

Salivary Progesterone as a Biomarker in Pregnancy

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Abstract

Progesterone was measured in the saliva of pregnant women at the end of gestation. Samples were collected two times daily (morning and afternoon) during the last two week before the expected delivery date and 2 days after delivery. Analysis was performed using commercial ELISA kits after simple freezing, thawing and centrifugation of the samples. It appeared that during the period before delivery the level of progesterone was in a steady state, until about 2 days before delivery, at which point a rapid decrease could be detected. Immediately after delivery, levels decreased again to non-pregnant values. For comparison reasons, two different ELISA kits were used and evaluated for suitability. In addition, in a preliminary experiment, the applicability and usefulness of the additional determination of salivary estriol was investigated. In conclusion, salivary progesterone may be used as indicator for imminent delivery in pregnant women. Salivary estriol may be an additional parameter that supports the usefulness of salivary progesterone. Further, estriol is said to be a trigger to delivery and is also known to give information about the wellbeing of the fetus. Consequently, its analysis may further contribute to predict the time of delivery, to optionally take measures in the case of premature delivery or complicated pregnancies and give information about the health of the fetus. The results of this study are only intended to indicate the possibility to develop a biosensor for home-use that would greatly contribute to the health of mother and newborn.

Keywords: Saliva; Progesterone; Estriol; Pregnancy

Introduction

Progesterone is considered as the pregnancy hormone. Already in 1958 Short was able to determine progesterone in blood and found that during pregnancy its levels increase from $<0.1 \mu\text{g}/100 \text{ mL}$ (non-pregnant) up to $87 \mu\text{g}/100 \text{ ml}$ [1]. In addition, Eton and Short [2] found that in early abortion or premature delivery the concentration of progesterone was relatively low, which finding were confirmed by Wiest [3] for fetal death. The role of progesterone during pregnancy includes the preparation of the uterus for implantation, to keep the uterus quiescent during gestation and lower immune responses of the mother. Initially, it is produced by the corpus luteum and after about 8 weeks by the placenta. It is suggested that a decline in progesterone levels facilitates the onset of labor and triggers milk production. Premature labour is characterized by progesterone concentrations lower than normal [4,5] and may be prevented by the administration of progesterone, 17OH-progesterone or 17-hydroxyprogesterone-caproate [6-9]. An update of this subject was given in a lecture during 14th Annual Symposium at the University of Illinois, Chicago, by Grobman, October 31, 2014.

The drop in progesterone levels as a trigger to the onset of labor has been assessed in sheep and other mammals and called 'P-withdrawal' [6,10-14]. Attempts to confirm this mechanism in humans has been hampered by the fact that repeated blood sampling is too stressful and not feasible in situations that there is no health threat or medical indication. No decrease in serum progesterone could be assessed when taken samples in large intervals of 10 days [15] or a week [16,17]. The determination of progesterone in saliva may be a convenient alternative for blood. It has been assessed that steroid hormones in saliva reflect the free, unbound concentrations in blood [18-20]. This free fraction is regarded as the biologically active part of the total concentration [21-23]. Consequently, levels of progesterone in saliva may be a better indicator for its activity.

Estriol is only produced in significant amount during pregnancy by the placenta and its levels increase from about week 9 until delivery with a surge before the onset of birth [24]. Its functions include

antagonism of progesterone, ripening of the cervix and sensitization of the myometrium [25]. Analysis of estriol in blood or salivary has been used to assess fetal health and wellbeing [26,27]. It is also considered as a biological marker for premature delivery [28].

The aim of this study was to investigate the usefulness of salivary progesterone in the prediction of delivery based on levels found in daily collected samples. Estriol was also measured in a very small series of salivary samples to assess its suitability in combination with progesterone. Satisfactory results might lead to the development of a biosensor for home use to prevent fetal death at premature deliveries.

Materials and Methods

For the collection of salivary samples, SaliCaps from IBL (IBL International GmbH, Hamburg, and Germany) were used. ELISA kits for salivary progesterone and estriol were purchased from IBL and Demeditec (Demeditec Diagnostics GmbH, Kiel, Germany), and used according to the kit inserts. For comparison of the performance and suitability of the kits, several extra validation experiments were performed. Herein the control samples contained in the respective kits were used. In addition, a series of samples from one subject was measured with both kits.

After the ELISA procedure the plates were analyzed in a microplate reader (Spectramax M5, Molecular Devices, and Berkshire, U.K.).

Subjects

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Pregnant women in the third trimester were asked to volunteer in the study. They were enrolled after signing an informed consent form and handed out an instruction sheet. In total 40 women were recruited. However, ten of the women were not included in the study due to several circumstances, such a not compliant with the instructions or delivery before the start of the sampling.

