Salivary Estriol and Progesterone Levels as Predictors of Spontaneous Preterm Labor in Pregnant Women at Risk

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Abstract: Objective: The aim of the current study was to evaluate the value of the salivary concentrations of estriol and progesterone as predictors of preterm labor in pregnant women at risk. **Methods:** The study included women with a singleton pregnancy, at 26 weeks' gestation, presenting to the outpatient clinic for routine antenatal care, being at a high risk of preterm labor. The recruited women were instructed to collect 2-3 ml of saliva every week, starting from the gestation of recruitment (26 weeks) till delivery. Salivary samples were assayed for estriol and progesterone concentrations. **Results:** The mean age of included women was 28.9 ± 7.23 years (range: 18 – 40 years). The mean levels of salivary estriol, measured at 26-35 weeks' gestation, were significantly higher in women of group I [Preterm Delivery Group] when compared to women of group II [Term Delivery Group]. On the contrary, the mean levels of salivary progesterone, measured at 26-35 weeks' gestation, were significantly lower in women of group I [Preterm Delivery Group] when compared to women of group II [Term Delivery Group]. A salivary estriol level ≥ 2.7 ng/ml at 26-27 weeks' gestation was associated with preterm labor with a sensitivity of 97.8%, a specificity of 71.1%, a positive predictive value [PPV] of 77.2%, and a negative predictive value [NPV] of 97%. A salivary progesterone level ≤ 1.6 ng/ml at 26-27 weeks' gestation was associated with preterm labor with a sensitivity of 100%, a specificity of 97.8%, a PPV of 97.8%, and an NPV of 100%. **Conclusion:** Salivary estriol and progesterone concentrations seem to be significant predictors of spontaneous preterm labor in pregnant women at a high risk for preterm labor.

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Key Words: Salivary estriol – salivary progesterone – preterm labor

1. Introduction:

Preterm labor is defined as spontaneous delivery of a live birth after 20 weeks' gestation and before completed 37 weeks' gestation. The incidence of preterm birth ranges between 10% and 12.5% of all births [1]. The annual rate of preterm birth shows a steady increase over the past two decades worldwide. It was estimated in some studies in the United States that annual rates of preterm birth have increased by 30% over only the past decade [2]. Preterm birth and its subsequent prematurity is the leading cause of neonatal and infantile mortality. Preterm birth is also the leading cause of cerebral palsy in the surviving children and is linked to other long-term developmental problems [3]. In addition, preterm births and their resulting premature newborns pose a major financial burden [4]. In Egypt, being a developing country, with limited neonatal facilities and low financial reserves, prematurity is considered a major health problem. The figures retrieved from unpublished local studies reveals significantly higher neonatal and infantile mortality rates, significantly poorer services and a significantly poorer overall outcome, when compared to the figures of other developing and developed countries. Despite the presence of tocolytic medications, the efficacy of such medications regarding long-term tocolysis has not been proven. Moreover, their use is associated with potentially harmful maternal and infant side effects [5]. For these reasons, strategies for prediction and prevention of preterm labor have attracted more attention over the past decade. Several biological, clinical and sonographic markers have been proposed as tools for predicting preterm labor [6]. The tested biological markers included cervico-vaginal fetal fibronectin level, human chorionic gonadotropin (hCG) level and serum corticotropin releasing hormone (CRH). Most of these tested markers lack either acceptable validity or feasibility and availability. There is a need for a rapid, inexpensive, simple bedside test with high sensitivity and specificity for accurate prediction of preterm labor, so that unnecessary tocolysis can be avoided in women who are unlikely to have preterm birth, whereas an appropriate intervention or referral to a higher center can be done in women likely to have preterm delivery [7]. Estriol is the main and most abundant estrogen in the serum of pregnant women. Estriol, along with estradiol and estrone, play major roles in preparing the uterine tissues, possibly

