trichopoulos, D. Maternal age, parity, and pregnancy estrogens. Cancer Causes Control, *1:* 119–124, 1990.

17. Kaijser, M., Granath, F., Jacobsen, G., Cnattingius, S., and Ekbom, A. Maternal pregnancy estriol levels in relation to anamnestic and fetal anthropometric data. Epidemiology, *11:* 315–319, 2000.

18. Goldkrand, J. W. Unconjugated estriol and cortisol in maternal and cord serum and amniotic fluid in normal and abnormal pregnancy. Obstet. Gynecol., *52:* 264–271, 1978.

19. Haning, R. V., Barrett, D. A., Alberino, S. P., Lynskey, M. T., Donabedian, R., and Speroff, L. Interrelationships between maternal and cord prolactin, progesterone, estradiol, 13, 14-dihydro-15-keto-prostaglandin $F_{2\alpha}$, and cord cortisol at delivery with respect to initiation of parturition. Am. J. Obstet. Gynecol., *130:* 204–210, 1978.

20. Penny, R., Parlow, A. F., and Frasier, S. G. Testosterone and estradiol concentrations in paired maternal and cord sera and their correlation with the concentration of chorionic gonadotropin. Pediatrics, *64:* 604–608, 1979.

21. Hercz, P., Ungar, L., Siklos, P., and Farquharson, R. G. Unconjugated 17β-oestradiol and destriol in maternal serum and in cord vein and artery blood at term and preterm delivery. Eur. J. Obstet. Gynecol. Reprod. Biol., *27:* 7–12, 1998.

22. Perry, L., Hickson, R., Obiekwe, B. C., and Chard, T. Maternal oestriol levels reflect placental function rather than foetal function. Acta Endocrinol., *111:* 563–566, 1986.

23. Mathur, R. S., Landgrebe, S., Moody, L. O., Powell, S., and Williamson, H. O. Plasma steroid concentrations in maternal and umbilical circulation after spontaneous onset of labor. J. Clin. Endocrinol. Metab., *51:* 1235–1238, 1980.

24. Abraham, G. E., Tulchinsky, D., and Korenman, S. G. Chromatographic purification of estradiol-17 for use in radio-ligand. Biochem. Med., *3:* 365–368, 1970.

25. Cassidenti, D. L., Vijod, A. G., Vijod, M. A., Stanczyk, F. Z., and Lobo, R. A. Short-term effects of smoking on the pharmacokinetic profiles of micronized estradiol in postmenopausal women. Am. J. Obstet. Gynecol., *163:* 1953–1960, 1990.

26. Willett, W. Correction for the effects of measurement error. *In:* W. Willett (ed.), Nutritional Epidemiology, pp. 272–291. New York: Oxford University Press, 1990.

27. Abramovich, D. R., and Rowe, P. Foetal plasma testosterone levels at mid-pregnancy and at term: relationship to foetal sex. J. Endocrinol., *56:* 621–622, 1973.

28. Maccoby, E. E., Doering, C. H., Jacklin, C. N., and Kraemer, H. Concentrations of sex hormones in umbilical-cord blood: their relation to sex and birth order of infants. Child Dev., *50:* 632–642, 1979.

29. Simmons, D., France, J. T., Keelan, J. A., Song, L., and Knox, B. S. Sex differences in umbilical cord serum levels of inhibin, testosterone, oestradiol, dehydroepiandrosterone sulphate, and sex hormone-binding globulin in human term neonates. Biol. Neonate, *65:* 287–294, 1994.

30. Herruzo, A. J., Mozas, J., Alarcón, López, J. M., Molina, R., Molto, L., and Martos, J. Sex differences in serum hormone levels in umbilical vein blood. Int. J. Gynecol. Obstet., *41:* 37–41, 1993.

31. Antonipillai, I., and Pearson Murphy, B. E. Serum oestrogens and progesterone in mother and infant at delivery. Br. J. Obstet. Gynaecol., *84:* 179–185, 1977.

32. Furuhashi, N., Suzuki, M., Fukaya, T., Kono, H., Shinkawa, O., Tachibana, Y., and Takahashi, T. Concentrations of luteinizing hormone-human chorionic gonadotropin, beta subunit of human chorionic gonadotropin, follical-stimulating hormone, estradiol, cortisol, and testosterone in cord sera and their correlations. Am. J. Obstet. Gynecol., *143:* 918–921, 1982.

33. Yuen, B. H., and Mincey, E. K. Human chorionic gonadotropin, prolactin, estriol, and dehydroepiandrosterone sulfate concentrations in cord blood of premature and term newborn infants: relationship to the sex of the neonate. Am. J. Obstet. Gynecol., *156:* 396–400, 1987.

34. Vatten, L. J., Romundstad, P. R., Ødegård, Nilsen, S. T., Trichopoulos, D., and Austgulen, R. Alpha-foetoprotein in umbilical cord in relation to severe pre-eclampsia, birth weight and future breast cancer risk. Br. J. Cancer, *86:* 728–731, 2002.