Platelet size and glycoprotein Ib and IIIa expression in normal fetal and maternal blood

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OBJECTIVE: Our purpose was to study platelet size and surface glycoprotein expression in normal fetal and maternal blood throughout pregnancy.

STUDY DESIGN: A cross-sectional study was performed at the Harris Birthright Research Centre for Fetal Medicine, King's College Hospital Medical School, London. Fetal and maternal blood samples were obtained from uncomplicated pregnancies at 8 to 42 weeks' gestation (n = 101 and n = 117, respectively) and from 30 nonpregnant controls. Flow cytometry was used to determine platelet size and glycoprotein Ib and IIIa expression both before and after stimulation with adenosine diphosphate.

RESULTS: Mean platelet size in both fetal and maternal blood was significantly lower than that of nonpregnant controls and decreased with advancing gestation. The surface density of glycoprotein Ib in maternal and fetal platelets was significantly lower than in nonpregnant controls but did not change with gestation. Adenosine diphosphate stimulation of maternal platelets resulted in increased percentage expression and surface density of all glycoproteins, whereas stimulation of control platelets resulted in increased surface density of glycoprotein Ib and percentage expression of glycoprotein IIIa. Adenosine diphosphate stimulation of fetal platelets resulted in increased surface density of glycoprotein Ib and IIIa.

CONCLUSION: Pregnancy is associated with increased thrombocytopoiesis in both the mother and fetus. Maternal platelet glycoprotein expression and responsiveness to adenosine diphosphate stimulation is increased. Fetal platelets are phenotypically mature from at least 12 weeks' gestation and respond in an adultlike fashion to stimulation with adenosine diphosphate. (Am J Obstet Gynecol 1994;171:791-6.)

Key words: Cordocentesis, platelet size, platelet glycoproteins

In normal fetuses the platelet count increases linearly with gestation, and at 15 weeks the levels are approximately 70% of those at term.[1, 2] The relatively high numbers of platelets from early pregnancy may be reconciled with the need to maintain vascular integrity during early fetal development. However, it is unknown whether platelet function also develops early in fetal life. Platelet maturity and function in adult life are reflected by platelet heterogeneity (variation in size) and by platelet surface glycoprotein expression.[3-5]

The aim of this study was to investigate platelet function in fetal and maternal blood by measuring platelet size, glycoprotein expression, and the up-regulation of these glycoproteins in response to stimulation by adenosine diphosphate.

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Patients and methods

A cross-sectional study was performed of 101 fetal blood samples obtained by (1) fetal cardiocentesis from women undergoing elective termination of pregnancy for social indications at 12 to 17 weeks' gestation (n = 7), (2) cordocentesis from women undergoing prenatal diagnosis at 18 to 36 weeks (n = 56), and (3) umbilical cord puncture at delivery from women undergoing elective cesarean section at 37 to 42 weeks (n = 38).

Fetal cardiocentesis was performed with the patient under general anesthesia and immediately before termination of pregnancy. Cordocentesis was performed without maternal sedation or fetal paralysis, and in all cases the fetal biometry and karyotype were normal. Kleihauer-Betke testing confirmed that all blood samples contained only fetal blood. Elective cesarean sections were performed either because of previous cesarean section and suspected cephalopelvic disproportion or for breech presentation. In all cases the infants were normal and their birth weights were >5th percentile for gestational age. There were no cases of maternal diabetes mellitus and preeclampsia.

Maternal blood samples (n = 117) were obtained at 8 to 42 weeks' gestation from women with singleton preg-

