

# Developmental Hematopoiesis in Normal Human Fetal Blood

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Using an easy and safe procedure for fetal blood sampling in utero, we studied 3,415 fetuses for prenatal diagnosis. Retrospectively, 2,860 normal blood samples, performed from the 18th week of gestation to the end of pregnancy, were selected. Differentials were evaluated in 732 cases. Burst-forming unit erythroid (BFU-E) and erythropoietin (Epo) were measured in 27 and 163 cases, respectively. Total nucleated cell and platelet counts did not change from the 18th to the 30th week of gestation. The lymphocytes represented the main population and the decrease of normoblastic cells made

up for the increase in neutrophils. The increase of red blood cells and hemoglobin was substantial during the studied period. At mid trimester threefold more BFU-E were obtained than at birth. Epo levels remained stable throughout the pregnancy and no correlation was found between Epo and gestational age. These normal values of fetal erythropoiesis will improve our knowledge of physiology and provide a better insight into developmental hematopoiesis.  
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**O**UR KNOWLEDGE of fetal hematology is limited and developmental hematopoiesis, once an entirely descriptive discipline, is now oriented toward mechanistic investigations. Most earlier studies were performed on aborted fetuses or under sampling conditions that may have altered hematologic values.

However, the recently developed technique of percutaneous umbilical cord sampling has improved on those investigations by providing a source of circulating blood cells from living human fetuses. We studied 3,415 fetuses for various prenatal diagnoses and retrospectively selected 2,860 normal fetuses from the 18th week of gestation to delivery in order to establish reference values for main hematologic parameters.

In a smaller group we studied burst-forming unit erythroid (BFU-E) and erythropoietin (Epo) level. These results provide useful data for future investigations.

## MATERIALS AND METHODS

*Sampling procedure.* Fetal blood sampling has been performed under ultrasound guidance and has been described in detail elsewhere.<sup>1</sup>

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*Patients.* Fetuses, 2,842, were studied and 2,860 fetal blood samplings were performed (18 twins) with the approval of the ethics committee of the institute, the medical expert panel, and the informed consent of the patients. Fetal samplings were performed for the prenatal diagnosis of toxoplasmosis<sup>2</sup> (n = 1,981), rubella<sup>3</sup> (n = 63), hemophilia<sup>4</sup> (n = 78) karyotyping (n = 482), and hemoglobinopathies (n = 76).

*Biologic investigation.* We usually obtained approximately 2 to 3 mL of blood and divided it into three aliquots. Five hundred microliters was anticoagulated in lyophilized EDTA K2 (Sarstedt, Nümbrecht, Germany), 400 µL was collected in .129 mol/L sodium citrate solution (ratio 1 + 4), and 1 mL was collected on heparin for progenitor studies. The remaining blood was not anticoagulated.

*Purity of the samples.* The assessment of fetal blood samples has been recently reviewed<sup>2</sup>: special assays included hematologic indexes, Kleihauer test, focusing of hemoglobin, blood smear, erythrocyte i/I antigens, β human chorionic gonadotrophin, and coagulation factor assays. Pure fetal blood was obtained in all cases, without dilution by amniotic fluid or contamination by maternal blood.

Main hematologic parameters were determined with a Coulter Counter S Plus II (Coulter, Hialeah, FL): all white blood cells (WBCs), platelets (PLT), red blood cells (RBCs), hemoglobin (Hb), hematocrit (Ht), and mean corpuscular volume (MCV) were measured from 44.7 µL of fetal blood.

The blood smears were stained using the May-Grünwald Giemsa method for the differential blood count. The number of normoblasts was calculated for 100 WBCs. The data of WBCs given by the Coulter Counter were overestimated due to the presence of normoblasts. Total WBC counts were analyzed separately.

