PLACENTAL / UMBILICAL CORD BLOOD COLLECTION & PROCESSING

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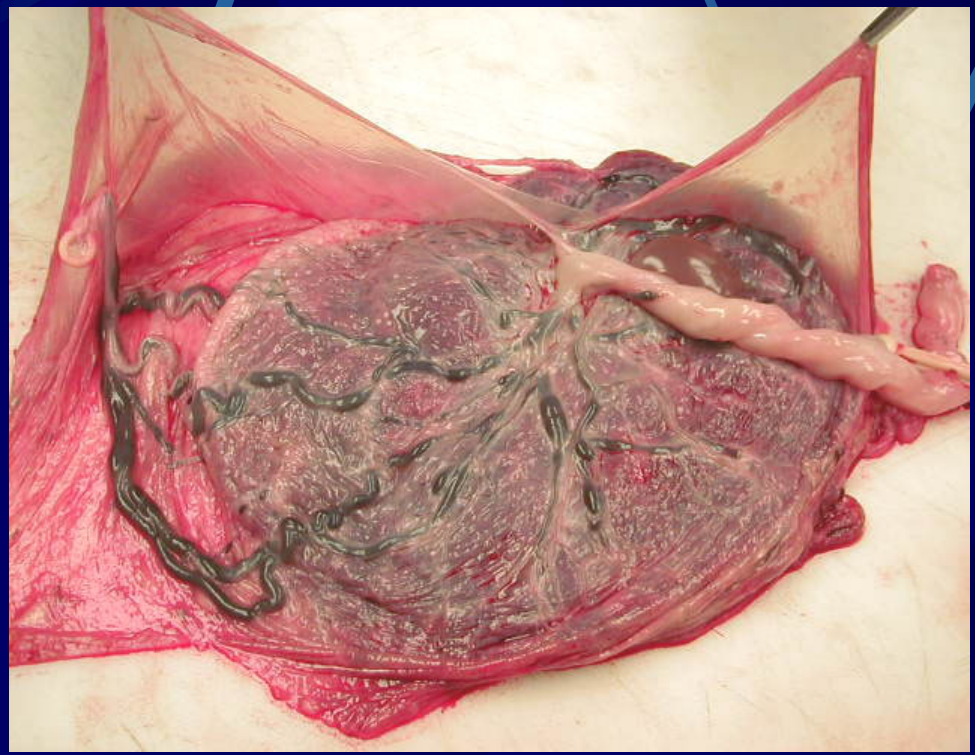
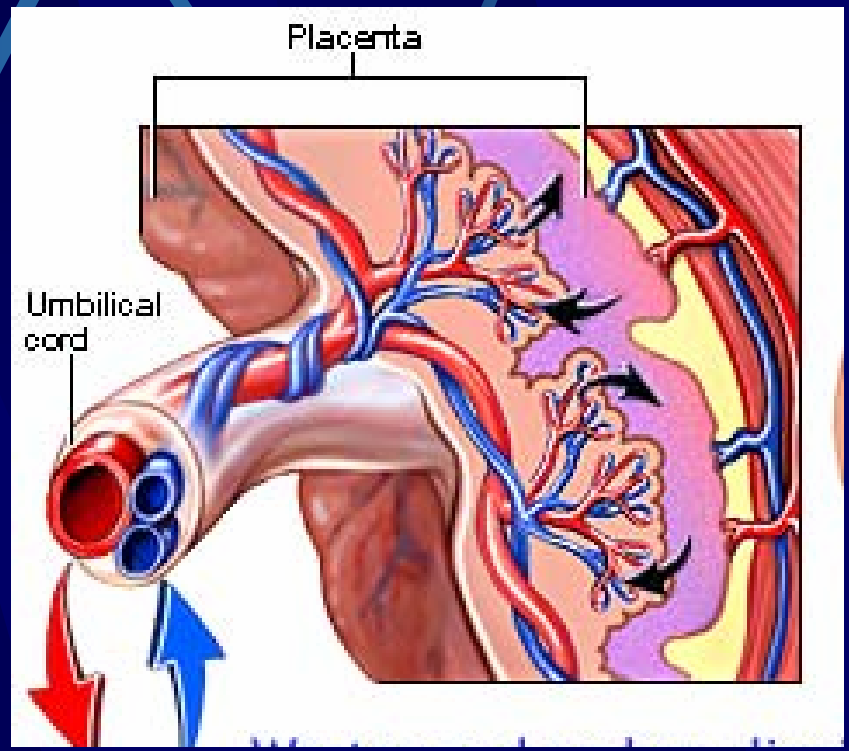
Placenta

Placenta = lat. Circular cake
(Ref., Williams Obstetrics, 18th ed.)





