Collection of fetal stem cells and newborn effects

C. Pafumi[1], G. Milone[2], A. M. Todaro[3], A. Carbonaro[1], G. Pernicone[1], S. Bandiera[1], A. Russo[1]

[1]Microbiology and Gynaecology Science Department; [2]Haematology Institute; [3]General Pathology Institute
S. Bambino Hospital, University of Catania, Catania (Italy)

Summary

The aim of this work was to test fetal stem cells (FSC) number modification in relation to clamping time and newborn effect. The results show that a fast sample, between 20 and 40 seconds, from umbilical cord after fetus birth and before placental detachment assured a greater quantity of blood useful for the transplants; and that it was necessary to enrich the collected blood in CD34+ cells with specific clonogenic culture, as this is otherwise a small number for a donation to an adult. In the "new donors" the effects of the unconscious donation always depend on the clamping time, which should be the shortest possible to avoid blood overload, which is very dangerous in the presence of heart malformation.

Key words: Umbilical Cord Blood; Fetal Stem Cells; Clamping Time.

Introduction

Gabutti [1] was the first to describe the presence of fetal stem cells (FSC) in umbilical cord blood (UCB); 13 years later, Gluckman [2] performed the first successful engraftment to cure a child affected by Fanconi anaemia with these "new old cells". Thus, interest in an alternative resource to the classical bone marrow is increasing more and more.

When bone marrow or peripheral blood stem cells were transplanted there were many GvHD possibilities, especially when the HLA compatibility was uncertain. This new source (UCB) of the same pluripotential cells guarantees self-renewal, capacity to differentiate into all hematopoietic lineage "but" has a low GvHD activity. The real FSC advantage is the easy availability and collection. During delivery it is possible, with the consent of the parturient, to collect a lot of FSC samples without any risk for the new "category of donors". Furthermore FSC from UCB is less open to CMV infection than bone marrow samples.

A large number of UCB banks have spread throughout Europe and the USA and the FSC engraftments are performed with the absolute concept of "Restitutio ad Integrum" for a great deal of diseases such as different kinds of leukemia, Thalassemia major, sickle cell disease, severe combined immune deficiency, Fanconi anaemia and many other inherited disorders.

UCB collected has to be purified to have a pure CD34+ sample. The low number of these nucleate cells is the only limit of this source. Thus the UCB samples have to be enriched in CD34+ using the classical clonogenic culture: progenitors obtained comprise a large majority of BFU-E (Burst Forming Unit Erithroid) and CFU-GM (Colony Forming Unit Granulocyte-Monocyte) and a minority of progenitors multipotent CFU-GEMM (Colony Forming Unit Granulocyte-Eosinophyle-Monocyte-Macrophages).

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The aim of this work was to test the ratio between FSC number modification and newborn effect in relation to clamping time.

Material and Methods

In our study clamping time of the umbilical cord and collection of UCB was entirely at the discretion of the operators. Umbilical cord blood collection had the local hospital ethics committee approval, and maternal consent was obtained in all cases. UCB is obtained in the Obstetrics and Gynaecological Department of Catania University, S. Bambino Hospital; the collection is made after delivery of an infant and ligation of the cord, and before the placental detachment and expulsion. After fetal expulsion, the cord is clamped by means of two hemostatic clamps a few cms from the umbilicus and the newborn is separated. The placental portion of the umbilical cord is cleaned with iodate alcohol (Betadin's solution and alcohol). The umbilical vein is cannulated and the sample is collected by gravity into a sterile set. The set is called Placental Blood Collection Bag (Maco Pharma Laboratoire Pharmaceutiques - France).

The quantity of blood collected using this procedure depends on period of gestation, placental weight, placental infarction, features of umbilical cord (size), placental weight/neonate weight ratio, time of bleeding after delivery, and fetal respiratory distress.

Results

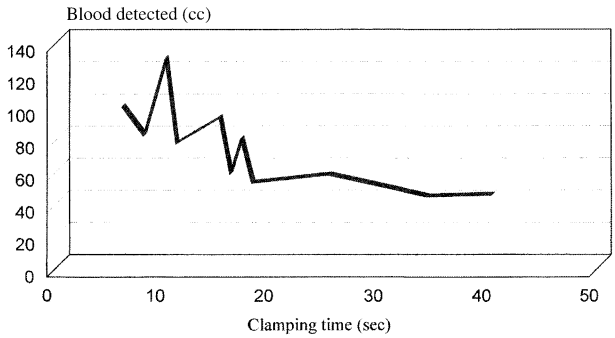
Table 1 shows that fetal blood volume increases when the clamping time rises, with a plateau between 20 and 30 sec. Subsequently UCB volume collected was equal in relation to the clamping time which increased because of the stasis and coagulation phenomena.

