Anti-K Mediated HDFN

The Role of the Transfusion Medicine Service in IUT

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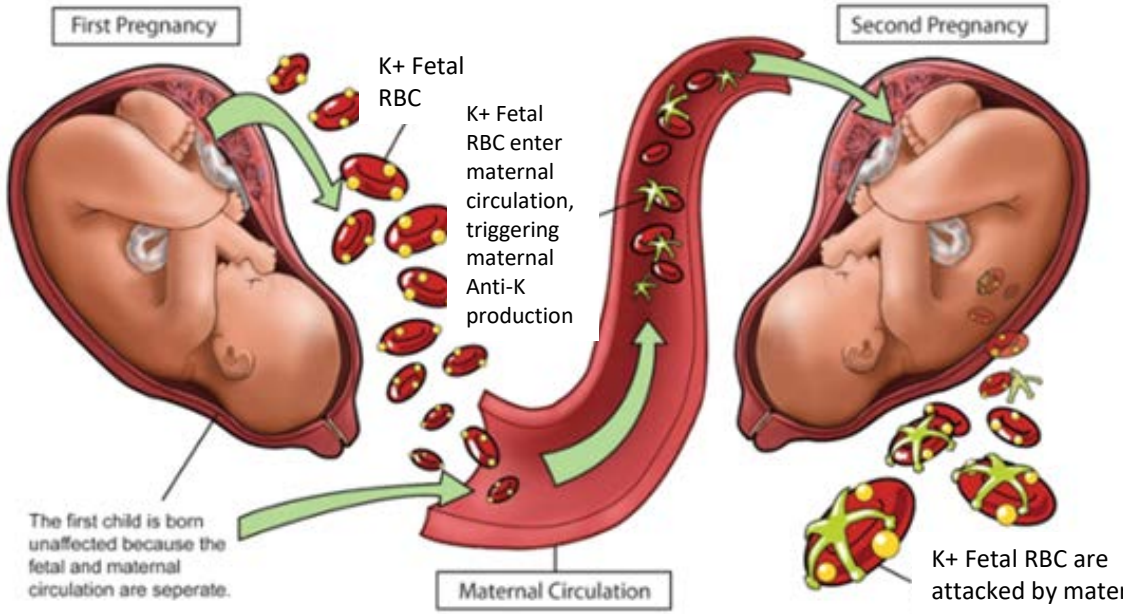
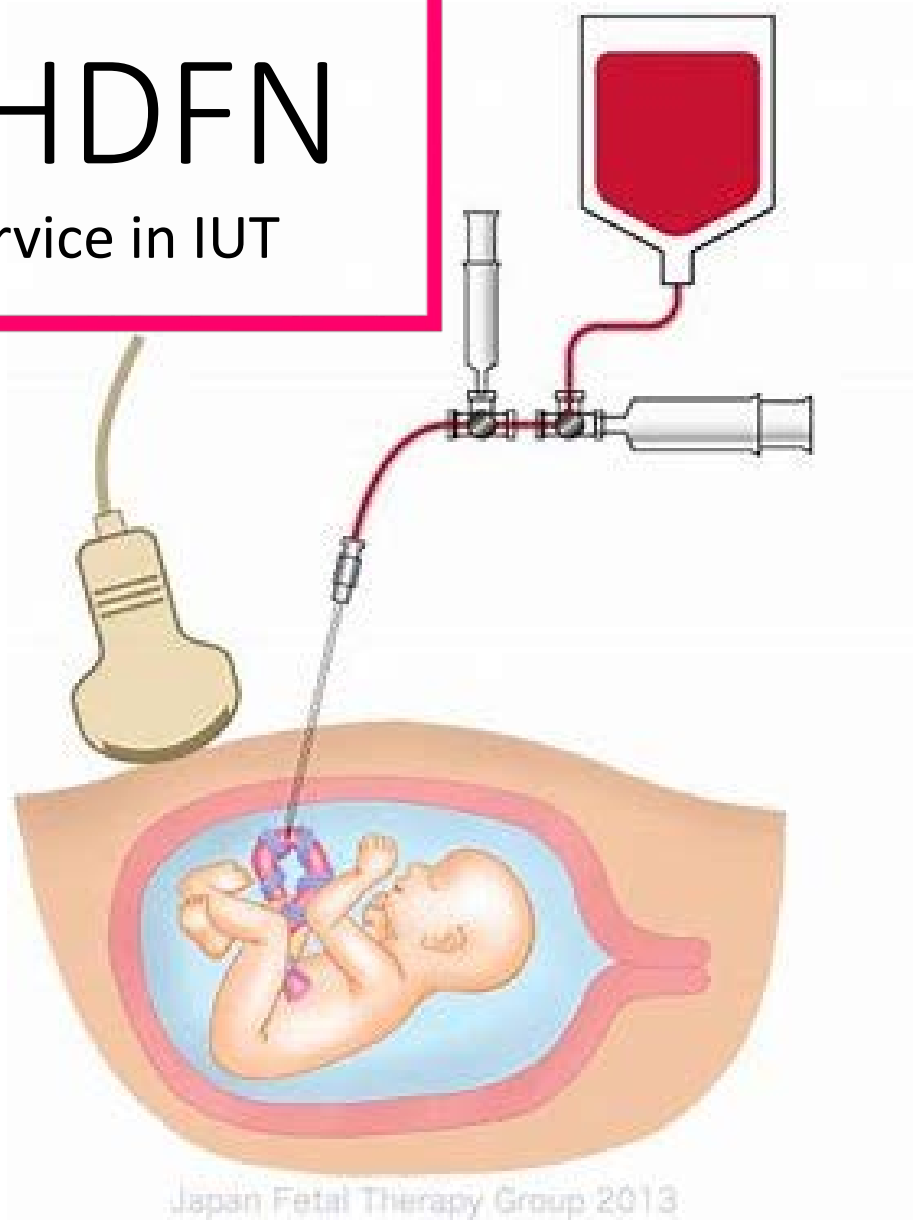