UCB – rich source of HSC

> 100 million births /year

10 million liters of umbilical cord blood

90% thrown away

Placental/Umbilical Cord Blood

- **Source of cells for transplantation**
  - **Hematopoietic Progenitor Cells**
    - blood cells
  - **Mesenchymal Progenitor Cells**
    - marrow stroma, bone, cartilage, muscle and connective tissues
  - **Other Stem/Progenitor Cells**
    - endodermal cells – hepatocytes
- **Source of cells for transfusion**

Prvi primer presaditbve avtologne popkovnične krvi pri otroku z levkemijo

First Report of Autologous Cord Blood Transplantation in the Treatment of a Child With Leukemia

Ammar Hayani, Eberhard Lampeter, David Viswanatha, David Morgan and Sharad N. Salvi

Pediatrics 2007;119;296-300
DOI: 10.1542/peds.2006-1009

History

- 1972

USA – first attempt to transplant

1. Ende M, Ende N. Hematopoietic transplantation by means of fetal (cord) blood: A new method. Va Med Mon, 1972; 99: 276-280.

- 1988

Paris – first UCB transplantation

- Gluckman E, Broxmeyer HA, Auerbach AD, Friedman HS, Douglas GW, Devergie A, Esperou H, Thierry D, Socie G, Lehn P, et al. Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. N Engl J Med. 1989 Oct 26;321(17):1174-8.

- 1991

New York - first Cord Bkood Bank

- Rubinstein P, Rosenfield RE, Adamson JW, Stevens CE. Stored placental blood for unrelated bone marrow reconstitution. Blood 1993;81:1679-1690.

Previous Opinions Against Autologous Cord Blood Banking

- American College of Ob and Gyn (ACOG)
  1997; Int J Gyn Ob, 58(2):257-259
- American Academy of Pediatrics
  July 1999; Pediatrics, 104:116-118
- Royal College of Ob and Gyn
  Oct. 2001; Opinion Paper 2
- European Group on Ethics in Science and New Technologies
  March 2004; Opinion No.19

Definition

- Hematopoietic Progenitor Cells, Cord Blood; **HPC-C** – are cells obtained from the umbilical cord and, occasionally, placental vessels at the time of delivery and immediately placed in an anticoagulant solution.

Circular of information; AABB; ARC; ABC; FACT;ISCT;NMDP;ASBMT

Placental/Umbilical cord blood transplantation

- feasibility of engraftment in children
- delayed time to engraftment of neutrophils and platelets
- lower incidence of acute and chronic GVHD

Transplantation of HPC-C

- Advantages
  - Limitless suppply
  - No donor attrition
  - Easy and safety of collection – rapid availability
  - Reduced GVHD
  - Reduced viral contaminantion
- Disadvantages
  - Insufficient cell dose
  - Uncertain GVT activity
  - Uncenrtain long-term graft durability (PTLD)
  - Risk of EBV-associated PTLD
  - Inability to obtain donor leukocytes