Table 1A shows that the increasing trend of FCB is proportional to increased clamping time.

Table 2 shows that CFU-GM concentrations increased in relation to the prolonged clamping time, with a plateau between 20 and 30 sec.

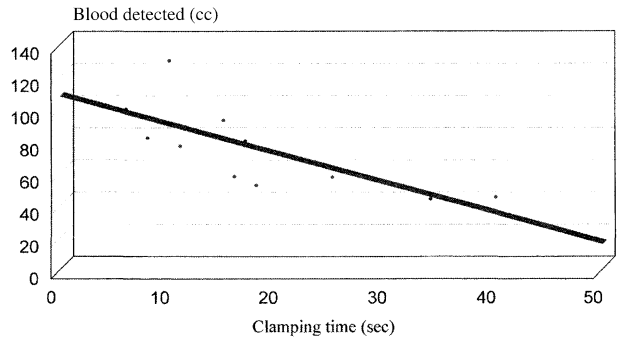
Table 2A shows that the trend is to increase in relation to the clamping time increasing.

Table 1. — *Clamping time and fetal blood volume collected (cc)*



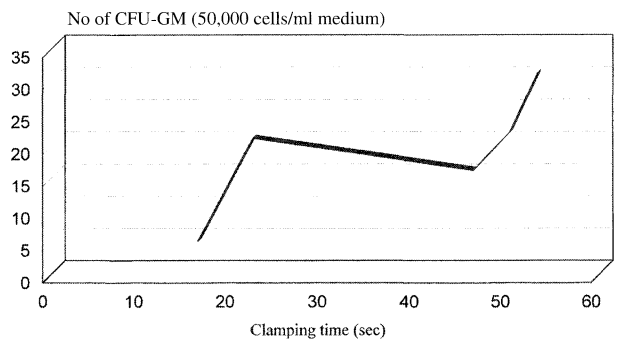
Fetal blood volume decreases when the clamping times rises, with a plateau between 20 and 30 sec. Subsequently UCB volume collected was equal in relation to increased clamping time because stasis and coagulation phenomena occurred.

Table 1A (Trend) — *Clamping time and fetal blood volume collected (cc.)*



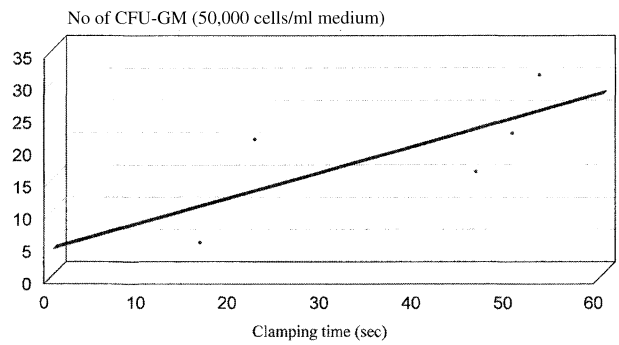
Decreasing trend of FCB is proportional to increased clamping time.

Table 2. — *Clamping time and no of CFU-GM*



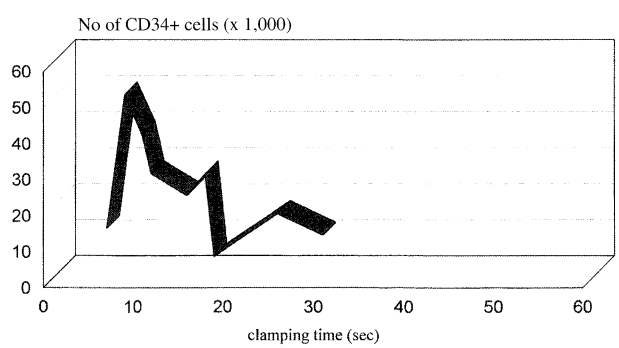
CFU-GM concentration increased in relation to the increase in clamping time, with a plateau between 20 and 30 sec.

