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Bile acids in a multicenter, population-based case-control study of stillbirth

Robert M. Silver, MD, Corette B. Parker, DrPH, Robert Goldenberg, MD, Uma M. Reddy, MD, MPH, Donald J. Dudley, MD, George R. Saade, MD, Carol J. Rowland Hogue, PhD, Donald Coustan, MD, Michael W. Varner, MD, Matthew A. Koch, MD, PhD, Deborah Conway, MD, Radek Bukowski, MD, PhD, Halit Pinar, MD, Barbara Stoll, MD, Janet Moore, MS, and Marian Willinger, PhD

Department of Obstetrics and Gynecology, University of Utah School of Medicine, Salt Lake City, UT (Drs Silver and Varner); RTI International, Research Triangle Park, NC (Drs Parker and Koch and Ms Moore); College of Physicians and Surgeons, Department of Obstetrics and Gynecology, Columbia University, New York, NY (Dr Goldenberg); Pregnancy and Perinatology Branch, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD (Drs Reddy and Willinger); University of Texas Health Science Center at San Antonio, San Antonio (Drs Dudley and Conway), and Department of Obstetrics and Gynecology, University of Texas Medical Branch at Galveston, Galveston (Drs Saade and Bukowski), TX; Rollins School of Public Health, Emory University (Dr Hogue) and Department of Pediatrics, Emory University School of Medicine (Dr Stoll), Atlanta, GA; and Departments of Obstetrics and Gynecology (Dr Coustan) and Pathology (Dr Pinar), Alpert Medical School, Brown University, Providence, RI.

Abstract

OBJECTIVE—We sought to compare bile acids in women with and without stillbirth in a population-based study.

STUDY DESIGN—The Stillbirth Collaborative Research Network conducted a multisite, population-based case-control study of stillbirth (fetal deaths ≥ 20 weeks). Maternal sera were obtained at the time of enrollment and frozen at –80° until assay for bile acids.

RESULTS—Assays were performed in 581 women with stillbirth and 1546 women with live births. Bile acid levels were slightly higher in women with stillbirth (geometric mean [95% confidence interval {CI}] = 3.2 [3.0–3.5]) compared to live births (2.9 [2.7–3.1], $P = .0327$). However, the difference was not significant after adjustment for baseline risk factors for stillbirth. The proportion of women with elevated levels (≥ 10 or ≥ 40 μmol/L) was similar in stillbirths and live births. Results were similar when the analysis was limited to subsets of stillbirths and live births. In women with stillbirths not associated with fetal anomalies or obstetric complications bile

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Reprints: Robert M. Silver, MD, Department of Obstetrics and Gynecology, University of Utah School of Medicine, 30 N. 1900 East, Room 2B308, Salt Lake City, UT 84132. bsilver@hsc.utah.edu..

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acid levels were higher than in women with term live births (geometric mean [95% CI] = 3.4 [3.0–3.8] vs 2.9 [2.7–3.0], $P = .0152$, unadjusted; $P = .06$, adjusted). However, a similar proportion of women in both groups had levels ≥ 10 mmol/L (10.7 vs 7.2%; odds ratio [OR], 1.54; 95% CI, 0.97–2.44; adjusted OR, 1.29; 95% CI, 0.78–2.15) and ≥ 40 μ mol/L (1.7 vs 0.7%; OR, 2.58; 95% CI, 0.85–7.84; adjusted OR, 2.28; 95% CI, 0.79–6.56).

CONCLUSION—Our data do not support testing for bile acids in cases of stillbirth in the absence of clinical evidence of intrahepatic cholestasis of pregnancy.

Keywords

bile acids; cholestasis; stillbirth

Intrahepatic cholestasis of pregnancy (ICP) has been associated with an increased risk of stillbirth.^{1–5} Indeed, the risk of stillbirth in women with expectantly managed ICP has been estimated as 2–11%.^{1–5} In addition to stillbirth, ICP has been linked to other adverse perinatal outcomes such as spontaneous preterm birth, meconium-stained amniotic fluid and aspiration, and abnormal fetal heart rate tracings.^{1–5}

