Serial Salivary Estriol to Detect an Increased Risk of Preterm Birth

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Objective: **To evaluate serial measurements of salivary estriol (E3) to detect increased risk of spontaneous preterm labor and preterm birth.**

Methods: **A masked, prospective, multicenter trial of 956 women with singleton pregnancies was completed at eight United States medical centers. Saliva was collected weekly, beginning at the 22nd week of gestation until birth, and tested for unconjugated E3 by enzyme-linked immunosorbent assay. Women were separated into high-risk and low-risk groups using the Creasy scoring system.**

Results: **A single, positive (at or above 2.1 ng/mL) salivary E3 test predicted an increased risk of spontaneous preterm labor and delivery in the total population (relative risk [RR] 4.0, $P < .005$), in the low-risk population (RR 4.0, $P \leq .05$), and in the high-risk population (RR 3.4, $P = .05$). Two consecutive positive tests significantly increased the RR in all study groups, with a dramatic improvement in test specificity and positive predictive value but only a modest decrease in sensitivity. In women who presented with symptomatic preterm labor, salivary E3 identified 61% of those who delivered within 2 weeks, using a threshold of 1.4 ng/mL.**

Conclusion: **Elevated salivary E3 is associated with increased risk of preterm birth in asymptomatic women and symptomatic women who present for evaluation of preterm labor. (Obstet Gynecol 2000;96;490–7. © 2000 by The American College of Obstetricians and Gynecologists.)**

Recent data show that 11% of women in the United States deliver before completing 37 weeks' gestation.[1] Sequelae of biologic immaturity at birth can persist for life and can engender immense direct and indirect costs. Efforts to prevent preterm birth have failed largely because of the heterogeneous nature of the condition and our inability to define which women would benefit from specific treatments. Interventions are most likely to be effective if pathophysiologic processes of preterm parturition can be identified and specifically remedied.

Biochemical markers directly linked to biologic mechanisms of parturition are needed so etiologic-based interventions can be tested. Because placental estrogens are important in mammalian parturition, their potential as biomarkers of impending term, preterm, or post-term labor and delivery has been explored.[2–6] Of the three major placental estrogens, estriol (E3), estradiol (E2), and estrone (E1), E3 is the most abundant during late pregnancy.[2,7–9] In humans, over 90% of E3 is derived from fetal and placental sources.[10] Maternal serum E3 is first detectable at 9 weeks' gestation.[2,7,11] Serum E3 levels increase gradually during the first and second trimesters. More rapid increases occur during the third trimester, with a characteristic surge preceding the onset of labor by several weeks.[12] The increase in E3 has been shown by direct measurement or by an increase in salivary E3: progesterone ratio. This increase occurs approximately 3 to 4 weeks before the onset of parturition, in term, preterm, and post-term pregnancies.[12–14] Additionally, women with prolonged pregnancies who showed increases in salivary E3 were more likely to deliver spontaneously than women who did not have E3 increases.[15]

Saliva is a useful matrix for measuring E3 steroid hormones because the unbound, unconjugated (free, biologically active) fraction of E3 in saliva correlates

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with free serum concentrations. We determined the feasibility of salivary E3 in detecting increased risk of preterm labor.[16] In that study, subjects typically had a surge or increase of salivary E3 approximately 3 weeks before parturition, regardless of whether delivery was preterm, term, or post-term. Using those data, a receiver operating characteristic (ROC) curve was constructed, and a salivary E3 level at or above 2.1 ng/mL predicted preterm labor more accurately than traditional risk assessment methods.[16]

In the current study we prospectively examined weekly saliva samples in women with singleton pregnancies at eight tertiary care sites in the United States to determine the utility of salivary E3 for identifying women at increased risk for preterm birth.

Materials and Methods

We conducted a prospective, longitudinal, masked, multicenter study to observe pregnant women from enrollment to delivery. It was approved by the research review board at each institution. Subjects gave informed written consent in English or Spanish. The trial was conducted between May 1995 and December 1996 at the antenatal clinics of eight United States medical centers. Neither care providers nor subjects had access to test results, and laboratory personnel were masked to clinical information.

Subjects were instructed to collect unstimulated saliva between 9 AM and 8 PM because no significant variation in E3 levels has been found between those hours.[17] Each subject was asked to rinse her mouth with water, wait 10 minutes, then drool 2 mL of saliva into the plastic tube that was provided. Subjects were asked to avoid eating, drinking, smoking, tooth brushing, mouth washing, or gum chewing for 1 hour before collection.

