Clinical Hemorheology and Microcirculation 22 (2000) 79–90
IOS Press

Rheological properties of fetal red cells with special reference to aggregability and disaggregability analyzed by light transmission and laser backscattering techniques

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Received 19 April 1999
Accepted 27 September 1999

Abstract. Blood viscosity factors and fetal erythrocyte aggregability were investigated with light transmission (Myrenne device) during a cross-sectional study of blood drawn *in utero* by cord venepunctures in 119 normal fetuses between 18 and 39 weeks gestation. There was a progressive increased blood viscosity at native hematocrit ($p < 0.01$) explained by a gradual increase in both hematocrit (from 33% to 40%, $p < 0.05$) and Dintenfass' 'Tk' RBC rigidity index ($p < 0.05$), while plasma viscosity remained constant at 1.18 ± 0.01 mPa.s as well as the h/η ratio (188.4 ± 2.7 $mPa^{-1}.s^{-1}$). The RBC aggregation index 'M' remained almost equal to zero (mean value: 0.04 ± 0.01) before 32 wk gestation and then increased ($p < 0.05$) until delivery. The upper physiological limit for this parameter before 32 wk (mean ± 2 SD) is 0.18. The RBC aggregation index 'M1' remained constant during pregnancy at 2.98 ± 0.26, i.e., the upper physiological limit for this parameter during the intrauterine life (mean ± 2 SD) is 7.85. Both fibrinogen ($r = 0.479$, $p < 0.05$) and albumin ($r = 0.494$, $p < 0.01$) correlated with time so that the albumin/fibrinogen ratio remained stable. We then studied with the laser retrodiffusion technique the venous blood of 20 women (18–43 yr, 37–40 wk gestation) and the cord blood of their newborns at birth, comparing RBC aggregation of: mothers (M), maternal RBCs resuspended on newborn plasma (MF), newborn RBCs resuspended on maternal plasma (FM), and newborns (F). Aggregability is higher in M (RBC aggregation time M < MF < FM < F; $p < 0.01$); RBC aggregation index at 10 s M > MF > FM > F; $p < 0.01$), with in turn the symmetric inverse picture for the partial disaggregation threshold (M > MF = FM > F). Thus RBC disaggregability is higher in newborns, and suspensions on maternal and newborn plasma suggest that half of this difference in aggregability (and disaggregability) between fetal and adult blood results from plasma factors and another half from erythrocytes.

Keywords: Fetal blood, cordocentesis, hemorheology, erythrocyte deformability, blood viscosity, erythrocyte aggregation, vascular resistance

1. Introduction

Tissue perfusion is mostly regulated by changes in vascular tone, but modifications of the rheologic properties of blood can also markedly influence it, mostly in situations like the intrauterine life where va-

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somotricity is immature [1]. Cord blood measurements made after delivery have given indirect evidence for marked differences between prenatal and postnatal blood rheology [2–5]. Fetuses had, compared to pregnant or nonpregnant adult women studied simultaneously, a significantly raised hematocrit [5,6], and reduced erythrocyte deformability [6], such a pattern resulting in an elevation of whole blood viscosity [2–5]. They have also reduced erythrocyte aggregation and lowered plasma viscosity [7]. This pattern has been suggested to play a physiological role in maintaining a sufficient O_2 supply to fetal tissues, since it reduces the tendency to increase viscosity despite increased RBC rigidity [7]. The direct study of fetal blood drawn *in utero* by cord venepunctures [8,9] has confirmed these data. A physiological description of the variations of blood viscosity during the intra-uterine life has been published [10]. However, one of the most intriguing hemorheologic properties of fetal blood is the low aggregability of erythrocytes, which remains uncompletely studied. A quantitative investigation with the laser backscattering technique was, to our knowledge, still lacking. In this study, we aimed at: (1) describing the physiological evolution of erythrocyte aggregability in a cross-sectional study of blood drawn *in utero* by cord venepunctures in 119 normal fetuses between 18 and 39 weeks gestation; (2) analysing disaggregability of these RBCs with the laser backscattering technique (AFFIBIO erythroaggregometer) which allows the measurement of disaggregability thresholds; (3) elucidating the respective role of the red cell and the surrounding milieu in these aggregation/disaggregation processes by *in vitro* incubation experiments on cord blood.

