

Non-DEHP storage of cord blood erythrocytes for transfusion in pre-term infants

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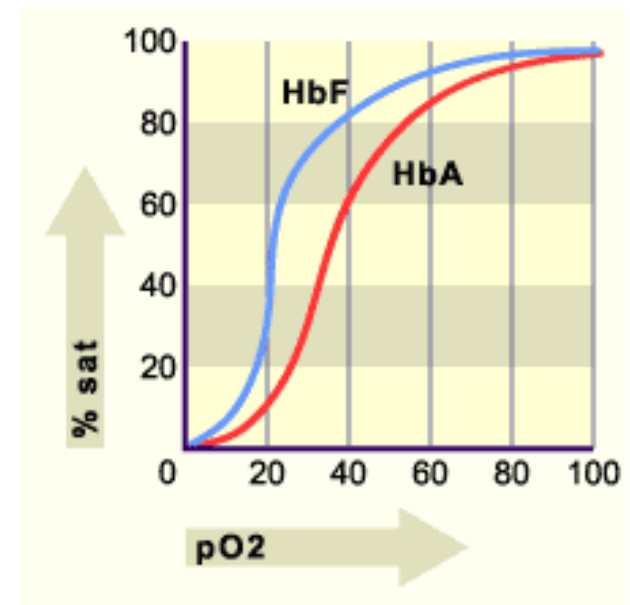
Disclosure

- No conflict of interest to declare



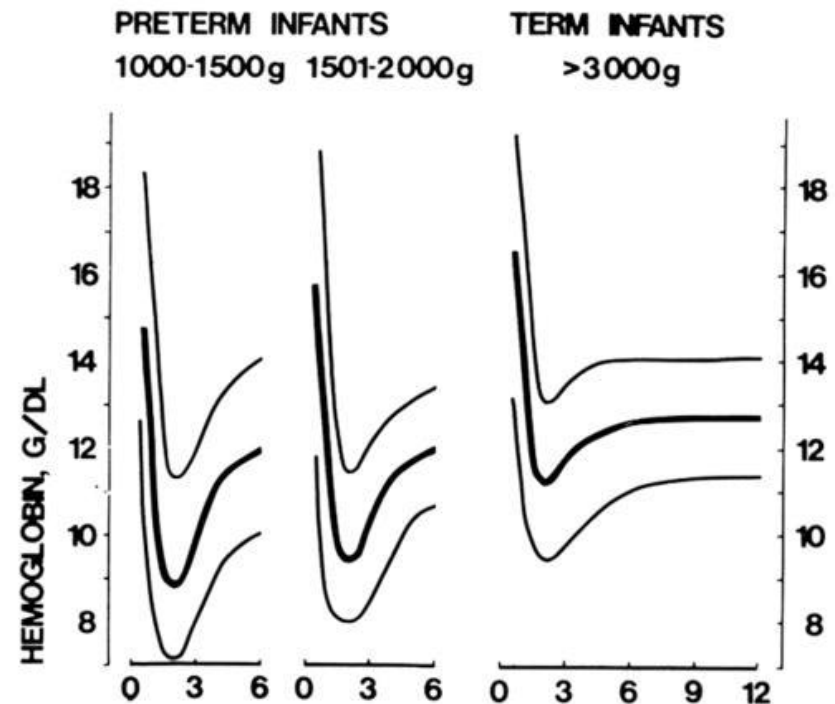
Background

- Normal infant Hb at birth: 15-20 gHb/dl
- Red cells express fetal hemoglobin (HbF)
- Higher oxygen affinity
- Hb switch “physiologic anemia”, problematic in pre-term infants



Background (1)

