Perinatal Transfusion Medicine – Determination of fetal blood group antigens in maternal blood“

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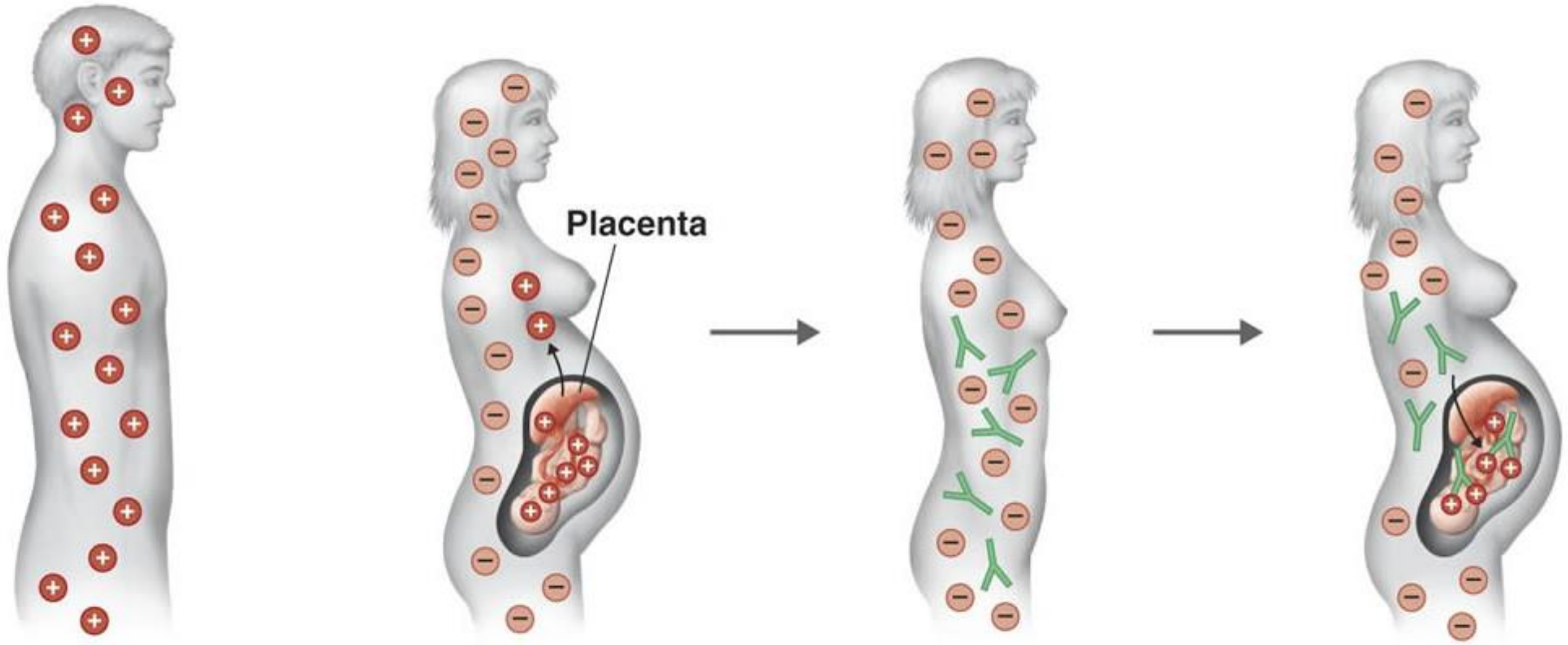
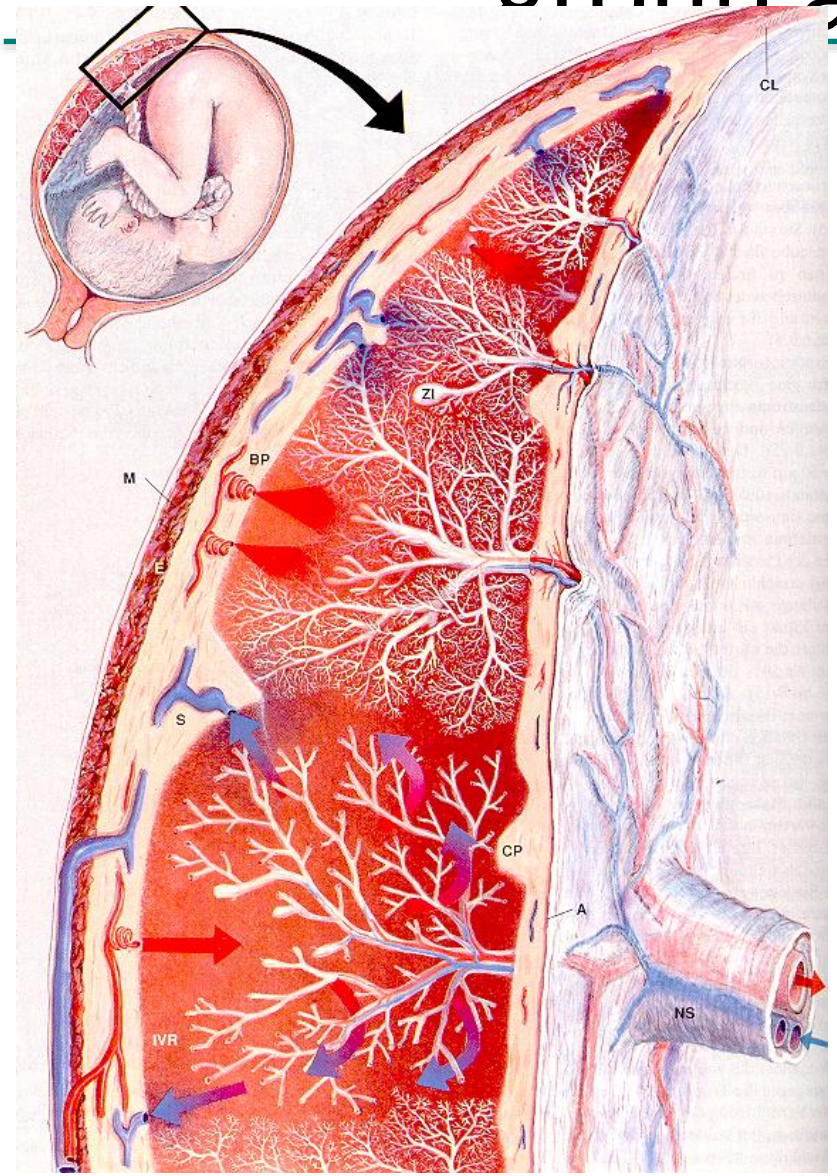
Timisoara – 29.10.2022