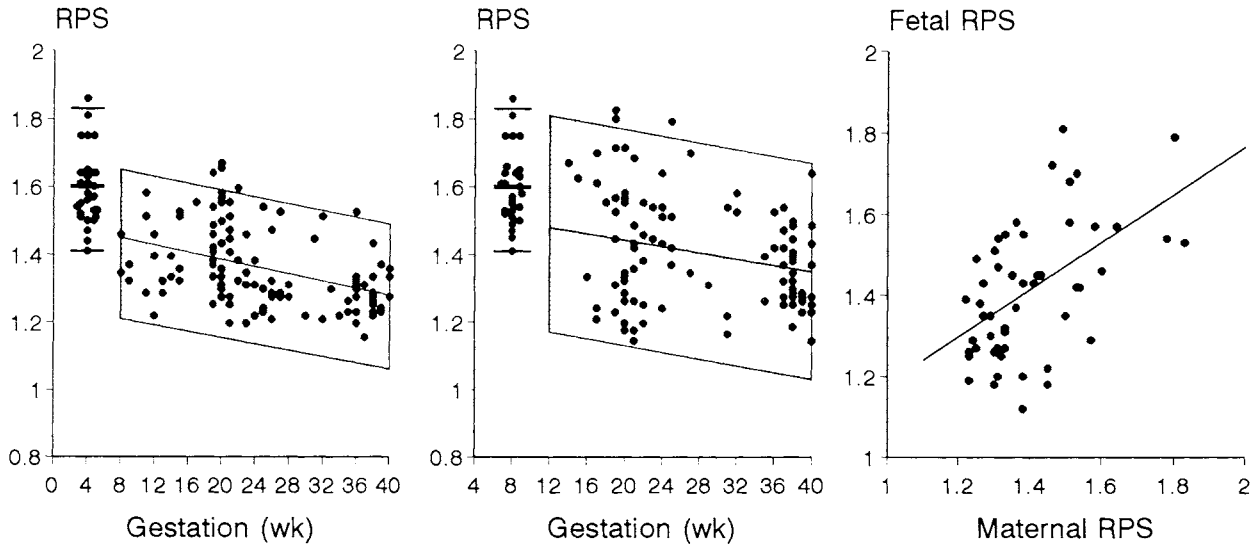

Fig. 1. *Left,* Maternal platelet size. *Middle,* Fetal platelet size. *Right,* Platelet size. Maternal and fetal platelet size *(RPS)* plotted as function of gestation. Sloping lines are mean and 2.5th and 97.5th percentile values. Maternal relative platelet size: mean 1.33, range 1.21 to 1.66; fetal relative platelet size: mean 1.40, range 1.17 to 1.72; nonpregnant controls relative platelet size: mean 1.56, range 1.41 to 1.83. *Points* and *horizontal lines* on left, Individual values, median, and range of nonpregnant controls.

Table I. Mean and range of percentage expression (percent) and surface density (relative fluorescence intensity) of platelet GPIb and GPIIIa in maternal, fetal, and nonpregnant adult blood both before and after stimulation with adenosine diphosphate

	Glycoprotein	*Unstimulated mean and range*	*Stimulated mean and range*	*Mean difference and 90% confidence interval*	*Student t test*
Maternal	GPIb (%)	83 (51-96)	84 (58-97)	1.8 (0.0-3.6)	4.08*
	GPIb (relative fluorescence intensity)	1.43 (1.09-2.61)	1.51 (1.08-2.69)	0.09 (0.02-0.15)	4.45*
	GPIIIa (%)	87 (55-98)	89 (68-98)	1.3 (−0.2-2.8)	4.2 *
	GPIIIa (relative fluorescence intensity)	2.11 (1.09-5.47)	2.44 (1.09-6.43)	0.34 (0.10-0.57)	6.30*
Fetal	GPIb (%)	76 (33-98)	77 (12-100)	1.1 (−2.2-4.4)	1.56
	GPIb (relative fluorescence intensity)	1.33 (1.00-1.88)	1.38 (1.07-1.99)	0.05 (0.01-0.10)	4.44*
	GPIIIa (%)	83 (56-100)	84 (58-100)	0.9 (−1.4-3.1)	1.58
	GPIIIa (relative fluorescence intensity)	2.06 (1.09-4.70)	2.18 (1.01-4.57)	0.12 (−0.09-0.33)	2.23†
Controls	GPIb (%)	80 (44-98)	82 (51-98)	2.0 (−3.3-7.2)	1.97
	GPIb (relative fluorescence intensity)	1.55 (1.09-2.46)	1.63 (1.10-3.34)	0.08 (−0.05-0.22)	2.72†
	GPIIIa (%)	86 (62-97)	89 (64-98)	2.7 (−1.05-6.45)	2.29†
	GPIIIa (relative fluorescence intensity)	2.25 (1.12-4.83)	2.45 (1.13-7.63)	0.21 (−0.22-0.63)	1.80

*$p < 0.001$.
†$p < 0.05$.

nancies attending routine antenatal clinics ($n = 65$) or at the time of fetal blood sampling ($n = 52$). Blood was also obtained from 30 nonpregnant, healthy female volunteers 25 to 39 years old, who were not using hormonal contraception or any other medication.