signaling parturition, in both human beings and great apes [8]. A rise in serum estriol level has been shown to precede labor, whether it was preterm, term or even post-term [9]. Progesterone has been known for its role in maintaining pregnancy, and the role of progesterone withdrawal in precipitating labor has been proven in animal models for decade [10]. Such a role in human beings remained unclear [11-13]. Nevertheless, there is some evidence that *functional* withdrawal of progesterone may initiate labor in human beings [14-15]. Administration of progesterone (suppositories or injections) has been shown, in two randomized trials, to reduce the risk of preterm labor in women at risk [16-17]. Concentrations of steroids in saliva have been shown to correlate well with their serum concentrations and reflect the unbound, unconjugated, and, therefore, the biologically active fraction of the plasma hormone profile. As saliva specimens are easy to collect and store, measurement of saliva hormones can be readily introduced into clinical practice when found to be of value [18]. The aim of the current study was to evaluate the value of the salivary concentrations of estriol and progesterone as predictors of preterm labor in pregnant women at risk.

2. Methods

The current study was conducted at Ain Shams University Maternity Hospital during the period between June 2010 and June 2011. The study included women with a singleton pregnancy, at 26 weeks' gestation, presenting to the outpatient clinic for routine antenatal care, being at a high risk of preterm labor. High risk of preterm labor was defined if prior history of spontaneous preterm labor and/or prior history of preterm prelabor rupture of the membranes (PPROM) were reported. Women with PPROM in the current pregnancy, antepartum hemorrhage, medical disorders (hypertension, diabetes mellitus, etc.), women on steroid or progestin therapy, and women who had local oral conditions interfering with saliva sampling were all not recruited in the study. The study was approved by the Ethical Research Committee, Obstetrics and Gynecology Department, Ain Shams University. Purposes and procedures of the study were explained to each participating woman. A written informed consent was taken from all included women before participating in the study.

Collection of Salivary Samples:

Women were instructed to rinse their mouth with water, and to wait for 10 minutes, then to allow saliva, without stimulus, to run freely into a standard plastic jars. Collection was made during the daytime hours (9 am – 8 pm), to avoid diurnal variation. Collection was made at least 60 minutes after a meal. Collected specimens were frozen within 4 hours after collection and stored at -20°C till the time of assay. All recruited women were followed-up at the outpatient antenatal clinic at Ain Shams University Maternity Hospital on weekly basis till delivery. The recruited women were instructed to collect 2-3 ml of saliva every week, starting from the gestation of recruitment (26 weeks) till delivery. Women who spontaneously delivered at gestation < 37 weeks' gestation were categorized as group I [Preterm Delivery Group]; weekly salivary samples, collected from women of this group, were assayed for both estriol and progesterone. An equivalent number of women were randomly selected from women who delivered at gestation > 37 weeks' gestation, and categorized as group II [Term Delivery Group]. Women who had induced delivery (whether induction of labor or Cesarean section) for reasons like medical disorders, obstetric complications or fetal compromise were not included in either group.

Salivary Estriol Assay:

Free estriol level in saliva was measured using the DRG® Salivary Free Estriol ELISA Kits (SLV-3653) [DRG-International, Inc., NY, USA]. The DRG Salivary Free Estriol ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding. The microtiter wells are coated with an anti-estriol IgG antibody. Endogenous unconjugated ("free") estriol of a sample competes with an estriol-horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration of estriol in the sample. After addition of the substrate solution, the intensity of color developed is inversely proportional to the concentration of free estriol in the sample [19].

Salivary Progesterone Assay:

Free progesterone level in saliva was measured using the DRG® Salivary Free Progesterone ELISA Kit (SLV-2931) [DRG-International, Inc., NY, USA]. The DRG Salivary Progesterone ELISA Kit is a solid phase enzyme-linked immunosorbent assays (ELISA), based on the principle of competitive binding. The DRG Salivary Progesterone ELISA KIT is based on the competition principle and the microplate separation. An unknown amount of progesterone present in the sample and a fixed amount of progesterone conjugated with horseradish peroxidase compete for the binding sites of a rabbit polyclonal progesterone-antiserum coated onto the wells. After one hour incubation the microplate is washed to stop the competition reaction. After adding the substrate solution, the concentration of progesterone is inversely proportional to the optical density measured [20].