Induction of antibodies against blood cells during pregnancy



1. Der Vater ist Träger von Blutzellen, die ein Antigen tragen, welches die Mutter nicht trägt
2. Der Foetus erbt dieses Antigen vom Vater
3. Eine kleine Anzahl foetaler Blutzellen tritt in die mütterliche Zirkulation ein
4. Die Mutter bildet Antikörper gegen das Antigen, welches der Foetus vom Vater geerbt hat.
5. Diese Antikörper greifen bei einer nächsten Schwangerschaft die Blutzellen des Foetus an.

Causes for induction of blood group antibodies



Exposition to allogenic blood:

1. Fetomaternal Hämorrhagia (FMH)

a) spontaneous

Trimenon			postpartal
1.	2.	3.	
3%	12,1%	45,4%	63,60%

J.M. Bowman et alt., Vox Sang 1986,51:117-121

b) iatrogen: invasive interventions

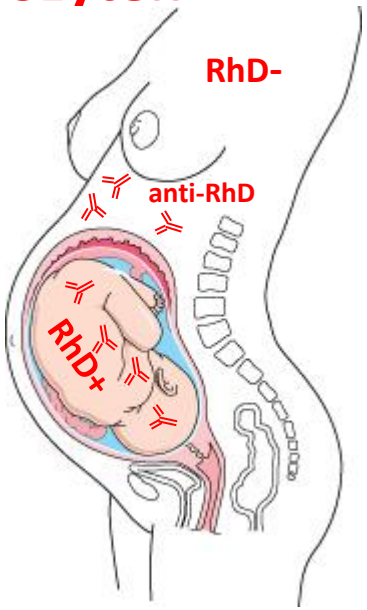
2. Transfusion

3. Rare Causes:

- Needle Sharing (i.v.-Drug addicts)

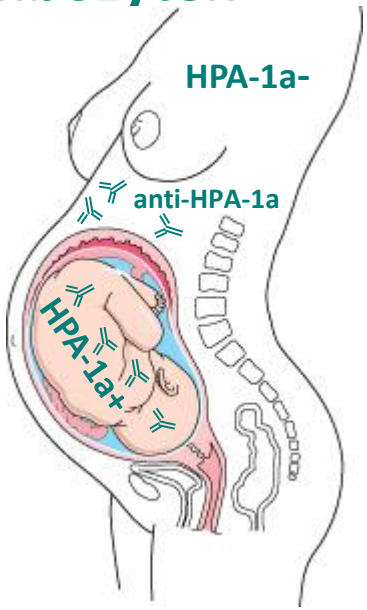
Alloantibodies against erythrocytes and platelets

Erythrozyten



- **Morbus Hemolyticus Neonatorum (MHN)**
  - Fetal anemia
  - Hydrops fetalis
  - Brain damage (Kern icterus)
  - Intrauterine death

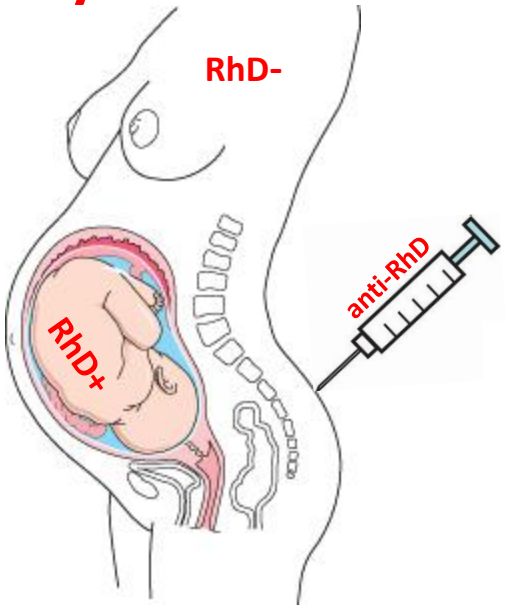
Thrombozyten



- **Fetal and neonatal Alloimmunthrombozytopenia (FNAIT)**
  - Fetale thromobcytopenia
  - Haemorrhagic Diathese
  - Brain damage (intracraniale Blutung)
  - Intrauterine death

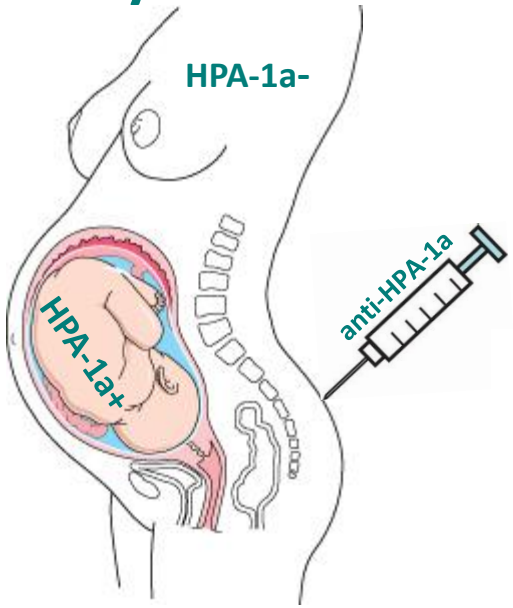
Prevention of alloimmunization in pregnancy

Erythorcytes



- **Standard of care since 40 years**
  - Immunisation with **anti-D Prophylaxis** to eliminate fetale RHD positive erythrocytes in the mothers circulation.