Image obtained from medicoaid.com



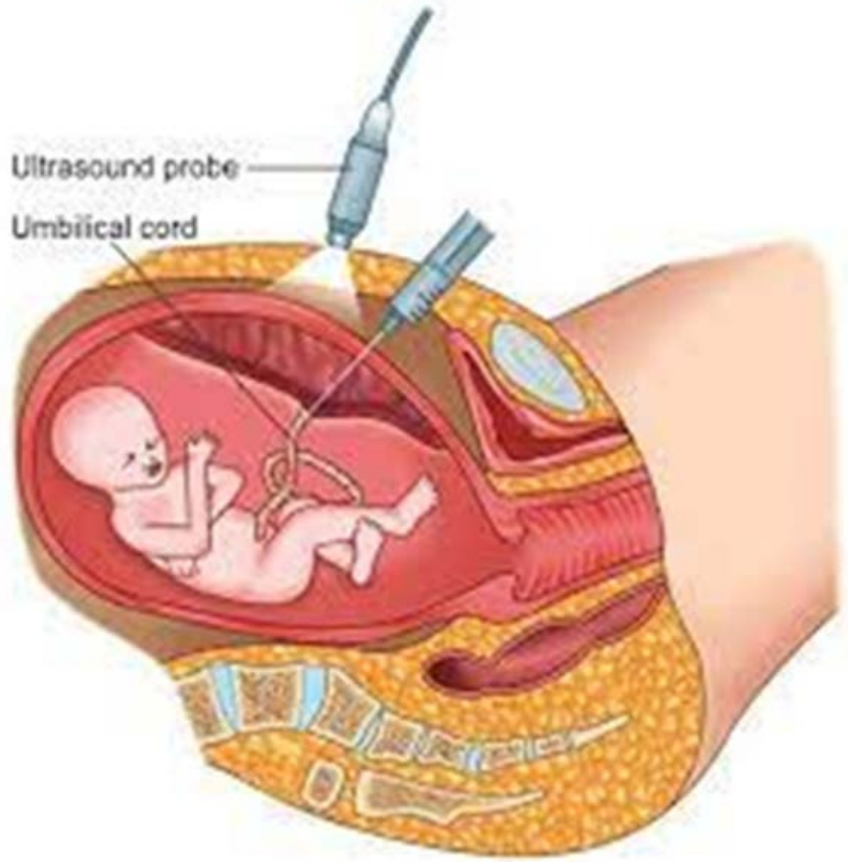
Objectives

Monitoring for a obstetric patient with Anti-K

Role of the Transfusion Medicine Service in an IUT

- Requirements for and preparation of RBC

Ways to treat/prevent HDFN due to Anti-K



Background

36 y/o female with known Anti-K referred to MSH

2021: Anti-K titre was 1:64
→ Patient was not pregnant at the time but titre was requested & done

2023: Patient is now pregnant
→Gravida: 4 Para: 1
→Partner is K antigen positive
→Positive antibody screen

			Rh-hr								KELL						DUFFY		KIDD		Sex Linked	LEWIS		MNS				P	LUTHERAN		Special AntigenTyping		Test Results
Cell #	Rh-hr	Donor Number	D	C	E	c	e	f	C^w	V	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Xg^a	Le^a	Le^b	S	s	M	N	P_1	Lu^a	Lu^b		Cell #	
1	R1R1	319509	+	+	0	0	+	0	0	0	+	+	0	+	/	+	+	+	0	+	+	0	+	+	0	+	0	+	0	+		1	3+
2	R2R2	328373	+	0	+	+	0	0	0	0	0	+	0	+	/	+	0	+	+	0	+	+	0	0	+	+	0	+s	0	+		2	0
3	rr	330214	0	0	0	+	+	+	0	0	0	+	0	+	/	+	+	0	0	+	+	0	+	0	+	+	+	+s	0	+		3	0
	Patient Cells																															

Shaded columns indicate those antigens which are destroyed or depressed by enzyme treatment.

"/" represents "Not Tested" for new donors.

Ortho Clinical Diagnostics

Reagent Red Blood Cells
0.8% Surgiscreen®
©Ortho Clinical Diagnostics 2010

LOT NO.
VSS434
EXP. DATE
2023-02-21

ANTIGRAM®
Antigen
Profile

Ortho Clinical Diagnostics

PATIENT NAME: _____

PATIENT ID: _____

DATE: _____ TECH: _____

CONCLUSION: Anti-K

Lot No. VRA424 Exp. Date 2023-02-21 CCYY-MM-DD

Panel A

Reagent Red Blood Cells
0.8% Resolve® Panel A
ANTIGRAM® Antigen Profile

6352012211

			Rh-hr									KELL				DUFFY		KIDD		Sex Linked	LEWIS		MNS				P	LUTHERAN		Special Antigen Typing		Test Results
Cell#	Rh-hr	Donor Number	D	C	E	c	e	f	C^w	V	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Xg^a	Le^a	Le^b	S	s	M	N	P1	Lu^a	Lu^b		Cell#	MTS
1	R1wR1	317993	+	+	0	0	+	0	+	0	0	+	0	+	/	+	+	0	+	+	+	0	+	0	+	+	0	+	0	+		1	0
2	R1R1	330228	+	+	0	0	+	0	0	0	+	+	0	+	/	+	+	+	0	+	+	0	+	+	+	+	0	+	0	+		2	3+
3	R2R2	313991	+	0	+	+	0	0	0	0	0	+	0	+	/	+	+	0	0	+	+	0	+	+	0	+	+	+	0	+		3	0
4	Ror	330060	+	0	0	+	+	+	0	+	0	+	0	+	/	+	0	0	+	+	0	0	0	0	+	0	+	+	0	+		4	0
5	r'r	330242	0	+	0	+	+	+	0	0	0	+	0	+	/	+	+	+	0	+	+	+	0	0	+	+	+	+	0	+	@	5	0
6	r''r	323655	0	0	+	+	+	+	0	0	0	+	0	+	/	+	0	+	0	+	+	0	+	+	+	+	+	+s	0	+	@	6	0
7	rr	325892	0	0	0	+	+	+	0	0	+	+	0	+	/	+	0	+	0	+	+	0	+	0	+	0	+	+s	0	+	@	7	3+
8	rr	330231	0	0	0	+	+	+	0	0	0	+	0	+	/	+	+	0	+	0	+	+	0	+	+	+	0	+	0	+	@	8	0
9	rr	323272	0	0	0	+	+	+	0	0	0	+	0	+	/	+	+	+	+	0	+	0	+	+	0	+	+	0	+	+		9	0
10	rr	322630	0	0	0	+	+	+	0	0	0	+	0	+	/	+	+	+	+	+	+	+	0	0	+	0	+	0	0	+		10	0
11	R1R1	327958	+	+	0	0	+	0	0	0	0	+	0	+	/	+	0	+	+	0	+	0	+	+	0	+	0	+s	0	+	HLA+	11	0
	Patient Cells																															0

Anti-K: Mechanism of Action

Antigens of the Kell blood group system are highly immunogenic, and Kell antibodies can cause severe HDFN