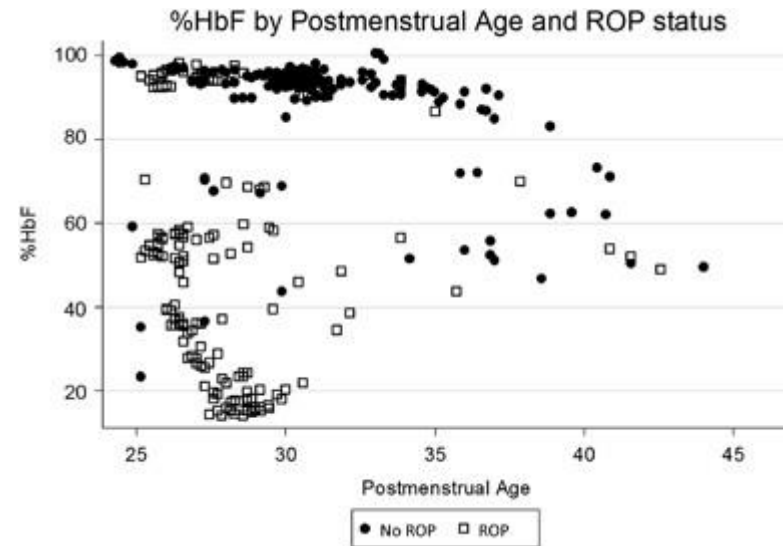
- <1000g, <29weeks, 90% receive 1 RCC transfusion
- 10-20ml/kg of a standard leucocyte-removed red cell concentrate from adult whole blood
- Hb corrects
- Transfusion associated with development of retinopathy of prematurity



*Adapted from Carroll et. al.
Ref range: 14-24 g/dl

Background (1)

- Earliest reports in 1970s
- Stutchfield et al. Correlation % HbF and ROP
- Lust et. al. 2018, Zhu et. al. 2020 PLOS; Tranfusion significantly associated with retinopathy.
- Cause?
- Retina in utero develops naturally in a state of hypoxia
- Bedrosian et al. (1953): Oxygen treatment of pre term infants: Abnormal growth of retinal blood vessels that are still developing, retinal detachment
 - High oxygen supplementation + fast withdrawal vs low oxygen supplementation + gradual withdrawal



Background (1)

Solution: no more oxygen and transfusions!



Background (1)

- Restrictive transfusion?
- Brooks et al., (1999): symptom-based transfusion vs continuous hct >40%
- Bell et al. (2005): *our finding of more frequent major adverse neurologic events in the restrictive RBC-transfusion group suggests that the practice of restrictive transfusions may be harmful to preterm infants.*
- Franz et al. (2020): *Do liberal vs restrictive transfusion strategies improve survival and neurodevelopmental outcome? 1013 infants... a liberal blood transfusion strategy did not reduce the likelihood of death or disability at 24 months compared with a restrictive strategy.*
- Wang et al., meta analysis (2021): *Restrictive transfusion does not increase the risk of all-cause mortality and does not increase the composite outcome of death or neurodevelopmental impairment or other serious adverse events*



ROP			
0	207/384 (53.9%)	237/382 (62.0%)	0.015
1	69/384 (18.0%)	59/382 (15.5%)	
2	58/384 (15.1%)	43/382 (11.3%)	
3	38/384 (9.9%)	41/382 (10.7%)	
4	12/384 (3.1%)	2/382 (0.5%)	

LTG liberal transfusion guidelines, *RTG* restrictive transfusion guidelines, *IVH* intraventricular hemorrhage, *HVI* Hemorrhagic venous infarction, *PVL* periventricular leukomalacia, *ROP* retinopathy of prematurity

- Restrictive transfusion may reduce ROP severity
- Can HbF RCC (higher oxygen affinity) further decrease ROP onset/severity?

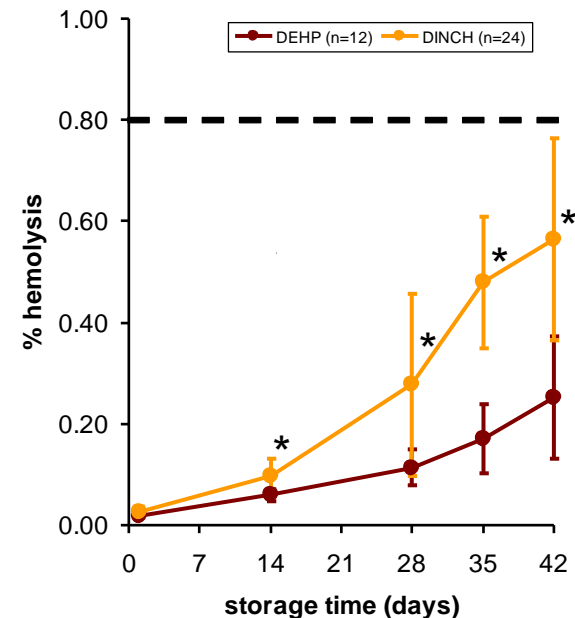
Aim:

- To standardize red cell concentrate (RCC) production from cord blood
- To optimize storage conditions for cord blood RCCs
 - Low level of hemolysis, high levels of ATP as to maximize recovery
- Ultimately, can transfusion of HbF RCC reduce retinopathy of prematurity?