*Cell cultures.* We used the method previously described by Iscove et al.<sup>6</sup> Mononuclear cells were recovered from Ficoll-Hypaque and washed in Iscove's Modified Dulbecco's Medium (IMDM). Mixtures containing 0.8% methylcellulose, 30% heat-inactivated fetal calf serum, 1% deionized bovine serum albumin, 10<sup>-4</sup> mol/L α thioglycerol, 100 U penicillin/mL, 100 µg streptomycin/mL, 2 × 10<sup>6</sup> nucleated cells, and 1 U of Epo/mL were used.

Dishes were incubated at 37°C in a humidified atmosphere with 5% CO<sub>2</sub>. For this study, 27 normal fetuses, 9 cord blood samples at

**Table 1. Evolution of Hematologic Values of 2,860 Normal Fetuses During Pregnancy (mean ± SD)**

Week of Gestation	WBC* (×10 <sup>9</sup> /L)	Total WBC Counts (×10 <sup>9</sup> /L)	PLT (×10 <sup>9</sup> /L)	RBCs (×10 <sup>12</sup> /L)	Hb (g/100 mL)	Ht (%)	MCV (fL)
18-21 (n = 760)	4.68 ± 2.96	2.57 ± 0.42	234 ± 57	2.85 ± 0.36	11.69 ± 1.27	37.3 ± 4.32	131.11 ± 10.97
22-25 (n = 1,200)	4.72 ± 2.82	3.73 ± 2.17	247 ± 59	3.09 ± 0.34	12.2 ± 1.6	38.59 ± 3.94	125.1 ± 7.84
26-29 (n = 460)	5.16 ± 2.53	4.08 ± 0.84	242 ± 69	3.46 ± 0.41	12.91 ± 1.38	40.88 ± 4.4	118.5 ± 7.96
> 30 (n = 440)	7.71 ± 4.99	6.40 ± 2.99	232 ± 87	3.82 ± 0.64	13.64 ± 2.21	43.55 ± 7.2	114.38 ± 9.34

\*Including normoblasts.

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**Table 2. Some Aspects of Fetal Differential Counts From 18 to 30 Weeks of Gestation of 752 Normal Fetuses**

Week of Gestation	Lymphocytes (%)	Neutrophils (%)	Eosinophils (%)	Basophils (%)	Monocytes (%)	Normoblasts (% WBCs)
18-21 (n = 186)	88 ± 7	6 ± 4	2 ± 3	0.5 ± 1	3.5 ± 2	45 ± 86
22-25 (n = 230)	87 ± 6	6.5 ± 3.5	3 ± 3	0.5 ± 1	3 ± 2.5	21 ± 23
26-29 (n = 144)	85 ± 6	8.5 ± 4	4 ± 3	0.5 ± 1	3 ± 2.5	21 ± 67
>30 (n = 172)	68.5 ± 15	23 ± 15	5 ± 3	0.5 ± 1	3 ± 2	17 ± 40

**Table 3. BFU-E in Normal Fetuses, Cord Blood (at birth), Adult Bone Marrow, and Peripheral Blood (mean ± SD)**

	Mean	SD	Extreme Values
Fetal blood (n = 27)	160	107	80-460
Cord blood at birth (n = 9)	48	19	12-96
Adult bone marrow (n = 10)	16	9	5-43
Adult peripheral blood (n = 10)	3.6	3	0-16

Results expressed per  $10^5$  cells.

birth, 10 adult bone marrows (healthy normal volunteers), and their corresponding peripheral blood were selected. Erythroid bursts were counted each day from day 9 to day 14. Considering the variation of the peaks of BFU-E, we scored fetal samples at 10 days, neonates at 13 days, and adults at 14 days.

Epo was measured with an enzyme immunoassay (EIA) kit from Becton Dickinson (Bio Mérieux Marcy l'Etoile, France) in 163 normal fetuses during the second and third trimesters of pregnancy according to the method of Cotes et al.<sup>7</sup>

## RESULTS

The means and standard deviations of the main hematologic parameters are shown in Table 1. To better assess the evolution of the hematopoietic system, four groups were considered: (1) 18 to 21 weeks of gestation, (2) 22 to 25 weeks of gestation, (3) 26 to 29 weeks of gestation, and (4) from the 30th week to birth.