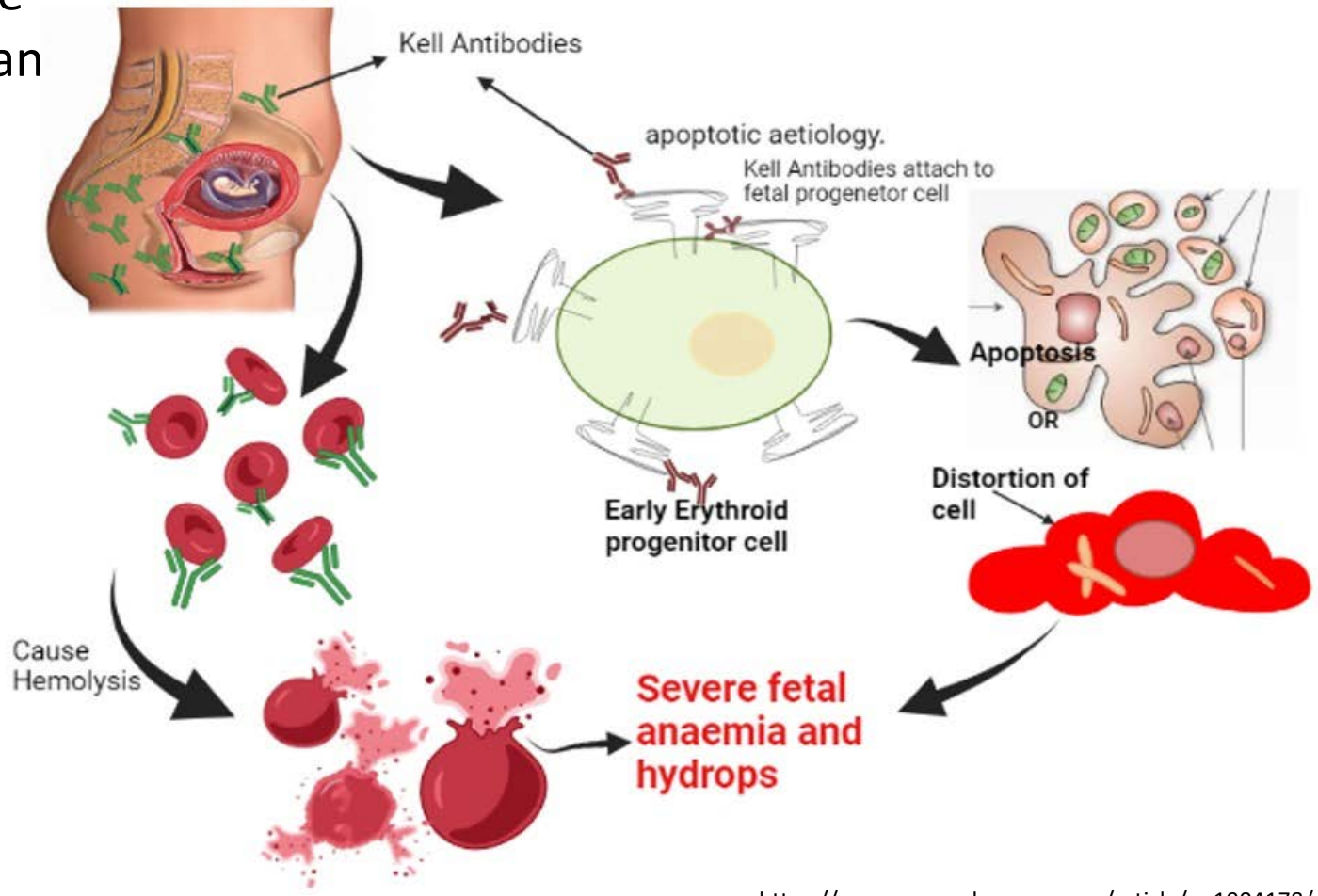
Maternal Anti-K targets Kell antigens expressed on the surface of

1. Mature fetal RBCs
2. RBC precursors***

Promotes the immune destruction of both types of K+ RBCs

Suppresses fetal RBC production

Potential for severe fetal anemia

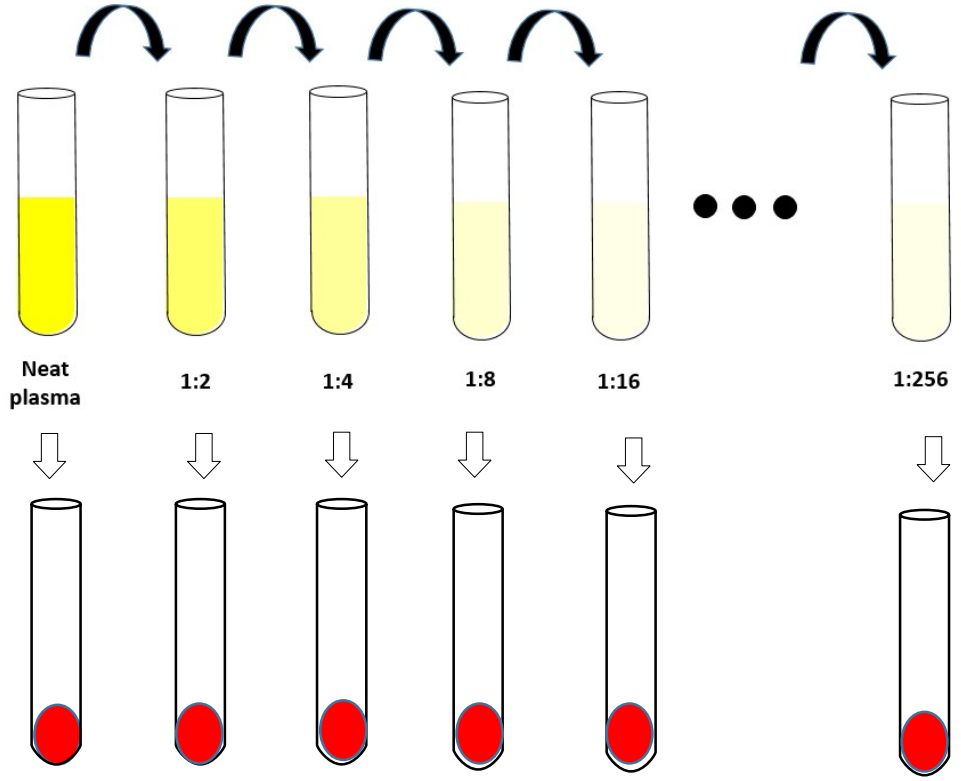


https://www.researchsquare.com/article/rs-1904178/v1

Close surveillance is required, especially if previous affected pregnancies

Titration of Anti-K

Serial dilutions of patient plasma



Antigen positive RBC (K+k+)

Estimate of strength/concentration of antibodies

SIAT
Tube technique

	Neat	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024
RESULT	4+	4+	3+	3+	2+	2+	1+	1+	0	0	0

Titre is reciprocal of highest dilution reacting at 1+ macroscopically

One month later: titre was still **1/128**
One month later: titre increased to **1/256**