Sample Size Justification:

Data from a previous study [21] showed that the sensitivity of salivary estriol in prediction of preterm labor was 71%. Calculation according to these values produced a minimal sample size of 42 cases in each group. Assuming a drop-out rate of 5%, the minimal sample size was estimated to be 45 in each group. Therefore, women fulfilling the eligibility criteria who accepted to participate in the study were recruited, till a number of 45 women who delivered preterm was reached. An equivalent number from the remaining women (who delivered at term) was included as a control group.

Statistical Methods:

Statistical analysis was performed using Microsoft Excel version 2010 and Statistical Package for Social Sciences (SPSS) for Windows version 15.0. Difference between two independent groups was estimated using independent student's t-test (for numeric parametric variables), Mann-Whitney's U-test (for discrete variables) and chi-squared test (for categorical variables). Receiver operator characteristics (ROC) curves were constructed for the measured salivary estriol and progesterone concentrations as predictors of preterm labor. Predictive validity was expressed in terms of sensitivity, specificity, positive and negative predictive values. Association between two variables was expressed in terms of relative risk and its 95% confidence interval. Significance level was set at 0.05.

3.Results

A total of 338 women were recruited in the study; of them 45 (13.3%) spontaneously delivered before 36 weeks' gestation, and were categorized as group I [Preterm Delivery Group]. Figure-1 shows the gestation of delivery in women of group I. The median gestational age at delivery was 32 weeks.

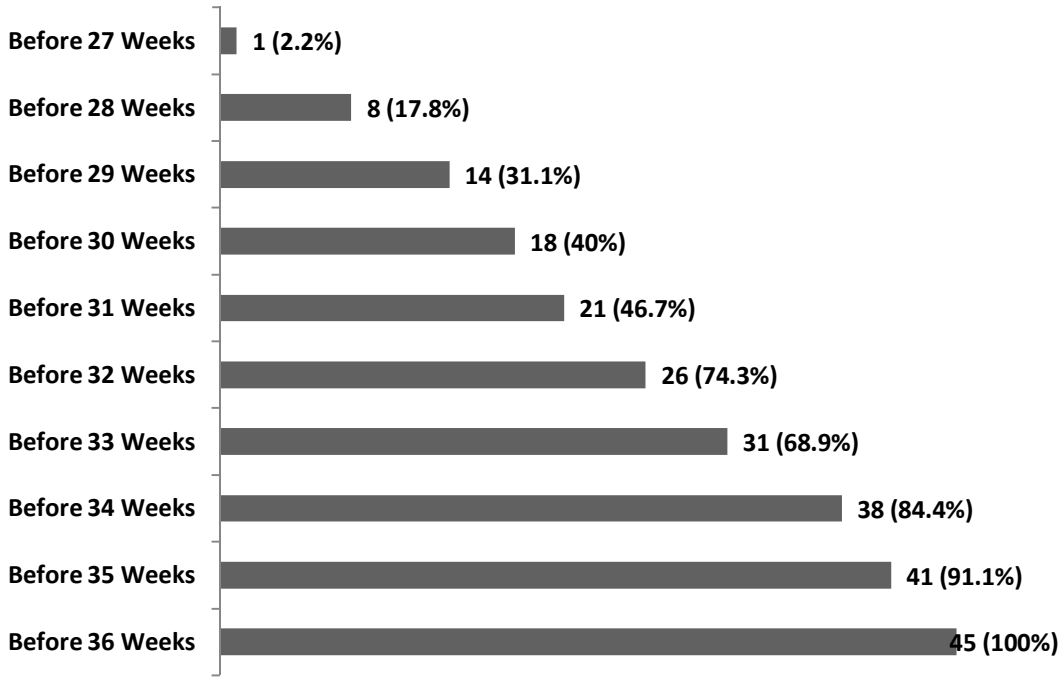


Figure-1 Bar-Chart showing Gestation of Delivery in Women of Group I [Preterm Delivery Group]