The pathophysiology of fetal death associated with ICP is unknown. It is not thought to be due to placental insufficiency since most fetuses are appropriately grown for gestational age and stillbirth may occur despite normal antenatal testing.^{3,6,7} Possible mechanisms include bile acid-induced cardiac arrhythmias and impaired contractility⁸ and chorionic vein constriction.⁹ The critical threshold of maternal serum bile acids that increases the odds of stillbirth is unclear. Although some investigators believe the risk is low if bile acids are <40 μ mol/L,¹⁰ stillbirths have been reported in cases with lower levels of bile acids.^{6,7}

Since a clear threshold for bile acid levels and stillbirth has not been established and since elevated levels of bile acids have been reported in asymptomatic individuals,¹¹ we hypothesized that elevated levels of bile acids are associated with stillbirth. Also, the association between maternal serum bile acids and stillbirth has not been systematically assessed. Thus, our objective was to compare maternal levels of bile acids in women with and without stillbirth in a large, population-based, racially and ethnically diverse cohort.

Materials and Methods

The Eunice *Kennedy Shriver* National Institute of Child Health and Human Development Stillbirth Collaborative Research Network (SCRN) conducted a population-based case-control study of stillbirth, with participant enrollment at the time of delivery from March 2006 through September 2008. The study design and methods have been described in detail.¹² The study was approved by the institutional review board of each clinical site and the data-coordinating center. An advisory board reviewed the progress and safety of the study. All participants gave written informed consent.

A stillborn fetus was defined by Apgar scores of 0 at 1 and 5 minutes, and no signs of life by direct observation. Deliveries resulting from the termination of a live fetus were excluded. Residents of a SCRN catchment area were eligible for participation. Catchment areas were defined by state and county boundaries and included portions of 5 states: Rhode Island,

Massachusetts, Georgia, Texas, and Utah. Daily surveillance and enrollment occurred at 59 tertiary care and community hospitals servicing the catchment areas. These hospitals deliver >80,000 infants per year.¹² Attempts were made to enroll all stillbirths and a representative sample of live births with oversampling of preterm births and non-Hispanic black women to ensure adequate numbers for stratified analyses.¹²

Study components included a comprehensive standardized fetal postmortem examination and uniform placental pathology evaluation performed by a perinatal pathologist in both stillbirths and live births.^{13,14} A standardized maternal interview during the delivery hospitalization and detailed chart abstraction of prenatal office visits, antepartum hospitalizations, and the delivery hospitalization was performed.

Biospecimens collected included maternal blood for serum and DNA, fetal blood from the umbilical cord (when available), placental samples, and fetal tissue (in cases). Maternal serum was stored at -80° for 2-5 years prior to assay.

Gestational age was determined by the best clinical estimate. Multiple sources were utilized including assisted reproduction with documentation of the day of ovulation or embryo transfer, first day of the last menstrual period, and obstetrical sonograms.¹⁵ Fetal deaths at 18 or 19 weeks and without good dating were included in the study to ensure inclusion of all possible eligible stillbirths.¹²

Maternal serum in cases and controls was assessed for total bile acids at ARUP Laboratories, Salt Lake City, UT. Deuterated internal standards were added to the serum and proteins were precipitated. Two-dimensional high-pressure liquid chromatography was performed, followed by electrospray ionization tandem mass spectrometry with negative ion monitoring. Bile acid levels ≥ 10 were considered to be abnormal by ARUP Laboratories. This is a commonly used threshold to define ICP.^{10,16} Bile acids ≥ 40 $\mu\text{mol/L}$ also were assessed since this level has been associated with an increase in the risk for stillbirth in some studies.¹⁰ Finally, receiver operating characteristic (ROC) analysis was done to determine if there is another threshold that might optimally differentiate between stillbirth and live birth.

The delivery, defined as a case if there were any stillbirths delivered and as a control if all live births were delivered, was the unit of analysis. The analyses were weighted for oversampling of live births and other aspects of the study design as well as for differential consent among the women with stillbirth and among the women with live birth using SUDAAN software, version 11.0.¹⁷ Construction of the weights has been previously described.¹³ Weighted samples of live births were used in the analyses to approximate random selection of live births in the catchment areas over the enrollment period since all live births could not be enrolled.¹³

Crude and adjusted stillbirth odds ratios and 95% confidence intervals (CIs) were calculated from univariate and multivariable logistic regression models using thresholds for bile acids. Additionally, analysis of variance was used to compare mean levels of bile acids for stillbirth to live birth groups with and without covariate adjustment. The distribution of bile acids was found to be highly skewed to the right. Thus, analysis of variance was conducted on the original scale (x), for completeness, and on a natural logarithmic scale ($\log_e [x+1]$).