Prior unpublished data showed no difference in mailed samples and those collected in clinic and frozen promptly. Samples collected at home were sent in mailers to the clinic, where they were stored (−20C). Samples were kept frozen at −20C until all subjects delivered. Samples were masked and randomized by computer-generated random number list at each study center, then mailed to Biex, Inc. (Dublin, CA), for batch assaying. Randomization of samples was done before mailing to guarantee masking of laboratory personnel. Samples were tested for unconjugated (free) E3 using a described competitive microplate enzyme immunoassay (SalEst; Biex, Inc., Dublin, CA).[16] Salivary E3 at least 2.1 ng/mL was designated as positive.

For study purposes, preterm labor and delivery were defined as spontaneous preterm labor with intact membranes that resulted in preterm birth within 72 hours and before 37 completed weeks' gestation, respectively. Symptomatic preterm labor was defined as presentation to labor and delivery for evaluation of symptoms consistent with preterm labor. Gestational age was determined by last menstrual period (LMP), ultrasound examination(s), obstetric assessment, and examination of the neonate. If there was more than a 14-day difference between the age determined by LMP and ultrasound, the earliest ultrasound measurement and examination of the neonate were used to assign gestational age at birth. Gestational age determinations were made before test specimens were processed. Women with singleton pregnancies were considered eligible if their pregnancies were between 21 and 25 weeks' gestation, they were at least 18 years of age, and gave informed consent. Exclusion criteria included symptoms of preterm labor; tocolytic therapy; placenta previa; cervical changes requiring cerclage; ruptured membranes in the current pregnancy; preeclampsia; medications known to affect hormone levels (eg, Dilantin, Haldol); planned cesarean delivery; presence of major congenital abnormalities, fetal growth restriction, erythroblastosis fetalis, or fetal death; an oral condition that would interfere with saliva collection; a maternal medical complication that requires specialized obstetric care (eg, chronic hypertension, renal failure, or any other serious medical condition); or history of drug or ethanol abuse.

Risk status for preterm labor was designated by the Creasy score: a score of at least 10 was considered high-risk and below 10 was considered low-risk.[1] Subjects were enrolled at 21–25 weeks' gestation and observed at weekly intervals until delivery. A baseline examination including assessment for signs and symptoms of preterm labor (abdominal pain, backache, flulike symptoms, pelvic pressure, cramping, change in vaginal discharge, vaginal bleeding, diarrhea); evaluation for contractions or rupture of membranes; and a digital vaginal examination to assess the position, consistency, length, and dilatation of the cervix was completed at enrollment. Demographic and obstetric information, including details of prior pregnancies and the current pregnancy, was obtained from patients' medical records. Subsequent clinic visits included questioning of subjects for signs or symptoms of preterm labor and performing a cervical examination every 4 weeks beginning at week 27, with recording of changes in medication, hospitalization, emergency department visits, and appreciation of uterine contractions. Inquiries intended to ensure that home saliva samples were collected and submitted properly were made at each visit.

In the event of hospitalization or outpatient evaluation for signs and symptoms of preterm labor or preterm rupture of membranes (PROM), saliva collection was continued, and data on symptoms of preterm labor

(abdominal pain, cramping, backache, flu-like symptoms, pelvic pressure, change in vaginal discharge, vaginal bleeding, contractions, and treatments), were recorded. Delivery and neonatal data were collected from hospital records.

Subjects were withdrawn from the study if they did not supply saliva samples for 3 consecutive weeks or did not attend two consecutive clinic visits (unless hospitalized and supplying weekly saliva samples). Women who received betamethasone or dexamethasone to induce fetal lung maturation were not included in the primary analysis because of known suppression of salivary E3 by corticosteroids.[19] The effects of tocolytics on salivary E3 are not known; therefore, those women also were excluded from analysis. Women with PROM and preterm births caused by obstetric indications were also excluded from primary analysis. Women who had preterm labor and term births also were excluded. These exclusions ensured focused evaluation of serial salivary E3 measurement for detecting women at risk for preterm birth.

We also evaluated the ability of salivary E3 to determine the risk of preterm birth within 2 weeks of admission in women admitted with signs and symptoms of preterm labor. Patients who had salivary E3 tests available within 2 weeks before admission and in whom delivery outcome was known were eligible for this separate analysis. Women subsequently treated with betamethasone or tocolytics were not excluded from analysis in this symptomatic group.