2. Materials and methods

2.1. Patients

Fetuses included in this study underwent a cord venepuncture for the detection of genetic abnormalities and/or recent infection by rubella or toxoplasmosis. Cordocentesis was performed *in utero* during pregnancy as previously reported [11]. The method allowed an ambulatory sampling during a hospital visit performed in an operating room with surgical preparation of the abdomen. No premedication (e.g., maternal sedation) was administered. Bladder filling was unnecessary. The ultrasound device was a 3.5 MHz sectorial transducer (Combison Kretz 320) manipulated through a sterile bag. After local anesthesia (1% xylocaine), a 22.5 gauge needle fixed on a syringe was introduced in the plane of the ultrasound section through the abdominal wall, the uterine wall, the membranes, into the amniotic cavity, and finally into the umbilical cord. The 22.5 gauge needle was chosen in order to reduce cord bleeding when the needle was withdrawn. Fetal blood was aspirated after changing the syringe in order to avoid contamination of samples with maternal blood or amniotic fluid. The duration of funicular bleeding was noted at the withdrawal of the needle. Two hours after sampling, patients were again examined ultrasonographically. Preventive antibiotic treatment consisting of 2 g of cefotaxime daily was administered for 5 days, as well as an injection of anti-D gamma globulin if the mother was Rh-negative and fetus Rh-positive. Two methods were used for verifying purity of blood samples: the Kleihauer test and the measurement of the mean corpuscular volume on a Coulter Counter S Plus II. During the study period, samplings were performed by the same obstetrician and ultrasound guidance was performed by the same specialist.

Pathologic cases (malformations, fetal distress) were excluded from the study. Finally, a group of 119 'normal' fetuses was constituted. Fetuses were studied between 18 and 39 weeks gestation. Twelve fetuses were studied between 18 and 21 wk, 21 between 22 and 23, 21 between 24 and 25, 15 between 26 and 27, 16 between 28 and 29, 10 from 30 to 31, 11 from 32 to 33, 5 from 34 to 35 and 8 between 36 and 39 wk. At the same time, maternal blood was also drawn (just before the cord puncture).

2.2. *In vitro study of fetal/maternal aggregation kinetics*

We then studied at birth the venous blood of 20 women (18–43 yr, 37–40 wk gestation) and the cord blood of their newborns. We compared RBC aggregation of: mothers (M), maternal RBCs resuspended on newborn plasma (MF), newborn RBCs resuspended on maternal plasma (FM), and newborns (F).

2.3. *Laboratory measurements*

Blood was collected into potassium EDTA. In fetuses, only 2–3 ml were drawn, while in mothers this volume was 7 ml. Measurements were performed within 2 h after venepuncture. Hematocrit (packed cell volume) was evaluated by a microhematocrit technique on a Hellige autocrit centrifuge. Blood viscosity and plasma viscosity were measured at very high shear rate ($\gamma = 1000\ s^{-1}$) with a micro-method. Measurements were performed on the MT90 falling ball viscometer (Medica-test, 37 rue de l'Ermitage, F-86280 Saint Benoit) [12,13]. We used for control quality and calibration a more sophisticated viscometer which unfortunately needed too much blood and could not be employed for fetal blood. The coefficient of variation of this method ranges between 0.6 and 0.8% (10 repetitive measurement of the same sample). The results of viscometric measurements were expressed as apparent viscosity at native hematocrit η_b, viscosity for corrected hematocrit 45% η_{45}, and RBC rigidity index 'Tk'. Correction of blood viscosity for hematocrit was calculated according to Quemada's equation [14]:

$$\eta_b = \eta_{pl}(1 - (1/2)kh)^{-2},$$

where η_{pl} is plasma viscosity, h hematocrit, and k a structural parameter of blood viscosity which depends at high shear rate on RBC flexibility. A viscometric index 'Tk' of red cell rigidity (as reflected by shear-induced erythrocyte elongation) was calculated from this viscometric measurement according to Dintenfass [15]. 'Tk' is given by the following equation:

$$\mathrm{Tk} = (\eta_r^{0.4} - 1)/(\eta_r^{0.4}h),$$

where η_r is relative blood viscosity (i.e., η_b/η_{pl}). The 'k' index of RBC rigidity was also calculated according to Quemada [14]:

$$k = 2(1 - \eta_r^{-0.5})h^{-1}.$$

Quality control of the measurements performed with the MT90 falling ball viscometer was regularly made with the Carrimed rheometer which allows a precise measurement of blood viscosity over a wide variety of shear rates (from less than 0.1 up to 2000 s^{-1}). This latter device was not used in the study because it needs more blood sample volume than available from fetuses.