Thrombocytes



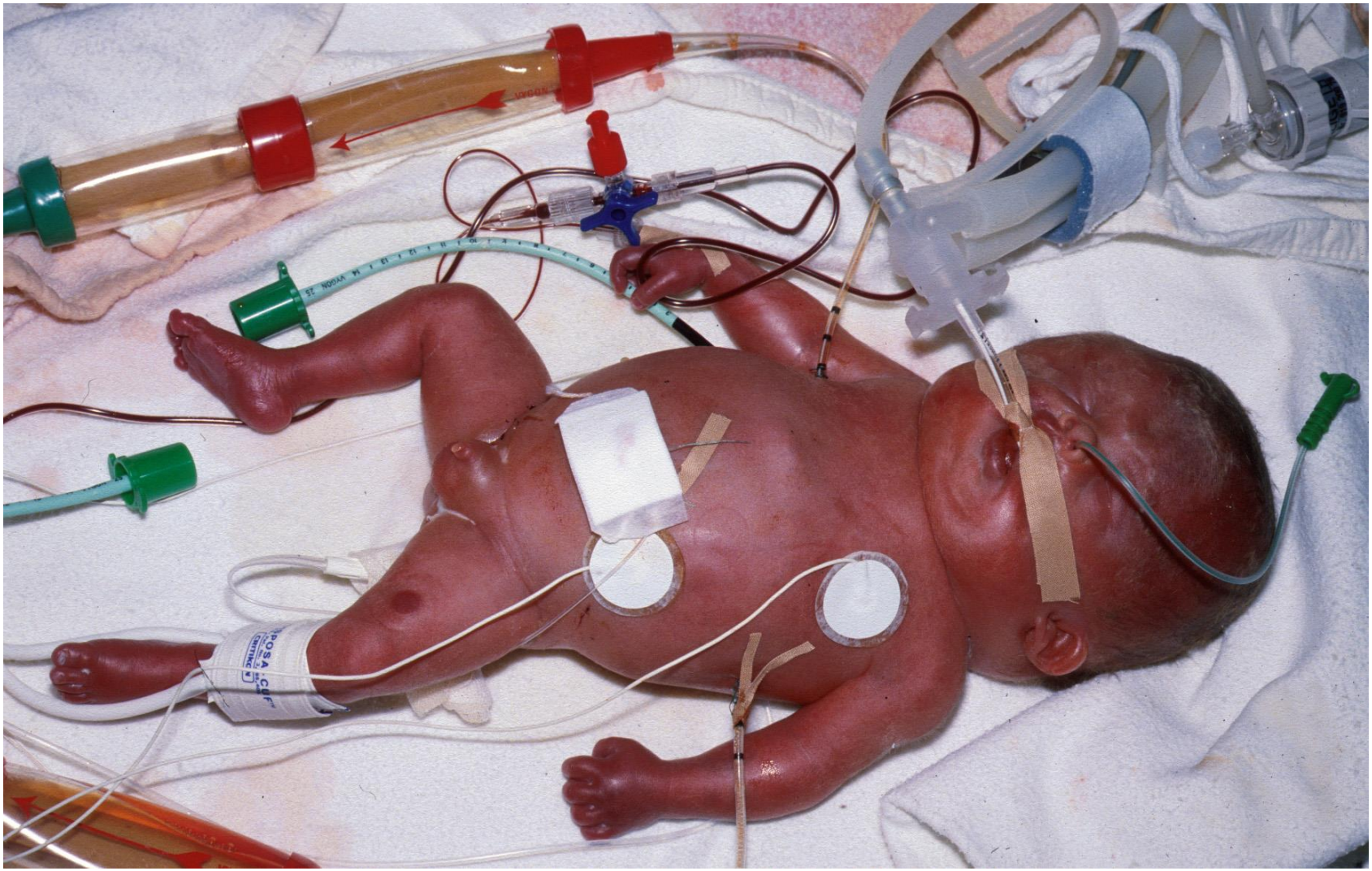
- **New concept**
  - Immunisation with anti-HPA-1a in order to eliminate fetal HPA-1a positive platelets from the mothers circulation.