2 weeks later: IUT was requested

MCA-PSV in detection of Fetal Anemia

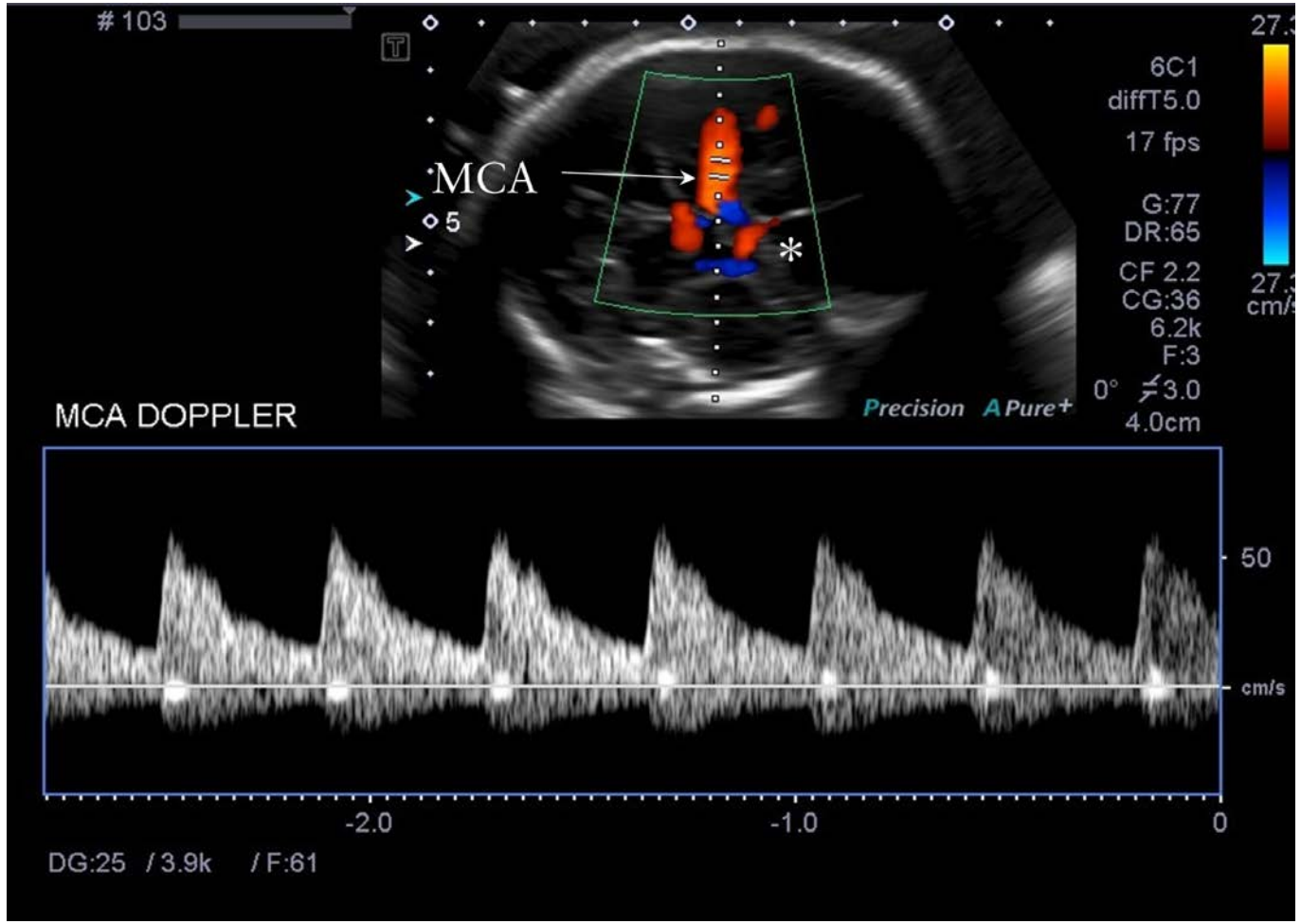
The correlation between Anti-K titre and severity of anemia is unclear

Fetal middle cerebral artery peak systolic velocity (MCA-PSV) is an additional diagnostic tool used to detect fetal anemia & indicate need for IUT

In fetuses with anemia, the velocity of bloodflow through this artery appears to increase because of decreased blood viscosity and increased cardiac output

⬆ velocity = ⬆ risk of fetal anemia

Non-invasive and has been shown to have high sensitivity and accuracy for prediction of severe fetal anemia



https://obgyn.onlinelibrary.wiley.com/doi/full/10.1002/uog.17555

Requirements for Blood

O Rh Negative

C-, E-, K- (and antigen negative for any additional clinically significant antibodies detected in mother's plasma)

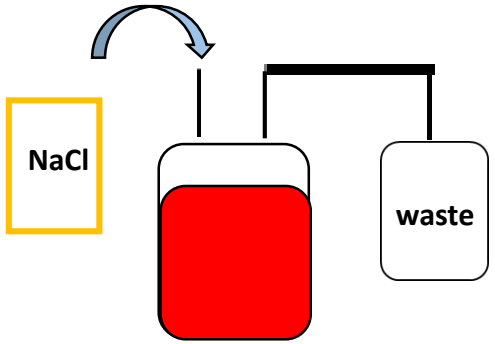
Fresh (less than 7 days old)

Units are crossmatched

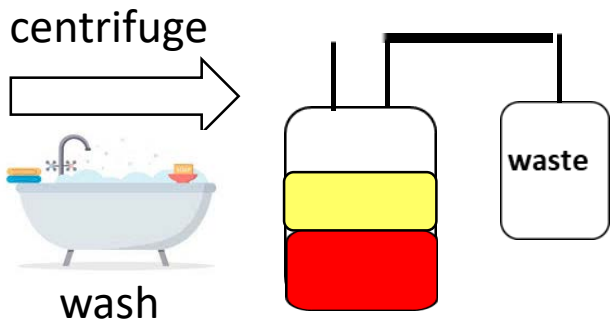
- Gel (MTS)

https://health.clevelandclinic.org/universal-blood-donor-type/

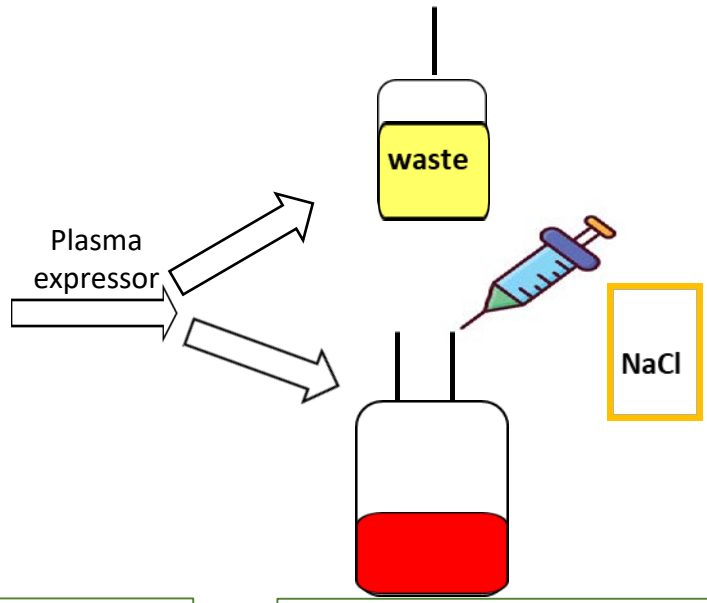
Preparation of RBC



Add ~ 250 mL 0.9% NaCl (saline) to wash unit and attach aliquot bag (for waste)

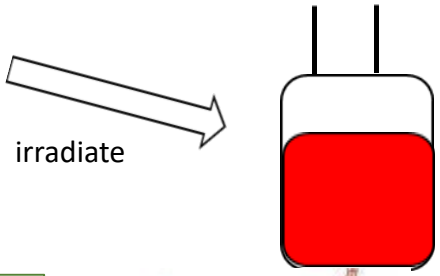


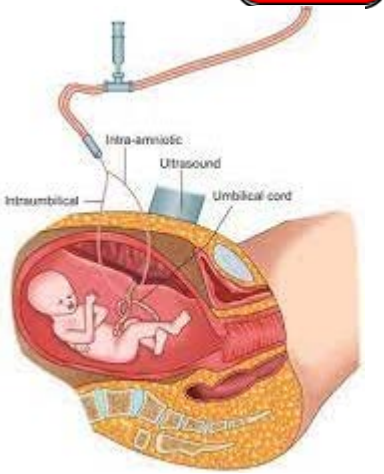
Spin unit in refrigerated centrifuge: 4000 rpm for 10 minutes @ 4°C