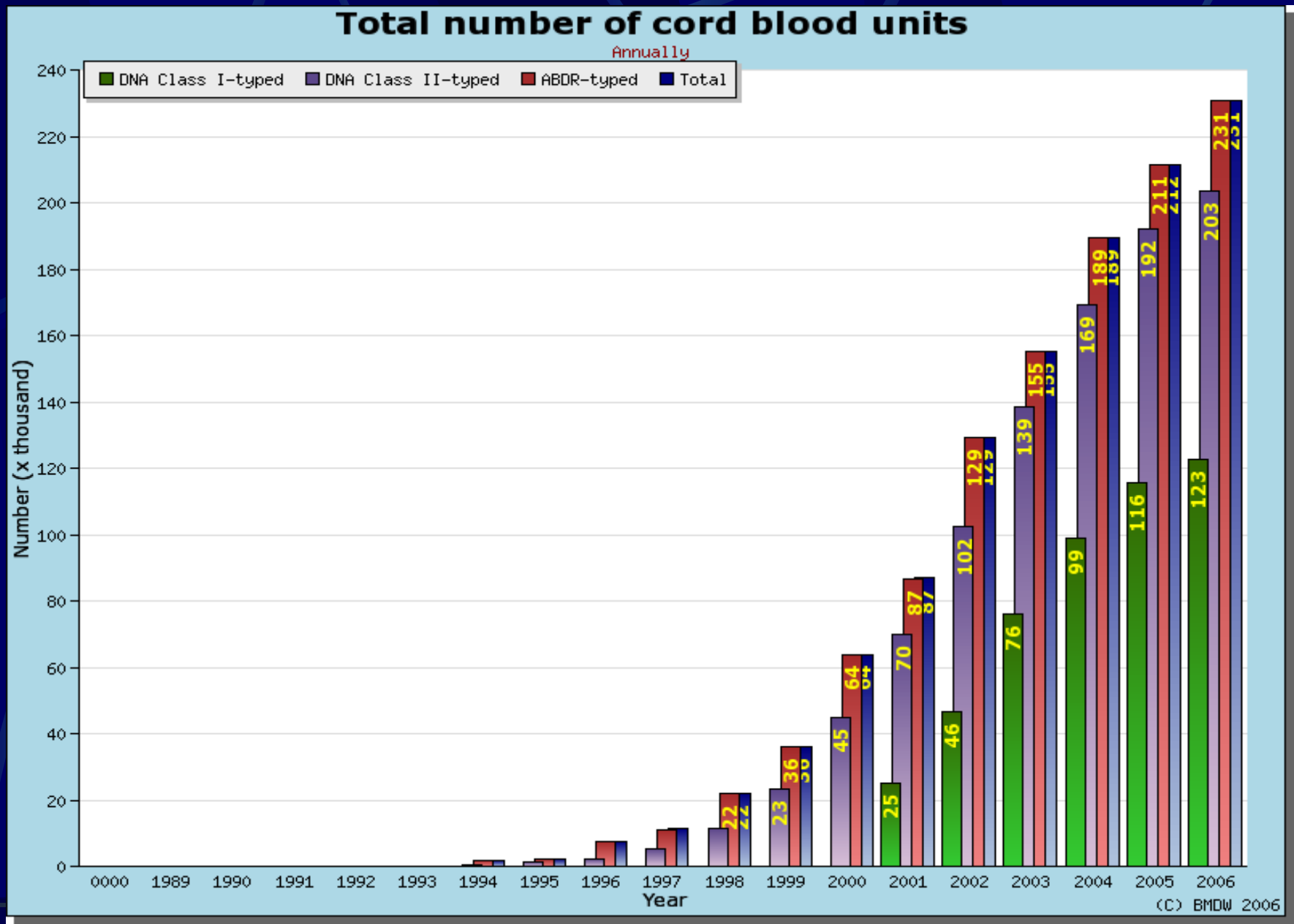
PTLD – post – transplant lympho proliferative disorder

Strategies to increase the cell dose for transplantation

- Two product transplantation
- Expansion in vitro
- In vivo stimulation with growth factors
- Optimization of collection, processing and cryopreservation
  - Increased cell yields
  - Minimal losses of cells

Placental/Umbilical Cord Blood Banking

- Cord Blood Banks
  - Public
  - Private
  - Research


Total number of cord blood units
Annually
DNA Class I-typed
DNA Class II-typed
ABDR-typed
Total
Number (x thousand)
Year
0000
1989
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
(C) BMDW 2006

Placental/Umbilical Cord Blood Banking Process

- Donor selection
- Collection
- Transporting
- Testing
- Processing
- Cryopreservation
- Storage
- Release for tranplantation

Initial Quality Parameter

- VOLUME.....> 60 ml
- TNC.....$>10^9$
- CD34+.....$>10^6$

Van Haute et al. Transfusion 2004;44:547-554.

Factors that influence the volume and cell yield

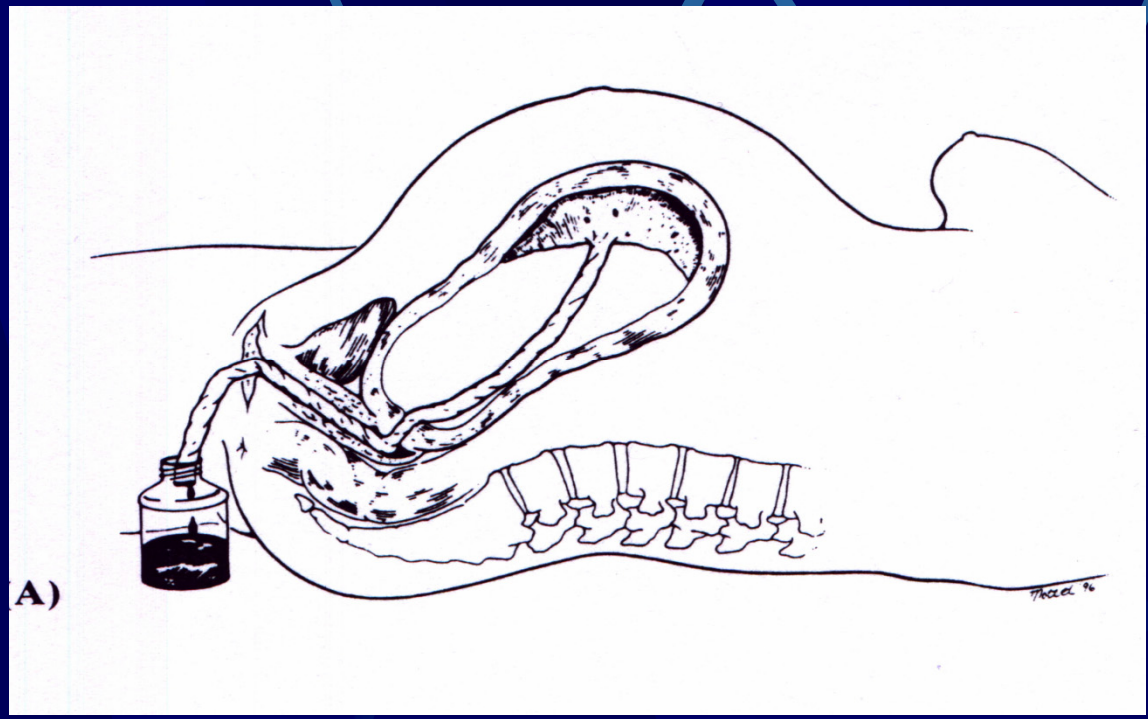
- Maternal
  - smoking, preeclampsia, No.of pregnancies
- Neonatal
  - Lenght of gestation, birth weight, bigger placenta and longer umbilical cord
- Obstetric
  - Cesarean section, clamping time, lenght of labour, labour stress, placing the newborn on the maternal abdomen
- Technical
  - Collection
  - Processing