Erythrocyte rigidity was measured by filtration of red cells resuspended at 8% hematocrit in Tris-albumin buffer, with the Hemorheometre MK-1 (from IMH, 2, allee du Jardin de la Cure, 95470 Saint Witz, France). This apparatus measures the the initial flow rate of a suspension of red cells [16] through 5 μm Nuclepore sieves. Results were expressed as a relative viscosity of filtration (η_{fr}) and corrected by hematocrit:

$$\eta_{fr} = (t_s/t_b)/h,$$

where t_s is the time of passage of the suspension of red cells at 8% hematocrit, t_b the time of passage of the buffer alone, and h the packed cell volume (%).

RBC aggregation in fetal blood drawn *in utero* was assessed with the Myrenne aggregometer [17] which gives two indices of RBC aggregation: 'M' (aggregation during stasis after shearing at 600 s^{-1}) and 'M1' (facilitated aggregation at low shear rate after shearing at 600 s^{-1}). On cord blood at birth we could employ a more quantitative technique which requires more blood, the AFFIBIO-SEFAM aggregometer which could thus provide a more precise assessment of RBC aggregation. This device is based upon the experiments of Mills [18] on cell disaggregation behavior in shear flow. This device measures the changes in backscattered light which are observed when sheared RBC suspensions are abruptly brought to a full stop. The decrease in the optical signal reflects the formation of RBC aggregates [19–21]. Some parameters are derived from the curve of light intensity as a function of time. The aggregation time is the reciprocal of the initial slope (calculated between 0.5 and 2 s after the shear has stopped). The aggregation index at 10 s is a measurement of the extent of erythrocyte aggregation and is the relative surface area above the curve calculated over the first 10 seconds. This device measures also disaggregation thresholds, by submitting blood to a succession of shear rates from 600 s^{-1} to 7 s^{-1}. The total disaggregation threshold is the shear rate below which the backscattered light intensity starts to decrease, indicating that the shear stress applied to aggregates is no longer sufficient for allowing complete dispersion of RBC aggregates. The partial disaggregation shear rate is defined as the shear rate corresponding to the intersection point of the two asymptotes drawn from the extremes (maximum and minimum shear rate). Hematocrit viscosity ratio (h/η) was calculated according to Stoltz [22], as an index of the contribution of blood rheology to O_2 supply to tissues.

2.4. Statistics

Correlations were tested by linear regression analysis. Results are presented as mean ± the SE of the mean. A value of $p < 0.05$ was considered as significant. Correlations were performed using the method of least squares. Variables in the two groups were compared using the two tailed nonparametric test of Mann–Whitney for unpaired data. Significance was defined as $p < 0.05$. The choice of nonparametric tests was due to the fact that hemorheological parameters usually appear to exhibit a non-normal distribution [23].

3. Results

Values of hemorheological parameters measured by cord venepunctures during the intrauterine life are shown in Table 1.

Whole blood viscosity at native hematocrit measured at high shear rate exhibits a highly significant progressive increase during the intrauterine life ($p < 0.01$). This increase is first explained by a gradual increase in hematocrit between 12 and 36 wk ($p < 0.05$), from an average of 33% to 40%. However when corrected for hematocrit (η_{45}) viscosity still increases very significantly during the period of the intrauterine life ($p < 0.01$). This is due to the Dintenfass' 'Tk' RBC rigidity index which undergoes a progressive increase during the intrauterine life ($p < 0.05$). Red blood cell rigidity was also measured by filtration with the Hemorheometre. In that case, the ANOVA did not detect a significant change during the intrauterine life, due to a large variability. By contrast, plasma viscosity η_{pl} remains constant during the intrauterine life at an average value of 1.18 ± 0.01, so that the physiological range for this parameter in fetus (mean ± 2 SD) is 0.99–1.37 mPa.s. The h/η ratio remains constant in fetal blood during the

Table 1
Values (mean ± SEM) of hemorheological parameters measured by cord venepunctures during the intrauterine life