WBCs started at  $4.68 \times 10^9/L$  at the 18th week of gestation to reach  $7.71 \times 10^9/L$  at the 30th week. We observed a gradual decrease of MCV during this same period from 131 to 114 fL, and an increase in Hb (11.69 in the first group v 13.64 g/100 mL in the fourth group). RBCs and Ht are progressively increased from 2.85 to  $3.82 \times 10^{12}/L$  and from 37.3% to 43.55%, respectively. We did not observe any change in the platelet counts, which remained comparable with adult values (around  $245 \times 10^9/L$ ).

The data of differential counts for 732 normal fetuses (Table 2) indicated that lymphocytes represented the main population (around 80%). The decrease in normoblastic cells, ranging from 45% to 17%, made up for the increase in neutrophils from 6% to 23%. Eosinophils, basophils, and monocytes remained stable in percentage.

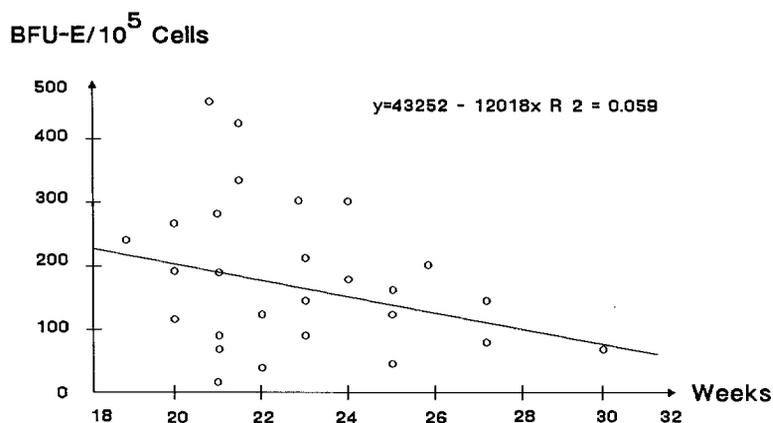
Data concerning BFU-E/ $10^5$  cells are shown in Table 3. We obtained  $160 \text{ BFU-E} \pm 107$  for the fetuses (mean ± SD),  $48 \pm 19$  at birth (cord blood),  $16 \pm 9$  in adult bone marrow, and  $3.6 \pm 3$  BFU-E in the adult peripheral blood. A graph of BFU-E versus gestation is presented in Fig 1. We did not observe any correlation between Hb, erythroblasts, or RBCs and BFU-E.

**Epo.** The extreme values of the measurements of 163 fetal Epo ranged from 0 to 12 with a mean of  $1.6 \pm 2.5$  mIU/mL, and Fig 2 represents Epo versus gestational age. No correlation was observed (Spearman coefficient: 0.062;  $P > .2$ ).

## DISCUSSION

Hematopoietic progenitors are first observed in the yolk sac.<sup>8</sup> Some experiments suggest that an intact yolk sac is required for subsequent fetal hematopoiesis, because progenitors from the vascular system of the yolk sac apparently migrate into and colonize the fetal liver.<sup>9</sup> Fetal bone marrow erythropoiesis begins between the 16th and 18th week,<sup>10</sup> as liver hematopoiesis is challenged by hepatocyte proliferation.<sup>11</sup> The first morphologically recognizable platelets appear in the fetal circulation at 8 to 9 weeks of gestation and the circulating platelet concentration is similar to those of adults and is achieved by or before 18 weeks of gestation.<sup>12,13</sup> Neutrophils are the last of the fetal blood cells to appear and the macrophages are probably the first.<sup>14-16</sup>