Collection

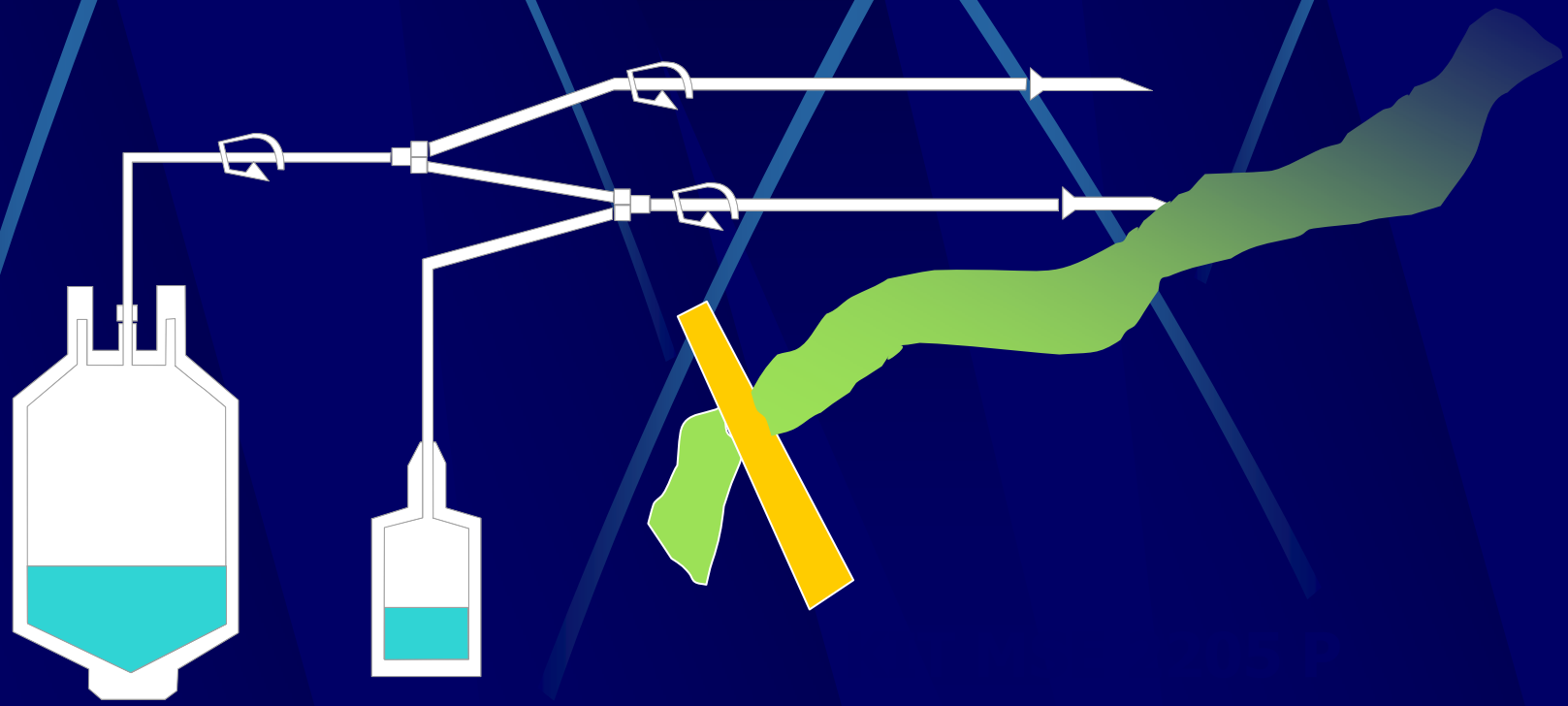
- Umbilical Cord Disinfection
- System used
  - Open system
  - Closed system