The mean age of included women was 28.9 ± 7.23 years (range: 18 – 40 years). The median parity was 1 (range: 0 – 4). The median no. of previous miscarriages was 1 (range: 0 – 6). There were no significant differences between women of both groups regarding age, parity and no. of previous miscarriages. Of the included 45 women of group I [Preterm Delivery Group], 29 (64.4%) had history of previous preterm labor, while 12 (26.7%) had history of previous miscarriage(s), in contrast to 23 (51.1%) and 17 (37.8%), respectively, in group II [Term Delivery Group]; these differences were not significant.

The mean levels of salivary estriol, measured at 26-35 weeks' gestation, were significantly higher in women of group I [Preterm Delivery Group] when compared to women of group II [Term Delivery Group] (Table-1).

Table-1 Difference between Both Groups regarding Salivary Levels of Estriol

	Salivary Estriol Level (ng/ml)		*P**
	Group I [Preterm Delivery Group]	**Group II [Term Delivery Group]**	
Week 26	900 - 1900 1284.44 ± 254.02	500 - 1100 768.89 ± 166.27	<0.001 HS
Week 27	1000 - 2200 1543.18 ± 314.31	600 - 1300 951.11 ± 176.61	<0.001 HS
Week 28	1300 - 2600 1737.84 ± 337.76	800 - 1500 1133.33 ± 209.98	<0.001 HS
Week 29	1400 - 2900 1919.35 ± 397	120 - 1700 1291.56 ± 283.21	<0.001 HS
Week 30	1600 - 3100 2107.41 ± 381.22	1100 - 1900 1522.22 ± 236.34	<0.001 HS
Week 31	1800 - 3300 2325 ± 369.78	1200 - 2200 1742.22 ± 272.60	<0.001 HS
Week 32	1900 - 3000 2494.74 ± 371.89	1400 - 2700 1957.78 ± 301.88	<0.001 HS
Week 33	2100 - 3400 2814.29 ± 414.83	1600 - 2900 2191.11 ± 330.17	<0.001 HS
Week 34	2600 - 3400 3100 ± 251.66	1800 - 3100 2440 ± 375.02	<0.001 HS
Week 35	3000 - 3800 3475 ± 340.34	200 - 3500 2704.44 ± 574.04	0.012 S

Data presented as range, mean ± SD
* Analysis using Independent Student's *t*-Test HS highly significant – S significant

On the contrary, the mean levels of salivary progesterone, measured at 26-35 weeks' gestation, were significantly lower in women of group I [Preterm Delivery Group] when compared to women of group II [Term Delivery Group] (Table-2).

