

# Low saliva progesterone concentrations are associated with spontaneous early preterm labour (before 34 weeks of gestation) in women at increased risk of preterm delivery

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Saliva progesterone and oestriol concentrations were determined weekly from 24 weeks of gestation in women at increased risk of preterm delivery. Samples were analysed from 28 women with spontaneous onset of labour and delivery before 37 weeks of gestation, and 64 who delivered at term. Saliva progesterone was lower in the 12 women delivering before 34 weeks than in those

delivering later, between 34 and 37 weeks ( $P = 0.007$ ) or at term ( $P = 0.009$ ). Measurement of saliva progesterone may be of value in the prediction of early preterm labour and in determining which women might benefit from progesterone supplementation.

**Keywords** Labour, oestriol, preterm, progesterone, saliva.

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## Introduction

The incidence of preterm birth in developed countries has remained unchanged over several decades, complicating approximately 7% of deliveries in developed countries.<sup>1</sup> While survival rates have improved dramatically, morbidity remains high. Identification of those women who are at greatest risk of preterm labour and delivery is inaccurate and still largely based on clinical history.

Labour in sheep is preceded by a decrease in progesterone and an increase in oestradiol (E2) plasma concentrations which result from conversion of progesterone to E2, mediated by placental  $17\alpha$  hydroxylase.  $17\alpha$  hydroxylase is not present in the human placenta and there is no evidence for a decrease in progesterone or an increase in E2 before the onset of term labour in women, although the concept of 'functional progesterone withdrawal' has been supported by investigations of the progesterone receptor and its isoforms.<sup>2</sup>

Concentrations of steroids in saliva 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. We have previously found no evidence of a change in the saliva E2:progesterone ratio before the spontaneous onset of labour in women.<sup>3</sup> There is, however, a marked increase in the saliva oestriol (E3) concentration and the saliva E3:progesterone ratio before term labour and in symptomatic women who deliver preterm.<sup>3,4</sup> Measurements of the saliva E3 concentration may therefore have clinical value in the prediction of preterm labour.<sup>5</sup> In this study, we have measured the saliva E3 and progesterone profiles in women known to be at risk of premature delivery.

For the purposes of this study, a subgroup of women at increased risk of preterm delivery, drawn from twelve centres, plus self-referrals, participating in a randomised controlled trial to assess the potential benefit of metronidazole in the prevention of preterm birth (The PREMETS study),<sup>1</sup> provided saliva specimens from 24 weeks of gestation until 34 weeks or delivery.

## Methods

In the PREMETS study, 892 women with singleton pregnancies, who had at least one risk factor for preterm delivery, were recruited between 23<sup>+0</sup> and 24<sup>+6</sup> weeks over a 4½-year period.<sup>1</sup> Eligibility included singleton pregnancy with a history of preterm birth or preterm prelabour rupture of the membranes before 37 completed weeks of gestation, previous late miscarriage, uterine anatomical abnormality, cervical surgery prior to the index pregnancy or current cervical cerclage. Women were screened for cervico-vaginal fetal fibronectin (fFN) at 24 and 27 weeks of gestation. The subgroup who were fFN positive ( $\geq 50$  ng/ml) were randomised to receive a 1-week course of metronidazole or placebo.

A cohort of women from 12 of the 14 centres in the PREMETS study, irrespective of their fFN status, provided weekly saliva samples from 24 weeks of gestation until 34 weeks or delivery. Samples were labelled with the study ID number and date of collection. These were analysed, blind to clinical outcome, for saliva E3 and progesterone by radioimmunoassay as previously described.<sup>3</sup> Briefly, aliquots (100  $\mu$ l) of saliva were mixed with 50  $\mu$ l sodium carbonate solution pH 10.5 and extracted with 10 volumes of diethyl ether. The mixture was frozen, the ether decanted and evaporated and the residue dissolved in 500  $\mu$ l phosphate buffered saline (PBS); 200  $\mu$ l aliquots of PBS were incubated with either tritium labelled E3 (NEN) and sheep antiE3 antiserum (Bioclin Services, Cardiff, UK), for E3 assays, or tritium labelled progesterone and sheep antiprogestosterone antiserum, for progesterone assays. Bound and free fractions were separated using dextran-coated charcoal. Interassay coefficients of variation for two control saliva pools were 7.2% and 5.8% for E3 concentrations of 0.96 and 2.28 nmol/l and 8.1% and 6.5% for progesterone concentrations of 1.15 and 2.43 nmol/l.