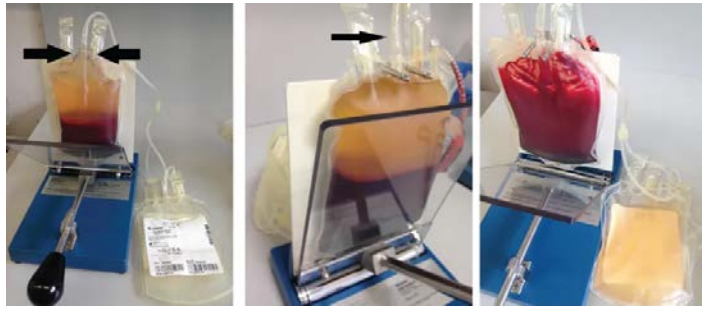


Separate supernatatnt (waste) from RBC using plasma expressor

Measure Hct & calculate volume of saline required to reduce Hct to 75% ±5%; add saline & repeat Hct to ensure it is in the required range







https://veteriankey.com/blood-component-processing-and-storage/

Intrauterine Transfusion (IUT)

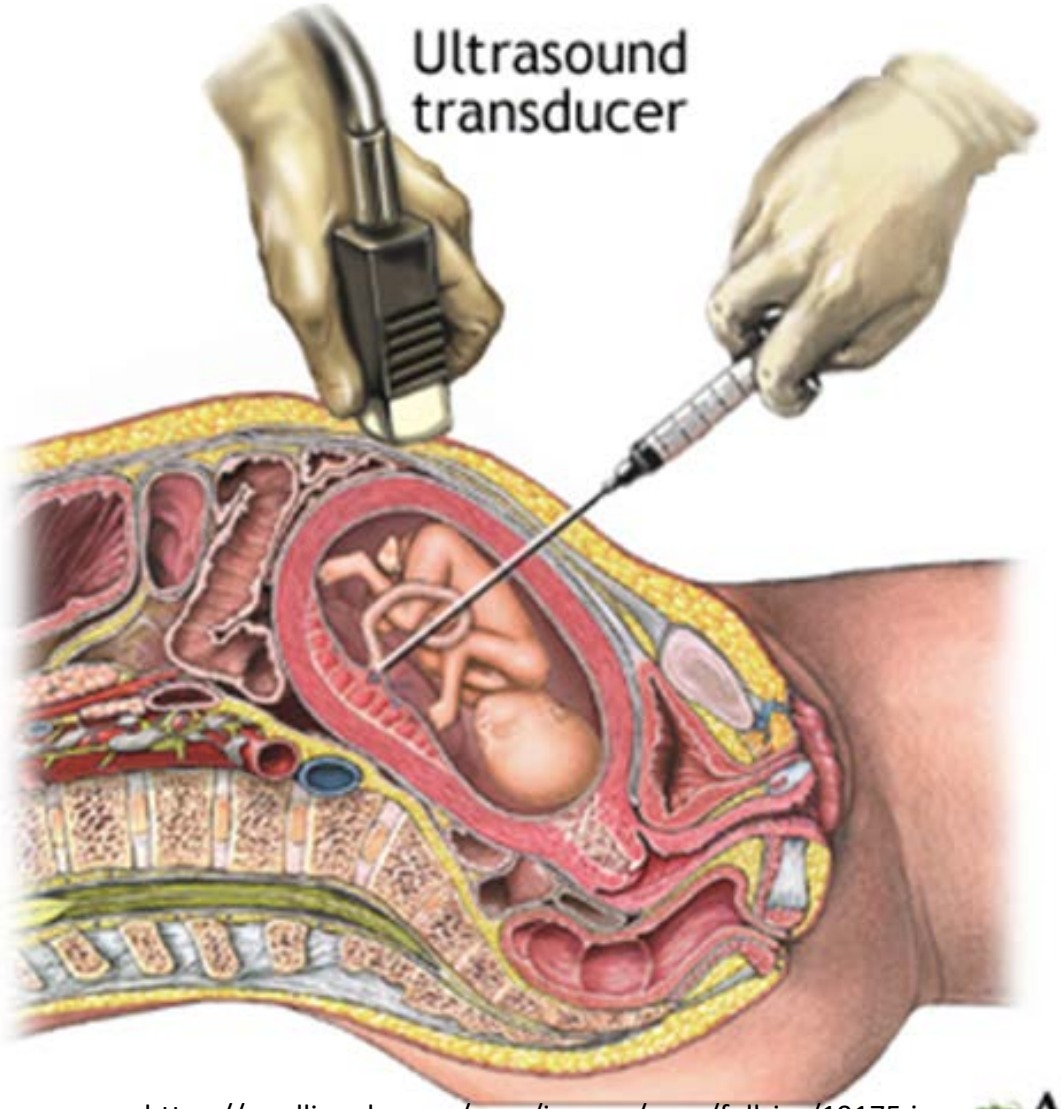
Can also be referred to as Percutaneous Umbilical Blood Sampling (PUBS)

A needle is inserted into either the placental cord insertion or the intrahepatic vein (~70%) to obtain a fetal blood sample

This procedure allows direct measurement of fetal hemoglobin, which provides insight into the presence/severity of fetal hemolytic disease

If fetal anemia is detected during the sampling procedure, the Perinatologist will likely perform intrauterine transfusion (IUT) concurrently

The fetus may not require transfusion if the haemoglobin is found to be acceptable, in which case the procedure is stopped



https://medlineplus.gov/ency/images/ency/fullsize/19175.jpg

During IUT

The team performing the IUT consists of:

- Perinatologist
- Nurses
- Respiratory Therapist/Anesthesia Assistant
- MLT

The perinatologist inserts the needle into the patient's abdomen and using ultrasound guidance, finds the appropriate sampling site

Syringes containing fetal blood are handed to the MLT for processing

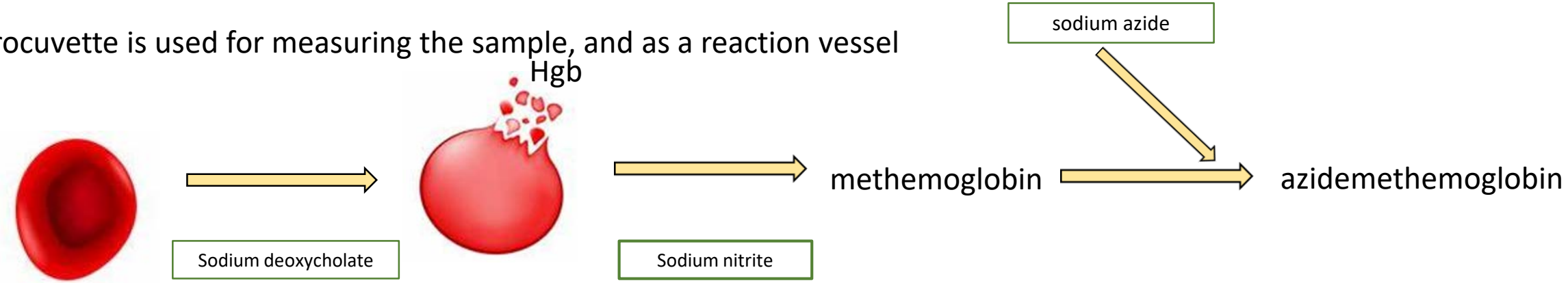
MLT will determine the hemoglobin using the HemoCue® photometer and immediately give the perinatologist a verbal result