Table 2A (Trend) — *Clamping time and no of CFU-GM*



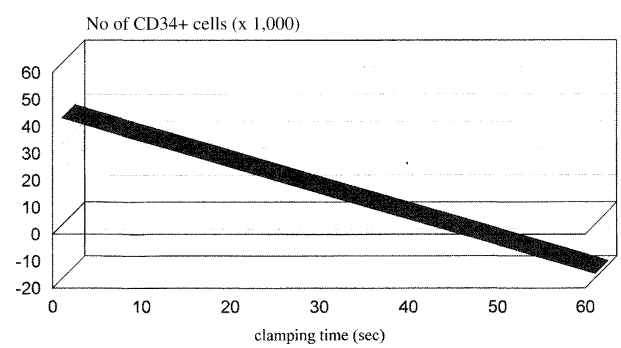
The trend is for an increase in relation to the clamping time increasing.

Table 3. — *Clamping time and no of CD34+ cells in umbilical cord blood*



CD34+ cells of UCB decreased in relation to the increase in clamping time, with the exception of clamping time between 5 and 10 sec.

Table 3A (Trend) — *Clamping time and no of cells CD34+ in umbilical cord blood*



The trend is that CD34+ cells increase is in relation to the clamping time decreasing.

Table 3 shows that CD34+ cells of UCB decreased in relation to the increase in clamping time, with the exception of clamping time between 5 and 10 sec.

Table 3A shows that the trend is to decrease in relation to the clamping time increasing.

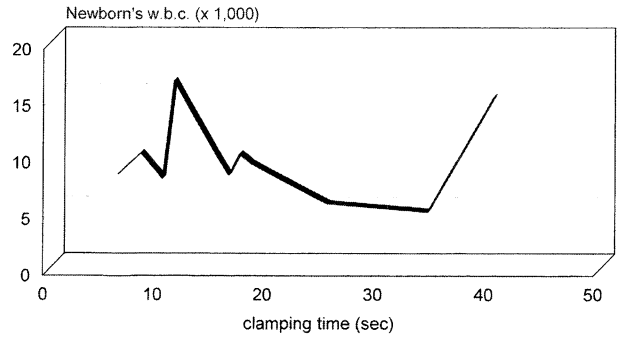
Table 4 shows that newborn white blood cells increased in relation to the increase in clamping time.

Table 5 shows that newborn red blood cells increased in relation to the increase in clamping time.

Table 6 shows that newborn haematocrit increased in relation to the increase in clamping time.

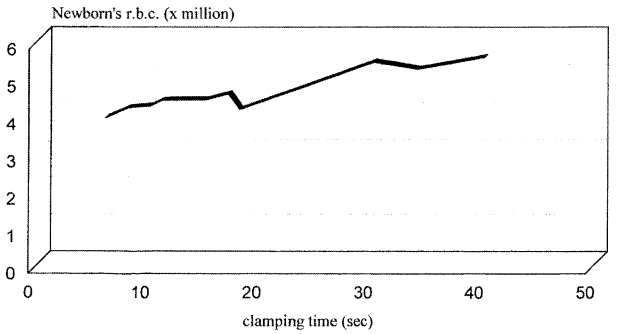
Table 7 shows that by purifying hematopoietic proge-

Table 4. — *Clamping time and newborn's white blood cells*



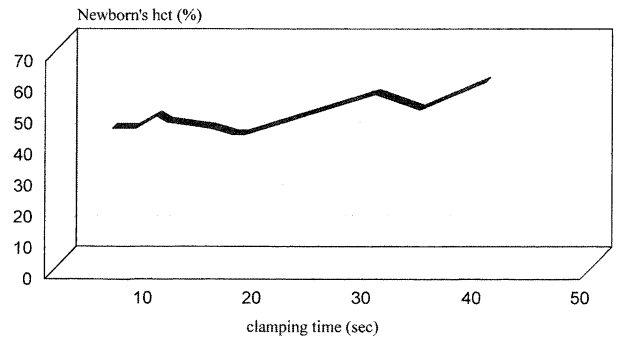
Newborn white blood cells increased in relation to increased clamping time.

Table 5 — *Clamping time and newborn's red blood cells*



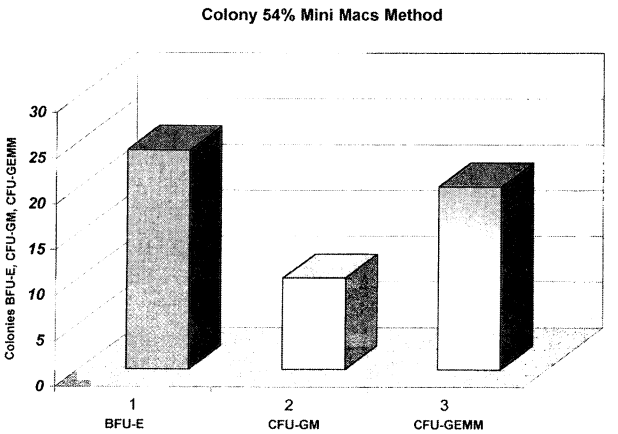
Newborn red blood cells increased in relation to increased clamping time.