Classification of Rh incompatibility

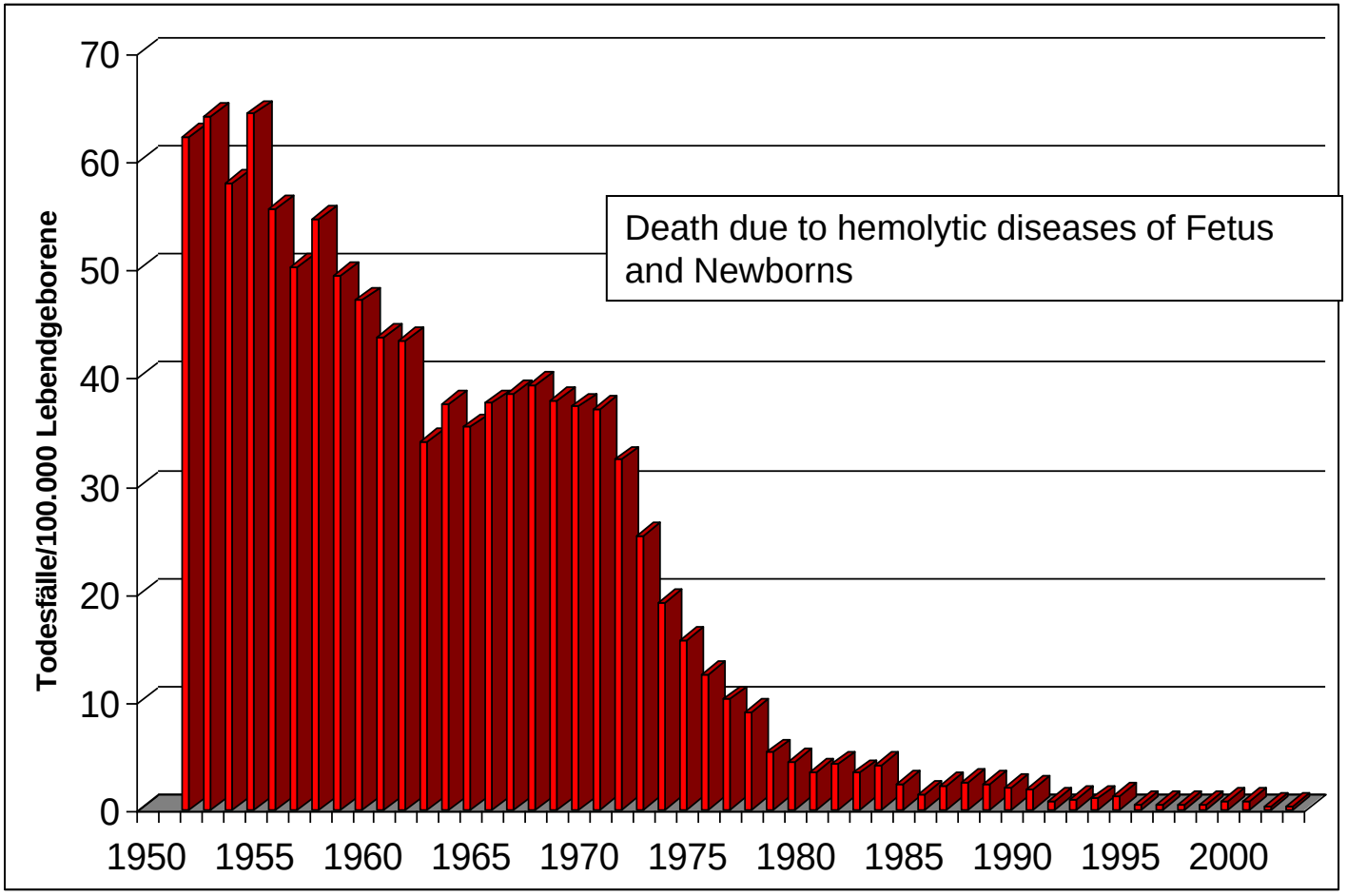
Ausprägung	Definition	Inzidenz (%)
mild	no Therapy necessary postpart. milde Anämie Hyperbilirubinämie	45 - 50 %
medium	postpartale Therapy necessary Ikterus gravis -> Kernikterus Austauschtransfusion	25 - 30 %
severe	Hydrops fetalis in utero before 34. SSW after 34. SS	20 - 25 % 10 - 12 % 10 - 12 %

Bowman, J.M, Pediatrics 1965;35:815-35

Fetale Erythroblastose



Mortality due to hemolytic diseases of newborns in Germany



Quelle: www.gbe-bund.de, Gesundheitsberichtserstattung des Bundes

Epidemiology

MHN

- **Ca. 16 % of cases relate to RhD negative mothers**
- **Before introduction of Anti-D Prophylaxis more then 40 years ago**
- MHN in **15 von 10,000 births**
- MHN in former times accounted for 10% of perinatale deaths
- **TODAY:**
- MHN is rare
- ***Post***-natale Anti-D Prophylaxis has reduced the risk for RhD-Immunisation to less then 2% .
- Introduction of ***ante***-natale Anti-D Prophylaxis reduced the risk by further ⅓ (in 28 SSW)

FNAIT

- **Ca. 2 % mothers are HPA-1a negative**
- **To date no possibility for prophylaxis**
- Ca. 10 % of HPA-1a negative pregnant women develop Anti-HPA-1a Antibody
- Ca. ⅓ of HPA-1a-immunised women will have a thrombopenic child
- Cerebral bleeding due to thrombocytopenia in 1 of 10.000 Newborns.
- In Europe and USA ca. **1000 cases of intracranial bleeding due to FNAIT yearly.**

Pathologic immune reactions during pregnancy

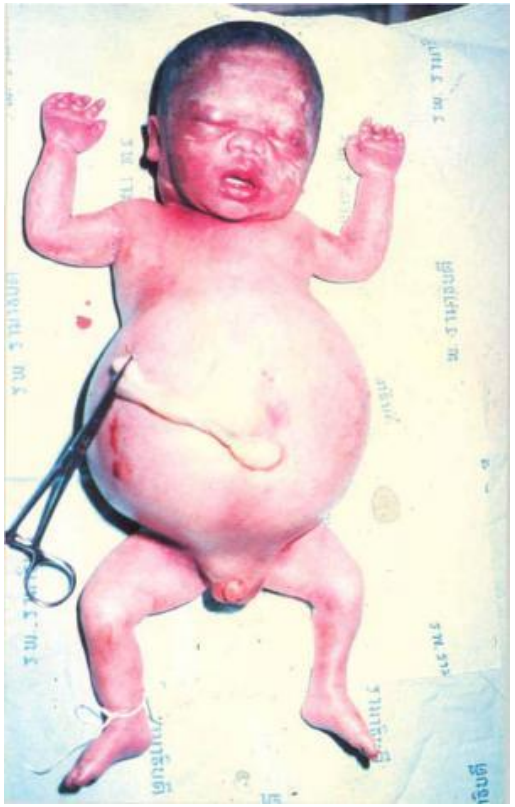
- **fetale Immunzytopenia**
  - **Alloantibodies against blood cells**
    - **Erythrocytes - Morbus Hämolyticus (MHN, HDN)**
    - Thrombocytes - Neonatal Alloimmunthrombopenia (NAITP)
    - Granulocytes - Neonatal Alloimmunneutropenia (NAINP)
- **Autoimmunerkrankungen**
- **Abortus?**

