

# Maternal Serum Progesterone-Induced Blocking Factor at 11–13 Weeks' Gestation in Spontaneous Early Preterm Delivery

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## Key Words

First-trimester screening · Preterm delivery · Progesterone-induced blocking factor · Pyramid of prenatal care

## Abstract

**Objective:** Progesterone-induced blocking factor (PIBF) may be the mediator of the pregnancy maintenance effects of progesterone. The aim of this study is to investigate the potential value of measuring the maternal serum concentration of PIBF at 11–13 weeks' gestation in the prediction of spontaneous early preterm delivery. **Method:** The maternal serum concentration of PIBF at 11–13 weeks was measured by enzyme-linked immunosorbent assay in 25 singleton pregnancies which subsequently delivered spontaneously before 34 weeks, and 75 controls who delivered at or after 37 weeks. The values in the 2 groups were compared by the Mann-Whitney U test. **Results:** The median maternal serum concentration of PIBF in women who subsequently delivered before 34 weeks (157.5, interquartile range 99.5–208.8 ng/ml) was not significantly different from the control group delivering at term (167.5, interquartile range 105.0–212.0 ng/ml;  $p = 0.519$ ). **Conclusions:** In women who have a spontaneous early preterm delivery, the maternal serum levels of PIBF are not altered at 11–13 weeks of gestation.

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## Introduction

Preterm birth is the leading cause of perinatal death and handicap in children, and the vast majority of mortality and morbidity relates to early delivery before 34 weeks [1, 2]. Delivery before 34 weeks occurs in about 2% of singleton pregnancies. In two thirds of cases this is due to spontaneous onset of labor or preterm prelabor rupture of membranes, and in the other third it is iatrogenic, mainly due to pre-eclampsia [3].

Progesterone is essential in establishing and maintaining pregnancy. It inhibits myometrial contractility and the onset of labor is thought to be the consequence of progesterone withdrawal [4–6]. In women with a previous history of preterm delivery and in asymptomatic women with a short cervix, the prophylactic use of progesterone halves the risk of spontaneous preterm delivery [7–9]. Some of the effects of progesterone are mediated by a lymphocyte derived protein, progesterone-induced blocking factor (PIBF). This factor has pregnancy protective effects which may be mediated by inhibition of NK lymphocytes and induction of an anti-inflammatory cytokine response by increasing interleukin (IL)-3, IL-4, and IL-10 and decreasing IL-12 and interferon- $\gamma$  [10–12]. In women presenting with threatened preterm labor at 24–37 weeks' gestation the maternal serum concentration of PIBF was lower in those delivering preterm than

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**Table 1.** Maternal characteristics and obstetric history in the screened population

Characteristics	Delivery $\geq$ 37 weeks (n = 75)	Delivery <34 weeks (n = 25)
Maternal age in years, median (IQR)	33.1 (29.5–36.0)	33.5 (25.6–36.1)
Maternal weight, median (IQR)	64.5 (60.0–74.0)	64.8 (58.3–73.0)
Maternal height in cm, median (IQR)	165.1 (160.0–170.2)	164.0 (160.0–170.6)
Racial origin		
Caucasian, n (%)	43 (57.3)	12 (48.0)
African, n (%)	31 (41.3)	11 (44.0)
South Asian, n (%)	0	2 (8.0)
Mixed, n (%)	1 (1.3)	0
Cigarette smoker, n (%)	5 (6.7)	2 (8.0)
Conception		
Spontaneous, n (%)	73 (97.3)	25 (100.0)
Ovulation induction drugs, n (%)	2 (2.7)	0
Parity		
Nulliparous, n (%)	35 (46.7)	13 (52.0)
Parous with previous preterm delivery, n (%)	2 (2.7)	7 (28.0)*
Parous without previous preterm delivery, n (%)	38 (50.7)	5 (20.0)*

Comparisons between the groups were carried out by  $\chi^2$  or Fisher's exact test for categorical variables and by Mann-Whitney U test for continuous variables. \*  $p < 0.05$ .

in those delivering after 37 weeks [13, 14]. A longitudinal study at 7–41 weeks' gestation reported that in women who subsequently miscarry or deliver preterm, the urine concentration of PIBF was significantly reduced compared to those delivering at term [15].