Blood samples (180 μl) were collected into 20 μl of isotonic edetic acid solution (0.5 mmol/L in 0.15 mmol/L sodium chloride), and the full blood count was determined with a Coulter S-Plus counter (Coulter Electronics, Luton, U.K.). Blood (500 μl) was also collected into 50 μl sodium citrate (3.8% wt/vol) for analysis by flow cytometry, which was performed on the same day of sampling.

Flow cytometry. Platelet-rich plasma was prepared

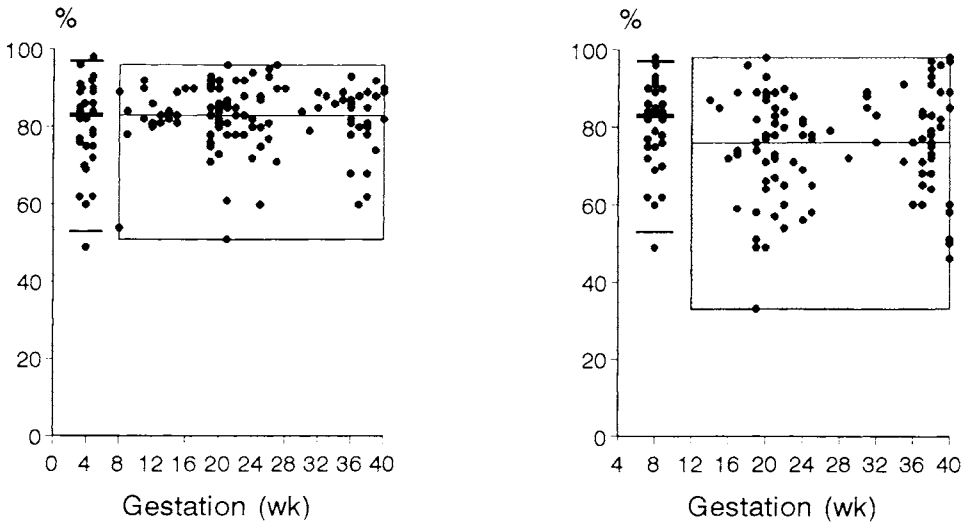

Fig. 2. Maternal *(left)* and fetal *(right)* (unstimulated) platelet GPIb percentage expression (%) plotted as function of gestation. *Lines* in shaded area, Mean and range; *points* and *horizontal lines* on left, individual values, median, and range of nonpregnant controls.

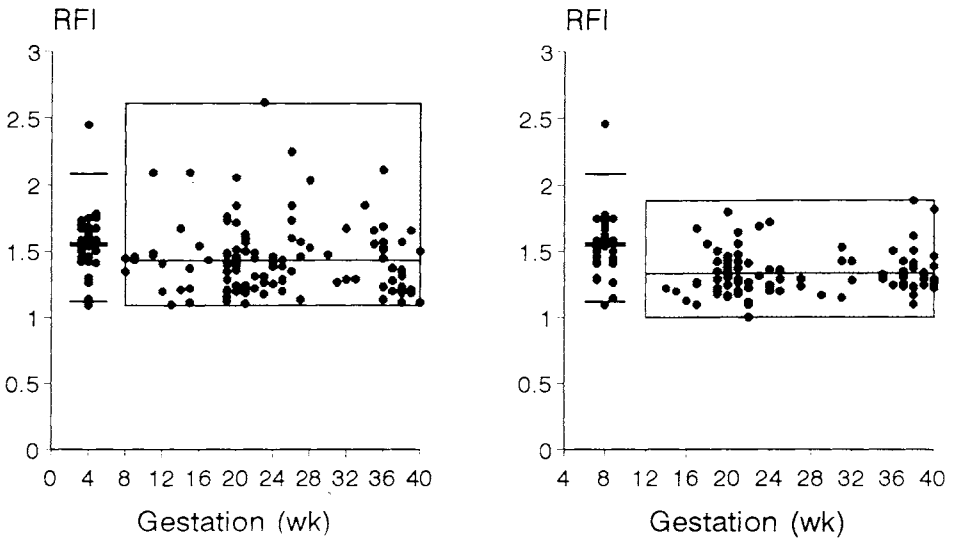

Fig. 3. Maternal *(left)* and fetal *(right)* (unstimulated) platelet GPIb surface density *(RFI)* plotted as function of gestation. *Lines* in shaded area, mean and range; *points* and *horizontal lines* on left, individual values, median, and range of nonpregnant controls.