The HemoCue® Hemoglobin analyzer

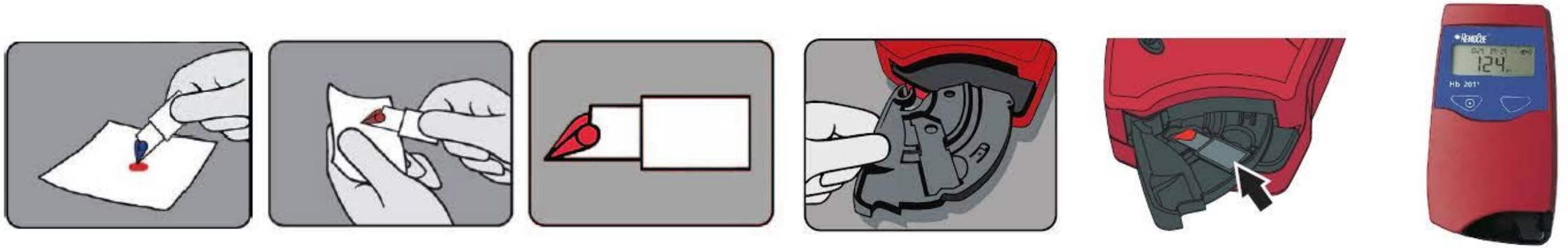
Point of care system that provides a rapid method of measuring the hemoglobin concentration

Consists of disposable microcuvettes containing reagent in dry form and a single purpose photometer where the reading of Hgb concentration takes place

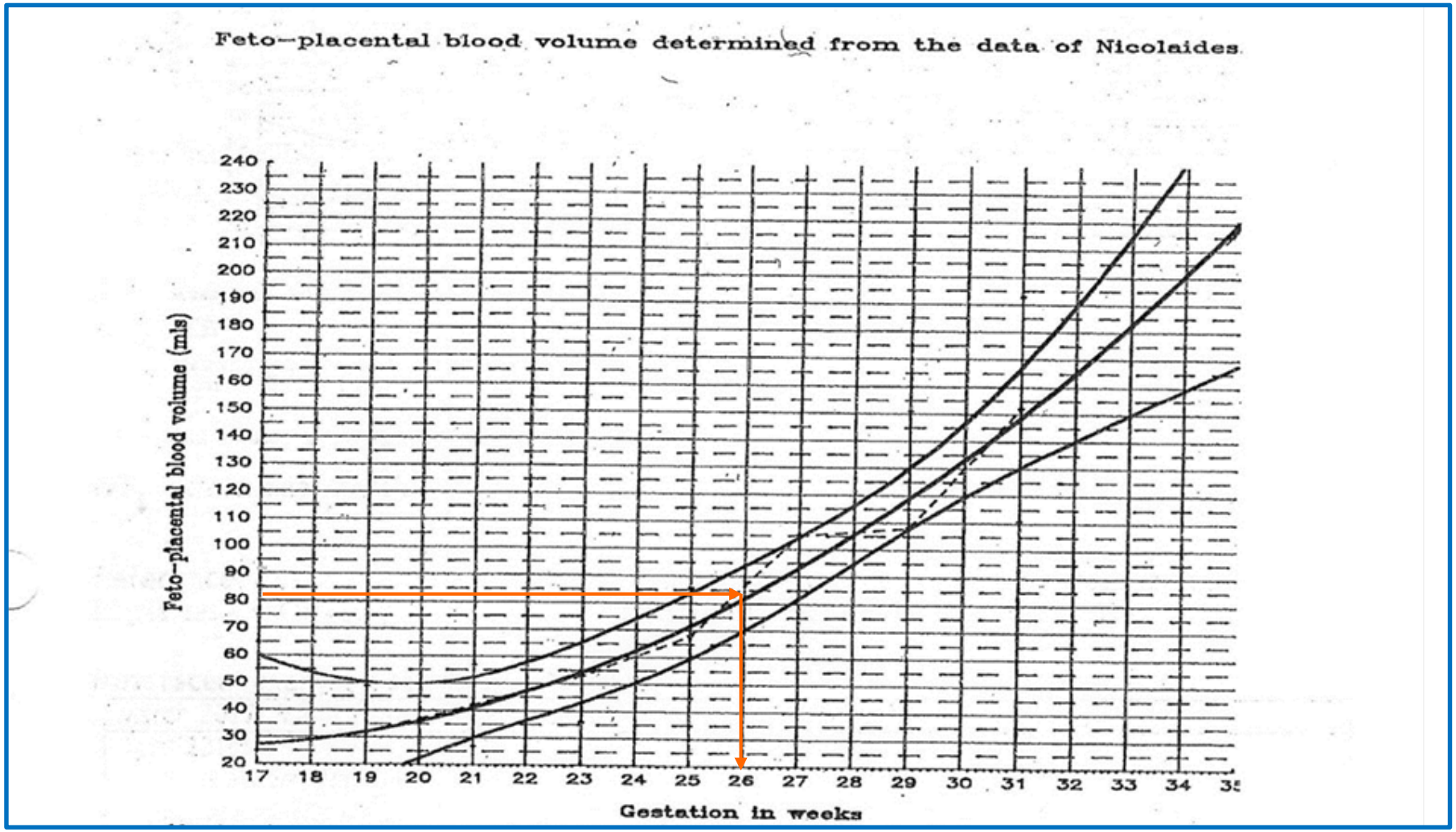
Microcuvette is used for measuring the sample, and as a reaction vessel



Hemoglobin concentration in blood is determined by measuring azidemethemoglobin



Nicolaides Graph



26 weeks GA= 82 mL Feto-placental blood volume

Fetal IVT Volumes

From CBC of donor RBC unit

From Nicolaides Graph

sion Service, Pathology and laboratory Medicine
Mount Sinai Hospital, Toronto, ON

Fetal IVT Volumes (mL)

Patient:

Donor Unit Hgb= 246 g/L

Fetal/Placental Blood Vol= 82 mL

Post-Tx'n Target Hgb

Pre-Tx'n Fetal Hgb	40	50	60	70	80	90	100	110	120	130	140	150	160	170
10	12	17	22	28	35	42	51	60	72	85	101	120	143	173
20	8	13	18	23	30	37	45	54	65	78	93	111	133	162
30	4	8	13	19	25	32	39	48	59	71	85	103	124	151
40	0	4	9	14	20	26	34	42	52	64	77	94	114	140
50		0	4	9	15	21	28	36	46	57	70	85	105	129
60			0	5	10	16	22	30	39	49	62	77	95	119
70				0	5	11	17	24	33	42	54	68	86	108
80					0	5	11	18	26	35	46	60	76	97
90						0	6	12	20	28	39	51	67	86
100							0	6	13	21	31	43	57	76
110								0	7	14	23	34	48	65
120									0	7	15	26	38	54
130										0	8	17	29	43
140											0	9	19	32
150												0	10	22
160													0	11
170														0

$$\frac{\text{Target Hgb - Fetal Hgb}}{\text{Donor Hgb - Target Hgb}} \times \text{Fetal-placental blood volume (FPBF)} = \text{volume of RBC to transfuse}$$

Hgb target is set by Perinatologist

MLT will use Pre-Tx Hgb to determine the blood volume required to raise Hgb to the target