MHN - Epidemiology

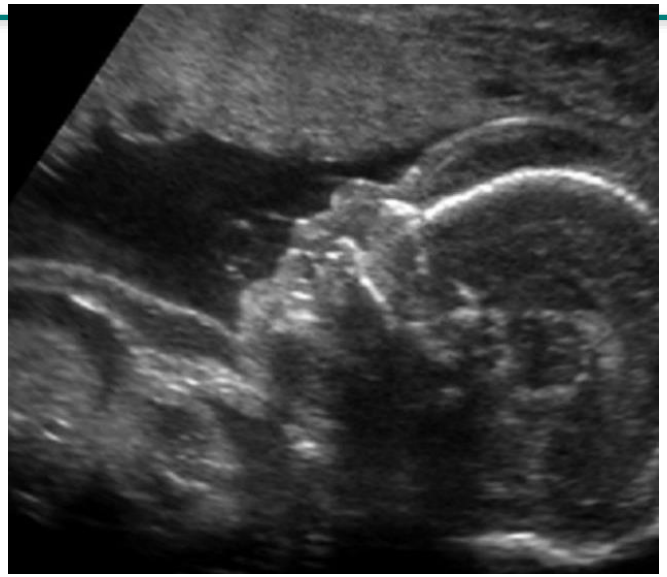
- bis in die 1960er-Jahre endemisch (Anti-RhD)
  - 7/1000 Schwangerschaften
    (4% Immunisierungsrate)
    - 60% therapiebedürftig
      - 12% Todesfälle
      - 25% irreversible neurologische Komplikationen

- Einführung der Anti-D IgG-Prophylaxe
  - postpartal
  - postpartal + antenatal
    -> 0.1% Immunisierungsrate

- *Antikörper gegen andere Antigene*
  - *Rhc, Kell, RhC, RhE, Fy^a, Jk^a, Rhe, ... (A, B)*
    keine Prophylaxe möglich, CAVE Trf. O RhD neg !



MHN – possible symptoms

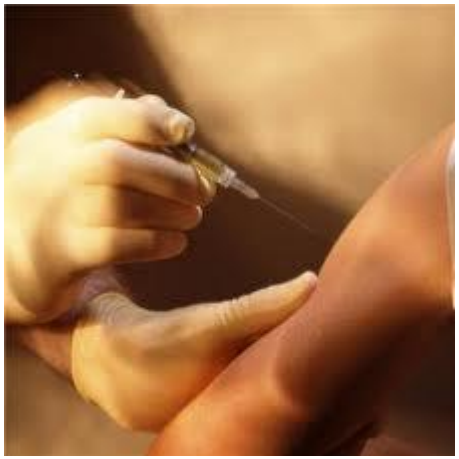


- Bilirubin increase (Ikterus) <24h
- Retikulocytosis and Erythroblastose
- Hämolysis/Anemia
- Liver -/Spleen enlargement
- Tissue hypoxia, Edema (Hydrops)
- post partum brain damage (Kernikterus, Bilirubinenzephalopathie)
- Death

MHN –Prophylaxis

- pooled human Hyperimmunglobuline Anti-D (IgG)

- ***Supply is getting more difficult***



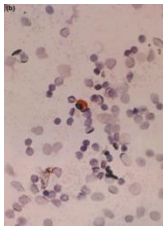
MHN - Diagnosis

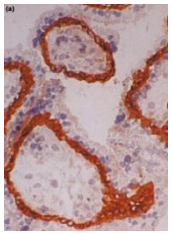
- Father
  - Blood group serology
  - Molekular genetic analysis (Zygotie)

- Non -invasive Prenatal diagnostic from maternal blood (NIPD)

Fetal cells in maternal circulation

Erythroblasten
Trophoblastische Zellen
Leukozyten



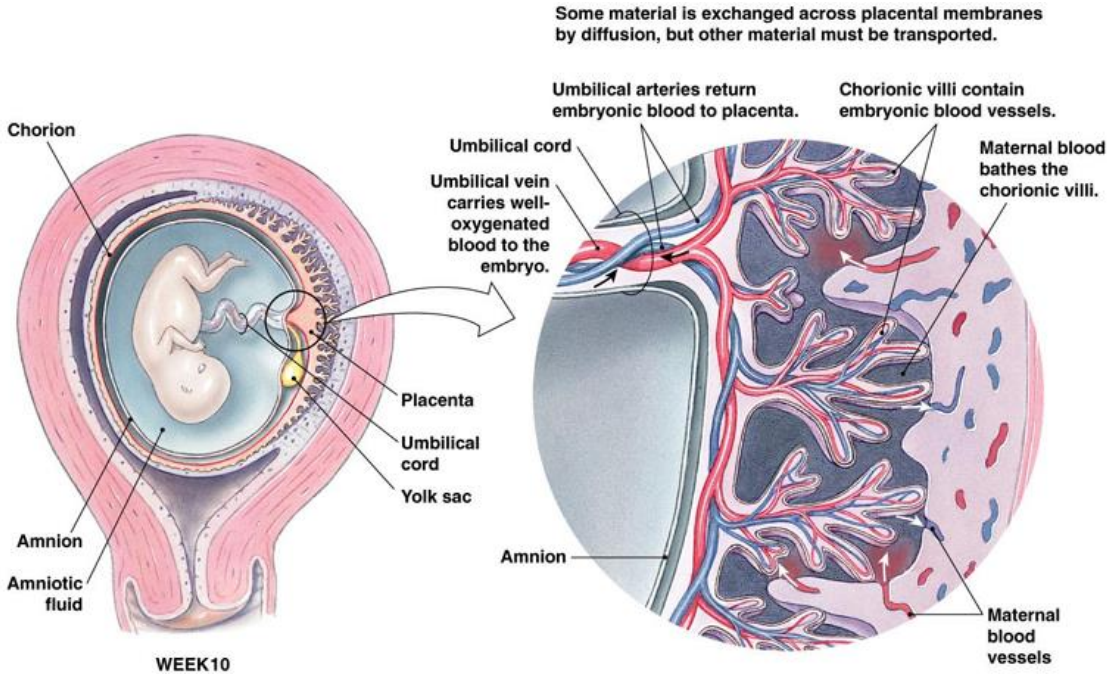


Difficult to isolate, but persist years after pregnancy in maternal cirkulation

Not suited for non invasive prenatal diagnosis

cellfree fetal DNA (cff-DNA)