- Before Delivery of Placenta
- After Delivery of Placenta
- Combined

Open system



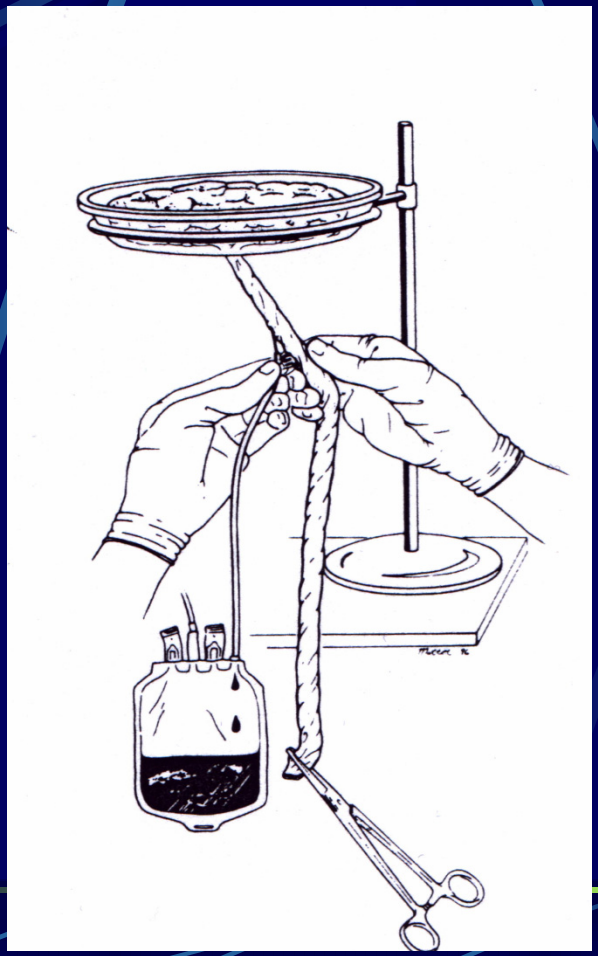
Closed System using the Placental/Umbilical Cord Blood Collection Kit



21 ml CPD

8 ml CPD

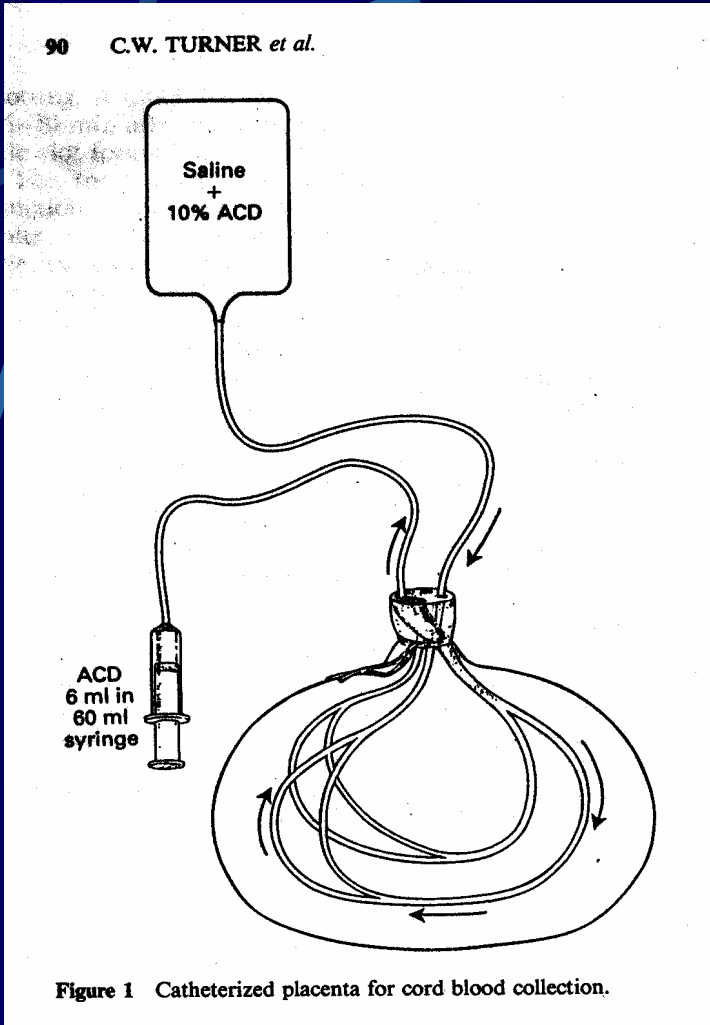
Collection after delivery of placenta



Combined system

- Collection from umbilical vein - placenta in utero
- Collection from placental vein
  - placenta ex utero