	Weeks gestation									Mother
	<22	22–23	24–25	26–27	28–29	30–31	32–33	34–35	>36 wk	
η_b (mPa.s)	1.79	1.85	1.87	1.94	1.88	2.23	1.99	2.04	2.33	2.13
	±0.04	±0.07	±0.05	±0.07	±0.07	±0.11**	±0.06*	±0.15	±0.16**	±0.07
η_{pl} (mPa.s)	1.23	1.17	1.13	1.13	1.21	1.16	1.16	1.23	1.23	1.326
	±0.07	±0.03	±0.03	±0.03	±0.02	±0.03	±0.03	±0.02	±0.02	±0.02
η_{45} (mPa.s)	1.96	2.06	2.24	2.16	2.11	2.38	2.24	2.36	2.42	2.55
	±0.05	±0.05	±0.1	±0.07	±0.05	±0.06	±0.06	±0.16	±0.08	±0.22
'Tk' RBC rigidity index	0.41	0.47	0.49	0.50	0.45	0.52	0.50	0.48	0.53	0.505
	±0.03	±0.03	±0.02	±0.02	±0.02	±0.02	±0.02	±0.03	±0.02	±0.04
'η_{fr}' RBC rigidity index	0.41	0.47	0.49	0.50	0.45	0.52	0.50	0.48	0.53	0.4
	±0.03	±0.03	±0.02	±0.02	±0.02	±0.02	±0.02	±0.03	±0.02	±0.04
h/η	202	181.3	191.6	197.1	178	186.2	189.8	178.8	179.5	170.6
	±6.8	±8.1	±7.7	±9.1	±13.8	±10.2	± 4.5	±6.4	±6.2	±11.4
Hematocrit (%)	33.7	33.9	34.8	36.3	37	39.8	37.4	36.6	40.2	36.35
	±1.65	±0.65	±1.24	±1.7	±1.97	±2.07	±0.96	±2.16	±2.55	±0.8
RBC aggregation 'M'	0.01	0.04	0.02	0.06	0.04	0.05	0.159	0.350	0.740	11.73
	±0.01	±0.02	±0.01	±0.03	±0.03	±0.02	±0.12	±0.07	±0.15	±0.45
RBC aggregation 'M1'	3.3	3.04	2.67	3.14	2.82	1.22	3.05	3.55	4.84	18.05
	±0.72	±0.57	±0.64	±0.80	±0.52	±0.36	±1.06	±0.85	±0.89	±0.58

*$p < 0.02$, **$p < 0.05$ vs preceding value.
For comparison values in maternal venous blood from 34 women (mean age 29.6 ± 0.7 yr) seen at 30–35 wk gestation are also given in the last column (comparison with fetal blood not indicated: all values significantly differ from fetal blood at almost any time).

intrauterine life at an average value of 188.4 ± 2.7. Therefore, the physiological range for this parameter in fetus (mean ± SD) is 132–244 $mPa^{-1}.s^{-1}$. As shown in Fig. 1, the RBC aggregation index 'M' before 32 wk gestation remains almost equal to zero arbitrary units (mean value: 0.04 ± 0.01). However, at 32–33 wk, they begin to slowly increase ($p < 0.05$) until delivery. The upper physiological limit for this parameter before 32 wk (mean ± 2 SD) is 0.18. The RBC aggregation index 'M1' is somewhat higher and remains constant during all the pregnancy at 2.98 ± 0.26, i.e., the upper physiological limit for this parameter during the intrauterine life (mean ± 2 SD) is 7.85.

Fibrinogen was assayed in 22 subjects. As shown in Fig. 2, it correlated with time ($r = 0.374$ non-significant tendency) but the regression became significant when an exponential relationship was tested ($r = 0.479$, $p < 0.05$). Albumin was measured in 27 subjects and was linearly correlated with time ($r = 0.494$, $p < 0.01$). The albumin/fibrinogen ratio (17 subjects) and the total fetal plasma protein concentration (22 subjects) were not significantly related to time (respectively $r = 0.322$ and $r = 0.273$), suggesting that this ratio is almost constant during the intrauterine life.