- Released from Chorionic vili in maternal circulation (`shedding`)



Characteristics von cff-DNA

- Extreme short fragments (max. 150bp)
- *Fast degradation (lt.Lit 24h post partum)*

- max. ~10% of Plasma-DNA of the mother

- Suitable to detect fathers genes in the circulation of the mother by RT-PCR

NIPD using RT-PCR possible indications

- Rh-D (RHD)

- Other blood group antigens
  - RHCE, Kell, Fy, PLA1, ...

- SRY-determination for sex associated diseases
  - CAH (congential adrenal hyperplasia)
  - X-linked diseases

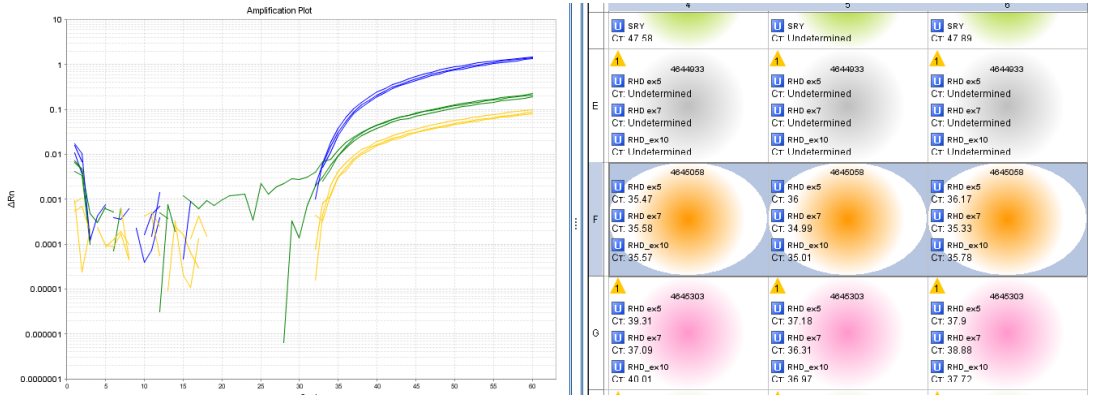
- *Other monogenetic inherited diseases inherited by the father*
- *ß-Thalassaemie*
  - *myotone Dystrophie*
  - *Achondroplasie*
  - *Cystische Fibrose*
  - *Chorea Huntigton*

NIPD-RHD in Europe

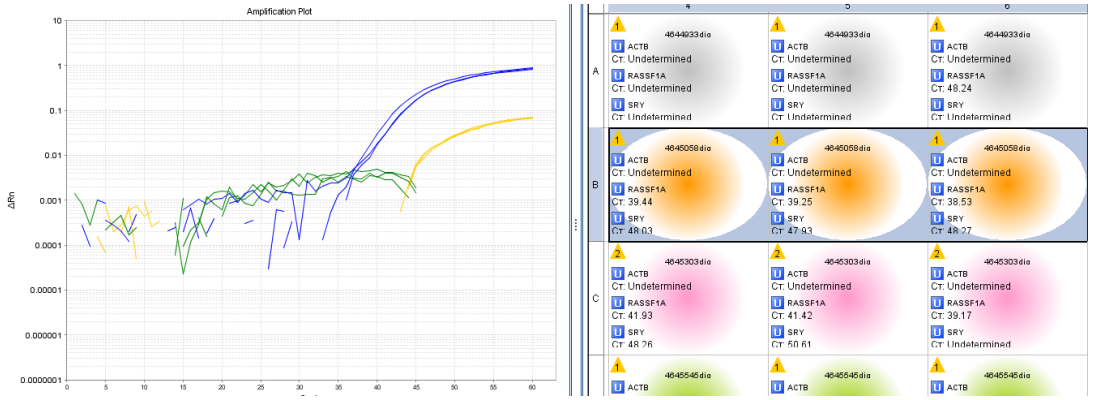
- In already alloimmunised women against for example RhD :
  - UK, NL, S, DK, B, D, PL, F, CH, A, SLO, P, E, I

- In order to decide if a Rh-D negative pregnant woman would need a RhD prophylaxis during this pregnancy :
  - NL, DK (nationale Programs)
  - B, F, CH, A (regional)