Table-2 Difference between Both Groups regarding Salivary Levels of Progesterone

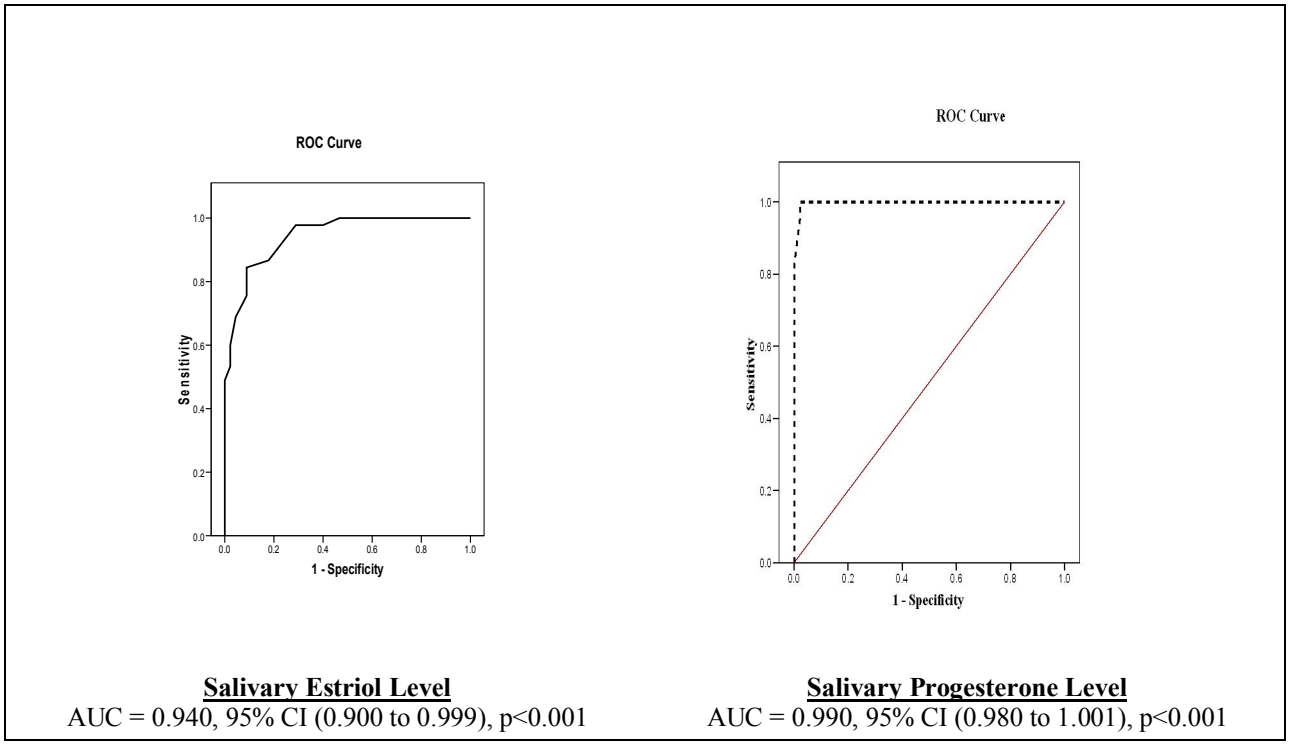
	Salivary Progesterone Level (ng/ml)		*P**
	Group I [Preterm Delivery Group]	**Group II [Term Delivery Group]**	
Week 26	450 - 950 701.11 ± 133.35	950.00 - 1450.00 1257.78 ± 145.76	<0.001 HS
Week 27	600 - 1100 814.77 ± 128.76	1100.00 - 1450.00 1276.67 ± 104.77	<0.001 HS
Week 28	650 - 1300 883.78 ± 144.36	1300.00 - 1600.00 1376.67 ± 71.98	<0.001 HS
Week 29	800 - 1150 946.77 ± 117.57	1300.00 - 1650.00 1462.22 ± 109.83	<0.001 HS
Week 30	850 - 1300 1001.85 ± 131.18	1350.00 - 1800.00 1547.78 ± 140.60	<0.001 HS
Week 31	850 - 1300 1064.58 ± 108.83	1400.00 - 1850.00 1632.22 ± 145.83	<0.001 HS
Week 32	950 - 1350 1155.26 ± 111.67	1450.00 - 1950.00 1724.44 ± 131.69	<0.001 HS
Week 33	1100 - 1350 1214.29 ± 94.93	1600.00 - 2100.00 1808.89 ± 128.05	<0.001 HS
Week 34	1150 - 1350 1264.29 ± 80.18	1650.00 - 2400.00 1896.67 ± 146.32	<0.001 HS
Week 35	1300 - 1400 1350 ± 40.82	1800.00 - 2400.00 1978.89 ± 160.43	<0.001 HS

Data presented as range, mean ± SD; * Analysis using Independent Student's *t*-Test; HS highly significant

Receiver operator characteristics (ROC) curves were constructed for salivary estriol and progesterone levels as predictors of preterm labor. A significant association was found between preterm labor and both variables, as denoted by the significantly large areas under the curves (AUCs) [AUC =0.940, 95% CI (0.900 to 0.999), p<0.001, and 0.990, 95% CI (0.980 to 1.001), p<0.001, respectively] (Figure-2).