The aim of this study was to investigate whether the maternal serum concentration of PIBF is altered in the first trimester of pregnancy in patients who subsequently deliver spontaneously before 34 weeks.

## Methods

### Screening Study Population

The data for this study were derived from a prospective screening study for adverse obstetric outcomes in women attending for their routine first hospital visit in pregnancy. In this visit, which is held at 11<sup>+0</sup> to 13<sup>+6</sup> weeks of gestation, we record maternal characteristics and medical history and perform an ultrasound scan to: (1) determine gestational age from the measurement of the fetal crown-rump length; (2) diagnose any major fetal abnormalities, and (3) measure fetal nuchal translucency thickness as part of screening for chromosomal abnormalities [16, 17]. In addition, the maternal serum pregnancy-associated plasma protein-A (PAPP-A) and free  $\beta$ -human chorionic gonadotropin are determined, and the results are combined with the fetal nuchal translucency to calculate the patient-specific risk for trisomy 21 [18]. Samples of serum were stored at  $-80^\circ\text{C}$  for subsequent biochemical analysis. Written informed consent was obtained from the women agreeing to participate in the study, which was approved by the King's College hospital ethics committee.

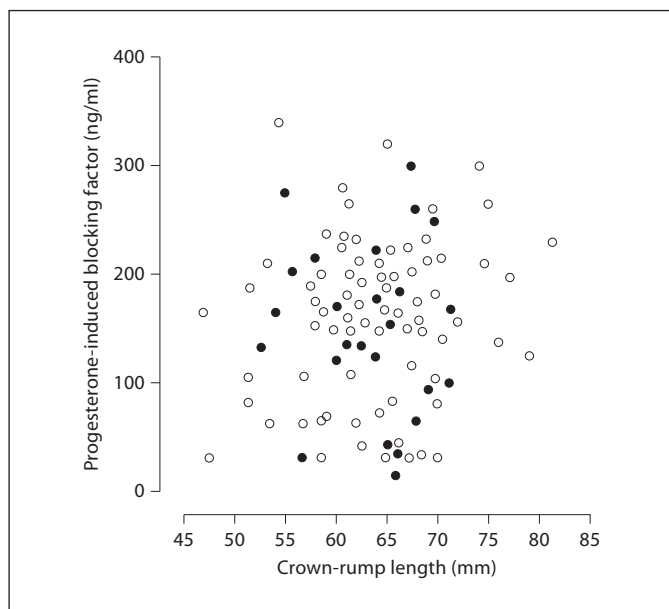
This case-control study involved measurement of maternal serum concentration of PIBF at 11–13 weeks' gestation in pregnancies complicated by spontaneous early preterm delivery and controls delivering after 37 weeks. The cases were drawn at random from the screening study population with available stored serum. The controls were from pregnancies with no complications and normal outcome matched to the cases for storage time. None of the samples were previously thawed and refrozen.

### Diagnosis of Spontaneous Early Preterm Delivery

Data on pregnancy outcome were obtained from the maternity computerized records or the women's general medical practitioners, and were also recorded in our database. The obstetric records of all patients delivering before 34 weeks were examined to determine if the preterm delivery was medically indicated or spontaneous. The latter included those with spontaneous onset of labor and those with preterm pre-labor rupture of membranes.

### Sample Analysis

The serum concentration of PIBF was measured by enzyme-linked immunosorbent assay (ELISA) as previously described [13]. The samples were pre-incubated with biotin-labeled anti-PIBF antibody to allow the binding of PIBF to the antibody, and then the remaining free antibody was reacted with the PIBF-coated plates. In brief, 96-well microtiter plates were coated with 100  $\mu\text{l}$  of recombinant PIBF (0.125  $\mu\text{g}/\text{ml}$  in 0.5 M Tris buffer, pH 6.5). After overnight incubation at  $4^\circ\text{C}$ , the plates were washed 3 times with 200  $\mu\text{l}$  PBS Tween. The recombinant PIBF standard (1.24, 5, 50, 100, 1,000 ng/ml) was diluted in 0.5 M phosphate buffer (pH 7.3–7.4) and then mixed with the same volume of 0.4  $\mu\text{g}/\text{ml}$  biotin-labeled anti-PIBF 1:25, and 1:50 diluted serum samples were mixed 1:1 with diluted biotinylated anti-PIBF IgG in 0.5 M PBS to achieve a final dilution of 1:50 and 1:100. Standards and diluted