Modified collection technique



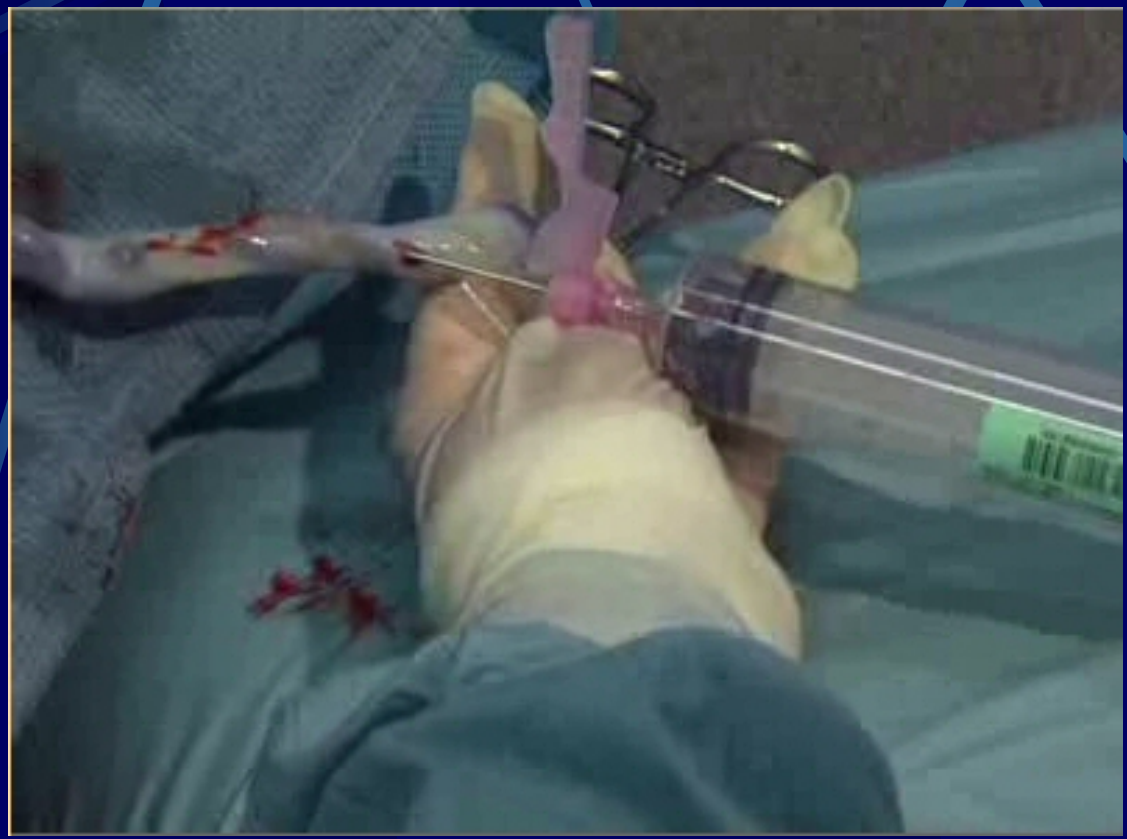
Needle exposure avoided

Low bacterial contamination

High volume and cell yield collected

Turner et al. Bone Marrow Transplant 1992;10:89-91

Collection before delivery of placenta



- Sirynge
- Plastic bag system

FAQs

- Early vs. delayed time of umbilical cord clamping
- Collection with placenta in utero vs. placenta ex utero
- Performance stuff - Blood Bank vs. Obstetricians and midwives

Two cord blood collection strategies

	Volume (ml)		TNC ($x10^8$)		CD34+ ($x10^5$)	
	ex utero	in utero	ex utero	in utero	ex utero	in utero
Surbek et al.1998	48.42 ± 4.07	83.26 ± 7.9	n.p.	n.p.	n.p.	n.p.
Pafumi et al.2002	60.9 ± 13.7	90.7 ± 6.0	7.1 ± 0.8	10.1 ± 1.2	1.64 ± 2.4	2.0 ± 0.6
Sparrow et al. 2002	62	67	10	12.1	2.9	3.8
Solves et al. 2003	98 ± 28.47	108.8 ± 28.6	8.55 ± 3.52	10.54 ± 4.15	2.96 ± 2.25	3.65 ± 3.38

Processing of Placental/Umbilical cord blood

- Volume reduction
  - Storage capacities enlargement
  - Lower quantities of DMSO infused
  - Lower quantities of hemoglobin infused
  - AB0 incompatibility