A salivary estriol level ≥ 2.7 ng/ml at 26-27 weeks' gestation was associated with preterm labor with a sensitivity of 97.8%, a specificity of 71.1%, a positive predictive value [PPV] of 77.2%, a negative predictive value [NPV] of 97%, a false positive rate of 28.9% and a false negative rate of 2.2%. A salivary estriol level ≥ 2.7 ng/ml at 26-27 weeks' gestation significantly increased the risk of preterm labor almost 25-folds [RR 25.5, 95% CI (3.7 to 166.7)] (Table-3).

A salivary progesterone level ≤ 1.6 ng/ml at 26-27 weeks' gestation was associated with preterm labor with a sensitivity of 100%, a specificity of 97.8%, a PPV of 97.8%, an NPV of 100%, a false positive rate of 2.2% and a nil false negative rate. A salivary progesterone level ≤ 1.6 ng/ml significantly reduced the risk of preterm labor almost 2-folds [RR 0.49, 95% CI (0.41 to 0.61).



Salivary Estriol Level
AUC = 0.940, 95% CI (0.900 to 0.999), p<0.001

Salivary Progesterone Level
AUC = 0.990, 95% CI (0.980 to 1.001), p<0.001

Figure-2 ROC Curve for Salivary Estriol and Progesterone as Predictors of Preterm Labor

Table-3 Accuracy of Salivary Estriol and Progesterone Levels in Prediction of Preterm Labor

	Sensitivity	Specificity	PPV	NPV	FP	FN
Salivary Estriol ≥ 2.7 ng/ml	97.8%	71.1%	77.2%	97%	28.9%	2.2%
Salivary Progesterone ≤ 1.6 ng/ml	100%	97.8%	97.8%	100%	2.2%	0%

PPV positive predictive value; NPV negative predictive value; FP false positive rate; FN false negative rate

Survival analysis showed significant shortening of gestation in women with a salivary estriol level ≥ 2.7 ng/ml and a salivary progesterone level ≤ 1.6 ng/ml at ≤ 27 weeks' gestation (figure-3).