Background (2)

- DEHP (di-ethylhexyl phthalate)
- Potentially toxic, reproductive impairment in rodents
- MDR, May 2021
- REACH (EU chemical agency): DEHP endocrine disruptor in the environment
- Sunset date 2025
- DEHP stabilizes the red blood cell membrane → higher hemolysis during storage → next generation storage media



Background (2)

	SAGM		PAGGSM	
WB collected in	DEHP-PVC	DINCH-PVC	DINCH-PVC	DINCH-PVC
RCC stored in	DEHP-PVC	BTHC-PVC	BTHC-PVC	DINCH-PVC
N	30	20	88	37
Quality parameters				
Day 1				
Hb (g/L)	193 ± 10	198 ± 6.5	194 ± 5.9	192 ± 7.5
Hct (L/L)	0.61 ± 0.03	0.64 ± 0.01 ^a	0.63 ± 0.02 ^a	0.61 ± 0.01 ^b
MCV (fL)	95 ± 6.0	101 ± 3.3 ^a	99 ± 4.0	96 ± 3.3 ^b
ATP (µmol/g Hb)	5.7 ± 0.6	5.9 ± 0.5	5.1 ± 0.5	5.3 ± 0.5
Day 42				
Hct (L/L)	0.65 ± 0.02	0.65 ± 0.01	0.64 ± 0.02	0.62 ± 0.02
MCV (fL)	103 ± 5.4	106 ± 3.5 ^a	101 ± 4.2 ^a	98 ± 2.6 ^{ab}
Hemolysis (%)	0.36 ± 0.17	0.66 ± 0.18	0.38 ± 0.12	0.48 ± 0.17^b
ATP (µmol/g Hb)	3.3 ± 0.5	3.5 ± 0.5	3.9 ± 0.5 ^a	4.0 ± 0.5 ^a

Aim:

- To optimize storage conditions for cord blood RCCs in non-DEHP



Methods

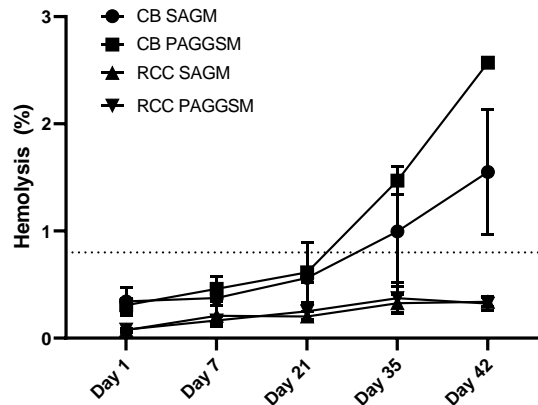
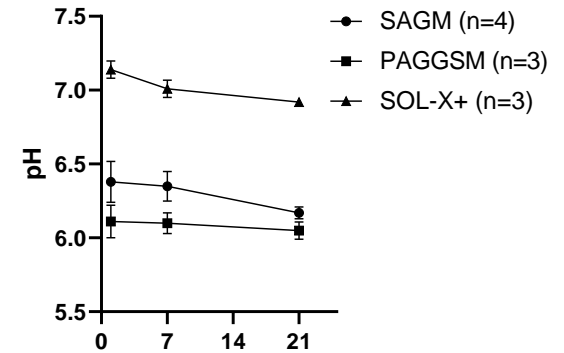
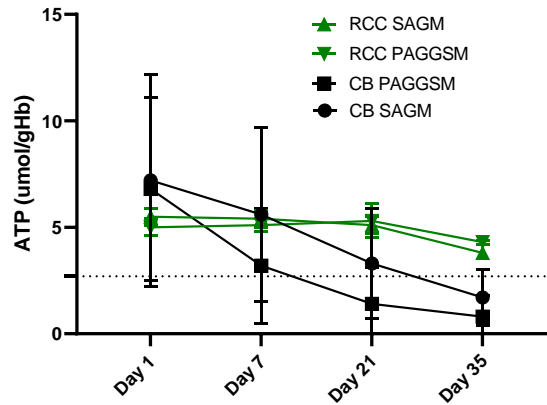
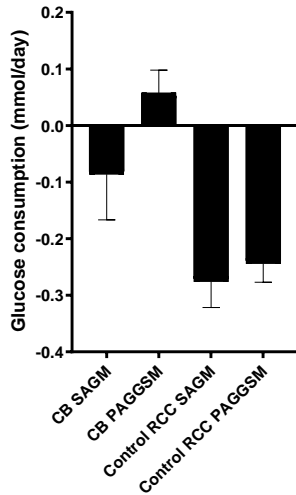
- We started off with a set of pilot experiments and started optimizing production strategy along the way
- SAGM vs PAGGSM
- DEHP vs DINCH
- 20 vs 50 ml