Minimally Manipulated Hematopoietic Progeniotor Cell Products

- HPC-C Plasma Reduced
- HPC-C Red Cell Reduced
- HPC-C Buffy Coat Preparation
- HPC-C Density Separated
- HPC-C CD34 Selected

Circular of information; AABB; ARC; ABC; FACT;ISCT;NMDP;ASBMT

Processing

- Manual
- Semi-automated
- Automated

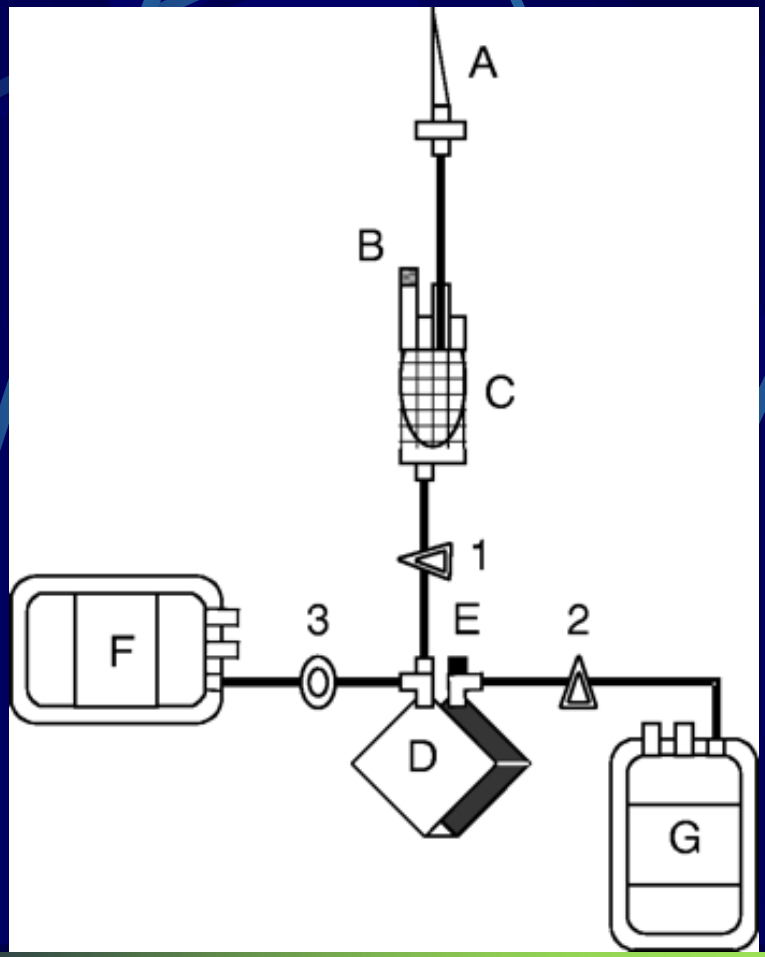
Manual processing

- Sedimentation
- Differential centrifugation and buffy coat removal
- Double centrifugation
- Addition of agents for
  - Better sedimentation of RBCs (galetine)
  - Density gradient formation (Ficoll,Percoll, polygelin,)
  - Rouleaux formation (HES)

Device for semi-automated processing

Multiple plastic bag system
for top and bottom separation of RBC and Plasma using HES

Placental / Umbilical Cord blood processing by filtration StemQuick™E filter device



A. needle for connection to the UCB bag
B. air vent filter
C. mesh chamber
D. filter
E. port for the application of flushing solution
F. recovery bag
G. drain bag

- 1, 2, 3 clamps

Eichler et al. Stem Cells. 2003;21(2):208-16.

Automated cord blood processing device Sepax S-100 (Biosafe S.A.)