NIPD-RHD: RHD positive male Fetus

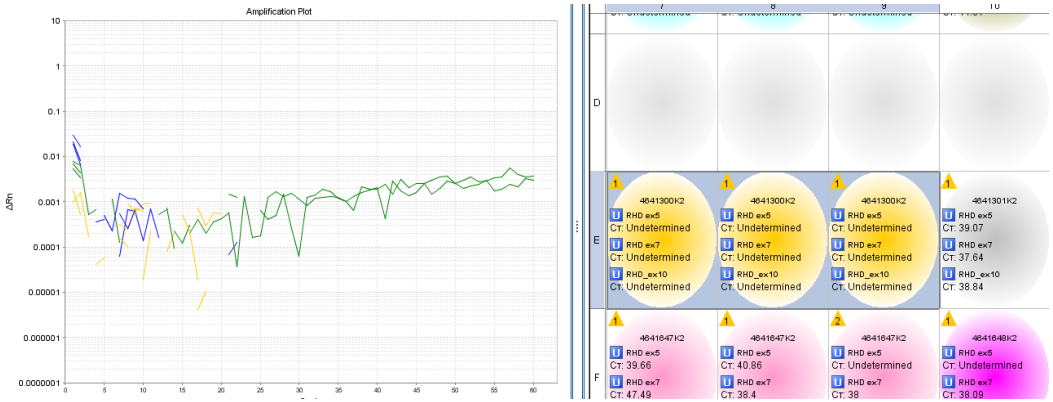


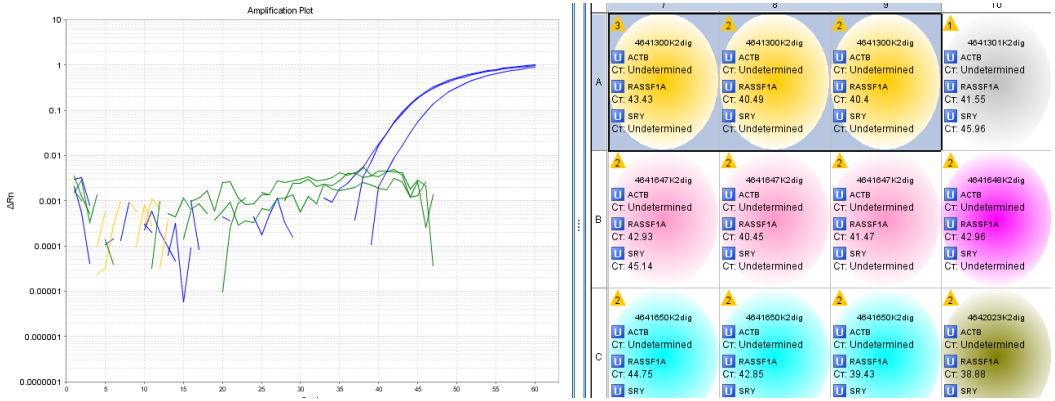
RHD exon 5
RHD exon 7
RHD exon 10



SRY
RASSF1A
ACTB

NIPD-RHD: RHD negative female Fetus



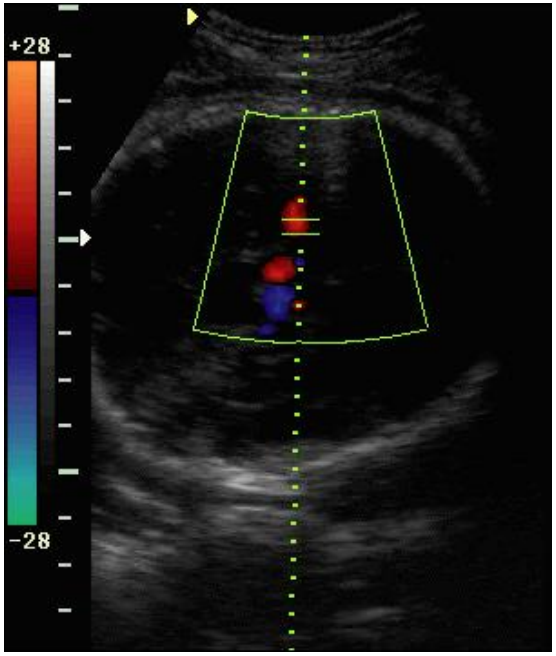


RHD exon 5
RHD exon 7
RHD exon 10

SRY
RASSF1A
ACTB

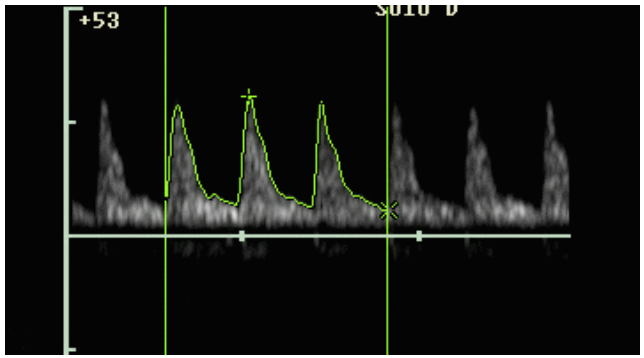
MHN - Therapy

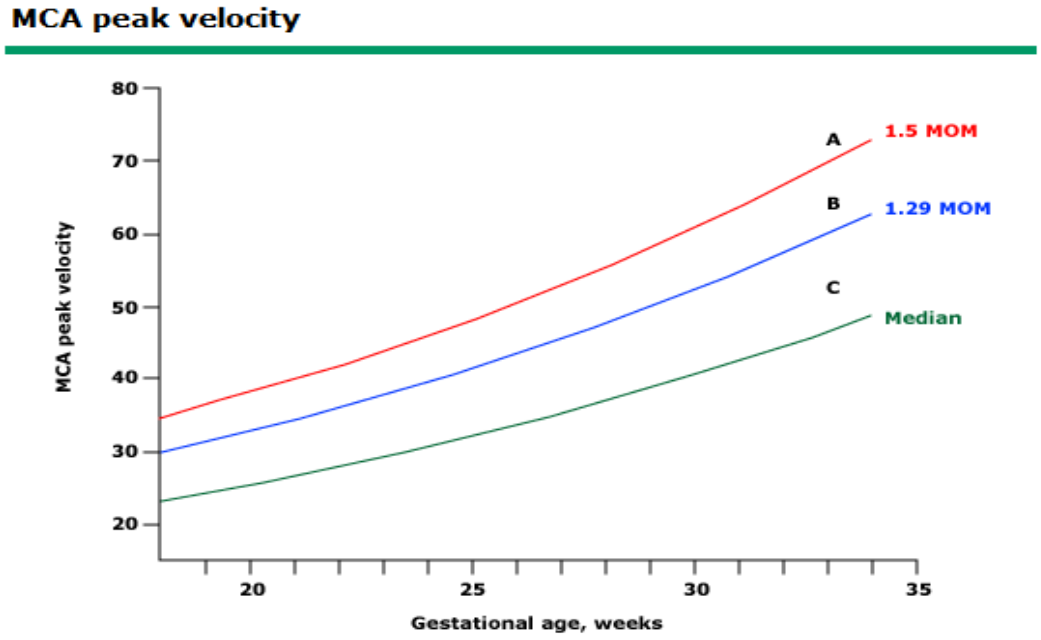
- ab 20.SSW
  - Regular antibody Titre controls
    - (Traditon)
  - **Regelular repeated ultrasonic controls**
    - **Doppler A.cerebri media**
  - intrauterine puncture of cord vein
    - *diagnostisch*
    - *intrauterine Transfusion*
- postnatal
  - Phototherapie
  - ivIgG
  - (exchange-) Transfusion