Results on the logarithmic scale were back transformed to geometric means and 95% CI to reflect the central tendency of the data. Conclusions from the analysis of variance are appropriately drawn from the results on the logarithmic scale and the corresponding geometric means. All tests were performed at a nominal significance level of $\alpha = 0.05$. All single degree of freedom tests were 2-sided.

The adjusted analyses accounted for stillbirth risk factors known at pregnancy confirmation (baseline) using a risk factor score for stillbirth that was developed on the logit scale using the coefficients from a logistic regression model. The model used data on all SCRNL deliveries where a maternal interview was conducted and a prenatal chart was abstracted. Variables contributing to the baseline risk factor score were those described previously¹⁸ and included the following maternal characteristics: age, race and ethnicity, marital status, education, pregnancy history, body mass index, smoking status, alcohol use, illicit drug use, hyper-tension, diabetes, seizure disorder, blood type, Rh factor, and multiple gestation in current pregnancy, as well as paternal age, family income, insurance and method of payment, and clinical site. All variables included in the score were categorical and an “average” of the regression coefficients associated with the categories was used when a variable was missing for an observation. The average was based on the sample-weighted proportion of live births by category.

Subanalysis was also performed in stillbirths excluding those with major anomalies and obstetric complications as previously described.¹⁸ The Initial Causes of Fetal Death (INCODE) system developed by the SCRNL was used to assign causes of death to each case.¹⁹ Some analyses were performed on subsets of cases and controls, including non-anomalous stillbirths and stillbirths without obstetric complications. Non-anomalous stillbirths excluded those stillbirths with possible or probable causes of stillbirth that included fetal genetic, structural, and karyotypic abnormalities. Cases with possible or probable causes of stillbirth including fetal maternal hemorrhage, cervical insufficiency, preterm labor, preterm premature rupture of membranes, clinical chorioamnionitis, intrapartum death, abruption, complications of multiple gestation, and uterine rupture were excluded in the subset of stillbirths without obstetric complications.

Results

The flow of patients included in the study is shown in the Figure. There were a total of 581 women with stillbirth and 1546 with live births who had adequate data and blood available for assessment of bile acid levels. Cases that did and did not enroll in the study were similar with regard to maternal age, maternal race/ethnicity, insurance/method of payment, and gestational age at delivery. Women with live births who did not enroll differed from those who enrolled by maternal race/ethnicity and gestational age at delivery.¹⁸

The characteristics of women enrolled in the study with and without testing for bile acids are shown in Table 1. Among women with stillbirth, those without testing (eg, refusing blood draw) were more likely to be non-Hispanic black and nulliparous than those with testing. Women with live births who were non-Hispanic black, <20 or 40 years of age, and did not receive early prenatal care were less likely to have test results.

Table 2 depicts levels of maternal bile acids in all stillbirths compared to all live births and to term live births. Bile acids tended to be slightly higher in women with stillbirth (geometric mean [95% CI] = 3.2 [3.0–3.5]) compared to live births (2.9 [2.7–3.1], $P = .0327$). However the difference was not significant after adjustment for baseline risk factors for stillbirth ($P = .23$). The proportion of women with bile acid levels ≥ 10 mmol/L was similar in stillbirths and live births and the same was true for women with levels ≥ 40 μ mol/L. Similar results were noted when all women with stillbirths were compared to women with term live births.

Comparisons of nonanomalous stillbirths and term live births and nonanomalous stillbirths without obstetric complications and term live births are shown in Table 3. When just the subset of nonanomalous stillbirths were compared to term live births, higher levels of bile acids were noted in mothers delivering stillbirths (geometric mean [95% CI] = 3.3 [3.0–3.6]) compared to live births (2.9 [2.7–3.0], $P = .0202$). Again, the difference was not significant after multivariable analysis ($P = .14$) and groups were similar with regard to the proportion of women with bile acid levels ≥ 10 and ≥ 40 μ mol/L. Finally, women with stillbirths not associated with either fetal anomalies or obstetric complications were compared to those with term live births. Mean levels of bile acid were higher in women with stillbirth compared to live birth (geometric mean [95% CI] = 3.4 [3.0–3.8] vs 2.9 [2.7–3.0], $P = .0152$, unadjusted; $P = .06$ adjusted). However, the percentage of women in both groups with bile acid levels ≥ 10 and ≥ 40 μ mol/L were similar.