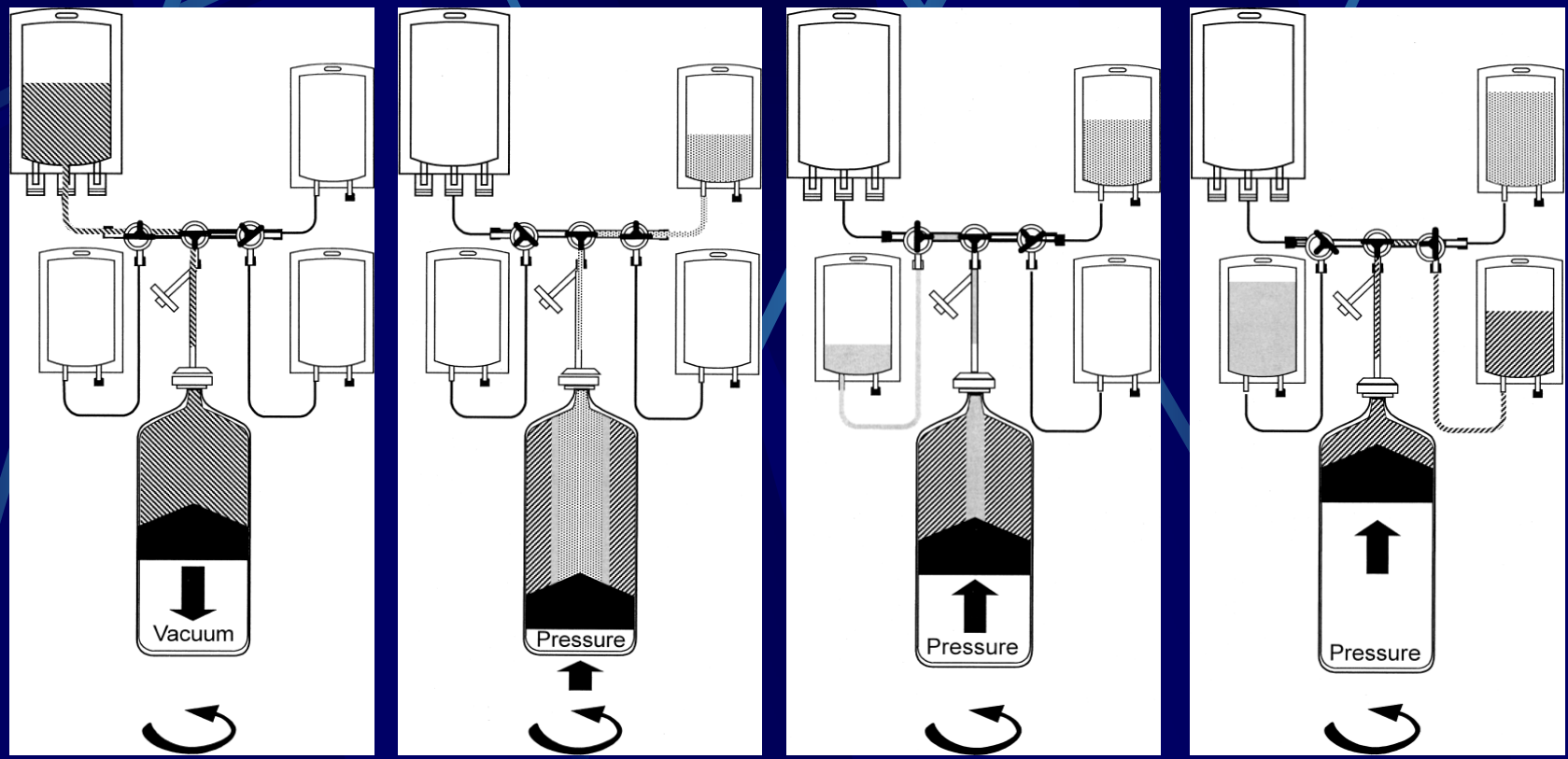
Closed system

Standardized procedure

Short processing time

Automated cord blood processing

Sepax S-100 (Biosafe S.A.)



Priming | Separation & plasma collection | Buffy coat collection | RBC collection

Zingsem et al.Transfusion 2003;43:806-812.

Thermogenesis AutoExpress AXP™

thermogenesis

Different approaches to processing of Placental/Umbilical cord blood

	Automated (HES) Zingsem et al. (37)	Filtration Rebulla et al.(36)	Semiautometed (HES) Bertolini et al. (35)	Manual (no HES)	
				Armitage at al. (32)	Sousa et al (31)
Volume (ml)	**32.6 ± 7.6**	**21.5**	**n.a.**	**24.5 ± 1.5**	**45 (19–63)**
Volume depletion (%)	**65.1 ± 15,8**	**n.a.**	**n.a.**	**67**	**56**
WBC Recovery (%)	**78.6 ± 24.9**	**49 ± 17**	**85.8 ± 7.9**	**83.3 ± 16.8**	**72 (52-90)**
CD34+ recovery (%)	**83.6 ± 32.5**	**85.3 ± 8.5**	**83.4 ± 5.6**	**98.9 ±15.6**	**87 (63-99)**

Selection of CD34+ cells from HPC-C

Isolex 300i device for immunomagnetic selection of CD34+ cells

Conclusion

- Collection and processing are the factors that can influence the volume and the yield of cells in the HPC-C products. In the absence of adopted standards, the strategies for collection and processing of HPC-C is to collect maximum volume with the minimal cell losses.

HPC-C collections at BTC

Related allogeneic collections .....No= 4
Test collections..... No = 12

Volume (ml)	WBC x10e9/L	WBC x10 e9	% CD34+ cells
88 +/-27	11 +/- 3	1 +/- 0,3	0.50