Our data confirmed that previously published,<sup>17</sup> but they



**Fig 1. Representation of the BFU-E/ $10^5$  cells obtained in 27 normal fetal blood samples versus gestation (expressed in weeks).**

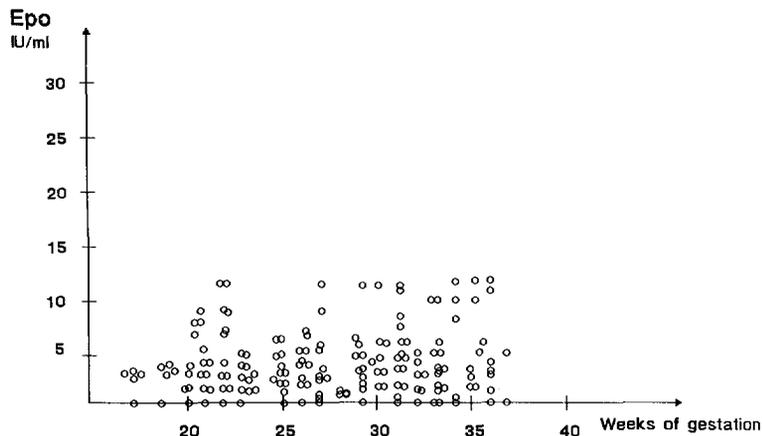


Fig 2. Representation of Epo versus gestation age in 163 normal fetuses.

were measured in a larger population. WBCs and neutrophils are much lower at 18 to 21 weeks than at the end of pregnancy ( $4.68 \pm 2.96/7.71 \pm 4.9 \times 10^9/L$ ), and that may suggest that, should such subjects be exposed to bacteria or parasites, their defenses would be deficient because of the very low neutrophil count, ie,  $28 \times 10^7/L$  at the beginning of pregnancy.

Fetal hematopoietic progenitors differ in several aspects from the marrow-derived adult progenitors.<sup>18</sup> These differences include their rapid cycling rate, the constantly and rapidly expanding pool size, in vivo differentiation almost exclusively along the erythroid pathway, accelerated in vitro maturation time, greater sensitivity to Epo, and decreased sensitivity to granulocyte-macrophage colony-stimulating factor.<sup>19</sup>

There have been few studies on BFU-E in fetuses and the data are heterogeneous due to the methods used for cell isolation, culture,<sup>20,21</sup> and to the way data are expressed. Some results are given for  $10^5$  seeded cells and others per milliliter of blood.<sup>22-25</sup> Our results are in good agreement with those obtained by Partanen et al,<sup>26</sup> using the same method with adult bone marrow; by Migliaccio et al,<sup>8</sup> who cultured 20 to 30 BFU-E per  $10^5$  mononucleated cells in

perinatal blood; and by Shekhter-Levin et al<sup>21</sup> in neonatal cord blood. It appears that the early second trimester has the highest BFU-E level. At mid-gestation, the proportion of BFU-E is approximately three times that of neonatal cord blood and 10 times that of adult bone marrow.

The level of Epo in fetal sera during the second and third trimesters of pregnancy is extremely low and, surprisingly, we did not observe any increase in Epo level at different gestational ages. We only observed elevations of Epo in cases of Rh alloimmunization and in some cases of severe intrauterine growth retardation (data not shown). All these new data confirm the fundamental differences between the hematopoietic systems of fetal<sup>27-29</sup> and adult subjects.<sup>30,31</sup>

In conclusion, it has been possible to establish normal hematologic reference values from a very large group of 2,680 normal fetuses during the second and third trimesters of pregnancy. We confirmed our previous data (obtained from a smaller group) and we added some new information about erythroid progenitors and Epo levels. It may provide better understanding of fetal hematopoiesis,<sup>32-34</sup> wiser usage of blood retrieved from the umbilical cord at delivery to restore abnormal hematopoiesis,<sup>35-37</sup> and directions for the administration of Epo in premature newborns.<sup>38</sup>

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