by centrifugation (225*g*, 10 minutes). Twenty microliters of platelet-rich plasma and a saturating volume (5 μl) of fluorescent antibody were incubated in the absence or presence of 1 mmol/L adenosine diphosphate (Sigma, Poole, U.K.). Fluorescein isothiocyanate–conjugated monoclonal mouse antihuman antibodies (Dako, High Wycombe, U.K.) were used for determination of cells positive for CD42b (glycoprotein Ib [GPIb]) and CD61 (glycoprotein IIIa [GPIIIa]). To prevent platelet aggregation the reaction mixture was diluted with phosphate-buffered saline solution (Sigma) to give a final volume of 100 μl. The tubes were incubated for 30 minutes in the dark at room temperature. Phosphate-buffered saline solution (500 μl) was then added to each tube before flow cytometry, which was carried out with a fluorescence-activated cell sorter (FACscan) and Consort 32 software (Becton Dickinson, Oxford, U.K.). The flow cytometer was calibrated for size and fluorescence before each analysis with a flow cytometry fluorescence intensity standardization kit (Coulter, Luton, U.K.). Samples were gated with forward angle and 90-degree light-scattering properties to exclude leuko-

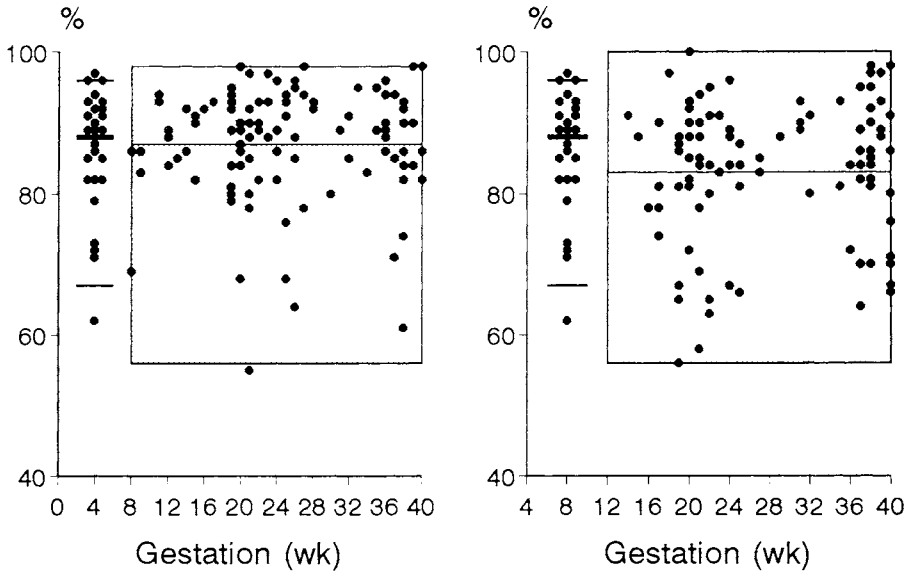

Fig. 4. Maternal *(left)* and fetal *(right)* (unstimulated) platelet GPIIIa percentage expression (%) plotted as function of gestation. *Lines* in shaded area, Mean and range; *points* and *horizontal lines* on left, individual values, median, and range of nonpregnant controls.