A power analysis was done to determine the sample required to detect a difference in the incidence of preterm labor and delivery between women with a salivary E3 level of at least 2.1 ng/mL and those with salivary E3 less than 2.1 ng/mL. Based on results of a previous study, a sample of 150 high-risk women and 110 low-risk women was estimated assuming a two-tailed significance level of .05, a power of 80%, and the ability to detect a difference of 5–20% for an effect size of approximately 0.5. This sample was based on preterm birth prevalence of 18% for high-risk and 19% for low-risk groups in our previous study, and we assumed that there would be 12 spontaneous preterm births in each group. The high background prevalence of preterm birth in the low-risk group and the exclusion criteria for betamethasone and tocolytic use made us decide to enroll women until approximately 12 evaluable preterm births were available for analysis in both groups.

Data for the primary endpoint, difference in incidence of preterm labor followed by preterm delivery (before 37 weeks) between women with salivary E3 at least 2.1 ng/mL and those with salivary E3 less than 2.1 ng/mL, were analyzed using Fisher exact test (two-tailed), with alpha at 0.05. Relative risks (RRs) and 95% confidence intervals (CIs) were calculated. The high- and low-risk groups were combined and evaluated using the Mantel-Haenszel χ^2 test and Woolf test. The Woolf test was used with the Mantel-Haenszel to test the heterogeneity of the odds ratio (OR) between low- and high-risk studies. An insignificant finding suggests that intrastudy results were equivalent; therefore, results from the combined analysis can be taken at face value. Center-to-center variation was assessed with a three-factor (gestational age, clinical center, preterm delivery) repeated measures analysis of variance with repeated analysis on the maximum intrapatient salivary E3 value collected during each gestational week. To avoid equivocal definitions of preterm labor, the primary analysis compared salivary E3 findings for subjects with preterm labor and delivery with those of subjects who delivered at term without preterm labor. Survival curves were constructed using data from all evaluable subjects to determine time to delivery after a single positive test at varying gestational ages using Kaplan-Meier estimates of survival. Survival curves were compared by log-rank test.

We also evaluated the utility of salivary E3 for predicting delivery intervals in women who presented with symptoms of preterm labor. Absolute levels in women who delivered within 2 weeks of presentation were compared with those who delivered after 2 weeks by Student *t* test. Receiver operating characteristic curves were generated to determine optimal salivary E3 value that determined risk of preterm birth within 2 weeks. Descriptive statistics were calculated on the value with the optimal combination of sensitivity and specificity.

Results

We enrolled 956 pregnant women, 302 (31.6%) of whom were designated high risk and 654 (68.4%) as low risk. Demographic characteristics of eligible women at baseline were similar among test centers with respect to age distribution, prepregnancy weight, obesity, weight at enrollment, height, number of children, gravidity, number of abortions, and number of preterm and term pregnancies. Most women were 20–35 years old (78%), weighed over 100 pounds (92%), were married or living with a mate (67%), had not had other preterm pregnancies (81%), had not had abortions (53%), and had at least one child (59%). Most (83.0%) high-risk women had one risk factor worth 10 points (eg, premature delivery, uterine anomaly, second-trimester abortion). Demographic characteristics of women who were salivary E3–positive versus negative are presented in Table 1. No differences were noted between groups. The dispo-

Table 1. Demographic Characteristics of Subjects With High and Low Salivary Estriol Levels

	Salivary E3 ≥2.1 ng/mL (n = 187)	Salivary E3 <2.1 ng/mL (n = 769)	Combined (n = 956)
Age (y)	27.3	26.1	26.4
Race (%)			
White	49.7	61.6	59.3
Black	13.4	18.1	17.2
Asian	5.3	4.4	4.6
Hispanic	26.2	13.0	15.6
Other	5.4	2.9	3.3
Prepregnancy weight (lbs)	142.9	148.5	147.4
Weight at enrollment (lbs)	155.6	164.8	163.0
Education (y) (%)			
≤12	49.7	48.4	48.6
>12-≤16	38.5	37.7	37.9
>16	9.1	9.5	9.4
Not reported	2.7	4.4	4.1
Marital status (%)			
Single	26.2	30.4	29.6
Married	54.0	51.6	52.0
Live-in partner	17.7	14.4	15.1
Divorced or separated	2.1	3.6	3.4
Gravida (%)			
1	24.1	23.8	23.9
>1	75.9	76.2	76.1
Abortions (%)			
0	56.7	51.6	52.6
1	26.2	29.4	28.8
2	8.0	11.8	11.1
≥3	8.0	6.2	6.6
Not reported	1.1	1.0	0.9
Prior preterm births (%)			
0	82.4	80.7	81.1
1	14.4	13.8	13.9
≥2	2.6	4.5	4.2
Not reported	0.5	1.0	0.8

E3 = estriol.
P = not significant for all comparisons.

sition of all subjects is shown in Figure 1. There was no center-to-center variation in salivary E3 results.