## Statistical analysis

After consideration of distributional plots, concentrations of progesterone and E3, and the E3:progesterone ratio were log-transformed to minimise non-normality. As a *post-hoc* analysis, comparisons were made between women delivering at term, between 34 and 37 weeks, and before 34 weeks of gestation. Regression models adjusted for weekly changes with gestation were fitted. To allow for repeated measurements by subject, generalised estimating equations were used, with standard errors adjusted for heterogeneity and residual non-normality using the sandwich estimator. Overall tests for differences between groups were used before comparing individual groups.

The performance of the measures as predictors of early delivery was evaluated using receiver operator characteristic curve (ROC) areas, based on sensitivity and specificity. Logistic regression, with the modifications described above,

was used to consider the combined role of saliva progesterone and a positive fFN test as predictors of early delivery. Absolute values of fFN and saliva progesterone at the same gestation were compared using Spearman's rank correlation. The analysis was conducted in Stata (version 9.2; StataCorp, College Station, TX, USA).

## Results

One hundred and eleven women supplied saliva samples for analysis between 24 and 34 weeks of gestation. Nineteen women were excluded from the analysis (four withdrew consent after a positive fFN test; five who delivered at term provided too few samples; one was treated with progesterone; seven had a caesarean section before the onset of labour; one underwent induction of labour at 36 weeks of gestation and for one the outcome of pregnancy was unknown). Samples from 92 women were analysed. Sixty-four women who delivered at term (at or after 37 completed weeks of gestation; mean 39.7, SD 2.0) provided an average of 10.8 samples. The 28 women who delivered before 37 weeks of gestation (mean 34.4, SD 2.6), following the spontaneous onset of labour, provided an average of 7.2 samples.

Nine women delivering before 37 weeks of gestation and eight delivering at or after 37 weeks of gestation were treated with metronidazole. There was no difference in the concentrations of saliva steroids between women who were, or who were not, treated with metronidazole in either the term or preterm groups. Although metronidazole treatment was associated with a small but significant increase in preterm delivery in the PREMETS study, there was no significant difference in gestational age at delivery between the 24 women randomised to treatment or placebo in the subgroup of 92 PREMETS trial participants providing saliva samples for the purposes of this study (36.4 weeks in seven women given placebo, 35.6 in 17 given metronidazole,  $P = 0.73$ ). Among this subgroup of PREMETS trial participants, the predominant risk factors for entry were previous preterm delivery and previous preterm rupture of the membranes. There were no significant differences in age, ethnicity, socio-demographics or obstetric history in the women who delivered preterm (before 37 weeks) compared with those who delivered after 37 weeks of gestation. The 92 women considered here were older, more likely to have had a previous second trimester abortion or a cervical cerclage but were otherwise similar to the other 704 women in the PREMETS study who delivered at term or as a result of preterm labour.

There was no significant difference in the concentration of saliva E3 or progesterone or in the E3:progesterone ratio between women who delivered before 37 weeks of gestation and those who delivered at term. In women who delivered

before 37 weeks of gestation, the progesterone concentration was 89% (CI 68–115%,  $P = 0.370$ ) of the value in the women delivering at term; E3 was 99% (CI 82–121%,  $P = 0.934$ ) and the ratio was 111% (CI 85–145%,  $P = 0.454$ ), based on ratios of geometric means.

Delivery before 34 weeks is associated with greater morbidity than delivery between 34 and 37 weeks and has been suggested to be of differing aetiology. Women who delivered before 34 weeks of gestation, between 34 and 37 weeks of gestation and at term (>37 weeks of gestation) were therefore considered separately. Systematic comparisons of the three groups showed a significant difference in the saliva progesterone concentrations between the three groups (<34 weeks, 34–37 weeks and term), but not in the E3 concentration or in the E3:progesterone ratio between groups (progesterone  $\chi^2 = 8.26$ ,  $P = 0.016$ ; E3  $\chi^2 = 0.22$ ,  $P = 0.896$ ; ratio  $\chi^2 = 4.63$ ,  $P = 0.099$ ).