3.1. *Aggregability of fetal and maternal red cells in vitro resuspended on fetal and maternal plasma*

The laser retrodiffusion technique, which requires too much blood to be employed for the study of fetal blood drawn *in utero*, was employed for a quantitative evaluation of aggregability and disaggregability of fetal red blood cells obtained from cord blood at birth. These experiments on RBC aggregation on cord blood at birth and maternal blood are shown in Figs 3, 4 and 5. When comparing RBC aggregation

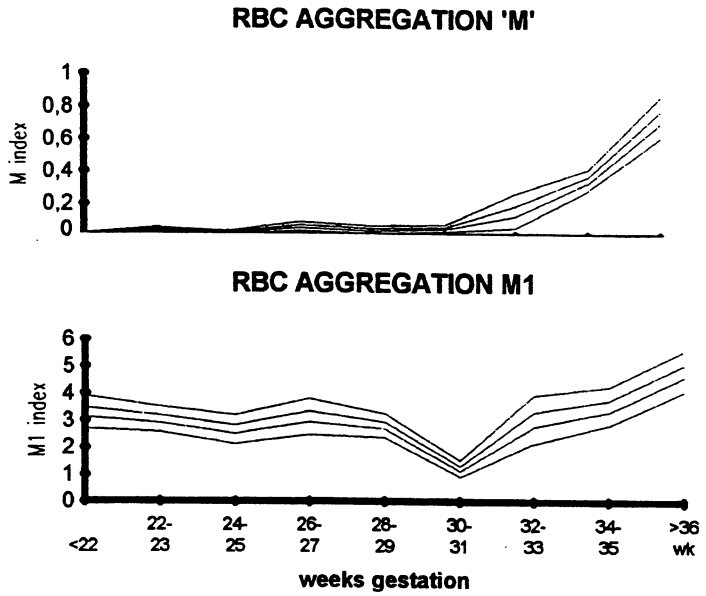

Fig. 1. Reference range of values for the RBC aggregation indexes 'M' and 'M1' measured by light transmission analysis (Myrenne Aggregometer) during the intrauterine life. Lines represent the boundaries for quintiles of distribution. Mean and SEM for values are given in Table 1.

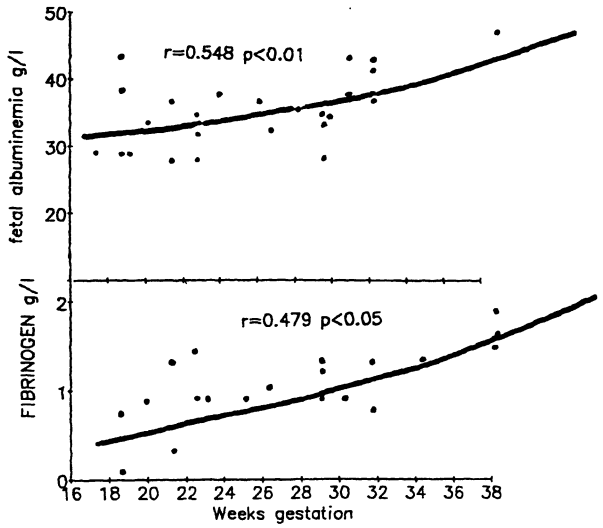

Fig. 2. Regression plots of plasma fibrinogen and albumin against time during the intrauterine life. A gradual increase can be observed, so that the albumin/fibrinogen ratio remains almost constant.

of maternal RBCs in their native plasma (M), maternal RBCs resuspended on newborn plasma (MF), newborn RBCs resuspended on maternal plasma (FM), and RBCs from newborns in their own plasma (F), this technique showed that aggregability is higher in M than the other combinations (M > MF > FM > F), with in turn the symmetric inverse picture for disaggregability, which is lower in M. Figure 3 shows this relationship for the RBC aggregation time (M < MF < FM < F) ($p < 0.01$). Figure 4 shows this pattern for the RBC aggregation index at 10 s (M > MF > FM > F) ($p < 0.01$). Figure 5 shows