Numbers of enrolled and analyzed subjects and subjects excluded from analysis of primary endpoints are shown in Table 2. No demographic differences were statistically significant between analyzed and excluded subjects. Noncompliance was the most common reason for discontinuation from the study (147 of 242), followed by development of exclusion criteria after enrollment (49 of 242), fetal death (four of 242), and those lost to follow-up (42 of 242). Seven hundred fourteen women were initially eligible for the primary analysis. We excluded 113 of them because of the reasons listed in Table 2. Primary analysis was done on two groups (n = 601), preterm labor followed by preterm delivery,

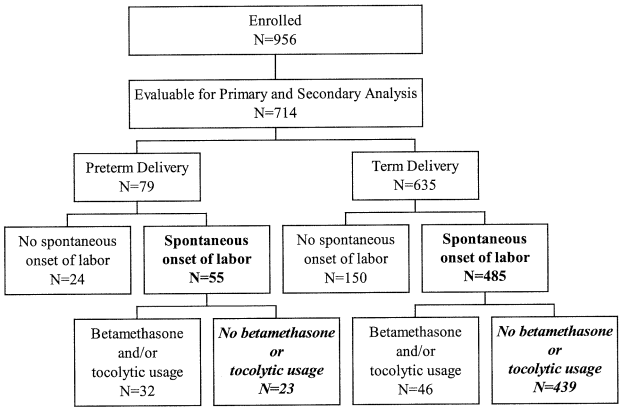

Figure 1. Outcomes of study subjects.

and term deliveries. Among 601 women, 449 were low risk and 152 were high risk. There were 578 term deliveries (141 high risk and 437 low risk) and 23 preterm deliveries (11 high risk and 12 low risk) in the primary analysis. Median gestational age of delivery in women who delivered preterm was 36 weeks (range 34–36 weeks).

A total of 13,347 samples were submitted. Thirty-one (0.23%) could not be analyzed because of insufficient saliva volume or tubes broken in mailing. The mean (± standard deviation [SD]) interval between samples was 1.1 ± 0.1 weeks for evaluable subjects.

Initially we validated the cut-off point of 2.1 ng/mL comparing ROC curves generated from women in the current study to curves from the pilot study. As shown in Figure 2, there was a slight decrease in overall test sensitivity in the current study, but a cutoff of 2.1 ng/mL generated optimal sensitivity and specificity. If

Table 2. Numbers of Subjects for Primary Endpoints

Subjects	Enrolled	Analyzed
High risk	302 (31.5%)	152 (25.3%)
Low risk	654 (68.5%)	449 (74.7%)
Combined groups	956	601
Preterm births <37 weeks	121 (12.6%)	23 (3.8%)
Spontaneous preterm births	71 (7.4%)	23 (3.8%)
Preterm PROM	21 (2.2%)	0
Medically indicated	29 (3.0%)	0
Subjects excluded from the primary analysis		355
Treated with betamethasone/dexamethasone or tocolytics		35
Preterm PROM		13
Medically indicated preterm births		10
Term with preterm labor		55
Subjects not eligible		242

PROM = premature rupture of membranes.

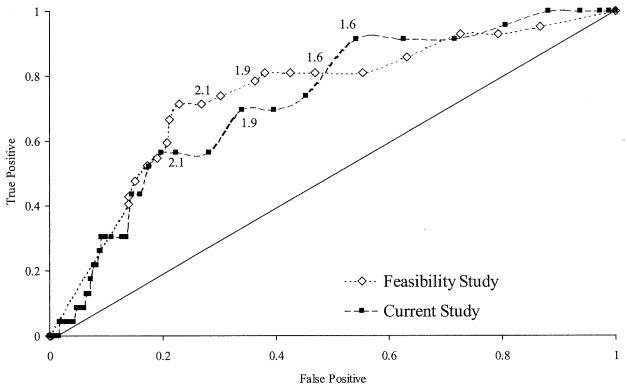

Figure 2. Receiver operating characteristic analysis: maximum intrapatient salivary estriol value and its ability to predict preterm birth, considering values collected at 24–36 weeks' gestation ($n = 601$) in our feasibility study and the current study.

the cut-off point level of salivary E3 was lowered to 1.9 ng/mL, sensitivity increased to 70%, but the false-positive rate increased from 22% to 34%. A value of 1.6 ng/mL detected 91% of women who delivered preterm with a false-positive rate of 54%. No women delivered prematurely below a value of 1.2, but the false-positive rate at that level was 88%.