**Fig. 1.** Maternal serum concentration of progesterone-induced blocking factor at 11–13 weeks in pregnancies delivering spontaneously before 34 weeks (closed circles) and those delivering at term (open circles).

samples were incubated for 1 h at 37°C. At the same time, the coated ELISA plate was blocked with 200 µl of 0.1% BSA, 0.5% gelatine in PBS-Tween (1 h, 37°C). One hundred microliters of the diluted standard and samples were added to the ELISA plate and incubated for 1 h at 37°C. After 3 washes with PBS-Tween, 100 µl/well of horse radish peroxidase conjugated streptavidine (Amersham-Pharmacia, Hungary) diluted 1:1,000 in 0.1% BSA PBS-Tween was added and incubated for 30 min at 37°C. Then the plate was washed 3 times with PBS-Tween and 100 µl TMB solution (BD OptEIA) was added to each well and incubated for 30 min at room temperature. Then the reaction was terminated by adding 100 µl 1 M H<sub>2</sub>SO<sub>4</sub> per well. Absorbance was determined at 450 nm, and the concentrations of sample PIBF were calculated from the corresponding standard curve, which ranged from 0.62 to 500 ng/ml.

#### Statistical Analysis

Comparisons between the spontaneous early preterm delivery group with those delivering at or after 37 weeks were carried out by  $\chi^2$  or Fisher's exact test for categorical variables and by Mann-Whitney U test for continuous variables.

The distribution of maternal serum concentration of PIBF was examined for normality using probability plots and Shapiro-Wilk test. Multiple regression analysis in the unaffected group was used to determine whether there was a significant contribution to the maternal serum PIBF levels by maternal characteristics and gestation. Mann-Whitney U test was used to compare values of PIBF between the outcome groups.

The statistical software package SPSS 16.0 (SPSS Inc., Chicago, Ill., USA) was used for data analyses.

## Results

The maternal characteristics of the outcome groups are compared in table 1.

Multiple regression analysis in the unaffected group demonstrated that for maternal serum PIBF there were no significant independent contributions from maternal age ( $p = 0.158$ ), maternal weight ( $p = 0.268$ ), maternal height ( $p = 0.332$ ), racial origin ( $p = 0.126$ ), smoking status ( $p = 0.333$ ), use of ovulation-induction drugs ( $p = 0.951$ ), or fetal crown-rump length ( $p = 0.197$ ).

The median maternal serum concentration of PIBF in women who subsequently delivered before 34 weeks (157.5, interquartile range 99.5–208.8 ng/ml) was not significantly different from the control group delivering at term (167.5, interquartile range 105.0–212.0 ng/ml;  $p = 0.519$ ) (fig. 1).

## Discussion

The findings of this study demonstrate that at 11–13 weeks' gestation, the maternal serum concentration of PIBF is not significantly different in pregnancies resulting in spontaneous early preterm delivery compared to those delivering at term. This finding suggests that the possible mechanism through which PIBF acts in maintaining the pregnancy is not altered in the first trimester of pregnancy.

In the pregnancies delivering at term the serum concentration of PIBF was unaffected by maternal characteristics or fetal crown-rump length. Similarly, a longitudinal study of 209 singleton pregnancies at 6–40 weeks reported no change in serum levels with gestational age [13]. In women presenting with threatened preterm labor the levels of PIBF were 70% lower in those progressing to preterm delivery compared to those delivering at term [14]. Our finding that spontaneous delivery before 34 weeks is not associated with a reduction in serum levels of PIBF at 11–13 weeks suggests that the reported decrease in serum PIBF in women in preterm labor [14] may be the consequence rather than the cause of an inflammatory process accompanying such labor. Alternatively, the decrease in maternal PIBF precedes the onset of labor but this decrease is not manifested in serum from the first trimester.

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