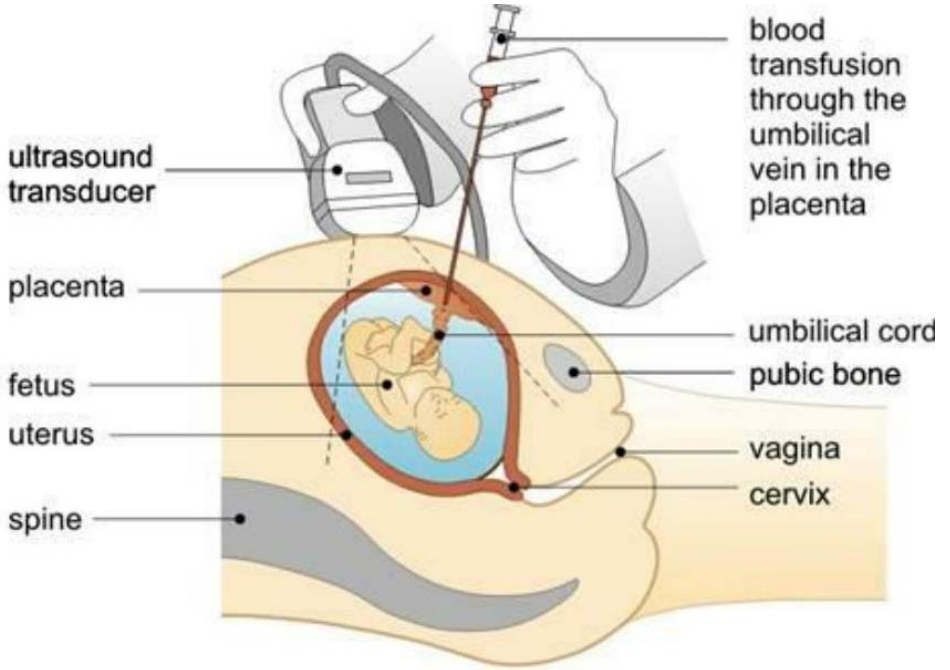
IVT chart for quick reference (based on formula)

Report the result verbally to the Perinatologist

Back to the IUT…

Once the target volume has been transfused, the Perinatalogist may perform post-transfusion sampling to ensure the hemoglobin value is satisfactory

If not, additional volumes of RBCs are transfused until the target is reached



IUT #1

Target Hgb was 100 g/L

Volume required to be transfused was calculated to be 45 mL

Sample #	Hgb (g/L)	Pre/Post Tx
1	20	Pre Tx
2	20	Pre Tx
3	55	Post Tx 20 mL
4	56	Post Tx 20 mL
5	98	Post Tx 40 mL
6	97	Post Tx 40 mL

After PUBS :

- A volume of the sample from the syringe is sent to Hematology for a full fetal CBC
- Samples may also be sent for Chemistry and other tests (Cytogenetics, Microbiology) as requested by the physician
- Sample left in the syringe (pre-transfusion) is saved for testing in Blood Bank

Blood Bank Testing

ABO and Rh

- O Positive

DAT

- Positive

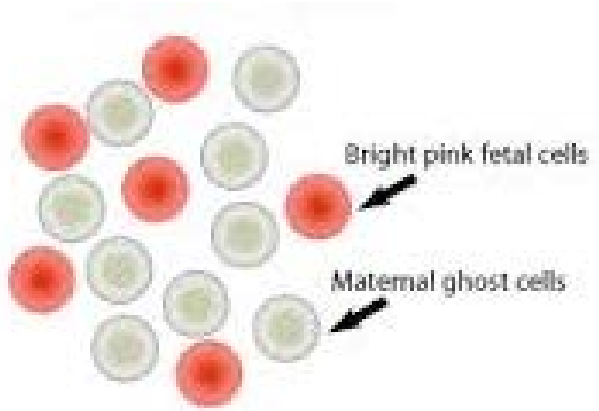
Betke-Kleihauer

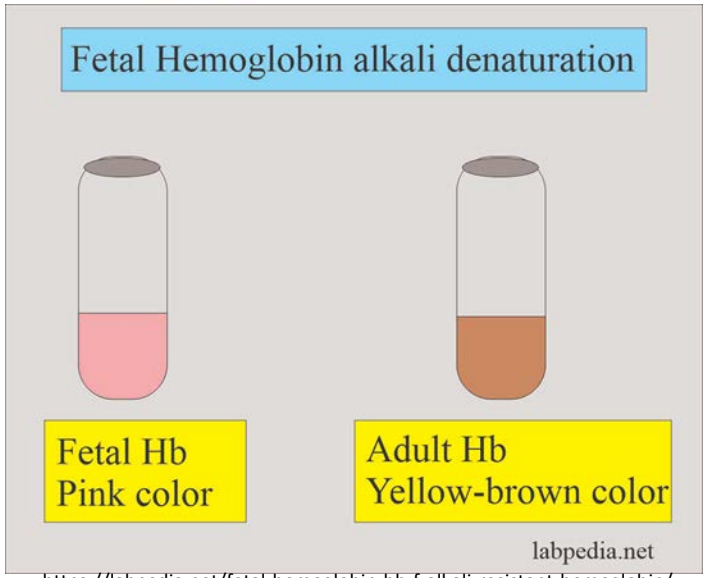
- 100% fetal cells

Alkaline Denaturation

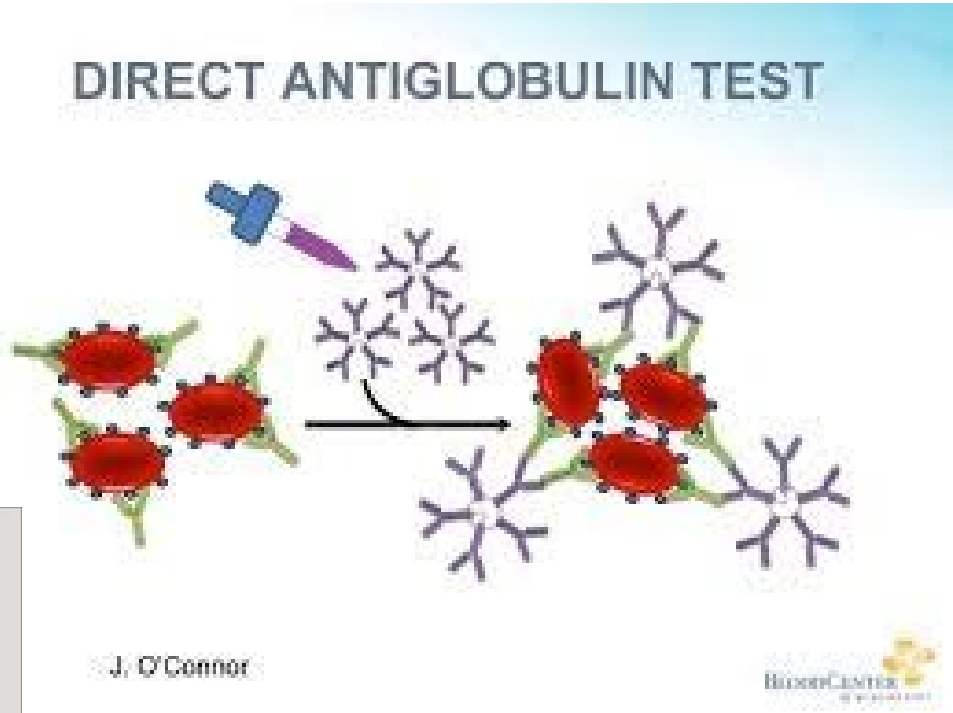
- Fetal

Hemoglobin F (Hb-F; fetal hemoglobin) is more resistant to denaturation by alkaline solutions than other types of hemoglobin