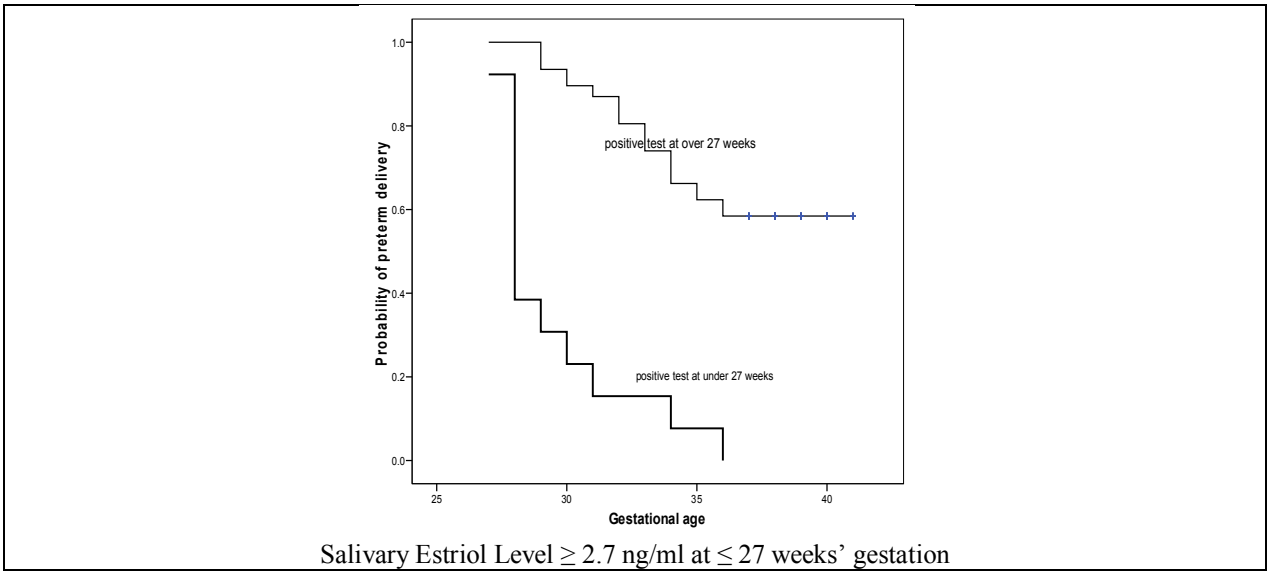


Figure-3 Kaplan Meier Curves for Survival Analysis for Preterm Labor in Women with a Salivary Estriol Level ≥ 2.7 ng/ml at or below 27 Weeks' Gestation

4. Discussion

The current study showed a significantly higher salivary estriol level and a significantly lower salivary progesterone level in women who delivered preterm when compared to women who delivered at term among pregnant women at a high risk for preterm labor. Both biomarkers were significant predictors of preterm labor in such women. High salivary estriol level and low salivary progesterone level were both significantly associated with preterm labor.

The correlation between salivary and serum concentrations of steroid hormones and of estriol and progesterone, in particular, have been well-established. Indeed, salivary concentrations reflect the free unconjugated biologically active form of these steroid hormones [22-24].

The role of estriol, progesterone and their ratio in initiation of labor has been proposed several decades ago. In 1978, Boroditsky *et al.* obtained serum concentrations of estrone, estradiol, estriol and progesterone from five normal women in the last 10 weeks of pregnancy and postpartum. They noticed a steady rise in the estriol level 14-28 days prior to the onset of labor [25]. In a subsequent study conducted by McGarrigle and Lachelin on six pregnant women, the salivary estriol-to-progesterone ratio rose from 0.8 to 1.43 during the last 28 days; this rise was attributable mainly to the rise in estriol level with nearly plateauing progesterone level. They concluded that estriol may have a role in triggering labor [26]. This estriol pre-labor rise or 'surge' (whether preterm, term or post-term labor) has been shown by other several subsequent studies [9, 18, 21, 27]. On the other hand, most of the published data showed no change in serum progesterone level prior to the onset of labor at term in human pregnancy [28-29]. In agreement to the results of the current study, however, Fonteini *et al.* showed that serum progesterone level declined by almost 30% at 28 to 34 weeks' gestation in women who delivered prematurely than in women who delivered at term [30]. In another study, Lachelin *et al.* showed that salivary progesterone level between 24 and 34 weeks' gestation was significantly lower in women who went into spontaneous preterm labor prior to 34 weeks' gestation when compared to women who delivered after 37 weeks' gestation [18]. The results of the current study and of the latter two studies, therefore, suggest a role for declining progesterone level prior to onset of spontaneous preterm labor.

These observations and findings suggested a role for progesterone supplementation (whether injections or suppositories) in prevention of preterm labor in pregnant women at risk [31]. Moreover, it has been shown by few studies that betamethasone, when given intramuscularly to enhance lung maturity in women with threatened preterm labor, caused

reduction in salivary estriol level, which might have had an impact in prolongation of pregnancy in such women [32-33].

In conclusion, salivary estriol and progesterone concentrations were shown to be significant predictors of spontaneous preterm labor in pregnant women at a high risk for preterm labor. The availability of such significant predictors may help direct the prophylactic therapy and preventive measures to those women who are more likely to have spontaneous preterm labor, thus reducing the cost as well as maternal and neonatal side effects of the tocolytic treatment.

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