As shown in Table 3, a salivary E3 level of at least 2.1 ng/mL associated significantly with preterm birth in each group ($P < .05$) with risk ratios ranging from 3.4 to 4.2. In the low-risk population, a single positive salivary E3 test before 36 completed weeks' gestation was a significant risk indicator for preterm delivery ($P = .018$) identifying 50% (95% CI 21.1%, 78.9%) of the spontaneous preterm births. A single positive test also identified 64% (95% CI 30.8%, 89.1%) of women who delivered preterm in the high-risk group. In the combined population, 57% (95% CI 34.5%, 76.8%) of women who had preterm deliveries had at least one elevated salivary E3 test.

The predictive accuracy of the salivary E3 test was enhanced by a second positive test of new saliva sample collected 1 week after the first positive test (Table 4). The occurrence of spontaneous preterm labor and delivery in women with a second elevated E3 level was 19%. Only 2% of women who had a salivary E3 below 2.1 ng/mL on the second test had spontaneous preterm labor and delivery. The number of women identified as at risk for preterm labor was reduced from 23.6% (142 of 601, overall incidence of having a single positive test) to 9.0% (54 of 601, the incidence of having a positive second test). The incidence of preterm labor with delivery in women with two consecutive salivary E3 tests of at least 2.1 ng/mL compared with incidence of women with salivary E3 under 2.1 ng/mL was 14.2% versus 1.7% in the low-risk population, 26.3% versus 4.5% in the high-risk population, and 18.5% versus 2.4% in the combined population.

Analyses were done to determine time to delivery from a positive test. Among eligible women, 22 had at least one positive salivary E3 test and delivered preterm. The median time to delivery in that group was 3.6 weeks. Among women who were eligible for the primary analysis and had at least one positive salivary E3 test, the median time to preterm birth was 2.3 weeks. Eighty-six percent of those women delivered in fewer than 3 weeks (Table 4). All women who had preterm births and a second positive test delivered in less than 3 weeks.

Table 4. Time Interval to Delivery in Women With a Single Positive or a Second Positive Test

Weeks to delivery	Single test ($n = 13$)	Second positive test ($n = 10$)
<1	23.1% (5.0, 53.8)	70.0% (34.8, 93.3)
<2	53.9% (25.1, 80.8)	90.0% (55.5, 99.8)
<3	84.6% (54.6, 98.1)	100.0% (69.2, 100.0)
<4	92.3% (64.0, 99.8)	100.0% (69.2, 100.0)
<5	92.3% (64.0, 99.8)	100.0% (69.2, 100.0)

Data are given as % (95% confidence interval).

Table 3. Predictive Accuracy of Salivary Estriol

	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Relative risk
Combined population					
Single positive test*	57 (34.5, 76.8)	78 (74.1, −81.0)	9 (5.0, 15.2)	98 (96.0, 99.0)	4.2 (1.9, 9.4)
Second positive test*	44 (23.2, 65.5)	92 (89.9, 94.4)	19 (9.3, 31.4)	98 (96.0, 98.7)	7.8 (3.6, 16.9)
Low-risk population					
Single positive test†	50 (21.1, 78.9)	81 (76.8, 84.4)	7 (2.5, 14.0)	98 (96.4, 99.4)	4.0 (1.3, 12.1)
Second positive test*	42 (15.2, 72.3)	93 (90.0, 95.3)	14 (4.8, 30.3)	98 (96.6, 99.3)	8.5 (2.8, 25.2)
High risk population					
Single positive test†	64 (30.8, 89.1)	68 (59.7, 75.7)	14 (5.6, 25.8)	96 (90.1, 98.9)	3.4 (1.0, 11.0)
Second positive test*	46 (16.8, 76.7)	90 (83.9, 94.5)	26 (9.2, 51.2)	96 (90.4, 98.3)	5.8 (2.0, 17.3)

Data are given as % (95% confidence interval).
* $P \leq .05$.
† $P \leq .005$.