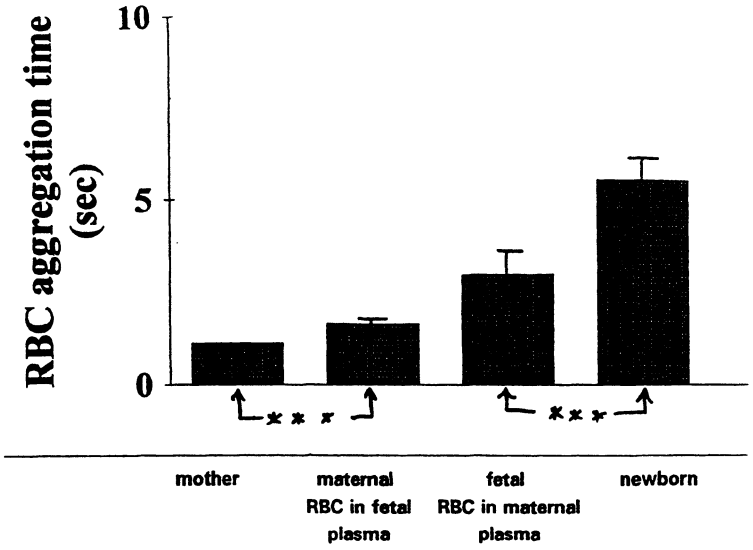

Fig. 3. Measurement with laser backscattering analysis of the RBC aggregation time of erythrocytes: from maternal systemic venous blood in their native plasma (M), from maternal systemic venous blood resuspended on newborn plasma (MF), newborn RBCs resuspended on maternal plasma (FM), and newborn erythrocytes in their native plasma (F). ***$p < 0.01$.

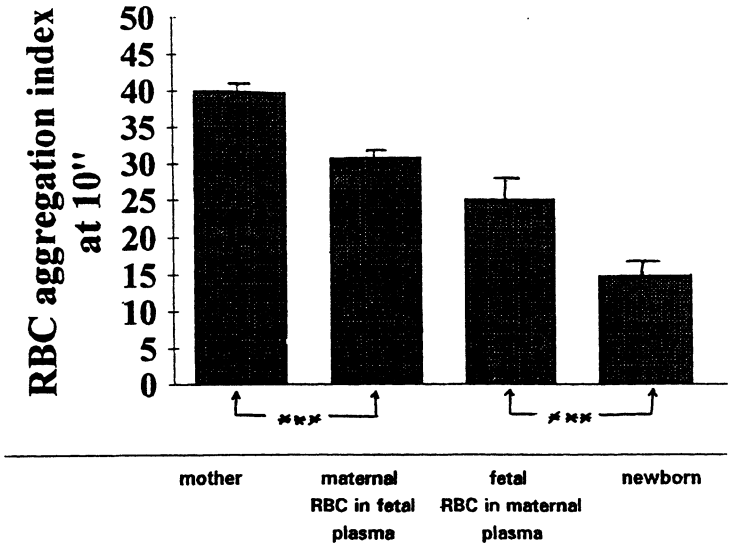

Fig. 4. Measurement with laser backscattering analysis of the RBC aggregation index at 10 s of erythrocytes: from maternal systemic venous blood in their native plasma (M), from maternal systemic venous blood resuspended on newborn plasma (MF), newborn RBCs resuspended on maternal plasma (FM), and newborn erythrocytes in their native plasma (F). ***$p < 0.01$.

that the highest partial disaggregation threshold is found in mothers (M > MF = FM > F) but the value of the partial disaggregation threshold (γ_D) for MF and FM was quite the same (62.56 ± 4.97 vs 59.16 ± 4.71 s^{-1}). This intermediate value of γ_D was higher than in F (41.53 ± 0.98, $p < 0.01$) and lower than in M (102.55 ± 5.34, $p < 0.001$).

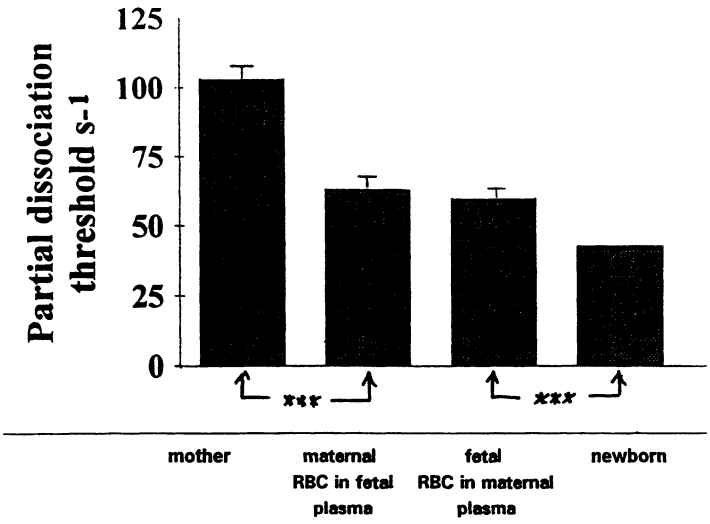

Fig. 5. Measurement with laser backscattering analysis of the RBC partial disaggregation threshold of erythrocytes: from maternal systemic venous blood in their native plasma (M), from maternal systemic venous blood resuspended on newborn plasma (MF), newborn RBCs resuspended on maternal plasma (FM), and newborn erythrocytes in their native plasma (F). $***p < 0.01$.