The area under the curve in the ROC analysis is 0.5306 (95% CI, 0.5019–0.5593); and the threshold that optimizes the distinction between stillbirth and live birth is 5 μ mol/L. Use of this data-driven cutoff is given in Tables 2 and 3, along with the cutoffs of 10 and 40.

In the entire SCRN cohort, there were 4 women with stillbirth and 3 women with live births who had clinical ICP during the current pregnancy. However, only 5 of these (4 women with stillbirth and 1 with a live birth) had sera available for analysis and were included in the current study. Of these, only 2 with stillbirth had elevated levels (29 and 59 μ mol/L) of bile acids when assessed as part of the current study. Both had ICP noted as a possible cause of stillbirth in the parent study (Table 4).

Comment

Maternal bile acid levels were not significantly higher in women with stillbirth after adjustment for potential confounding variables. We did not find a significantly greater proportion of women with elevated levels of bile acids using a threshold of >10 mmol/L. This is the generally accepted threshold for defining ICP.^{10,16} Of course, each laboratory has its own reference range for normal and varying methodologies may influence levels. However, most reference populations do not include pregnant women. A recent Irish study noted normative values of 0.3–9.8 μ mol/L with no change in bile acids over different gestational ages and within 72 hours postpartum.²⁰ Accordingly, this threshold may stand up during pregnancy.

In addition, similar percentages of women with stillbirth and live birth had bile acid levels $>40 \mu\text{mol/L}$. This threshold has been reported to be more strongly associated with stillbirth than $10 \mu\text{mol/L}$.¹⁰ ROC analysis identified $5 \mu\text{mol/L}$ as the threshold with the greatest discrimination between stillbirths and live births in our study, but the area under the ROC curve was very modest, showing little discrimination of the bile acids measurement. Accordingly, our data do not support the assessment of bile acids in asymptomatic patients in the evaluation of stillbirth.

Whether modest differences in bile acid levels are clinically meaningful is not clear. Indeed, slight elevations in the “normal range” are unlikely to have any adverse clinical effects. Another possibility is that stillbirth itself causes slight elevations in maternal serum bile acids. We were unable to precisely ascertain the timing of stillbirth in relationship to the time of blood draw in enough participants to exclude this possibility. Also, women with clinical symptoms (pruritus) may comprise a different population than asymptomatic women with elevated levels of bile acids with regard to stillbirth risk.

The mechanism(s) by which bile acids might increase the risk of stillbirth remains uncertain. There is a strong association between increasing levels of bile acids and in utero meconium passage.^{2,3,21} In turn, there is a strong association between meconium passage and stillbirth in the setting of ICP.¹⁶ In fact, in utero meconium has been reported in $>85\%$ of cases of ICP-associated stillbirth.¹⁶ Nonetheless, it is unclear whether meconium passage is a cause of stillbirth or simply a marker for a distressed fetus.

An attractive hypothesis to explain stillbirth associated with ICP is that bile salts are deposited in the placenta leading to inflammation, ischemia, placental insufficiency, and ultimately stillbirth. However, available data do not support chronic placental insufficiency as a mechanism for ICP-related stillbirth. First, most cases of fetal death associated with ICP are of normally grown infants.³ Second, antepartum testing such as nonstress testing that usually identifies placental insufficiency is unreliable in the prevention of stillbirth in women with ICP.^{3,6,7}

One potential cause of fetal death is constriction of the chorionic vein. Sepulveda et al⁹ noted that bile acids (cholate) can induce vasoconstriction of the human chorionic vein in vitro. This may lead to acute hypoxia and stillbirth. Another potential mechanism of fetal death mediated by bile acids is direct myocardial toxicity. A series of experiments in rats show that taurocholate can lead to impaired myocardial contractility and arrhythmias.^{8,22} This may explain the sudden development of abnormal fetal heart rate tracings noted in cases of ICP.

Some studies suggest that there may be a critical threshold of bile acids with regard to stillbirth risk. Glantz et al¹⁰ reported no increase in the risk of stillbirth in women with bile acid levels $<40 \mu\text{mol/L}$. Similarly, Lucangioli et al²³ noted an increased risk of asphyxia (defined by low 5-minute Apgar scores) associated with higher levels and exposure time to higher levels of bile acids. In contrast, stillbirths have been reported despite normalization of bile acids.^{6,7} Thus, there is no precise bile acid threshold for stillbirth in ICP.