https://labpedia.net/fetal-hemoglobin-hb-f-alkali-resistant-hemoglobin/



Subsequent IUTs

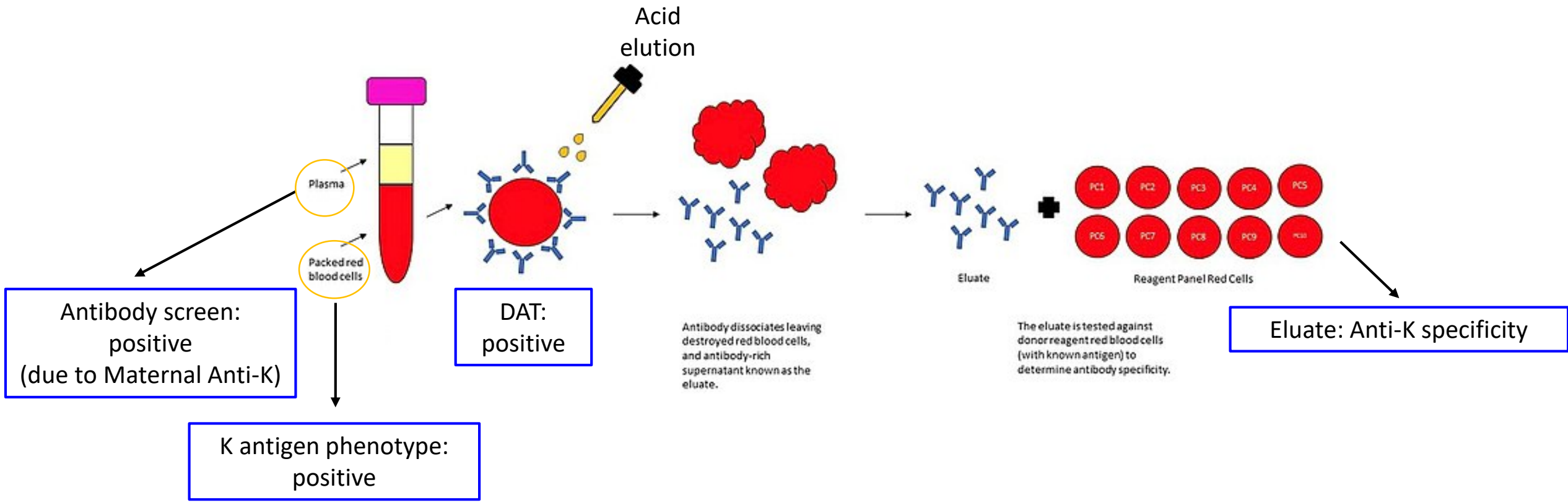
	Weeks Gestation	Starting Hgb (g/L)	Volume Transfused(mL)	Final Hgb (g/L)	Drop in Hgb (g/L)
IUT #2	27	62	100	141	5
IUT #3	28 + 6 days	127	70	157	14
IUT #4	31 + 5 days	81	110	158	76
IUT #5	35 + 1 days	114	120	155	44

Neonatal Testing

Baby was born at 37 weeks +2 days

Cord blood sample was received for testing

ABO/Rh- O Positive



Antibody screen: positive (due to Maternal Anti-K)

DAT: positive

Eluate: Anti-K specificity

K antigen phenotype: positive

https://en.wikipedia.org/wiki/Antibody_elution

Neonatal Outcomes

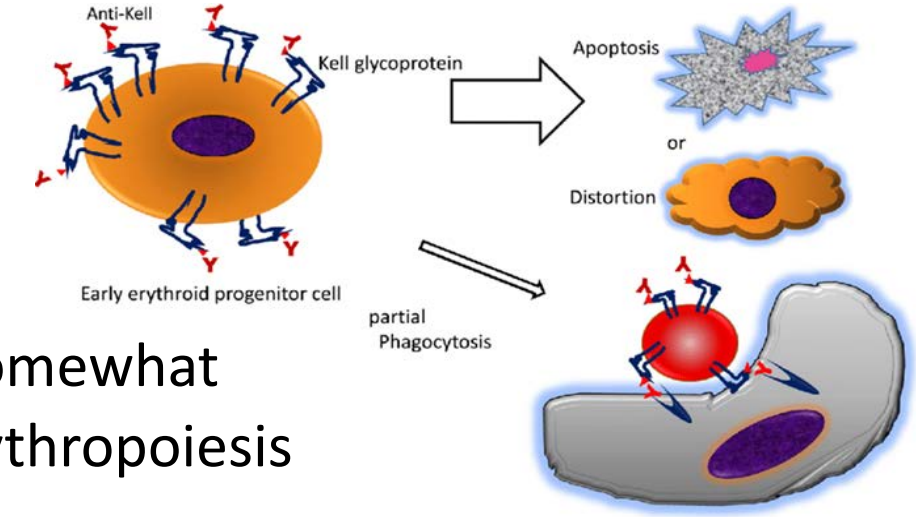
Hemoglobin at birth was 180 g/L

No neonatal transfusions were done

Discharged after 5 days- Hgb was 163 g/L

Came into postnatal unit approximately 1 month after discharge- Hgb was 167 g/L and no other abnormal findings in bloodwork

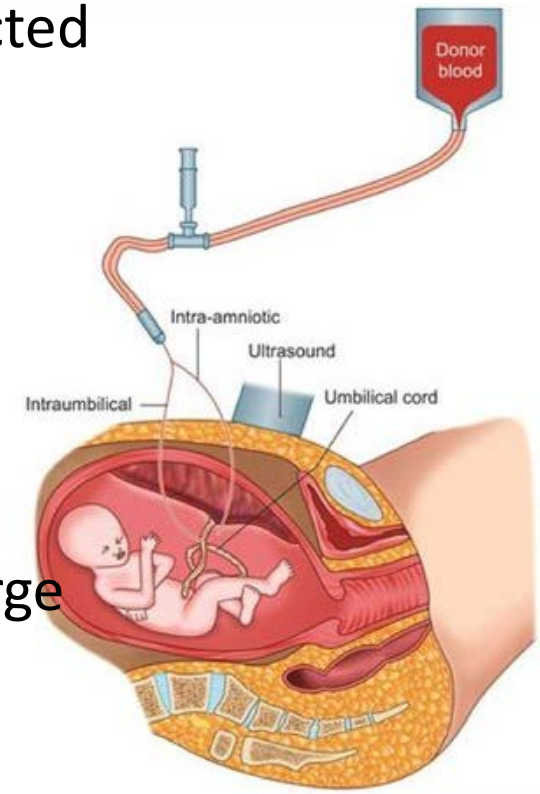
Summary



The mechanism of fetal anemia in Anti-K mediated HDFN is somewhat unique in that anti-K antibodies cause suppression of fetal erythropoiesis

Monitoring is crucial by a specialized centre once an Anti-K antibody is detected

- Titration of Anti-K at regular intervals by Mount Sinai Hospital
  - Closer/more frequent monitoring when titre reaches 1/32
- Doppler MCA-PSV
  - Intervention (IUT) triggered when velocity reaches specified level, suggesting severe fetal anemia



Monitoring of neonates who receive IUT for at least one month after discharge

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