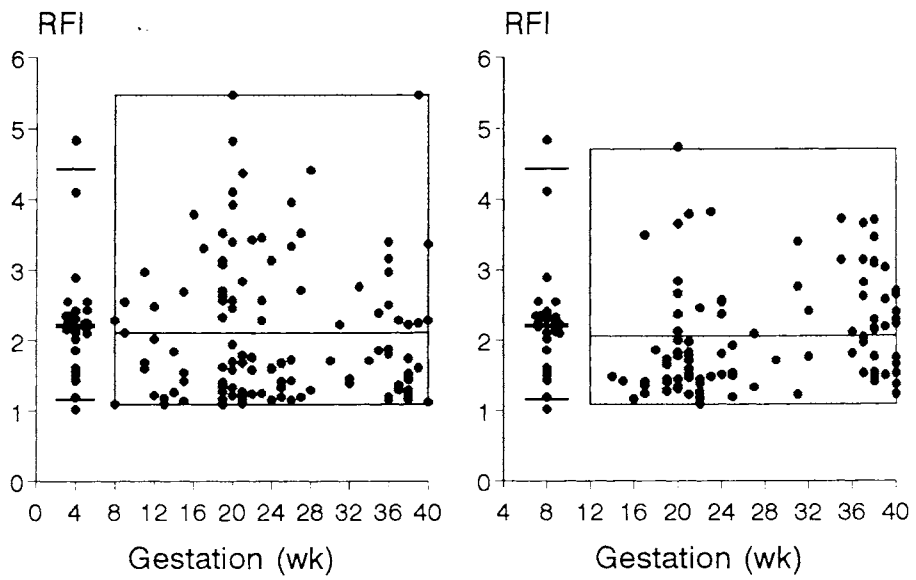

Fig. 5. Maternal *(left)* and fetal *(right)* (unstimulated) platelet GPIIIa surface density *(RFI)* plotted as function of gestation. *Lines* in shaded area, Mean and range; *points* and *horizontal lines* on left, individual values, median, and range of nonpregnant controls.

cytes. Control staining of platelet-rich plasma with antimouse monoclonal immunoglobulin G_{2a}-phycoerythrin immunoglobulin G_1–fluorescein isothiocyanate was performed on each sample, and background readings of $< 1\%$ were obtained. A minimum of 5000 cells were acquired in the platelet fraction and analyzed to calculate the percentages and the mean fluorescence intensity of each sample. The density of surface glycoproteins was measured by calculating the relative fluorescence intensity with the formula Relative fluorescence intensity = Antilog (Mean fluorescence intensity/Number of channels per decade).[6] The platelet size was estimated by calculating the relative platelet size with the formula Relative platelet size = Antilog (Median forward scatter/Number of channels per decade).

Statistics. Regression analysis was used to determine the significance of any association between measured variables and gestational age. For comparison of the

Table II. Correlation coefficient for association with gestation (r) and significance of any differences in percentage expression (percent) and surface density (relative fluorescence intensity) of platelet GPIb and GPIIIa between maternal and nonpregnant adult blood both before and after stimulation with adenosine diphosphate

	r	*Mean difference from controls and 90% confidence interval*	*Student t test*
GPIb (%)	−0.063	2.6 (−0.6-5.8)	1.36
GPIb (relative fluorescence intensity)	−0.066	0.12 (−0.21-0.03)	2.22*
GPIIIa (%)	0.013	1.3 (−1.3-3.9)	0.81
GPIIIa (relative fluorescence intensity)	−0.037	−0.14 (−0.46-0.19)	0.70
Stimulated GPIb (%)	−0.034	29.9 (24.6-35.2)	9.33†
Stimulated GPIb (relative fluorescence intensity)	0.027	0.52 (0.42-0.62)	8.47†
Stimulated GPIIIa (%)	0.064	30.9 (25.3-36.4)	9.13†
Stimulated GPIIIa (relative fluorescence intensity)	−0.055	0.86 (0.68-1.04)	7.86†

None of variables changed significantly with gestational age.
*$p < 0.05$.
†$p < 0.001$.

Table III. Correlation coefficient for association with gestation (r) and significance of any differences in percentage expression (percent) and surface density (relative fluorescence intensity) of platelet GPIb and GPIIIa between fetal and nonpregnant adult blood both before and after stimulation with adenosine diphosphate

	r	*Mean difference from controls and 90% confidence interval*	*Student t test*
GPIb (%)	0.034	−4.3 (−8.9-0.3)	1.56
GPIb (relative fluorescence intensity)	0.095	−0.21 (−0.28-0.15)	5.49*
GPIIIa (%)	0.103	−2.5 (−5.9-0.9)	1.22
GPIIIa (relative fluorescence intensity)	0.275	−0.18 (−0.46-0.10)	1.09
Stimulated GPIb (%)	0.114	21.4 (16.4-26.3)	7.09*
Stimulated GPIb (relative fluorescence intensity)	0.201	0.37 (0.28-0.46)	6.55*
Stimulated GPIIIa (%)	0.040	23.6 (18.3-29.0)	7.29*
Stimulated GPIIIa (relative fluorescence intensity)	0.325	0.59 (0.43-0.75)	6.11*

None of variables changed significantly with gestational age.
*$p < 0.001$.

means of measured variables between groups, the unpaired Student t test (two-tailed) was used. The paired Student t test (two-tailed) was used to examine the effect of adenosine diphosphate stimulation on measured variables.