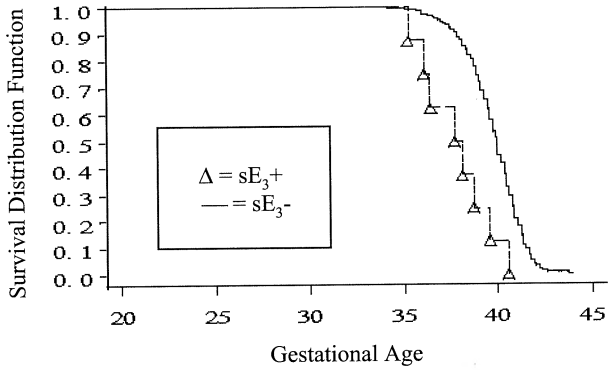

Figure 3. Survival analysis for subjects with single positive salivary estriol (sE3) (at or below 2.1 ng/mL) at under 28 weeks' gestation compared with all subjects.

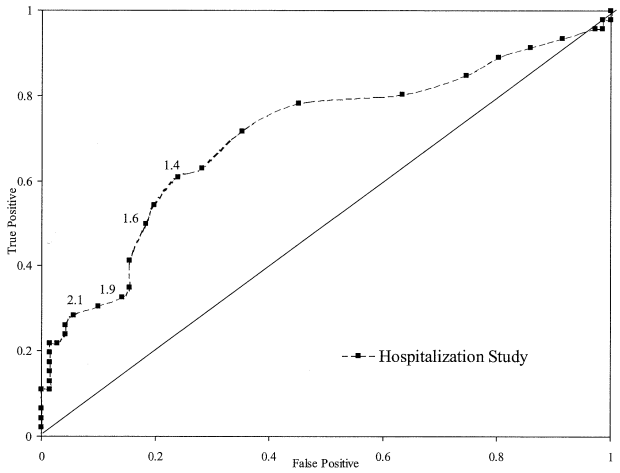

Figure 4. Estriol value collected no more than 1 week before initial hospital admission. Positive result defined as delivery at or less than 2 weeks from the date of admission.

Survival analysis that represented time from a single positive or negative test at a specific gestational age to delivery was done on the entire population ($n = 714$), including preterm deliveries treated with betamethasone or tocolytics, PROM, and medically indicated preterm births. Survival analysis found significant shortening of gestation in women with a single positive test at under 28 weeks' (Figure 3), 32 weeks', or 34 weeks' gestation (data not shown) compared with all subjects ($P < .05$).

Among the 714 subjects, 158 presented with signs or symptoms of preterm labor. Of those, 115 met criteria for preterm labor diagnosis. The remaining 43 were evaluated for preterm contractions. The incidence values for symptomatic women were done on an intent-to-treat basis. The average time to delivery was 5.4 ± 4.9 weeks after presentation. Delivery within 2 weeks occurred in 35% of subjects. The mean ±SD salivary E3 value within 1 week before admission was 1.95 ± 1.44 ng/mL in those who delivered within 2 weeks versus 1.16 ± .60 ng/mL in those who did not ($P < .001$). Two weeks before admission the values were 1.85 ± 1.40 and 1.18 ± 0.57 ng/mL, respectively ($P = .001$). Receiver operating characteristic curves to determine the ideal threshold of salivary E3 to determine the risk of preterm delivery are presented in Figure 4. A salivary E3 level of at least 2.1 ng/mL identified 29.4% of deliveries within 2 weeks with a false-positive rate of 7.8%. The ideal salivary E3 threshold appeared to be 1.4 ng/mL, which had a sensitivity of 61% and a specificity of 76%.

Discussion

In this prospective trial, incidence of preterm labor and delivery was greater in women who had at least one salivary E3 value of 2.1 ng/mL or greater on longitudinally collected salivary samples, which supports an established threshold value. Regardless of a woman's risk classification, the risk of preterm birth was substantially higher in women with at least one elevated salivary E3 test. Reduction of the threshold value significantly enhanced the sensitivity of the test (Figure 3), but the false-positive rate increased.