Our study had several weaknesses. First, bile acids were not assessed while participants were fasting. Although some authorities believe that bile acids should be assessed in the fasting state, it remains controversial.¹⁶ In addition, the Irish cohort did not note any differences in bile acids in fasting and nonfasting women. Second, we only measured total bile acids. Cholic acid:chenodeoxycholic acid ratios are elevated in patients with ICP.²⁴ However, 1 study noted that total bile acid level was superior to the cholic acid:chenodeoxycholic acid ratio for the diagnosis of ICP.²⁵ Third, we did not specifically query women about pruritus during their interview. Thus, mild symptoms may not have been ascertained from the medical record. Finally, we did not have precise data regarding the timing of blood draw and the presence or absence of labor. Although unlikely, labor could potentially influence bile acid levels.

There were numerous strengths of the study. Our study systematically evaluated both women with stillbirths and with live births for bile acids. The stillbirth cohort is large and well characterized, allowing us to focus on otherwise unexplained stillbirth. Both cases and controls are ethnically, racially, and geographically diverse, making these data somewhat applicable to the general population in the United States. Lastly, testing of bile acids was performed in a laboratory blinded to clinical status of the participants.

In summary, the proportion of women with elevated levels of bile acids was similar in women with stillbirth and live births using either 10 or 40 $\mu\text{mol/L}$ as a threshold. Thus, our data do not support testing for bile acids in cases of stillbirth in the absence of clinical evidence of ICP.

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FIGURE. Study enrollment and inclusion in cholestasis analysis

This analysis compares bile acid testing results from stillbirth and live birth pregnancies. Pregnancy was categorized as stillbirth pregnancy if there were any stillbirths delivered and as live birth pregnancy if all live births were delivered. Fetal death was defined by Apgar scores of 0 at 1 and 5 minutes and no signs of life by direct observation. Fetal deaths were classified as stillbirths if best clinical estimate of gestational age at death was ≥ 20 weeks. Fetal deaths at 18 and 19 weeks without good dating were also included as stillbirths. Analysis includes comparison of: all stillbirths to all live births; all stillbirths to term live births; nonanomalous stillbirths to term live births; and nonanomalous stillbirths without obstetric (OB) complications to term live births.

Silver. Bile acids and stillbirth. *Am J Obstet Gynecol* 2013.

TABLE 1

Comparison^a of women who were tested for bile acid levels in serum vs those who were not tested—separately for stillbirth and live birth pregnancies

Characteristic—weighted %	Stillbirths		P value	Live births		P value
	Testing	Testing		Testing	Testing	
	Yes	No		Yes	No	
Unweighted sample size, N	581	82		1546	386	
Weighted sample size, N _w	579	84		1183	256	
Maternal age at delivery, y						
<20	13.0%	14.3%	.6075	9.5%	13.9%	.0284
20-34	70.2%	66.1%		76.6%	71.4%	
35-39	11.8%	16.3%		12.2%	10.6%	
40	5.0%	3.2%		1.7%	4.1%	
Maternal race/ethnicity						
White, non-Hispanic	35.8%	17.5%	.0004	47.3%	38.9%	<.0001
African American, non-Hispanic	20.7%	41.2%		9.4%	22.5%	
Hispanic	36.9%	32.3%		36.1%	29.2%	
Other	6.6%	9.0%		7.2%	9.4%	
Marital status/cohabitating						
Not married or cohabitating	25.1%	27.3%	.5635	14.9%	17.3%	.4900
Cohabitating	25.3%	30.2%		23.7%	25.1%	
Married	49.6%	42.6%		61.4%	57.7%	
Maternal education						
0-11 (None/primary/some secondary)	23.4%	26.8%	.6022	18.3%	18.4%	.8478
12 (Completed secondary)	29.3%	32.5%		25.6%	27.3%	
13 (College)	47.4%	40.6%		56.1%	54.3%	
Insurance/method of payment						
No insurance	5.3%	10.0%	.2482	3.6%	3.1%	.4770
Any public/private assistance	53.7%	52.3%		47.9%	52.1%	
VA/commercial health insurance/HMO	40.9%	37.8%		48.5%	44.8%	
Income						
Only public/private assistance	9.3%	3.7%	.3708	5.9%	5.9%	.7436
Assistance and personal income	37.9%	37.7%		37.0%	39.6%	
Only personal income	52.8%	58.7%		57.1%	54.4%	
No first- or second-trimester prenatal care	7.3%	12.5%	.1292	2.5%	4.7%	.0328
Gestational age, mo						
18-19	2.3%	3.7%	.1593	0.0%	0.0%	.2648
20-23	32.0%	46.2%		0.4%	0.3%	
24-27	15.9%	15.4%		0.7%	1.0%	