Sampling

Subjects collected saliva (± 1 mL) in the morning at about 08.00 h and in the afternoon at about 16.00 h, storing them in the freezer. The sampling period started at about 2 weeks before the expected day of delivery and ended at 2 days after giving birth. After this period the samples were brought to the lab for further processing and analysis.

Sample Pretreatment

The collected saliva samples were thawed and centrifuged at low speed for about 5 minutes. The clear supernatant was used in the respective ELISA. Pregnancy samples were diluted 10 times in the wells of the plate using 0.1 M PBS, pH 7.4.

Calculations

Concentrations were calculated using the 4-parametric log-log transformation. Values of the control samples contained in the kits were used for approval of the assay.

Results

Because there are several ELISA kits for progesterone and estriol, we choose two and evaluated the suitability for the measurement of large series of samples by performing an extra validation. The control samples (low and high concentration) were put in the plate immediately after the standard curve and before the samples as well as at the right end of the plate. The concentrations found should be similar and agree with the values on the kit insert. It appeared that for the kits from IBL there was good agreement between the concentrations found and those expected for both kits. However, for the Demeditec kits there was a large difference between these parameters. For example, for estriol low (range 87-204 pg/mL) was found a concentration of 155 pg/mL and 6432 pg/mL at the respective sites. For estriol high (range 429-998 pg/mL) was found 827 pg/mL and 15299 pg/mL. A 325 pg/mL control sample from the IBL kit measured in the Demeditec kit gave a concentration of 3071 pg/mL. Although the Demeditec progesterone kit performed somewhat better with regard to the low and high level control samples, a comparison of undiluted and diluted saliva samples resulted in values for the diluted samples after correction for the dilution factor were 2 times as high as expected.

The evaluation of the IBL kits was performed similarly. It appeared in both progesterone and estriol kits the control values were found within the range as specified on each vial with control samples. In addition, there was no statistically significant difference between the values at both sites on the plate.

Based on the above results only the ELISA kits from IBL were used in this study. The course of the salivary progesterone levels in the sampling period showed large differences between the subjects. An example for a normal course and natural delivery is given in Figure 1.

In this subject the expected date of delivery was 15 Oct and the delivery was 16 Oct. Values in the morning and afternoons were rather comparable and varied between appr. 1000-4500 pg/mL. In the last ten days the levels increase and 3-4 days before delivery they decrease

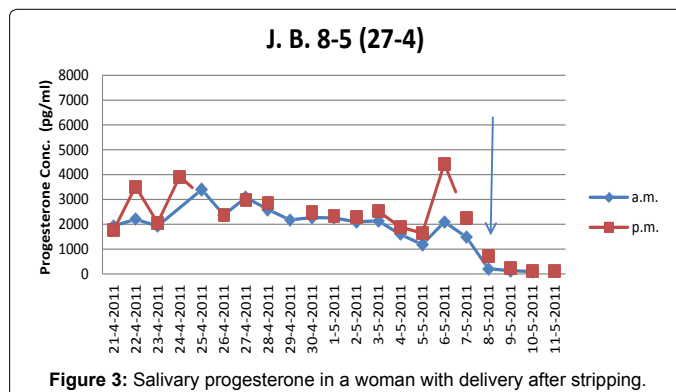
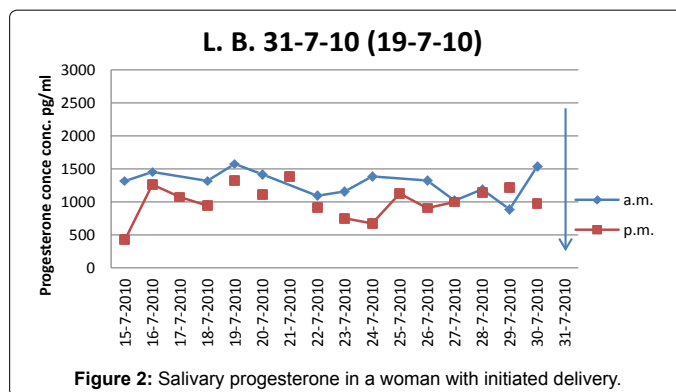
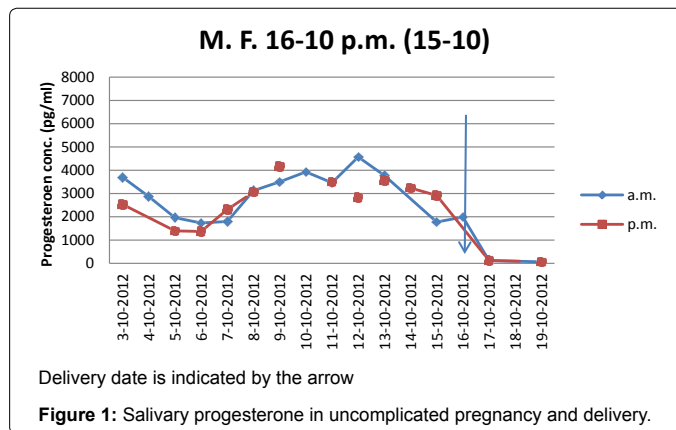
being in the non-pregnant range after giving birth. These findings were supported by the results of other women with natural delivery.