The ability to determine preterm birth in the single positive test group was offset by the low positive predictive values of 7–14%. Two consecutive, elevated salivary E3 levels improved the predictive accuracy of the test. In both study groups, the RR for preterm birth approximately doubled. Specificity and positive predictive value improved with a positive second test, with only slight diminution in test sensitivity. The negative predictive value for preterm birth after spontaneous preterm labor for single and second positive tests was 98%, which means that it is highly unlikely that preterm labor and delivery will occur in women with low salivary E3 values.

Among our population, 22% of women presented with symptoms of preterm labor. Most of those women delivered more than 5 weeks after presentation. Among women who delivered within 2 weeks of presentation, salivary E3 values were significantly higher at 1 and 2 weeks before admission. At our threshold of 2.1 ng/mL, salivary E3 identified only 29% of those who delivered within 2 weeks. That increased to 61% when the threshold salivary E3 value was decreased to 1.4 ng/mL, but specificity decreased from 92% to 76%.

Salivary E3 testing was similarly accurate in predicting preterm birth in low- and high-risk groups. Salivary E3 testing was accurate in primiparous women and those with no recognized risk factor. Strengths of this trial included the large number of subjects in geograph-

ically diverse populations and the masking. Weaknesses included the relatively few preterm births despite many enrollees, as a consequence of our stringent criteria, which excluded women with multiple gestations, PROM, and those treated with corticosteroids and tocolytics, except in the survival analysis and in our symptomatic patient analysis. Such exclusion criteria were designed to increase internal validity but might reduce external validity, ie, applicability to other groups of women.

A further limitation of the study was lack of experience with very early preterm births (ie, before 30 weeks' gestation). In this trial, all the E3-positive preterm births were after 32 weeks' gestation. This finding is not surprising because 80% of preterm births occur between 34 and 36 weeks. Most of our subjects who delivered at earlier gestations were evaluated for symptoms of premature labor but delivered on average more than 5 weeks after the initial event. Almost all received betamethasone at initial presentation, which suppresses E3 production, and tocolytics, which have unknown effects, making levels of E3 after initial presentation difficult to interpret.

Infection accounts for approximately 30% of preterm births at less than 32 weeks.[20,21] Estriol might not identify infection-mediated preterm birth at earlier gestations but theoretically would identify women with idiopathic preterm labor. Survival analysis, which found a shortening of gestational age if E3 was positive at less than 28, 32, or 34 weeks' gestation, supports that concept. The ability of salivary E3, albeit at a lower threshold of 1.4 ng/mL, to predict most but not all women who will deliver with symptoms of preterm labor, provides further support.

Potential explanations of the lower salivary E3 threshold required to identify symptomatic women who then deliver include time of collection relative to delivery and gestational age at collection. The salivary E3 specimens collected 1 to 2 weeks before preterm labor symptoms were up to 3 to 4 weeks from actual delivery and might represent initiation of the salivary E3 surge, which would have continued had treatment not been initiated. There also was a slight increase in salivary E3 normally seen with advancing gestation that suggests a lower threshold might be necessary at the earlier gestational ages at which those women presented.

Salivary E3 testing offers several advantages as a risk-assessment marker for preterm labor. Testing for elevated levels of salivary E3 (at least 2.1 ng/mL) is simple, convenient, and noninvasive. Samples can be collected at home or in the clinic. Serial salivary E3 determinations can also be easily integrated into common pregnancy management protocols. Physical signs and symptoms of preterm labor can be subtle, but if they escape recognition, the opportunity for early treatment might be missed.[9,22,23] Use of an objective biochemical test, such as salivary E3, can aid early detection of preterm labor before physical symptoms are manifest. Threshold levels of salivary E3 were achieved approximately 3 weeks before parturition, so there might be time to evaluate previously unproved interventions, such as decreased maternal activity or administration of prophylactic tocolytics, which might improve pregnancy outcomes.

Salivary E3 is a noninvasive biologic marker that can be used to assess risk for spontaneous preterm labor and delivery in asymptomatic and symptomatic women. Serial salivary E3 testing can improve accuracy in assessment of risk of preterm birth, especially in women at high risk of preterm labor and delivery using traditional scoring methods. Unproved interventions, such as weekly office visits with serial cervical examinations and discontinuation of work and family responsibilities, can be avoided with most women previously deemed at high risk. Future studies to evaluate potential treatments of women with elevated salivary E3 are required before its use can be recommended in the general low-risk obstetric population.