Characteristic-weighted %	Stillbirths			Live births		
	Testing			Testing		
	Yes	No	P value	Yes	No	P value
28-31	12.9%	11.3%		1.0%	1.2%	
32-26	19.8%	11.6%		8.2%	10.6%	
37	17.0%	11.8%		89.8%	86.9%	
Nulliparous	43.4%	56.1%	.0373	34.6%	38.7%	.2342
Multifetal pregnancy	6.5%	4.2%	.4069	1.7%	2.9%	.2440

HMO, health maintenance organization; VA, Departments of Veterans Affairs.

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^aResults shown are weighted for study design and differential consent based on characteristics recorded in screened population. Overall sample sizes are given, unweighted and weighted. Sample sizes for weighted percentages vary slightly by characteristic.

TABLE 2

Maternal serum bile acid levels in all stillbirths, all live births, and term^b live births^a

Bile acid levels, $\mu\text{mol/L}$	Stillbirths	Live births	Term live births	Stillbirths vs live births ^d		Stillbirths vs term live births ^d	
				Unadjusted	Adjusted ^e	Unadjusted	Adjusted ^e
Unweighted sample size, N	581	1546	1163				
Weighted sample size, N _w	579	1183	1062				
Arithmetic mean (95% CI)	5.3 (4.2–6.3)	4.4 (3.9–4.8)	4.2 (3.8–4.7)	<i>P</i> = .1211	<i>P</i> = .2947	<i>P</i> = .0801	<i>P</i> = .2133
Geometric mean (95% CI) ^c	3.2 (3.0–3.5)	2.9 (2.7–3.1)	2.9 (2.7–3.0)	<i>P</i> = .0327	<i>P</i> = .2271	<i>P</i> = .0171	<i>P</i> = .1387
Median (minimum-maximum)	2.5 (0–193)	2.0 (0–108)	2.0 (0–108)				
Cut points:				OR (95% CI)		OR (95% CI)	
5 $\mu\text{mol/L}$	32.3%	25.0%	24.6%	1.43 (1.14–1.79)	1.27 (1.00–1.61)	1.46 (1.16–1.84)	1.31 (1.02–1.68)
10 $\mu\text{mol/L}$	9.2%	7.9%	7.2%	1.19 (0.83–1.69)	1.01 (0.69–1.49)	1.30 (0.90–1.89)	1.10 (0.73–1.66)
40 $\mu\text{mol/L}$	1.0%	0.8%	0.7%	1.25 (0.46–3.42)	1.09 (0.42–2.82)	1.43 (0.50–4.07)	1.12 (0.41–3.09)

CI, confidence interval; OR, odds ratio.

Silver. Bile acids and stillbirth. Am J Obstet Gynecol 2013.

^aTerm = gestational age $\geq$ 37 wk at birth

^bResults shown are weighted for study design and differential consent based on characteristics recorded on all eligible pregnancies that were screened for study-overall sample sizes are given, unweighted and weighted

^cGeometric mean (95% CI) is based on natural logarithmic transformation of bile acids values, $\log_e(x+1)$, where bile acids value is denoted by x

^d*P* value from weighted analysis of variance

^eAdjusted *P* values and OR account for pre-pregnancy risk factors for stillbirth via score developed on logit scale and using data on all participants to Stillbirth Collaborative Research Network. Variables contributing to baseline risk factor score included following: maternal characteristics: age, race/ethnicity, marital status, education, pregnancy history, body mass index, smoking status, alcohol use, illicit drug use, hypertension, diabetes, seizure disorder, blood type, Rh factor, and multiple gestation in current pregnancy, as well as paternal age, family income, insurance/method of payment, and clinical site. Score is available on 552 of 581 stillbirth pregnancies, 1498 of 1546 live birth pregnancies, and 1131 of 1163 term live birth pregnancies.