Table 6. — *Clamping time and newborn's haematocrit*



Newborn hematocrit increased in relation to increased clamping time.

Table 7.



Purifying hematopoietic progenitors from normal cord blood using Mini-Macs separation, we obtained a final purification index of 54%. These progenitors (CFU-GEMM, BFU-E, and CFU-GM) give rise to large colonies in clonogenic culture. The purified UCB progenitors comprise a large majority of BFU-E and CFU-GM and a minority of multipotent progenitors (CFU-GEMM).

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Discussion

Modifications of umbilical cord blood depending on clamping time were investigated. Clamping time was calculated from the fetus birth till che clamp of the umbilical cord blood by a clip. Umbilical cord blood volume increases when the clamping time rises, with a "plateau" between 20 and 30 seconds. The volume of detected umbilical cord blood was equal when clamping time was over 30 seconds. The trend is increased UCB in relation to the increase in clamping time.

CFU-GM concentrations increased in relation to the decrease in clamping time, with a "plateau" between 20 and 30 seconds. The trend results in decreased concentrations in relation to the clamping time.

The trend of the cells CD34+ of umbilical cord blood decreased in relation to the increase in clamping time, with the exception of clamping time between 5-10 sec. White cells, red cells and haematocrit of the newborn increased in relation to the increase of clamping time.

Greater blood quantity can be obtained if the bleeding is before placental detachment, as in the vaginal delivery or cesarean section under ultrasonic guidance. Residual materno-placental circulation represent a "vis a tergo" that permits a flow from the fetal section to the placenta; in addition there is the contractility of the uterus during the placental stage. The blood quantity collection also depends on placental ablation (centric or limbic). Nonetheless, the precocity of the blood collection, under the same conditions, permits a larger collection quantity of blood, anticipating the early stasis phenomena and intravasal coagulation.

In our case histories of fetal blood collection (500 bags) alterations in placental stage or a delay have never been observed.

The umbilical cord cut could be made between 20 and 40 sec. after the delivery with or without hand-massage. A healthy neonate easily tolerates the abrupt circulatory overload; the greater volume of blood assures plentiful iron deposits. If the neonate is underweight (preterm), ailing or born with a difficult delivery it is necessary to immediately cut the umbilical cord to avoid risks related to abrupt circulatory overload and to permit reanimation as soon as possible. The immediate umbilical cord cut is necessary in materno-fetal red cell isoimmunization to reduce the transfer of blood containing red cells with superficial antibodies to the mother.

In addition, if the mother is Rh negative, it should be helpful to not occlude the umbilical cord near the placenta after the cut and to reduce the possible passage of fetal red cells in the maternal circulation. Moreover in many cases of fetal distress during labour a transfer of blood from placenta to fetus could happen before the delivery. In this case immediately after the delivery, the blood vessels of the umbilical cord appear scanty of blood; in fact, if the residual placental blood is measured by putting it in a graduate tube, it is frequently reduced. Finally in case of caesarean section, it is useful to cut the umbilical cord immediately to avoid raising the neonate above the placental level.

As for the umbilical cord cut method, generally it is cut between two hemostatic clamps, leaving a few cms of cord from the fetal implantation. While perfect occlusion of the fetal shoot is essential, placental shoot clamping is advisable to avoid drops of blood; however, it is inadvisable in Rh negative women. If the newborn is healthy the final occlusion of the fetal shoot can be performed immediately after the cut; otherwise it is better to wait for a few minutes in case intravenous injections are necessary. A particular clamp cam make the final occlusion approximately 1 cm from the fetal implantation. It is important to check the number of funicular blood vessels, which are normally, 3 or 2 arteries and 1 vein; sometimes the umbilical cord shows only 2 blood vessels, and in this case there is greater possibility of congenital malformations.

Conclusions

Modifications of UCB collected and the number of CD34+ cells depend on clamping time length. We have demonstrated that the best "time-window" for a perfect sample is between 20 and 40 sec in order to have a larger quantity of blood, more CD34+ cells and to avoid stasis phenomena that make the sample difficult and overload the blood, which is very dangerous in heart malformation.

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Address reprint requests to:
CARLO PAFUMI, M.D.
Faculty of Medicine and Surgery
University of Catania
Microbiological and Ob-Gyn Science Dept.
Via D'Annunzio, 125
95127 Catania (Italy)