However, pregnancy may be accompanied by various complications. In case delivery is overdue, the risk for mother and child increases and delivery is initiated. An example of such a case is given in Figure 2.

It appeared that in this situation the course of progesterone was rather flat and levels were low in comparison with a natural delivery as shown above.

An alternative to initiation is stripping. This intervention is also intended to initiate delivery when mother or fetus or both are at risk. An example is given in Figure 3.

Stripping (on 6 May) resulted in a sudden rise in progesterone levels, followed by a steep decline and delivery (8 May).



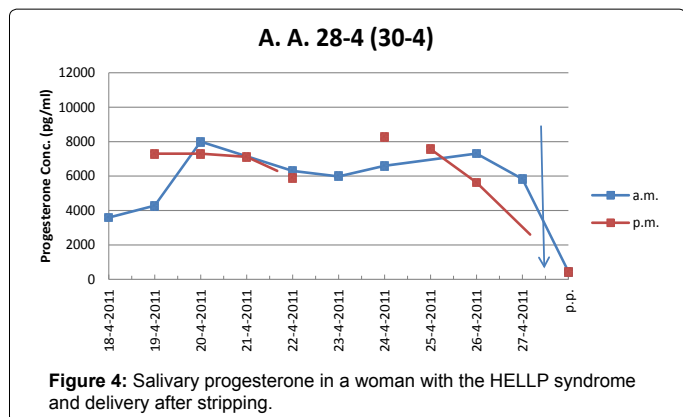


Figure 4: Salivary progesterone in a woman with the HELLP syndrome and delivery after stripping.

The HELLP syndrome is a serious complication in pregnancy characterized by a very high hypertension. In such cases, generally delivery is initiated. An example is given in Figure 4.

Initiation was performed on 27 April and followed by delivery on 28 April. Remarkable are the very high concentrations of progesterone during the sampling period.

Salivary estriol was measured in a very preliminary part of the study in only two subjects. One of the women delivered in a natural way (Figure 5a); also the ratio of estriol (E3)/progesterone (P) was calculated (Figure 5b). In the other subject delivery was initiated (Figure 6a and 6b)

From the above figures, it appears that in natural delivery estriol shows a surge at the time delivery should occur and that the ration E3/P is more indicative for the start of birth. In pregnancy where delivery has to be initiated there is no clear rise in salivary estriol, but the initiation leads to a high increase in the ratio of E3/P.

Discussion

The aim of this study was to assess the suitability of salivary progesterone as indicator for the time of delivery. Previously, many attempts have been made to find proof for the hypothesis of P-withdrawal to apply to humans; however with contradictory results. That may be explained by: a. the type of assay used; b. the sensitivity of the assay; c. the type of matrix (blood, urine, saliva); d. the period of sampling; e. the frequency of sampling. In our study, it appears that generally the levels are in the range of 1000-7000 pg/mL, with initially a surge, followed by a steady-state and then a clear decline at two days before delivery. In complicated pregnancy where initiation is indicated, the levels are much lower and no decline can be seen. Remarkably, in a pregnant women suffering from the HELLP syndrome, the levels of salivary progesterone were very high. The relation between progesterone and high blood pressure has been described in literature [29] and measuring progesterone in saliva during pregnancy may give indications of this syndrome already early in the third trimester and time to take measures.

The measurement of estriol in this study was only preliminary and included few samples. However, the surge in natural delivery is clearly seen as expected. Further, the ratio of estriol/progesterone shows a sudden surge at the time of birth and may even be a better indicator for the time of delivery than progesterone alone. This phenomenon has been described [23], but in that investigation only weekly sampling was performed. In pregnancies where initiation is indicated the levels of estriol in saliva do not show this sudden rise. However, the intervention of initiation does increase estriol as well as the ratio of

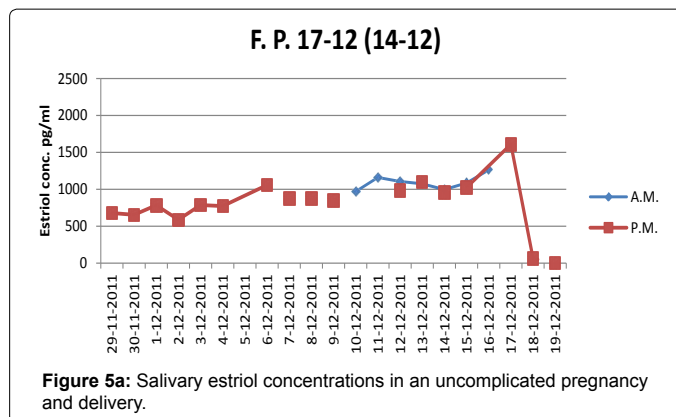


Figure 5a: Salivary estriol concentrations in an uncomplicated pregnancy and delivery.