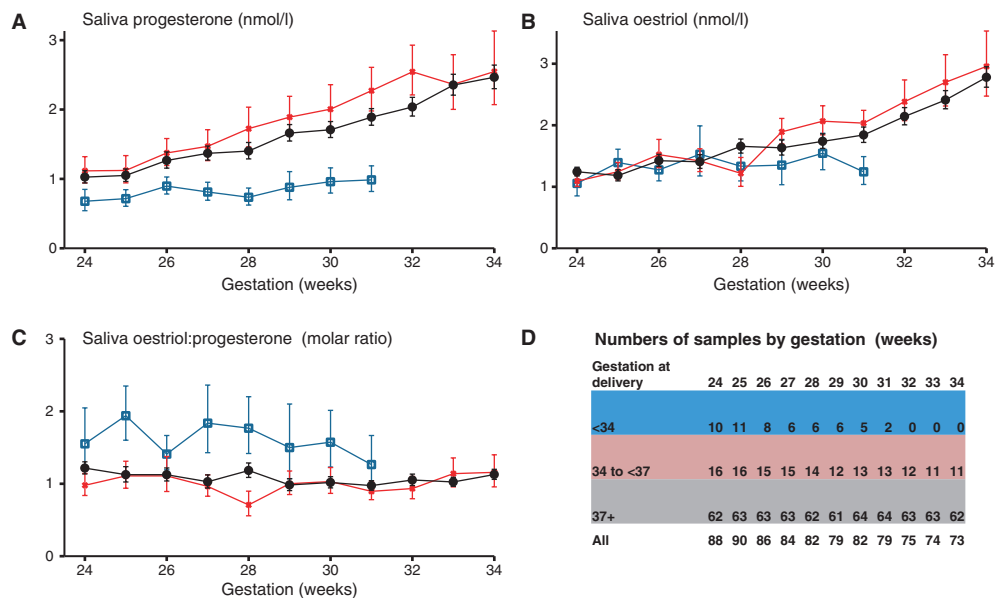
The gestational profiles between 24 and 34 weeks for saliva progesterone, E3 and the E3:progesterone ratio are shown in Figure 1. Figure 1D shows the numbers of samples provided at each week of gestation in the different groups. E3 and progesterone increased with gestation in women delivering after 37 weeks of gestation (Figure 1A, B). The saliva progesterone concentrations in the 12 women who delivered before 34 weeks (mean 30.7 weeks, SD 2.0) following the spontaneous onset of preterm labour

were significantly lower than those of the term group (63%, 95% CI 45–89%,  $n = 64$ ,  $P = 0.009$ ) or those delivering after the spontaneous onset of labour between 34 and 37 weeks of gestation (57%, CI 37–86%,  $n = 16$ ,  $P = 0.007$ ) (Figure 1A). The E3:progesterone ratio was higher in the women who went into spontaneous labour and delivered before 34 weeks than in those delivering between 34 and 37 weeks of gestation (161%, 95% CI 102–206%,  $P = 0.041$ ) or at term (148%, 95% CI 100.4–217%,  $P = 0.047$ ) (Figure 1C). In each case, results for the women delivering between 34 and 37 weeks (mean 35.8 weeks, SD 0.92) were similar to those delivering at term.

Considered alone, low saliva progesterone was a predictor of early preterm labour and delivery (before 34 weeks), compared with later delivery, with ROC areas between 0.64 and 0.69. When saliva progesterone at 24 weeks was used in conjunction with the fFN result, there was a nonsignificant increase in the ROC area from 0.71 for fFN alone to 0.79. There was no correlation between saliva progesterone and the fFN test result (Spearman's rank correlation =  $-0.159$  at 24 weeks,  $+0.093$  at 27 weeks).

## Discussion

The data from this exploratory study, which has shown lower concentrations of saliva progesterone in women at



**Figure 1.** Longitudinal determination of saliva progesterone (A) and saliva oestriol (B) and the saliva oestriol:progesterone ratio (C) in women at known risk of preterm labour and delivery who delivered before 34 weeks of gestation ( $n = 12$  □), between 34 and 37 weeks of gestation ( $n = 16$  ×) and after 37 weeks of gestation ( $n = 64$  ●). Saliva progesterone was significantly different between those who delivered before 34 weeks and those delivering between 34 and 37 weeks ( $P = 0.007$ ) and at term ( $P = 0.009$ ). There was no significant difference between groups in oestriol, but the oestriol:progesterone ratio was higher in the early preterm (<34 weeks) group than those delivering between 34 and 37 weeks ( $P = 0.041$ ) and at term ( $P = 0.047$ ). Values shown are geometric means with standard error bars. Panel (D) shows numbers of samples provided at each week of gestation in the different groups.

known risk of preterm birth who deliver before 34 weeks of gestation, add strength to the concept that measurement of saliva steroids could play a role in early identification of a subgroup of women at risk of preterm labour.