4. Discussion

Although a physiological description of whole blood and plasma viscosity evolution (providing reference ranges during the intrauterine life) has been previously published by others [10], this information was still lacking for RBC aggregation assessed by specific techniques, although this parameter was known to undergo profound modifications during this period [1–7]. Therefore, this study gives the first physiological description of RBC aggregation of fetal blood obtained from venepunctures during the intrauterine life. Data are in accordance with previous studies investigating more indirectly this question with cord blood at birth [2–6]. We confirm with the Myrenne erythroaggregometer that fetal RBCs are almost unaggregable until the last weeks of pregnancy, as previously established by Rampling and coworkers [7,24,25]. In addition, an *in vitro* experiment on maternal blood and fetal blood suggests that almost half of the difference in aggregability (and disaggregability) between maternal and fetal blood comes from the red cell and the other half from the suspending medium.

4.1. Methodological aspects

It is difficult, for obvious ethical reasons, to measure fetal blood rheology without medical reasons for performing a cordocentesis. Therefore, our sample of fetuses is not a truly 'physiological' one and is selected after the fetal pathologies have been ruled out: this represents an important cause of methodological bias which seems difficult to overcome. However, we think that this approach provides a more direct evaluation of what happens *in utero* than the previous studies using cord blood at birth. In our preliminary report on 29 fetuses [8,9] we recognized that the study of cord blood of prematures at birth, as performed by the first investigators in this field [1–7,10,24,25], was a good model for evaluating fetal rheology. Our present findings are in agreement with these previous statements. However, two major possibilities of artifacts exist for that method: (a) prematures are not 'normal' newborns and could be expected to have diseases which modify blood rheology; (b) labor and delivery are very stressful events which induce in

the mother dramatic increases in blood viscosity [26–29]. Moreover, for many physiological reasons, the newborn can no longer be considered as a fetus.

Concerning laboratory measurements, it is clear that only techniques requiring a very little volume of blood could be used in humans for this purpose. The Myrenne aggregometer was especially interesting for this, since it uses only a droplet (about 100 μl) of blood. The MT90 falling ball viscometer [12, 13] which has been largely used in our clinical studies, also offers the advantage of minimal volume requirements (about 800 μl). We should indicate for this technique a slight difference between the values presented here and our preceding ones [8,9] in preliminary papers on the same subject. The introduction of a quality control with the Carri-Med rheometer helped us to correct our results obtained with the falling ball viscometer by a proportionality factor. We think that the values given in this paper are more consistent with what would give classical Couette viscometry at the same shear rate [13].

4.2. *Particularities of fetal blood*

This study confirms that fetuses exhibit a peculiar hemorheologic pattern: lower plasma viscosity, lower blood viscosity, more rigid RBCs, higher hematocrit/viscosity ratio. RBC aggregation which could not be measured in our preceding preliminary reports [8,9] is very low (M index equal to zero before 20 wks). This latter finding is in agreement with the reports of the team of Rampling [7,24,25]. These authors interpret the increase in rouleaux formations in fetal blood at the end of pregnancy as a consequence of increases in concentrations of fibrinogen, α2 macroglobulin, IgG and a reduction in the degree of sialination of fibrinogen. Our *in vitro* experiment confirms that cellular factors which are not overcome by resuspension of fetal RBCs in adult plasma are also involved, as previously suggested [30]. Little is known about cellular factors explaining this "donor specific effect" whose importance has been emphasized by the team of Meiselman [31]. In addition, erythrocyte disaggregability, which to our knowledge, has never been studied for fetal blood, exhibits a similar pattern with high values reflecting a poor interaction among red cells, which is reduced by only 50% after resuspension in the maternal plasma, i.e., a surrounding medium which promotes hyperaggregability [1,4,6,26,32,33]. Therefore, half of this property of hypoaggregability–hyperdisaggregability of fetal red cells appears to depend upon the "donor-specific effect". Presumably, the specific membrane composition of fetal red cells [34] explains most of this rheologic property.