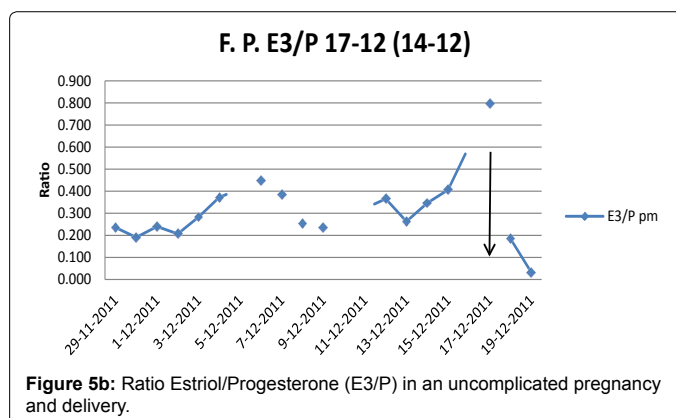


Figure 5b: Ratio Estriol/Progesterone (E3/P) in an uncomplicated pregnancy and delivery.

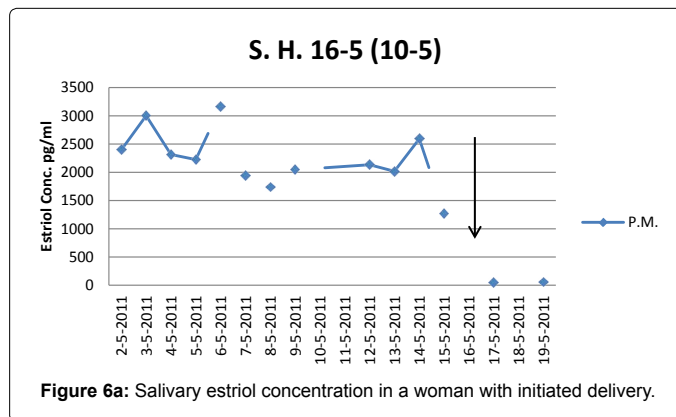


Figure 6a: Salivary estriol concentration in a woman with initiated delivery.

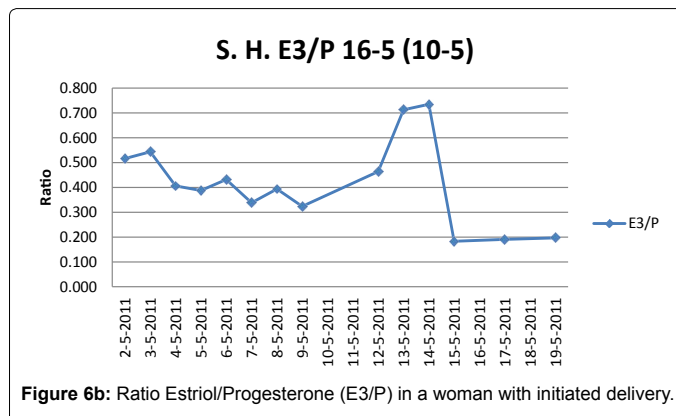


Figure 6b: Ratio Estriol/Progesterone (E3/P) in a woman with initiated delivery.

estriol/progesterone, finally leading to delivery.

It must be remarked that the large inter-individual differences prevented statistical evaluation of the results. In the figures only the course of the concentrations of selected women in the indicated time period is given.

In conclusion, the course of salivary levels of progesterone in pregnancy supports the hypothesis of P-withdrawal to apply to humans. It can be used to predict the time of delivery, but also indicate complicated pregnancies already early before delivery. This gives opportunities to take timely measures. Salivary estriol may present a factor that enhances the suitability of progesterone.

There is additional research required in order to statistically assess the findings of this study by including more subjects, both having natural deliveries and complicated pregnancies. This can be followed by the development of a biosensor for home-use for the on-site real-time determination of either one of both of these hormones. Coupled with an iPad, results may be directly sent to the attending healthcare professional. Taking well-timed measures may lower premature deliveries and their corresponding complications.

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