The concentrations of progesterone and oestriol, and the gestational profile were similar in the women who delivered after 37 weeks of gestation to those we have previously described in a cohort of 20 normal pregnant women providing saliva samples twice a week from 18 to 28 weeks of gestation and then daily until delivery.<sup>3</sup>

The lower progesterone concentration and higher E3:progesterone ratio in the women who delivered before 34 weeks support the theory that imbalance between these hormones may be associated with preterm labour in some women. To our knowledge, lower saliva concentrations of progesterone in women at increased risk of preterm labour have not been described previously. The absence of a relationship with the concentration of cervico-vaginal fFN may suggest a different aetiology and the potential for identification of a subgroup of women at risk. The lower saliva progesterone concentrations and higher ratio in the early compared with the late preterm labour groups also adds strength to the suggestion that early preterm delivery may be mechanistically different from late preterm spontaneous labour and delivery. We are aware of only one study which has addressed the use of saliva progesterone as a predictor of preterm labour. Klebanoff *et al.*<sup>6</sup> measured baseline saliva progesterone and oestriol in 386 and 413 women at high risk of premature delivery who took part in a randomised controlled trial of 17 $\alpha$  hydroxyprogesterone caproate, which showed significant benefit of the intervention. These authors found no association of progesterone measured at 16–20 weeks of gestation and gestational age at delivery. Although longitudinal samples were provided by 20 women who delivered preterm and 20 who delivered at term in the placebo group, no analysis in relation to outcome was presented. As noted by these authors, the saliva progesterone concentrations measured between 16 and 20 weeks of gestation may not reflect changes occurring later in pregnancy.

Progesterone is essential for the maintenance of pregnancy and promotes uterine relaxation by suppressing the formation of gap junctions and reducing prostaglandin synthesis and the concentration of oxytocin receptors in the myometrium. Progesterone also increases cellular calcium binding and may lower myometrial intracellular calcium.<sup>3</sup> Progesterone also has anti-inflammatory properties,<sup>2</sup> and lower than normal progesterone status, as identified in the early preterm group in the present study, could contribute to an exaggerated response to bacterial invasion, widely recognised as a cause of early preterm labour. Because of promising data from clinical trials, progestogen supplementation is now being widely investigated as a preventative intervention in women at risk of preterm labour

and it is suggested that anti-inflammatory properties of progesterone may contribute to the potential benefit.

On the basis of this study of a small cohort of women at increased risk of preterm delivery, further prospective studies with larger numbers of women are indicated to assess whether measurement of saliva oestriol and progesterone in women at risk for preterm labour could help to select those most likely to respond to progesterone treatment. Although there was no observed correlation between fFN and saliva, possibly because of small sample size, associations between these variables should be addressed in larger studies.

## Conclusions

Saliva progesterone concentrations between 24 and 34 weeks of gestation were significantly lower in women who went into spontaneous labour and delivered before 34 weeks of gestation than in those delivering after 37 weeks. Given the likely heterogeneous origins of preterm delivery, it is unlikely that a single test will serve to identify all women at risk, and that an algorithm which combines clinical risk factors and biomarkers is likely to provide better accuracy. We suggest that future investigations address the potential value of a combination of tests, including measurement of fFN and oestriol and progesterone concentrations, for the prediction of preterm labour. Measurement of baseline saliva progesterone in intervention studies assessing effects of progesterone supplementation may also determine whether this simple test can identify those women most likely to benefit from progesterone treatment.

## Disclosure of interests

None.

## Contribution to authorship

GCLL designed the study and contributed to writing the manuscript; HHMcG performed the assays and contributed to writing the manuscript; PTS performed the statistical analysis and contributed to writing the manuscript, AS and LP designed the PREMETS study and contributed to writing of the manuscript. AB contributed to trial management, recruitment of women, collection of samples and to review of the manuscript.

## Details of ethics approval

This study was approved by the South East Thames Multi-centre Research Ethics Committee.

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