- Ht of between 50-65%
- Thrombocytes $<50 \times 10^9/l$ (90%)
- Leukocytes $<1 \times 10^6/E$

- Hemolysis $<0.8\%$, preferably $<0.3\%$ (same as current RCC)
- ATP $\geq 2.7 \mu\text{mol/g Hb}$

Results

- 20ml CB, 150ml DINCH in SAGM vs PAGGSM



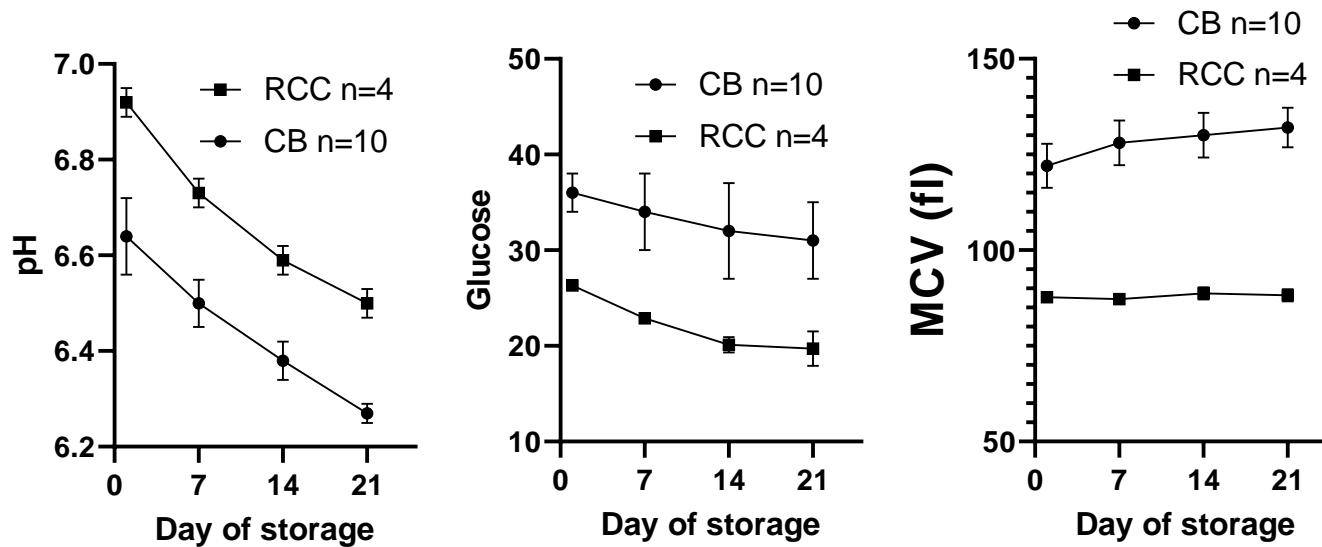
- Storage until?
- Cause initial hemolysis

Optimization of:

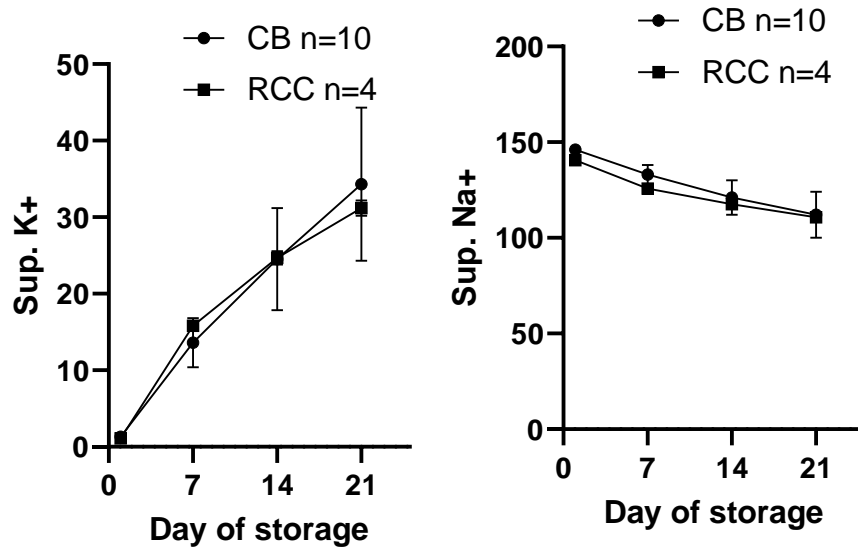
- Processing: no extra dilution prior to filtration
- Storage condition: examine volume storage bag/CB ratio
- Initial pH
- Plasma content optimization
- SAGM, 100ml DINCH

Results

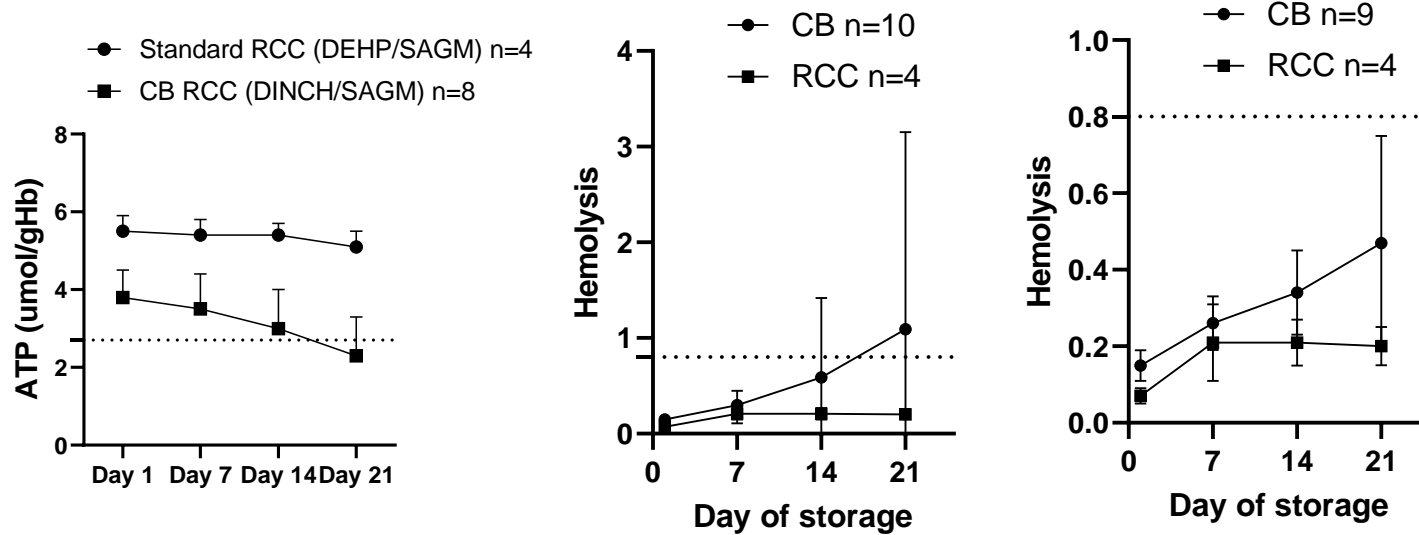
20ml CB-RCC in 100ml DINCH/SAGM



Results



Results



- Screen for hemolysis on day 7 (exclude those >0.5% (n=1/10))

Acknowledgements

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