References

1. The Centers for Disease Control and Prevention. Births: Final data for 1997. National Vital Statistics Reports. Vol. 47, no. 18. Hyattsville, Maryland: National Center for Health Statistics, 1999:2.
2. Speroff L, Glass RH, Kase NG. The endocrinology of pregnancy. In: Speroff L, Glass RH, Kase NG, eds. Clinical gynecologic endocrinology and infertility. 5th ed. New York: Williams & Wilkins, 1994:251–89.
3. Fuchs F. Endocrinology of parturition. In: Fuchs F, ed. Endocrinology of pregnancy. 3rd ed. New York: Harper & Row, 1983:247–70.
4. Schatz F, Markiewicz L, Gurpide E. Effects of estriol on $PGF_{2\alpha}$ output by cultures of human endometrium and endometrial cells. J Steroid Biochem 1984;20:999–1003.
5. Garfield RE, Hayashi RH. Appearance of gap junctions in the myometrium of women during labor. Am J Obstet Gynecol 1981; 140:254–60.
6. Fuchs AR, Fuchs F, Husslein P, Soloff MS. Oxytocin receptors in the human uterus during pregnancy and parturition. Am J Obstet Gynecol 1984;150:734–41.
7. Fuchs AR, Fuchs F, Stubblefield PG. Preterm birth. In: Fuchs AR, Fuchs F, Stubblefield PG, eds. Endocrinology of term and preterm labor. 2nd ed. New York: McGraw Hill, 1993:59–96.
8. Tulchinsky D, Hobel CJ, Yeager E, Marshall JR. Plasma estrone, estradiol, estriol, progesterone and 17-OH-progesterone in human pregnancy. Am J Obstet Gynecol 1972;112:1095–100.
9. Norwitz ER, Robinson JN, Challis JRG. The control of labor. N Engl J Med 1999;341:660–6.
10. Siiteri PK, MacDonald PC. Placental estriol biosynthesis during human pregnancy. J Clin Endocrinol 1966;26:751.
11. Buster JE, Carson SA. The endocrinology and diagnosis of pregnancy. In: Gabbe SG, Niebyl JR, Simpson JL, eds. Obstetrics normal

and problem pregnancies. 3rd ed. New York: Churchill Livingstone, 1996;31–64.
12. Darne J, McGarrigle HHG, Lachelin GCL. Saliva oestriol, oestradiol, oestrone and progesterone levels in pregnancy: Spontaneous labour at term is preceded by a rise in the saliva oestriol/progesterone ratio. Br J Obstet Gynaecol 1987;94:227–35.
13. Darne J, McGarrigle HHG, Lachelin CCL. Increased saliva oestriol to progesterone ratio before idiopathic preterm delivery: A possible predictor for preterm labour? BMJ 1987;294:270–2.
14. McGarrigle HHG, Lachelin GCL. Increasing saliva (free) oestriol to progesterone ratio in late pregnancy: A role for oestriol in initiating spontaneous labour in man? BMJ 1984;289:457–9.
15. Moran DJ, McGarrigle HH, Lachelin GCL. Lack of normal increase in saliva estriol-progesterone ratio in women with labor induced at 42 weeks' gestation. Am J Obstet Gynecol 1992;167:1563–4.
16. McGregor JA, Jackson GM, Lachelin GCL, Goodwin TM, Artal R, Hastings C, et al. Salivary estriol as risk assessment for preterm labor: A prospective trial. Am J Obstet Gynecol 1995;173:1337–42.
17. McGregor J, Barrett J, Hastings C. Diurnal variation of salivary estriol in pregnancy. Am J Obstet Gynecol 1999;180:S223–5.
18. Creasy RK, Herron M. Prevention of preterm birth. Semin Perinatol 1981;5:295–302.
19. Hendershott CM, Dullien V, Goodwin TM. Serial betamethasone administration and effect on maternal salivary estriol levels. Am J Obstet Gynecol 1999;180:S219–22.
20. Dudley DJ, Pre-term labor: An intra-uterine inflammatory response syndrome? J Reprod Immunol 1997;36:93–109.
21. Gibbs RS, Romero R, Hillier, Eschenbach DA, Sweet RL. A review of premature birth and subclinical infection. Am J Obstet Gynecol 1992;166:1515–28.
22. American College of Obstetricians and Gynecologists. Preterm labor. ACOG technical bulletin no. 133. Washington DC: American College of Obstetricians and Gynecologists, 1989.
23. American College of Obstetricians and Gynecologists. Preterm labor. ACOG technical bulletin no. 206. Washington DC: American College of Obstetricians and Gynecologists, 1995.

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