TABLE 3

Maternal serum bile acid levels in nonanomalous stillbirths, nonanomalous stillbirths without obstetric complications, and term ^a live births ^b

Bile acid levels, $\mu\text{mol/L}$	Nonanomalous stillbirths	Nonanomalous stillbirths without complications	Term live births	Nonanomalous stillbirths vs term live births ^d		Nonanomalous stillbirths without complications vs term live births ^d	
				Unadjusted	Adjusted ^e	Unadjusted	Adjusted ^e
Unweighted sample size, N	390	273	1163				
Weighted sample size, N _w	387	268	1062				
Arithmetic mean (95% CI)	5.7 (4.1–7.2)	6.2 (4.0–8.4)	4.2 (3.8–4.7)	P = .0846	P = .1796	P = .0829	P = .1214
Geometric mean (95% CI) ^c	3.3 (3.0–3.6)	3.4 (3.0–3.8)	2.9 (2.7–3.0)	P = .0202	P = .1443	P = .0152	P = .0614
Median (minimum–maximum)	2.5 (0–193)	2.5 (0–193)	2.0 (0–108)				
Cut points:				OR (95% CI)		OR (95% CI)	
5 $\mu\text{mol/L}$	32.2%	33.5%	24.6%	1.45 (1.12–1.89)	1.27 (0.96–1.69)	1.54 (1.15–2.07)	1.36 (0.99–1.86)
10 $\mu\text{mol/L}$	10.2%	10.7%	7.2%	1.47 (0.98–2.22)	1.20 (0.76–1.89)	1.54 (0.97–2.44)	1.29 (0.78–2.15)
40 $\mu\text{mol/L}$	1.4%	1.7%	0.7%	2.15 (0.75–6.12)	1.66 (0.60–4.58)	2.58 (0.85–7.84)	2.28 (0.79–6.56)

CI, confidence interval; OR, odds ratio.

Silver. Bile acids and stillbirth. Am J Obstet Gynecol 2013.

^aTerm = gestational age $\geq$ 37wk at birth

^bResults shown are weighted for study design and differential consent based on characteristics recorded on all eligible pregnancies that were screened for study—overall sample sizes are given, unweighted and weighted

^cGeometric mean (95% CI) is based on natural logarithmic transformation of bile acids values, $\log_e(x+1)$, where bile acids value is denoted by x

^dP value from weighted analysis of variance

^eAdjusted P values and OR account for prepregnancy risk factors for stillbirth via modification to score developed on logit scale and using data on all participants to Stillbirth Collaborative Research Network. Variables contributing to baseline risk factor score included following maternal characteristics: age, race/ethnicity, marital status, education, pregnancy history, body mass index, smoking status, alcohol use, illicit drug use, hypertension, diabetes, seizure disorder, blood type, Rh factor, and multiple gestation in current pregnancy, as well as paternal age, family income, insurance/method of payment, and clinical site. Score is available on 377 of 390 nonanomalous stillbirth pregnancies, 262 of 273 nonanomalous stillbirth pregnancies without obstetric complications, and 1131 of 1163 term live birth pregnancies.

TABLE 4

Cases of intrahepatic cholestasis of pregnancy documented during pregnancy

ID	Maternal age, y	Race/ethnicity	Parity	Plurality	Gestational age, wk	Bile acids value, micromol/L	ICP treatment during pregnancy	SB/LB status	SCRN cause of death
1	28	Hispanic	Multiparous	Singleton	30	29	Ursodeoxycholic acid	SB	Possible cause: ICP
2	24	Black, non-Hispanic	Multiparous	Singleton	18	1	None noted	SB	Probable cause: massive subchorionic hematoma, maternal floor fibrin deposition
3	27	White, non-Hispanic	Multiparous	Twins	27	1	Hydroxyzine	SB	Probable cause: complication of monochorionic multiple gestation
								LB	NA
4	20	Hispanic	Nulliparous	Singleton	34	59	Diphenhydramine	SB	Possible cause: ICP
5	25	White, non-Hispanic	Nulliparous	Singleton	41	4	Treatment not specified	LB	NA

ICP, intrahepatic cholestasis of pregnancy; LB, live birth; NA, not applicable; SB, stillbirth; SCRN, Stillbirth Collaborative Research Network.

Silver. Bile acids and stillbirth. Am J Obstet Gynecol 2013.