Results

Platelet size in both fetal and maternal blood was significantly lower than in nonpregnant controls ($t = -4.61$, $p < 0.0001$ and $t = -7.57$, $p < 0.0001$, respectively) and decreased with advancing gestation (Fig. 1, $r = -0.248$, $p < 0.05$ and $r = -0.406$, $p < 0.001$, respectively). There were significant associations between fetal platelet size and both fetal platelet count ($r = -0.293$, $n = 101$, $p < 0.01$) and maternal platelet size (Fig. 1, $r = 0.526$, $n = 52$, $p < 0.0001$).

The percentage expression and surface density of platelet glycoproteins, both before and after stimulation with adenosine diphosphate, in mothers, fetuses, and nonpregnant controls are compared in Table I. Figs. 2 through 5 illustrate the levels of unstimulated glycoprotein expression and surface density in maternal and fetal platelets compared with those of nonpregnant adults. Adenosine diphosphate stimulation of (1) maternal platelets resulted in increased percentage expression and surface density of both glycoproteins, (2) control platelets resulted only in increased surface density of GPIb and percentage expression of GPIIIa, and (3) fetal platelets resulted in increased surface density of GPIb and GPIIIa.

Tables II and III show the difference in percentage expression and surface density (relative fluorescence intensity) of platelet GPIb and GPIIIa both before and after stimulation with adenosine diphosphate between maternal and nonpregnant controls and between fetal and nonpregnant controls, respectively. The findings were (1) lower surface density (relative fluorescence intensity) of GPIb in both mothers and fetuses compared with nonpregnant controls, (2) no significant differences in any of the other variables in the unstimulated group, and (3) adenosine diphosphate stimulation of maternal and fetal platelets resulted in increased

percentage expression and surface density of both glycoproteins compared with nonpregnant controls.

Comment

Fetal platelet count increases, whereas platelet size decreases, with gestation, suggesting that with fetal maturation there is increased thrombocytopoiesis. In adults there is an inverse relationship between platelet count and size,[7] and thrombocytopoiesis is associated with increased megakaryocyte ploidy and decreased platelet size.[8, 9] Differences in ploidy between adults and fetuses may explain the finding that fetal platelets are smaller than those of nonpregnant controls. The additional finding of a significant correlation between fetal and maternal platelet size suggests that there may be a common factor influencing thrombocytopoiesis in both maternal and fetal compartments. A possible candidate is granulocyte-macrophage colony-stimulating factor, which is known to cross the placenta freely.[10]

The finding that the percentage expression of fetal platelet glycoproteins is not significantly different from adult controls is consistent with the hypothesis of this study that fetal platelets develop adult-like functional capacity from an early stage of intrauterine life. Supportive evidence is provided by the finding that stimulation of fetal platelets with adenosine diphosphate results in up regulation of both GPIb and GPIIIa of an equivalent magnitude to that seen in nonpregnant controls.

Maternal platelet size decreases with gestational age, presumably reflecting increased thrombocytopoiesis in the face of expanding plasma volume. However, previous studies of pregnant women have shown either no change or a slight increase in platelet volume with gestational age.[11, 12] Measurements of platelet volume in these studies were carried out on whole blood, which may produce abnormally high readings as a result of red blood cell or leukocyte fragmentation; the use of platelet-rich plasma, as in our study, prevents this inaccuracy.[13] Furthermore, previous studies used blood samples placed in ethylenediaminetetraacetic acid, which is known to alter platelet volume when it is measured electronically.[14]

Pregnancy is associated with a significant decrease in the surface density of maternal and fetal platelet GPIb compared with nonpregnant controls. Furthermore, when stimulated with adenosine diphosphate, maternal and fetal platelets were more responsive and produced significantly increased percentage and surface density of both GPIb and GPIIIa, suggesting that platelet responsiveness is increased in pregnancy. Previous studies of maternal serum levels of coagulation factors and intracellular platelet calcium concentration have reported increases with advancing gestation, supporting the hypothesis that a hypercoagulable state develops during pregnancy.[15, 16]

This study has demonstrated that pregnancy is associated with alterations in platelet size and glycoprotein expression in both the mother and the fetus. Furthermore, the data indicate that fetal platelets are phenotypically mature from at least 12 weeks' gestation. It remains to be established whether pregnancy complications that are known to affect maternal platelets, such as preeclampsia, growth retardation, and diabetes mellitus,[17-19] also affect fetal platelet heterogeneity and function.

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