Deformability of the erythrocytes was mostly assessed by calculations from viscometric measurements, for reasons of volume requirements. The Dintenfass' 'Tk' RBC rigidity index progressively increases during the intrauterine life. However, we previously reported [8] that RBC filterability measured with the hemorheometre seemed to exhibit a "U-shaped curve". While we have considerably expanded our population sample, we did not increase to a similar extent the number of hemorheometre measurements, because they required too much blood volume. Thus, RBC rigidity seems to undergo modifications during the intrauterine life, and these modifications remain to be described in more detail. The viscometric approach used in this paper was unable to detect them. However, filtration studies in fetuses should carefully avoid artifacts related to MCV changes throughout the intrauterine life [34]. The viscometric assessment only shows that red cell rigidity increases by 30% between 22 and 36 wk gestation, i.e., an average weekly increase of 2%. Thus, the low deformability of red cells classically observed on cord blood at birth is clearly a property which develops only at the end of the intrauterine life. Colin and coworkers [34] have also reported data on RBC filterability and RBC membrane lipids in 40 fetuses. They show, in accordance with other reports [8,35], that RBC deformability was lower in term fetuses (37–40 wks) than in earlier fetuses (18–24 wks).

We previously reported that the hematocrit/viscosity ratio is higher in fetuses than in their mothers [8, 9]. This parameter is believed to evaluate the contribution of blood rheology to O_2 supply to tissues [22]. In fact, in maternal organisms, vasodilatation is an important factor of increased blood distribution [36], despite lower hematocrit and slowly increased viscosity. By contrast, in fetus, O_2 content of blood is relatively low [36,37], as well as systemic blood pressure [37]. Thus, a low h/η ratio is probably important for maintaining a sufficient O_2 distribution to fetal tissues. Interestingly, in our study, this parameter remains constant with no tendency to increase during intrauterine life, as a result of a combination of the various changes observed during this period, suggesting that there is in fetuses some degree of homeostasis of the rheologic determinants of fetal blood circulation during the intrauterine life.

All these aspects of intrauterine evolution of the rheologic properties of blood show a progressive modification of them, from a very peculiar 'fetal' pattern towards a more "adult-like" one which is found in the newborn. Especially, the physiological meaning of a lack of RBC aggregation in the youngest fetuses remains unclear. As previously indicated [38] these aspects are important for therapeutic purposes, since transfusion of adult blood (with more viscous plasma and much more aggregable RBCs) may result in dangerous hyperviscosity states. Clearly, viscosity, in fetuses as well as adults, is an important regulator of endothelial function, since it determines the wall shear stress which triggers synthesis and release of nitric oxide, resulting in major alterations in tissue perfusion pressure [39]. Consistent with this assumption, we previously observed highly significant correlations between fetal viscosity (and hematocrit) and Doppler-derived indices of vascular resistance in umbilical arteries [40].

We present in this study (Fig. 1) reference ranges for RBC aggregation directly measured in fetal blood with a technique suitable for this purpose, requiring only a tiny amount of blood. In a previous paper, we evidenced that values of RBC aggregation above this control range are associated with pathologic situations [40]. Thus, aggregometry with the Myrenne erythroaggregometer is likely to be a marker of fetal pathologies, probably as a consequence of disturbed plasma protein pattern.

On the whole, the physiology of blood rheology in fetus, which remained uncompletely known until now, appears to be interesting to study for three reasons. First, it can help us to improve the accuracy of intrauterine fetal therapeutics (e.g., transfusion). Then, for the hemorheologist, our preliminary findings suggest that the fetus may be a model for studying the interactions of blood rheology with circulation [40]. Finally, some pathophysiological disorders of the fetoplacental unit may involve rheologic abnormalities, with possible hemorheologic treatment. Our more sophisticated experiment with laser retrodiffusion confirms the low value of RBC aggregation in newborns at birth (a classical model for fetal blood) and indicates that RBC disaggregability is also easier in newborns. Suspensions on maternal and newborn plasma suggest that half of this difference results from plasma factors and another half from erythrocytes.

Acknowledgements

This work was supported by Negma laboratories and has been presented in part as a poster at the 9th ECCH, Siena, 1995.

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