Doppler A.cerebri media

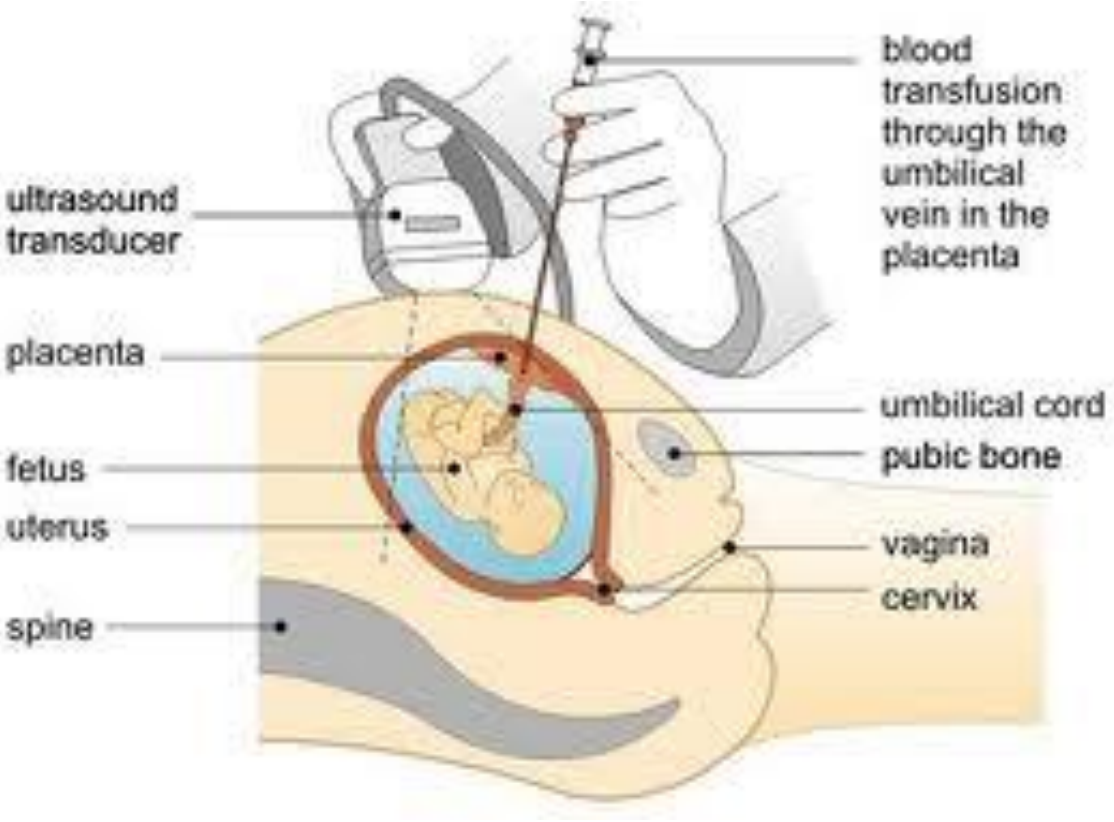
Peak systolic velocity (Vmax)

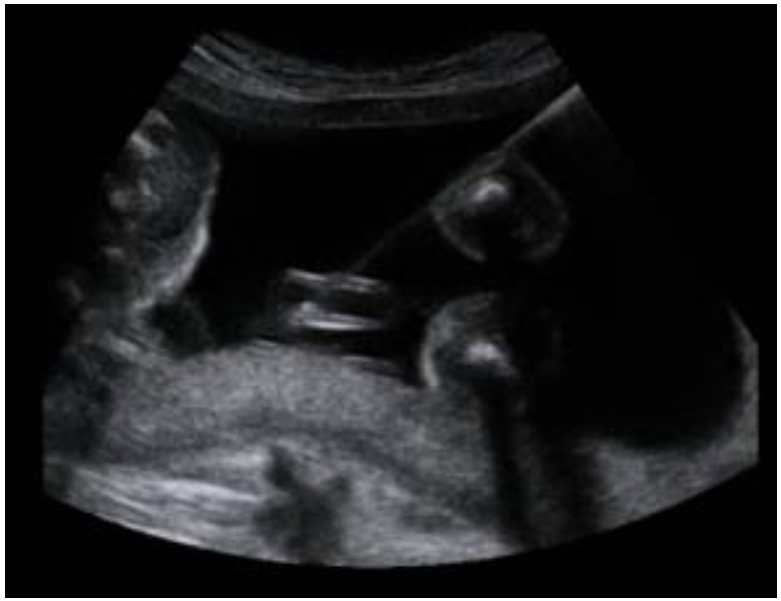




A: moderate to severe anemia; B: mild anemia; C: no anemia; MCA: middle cerebral artery; MOM: multiples of the median.
Data from: Mari, G, for the Collaborative Group for Doppler Assessment of the Blood Velocity in Anemic Fetuses. Noninvasive diagnosis by Doppler ultrasonography of fetal anemia due to maternal red-cell alloimmunization. N Engl J Med 2000; 342:9.

Intrauterine Transfusion





Blood products for intra uterine transfusion

- Goal: maximal substitution with minimal volume
  - Packed red blood cells: irradiated major compatible Erythrocyte concentrate (SAGM) with Hkt 70 (+/-5) (cross match with maternal blood)

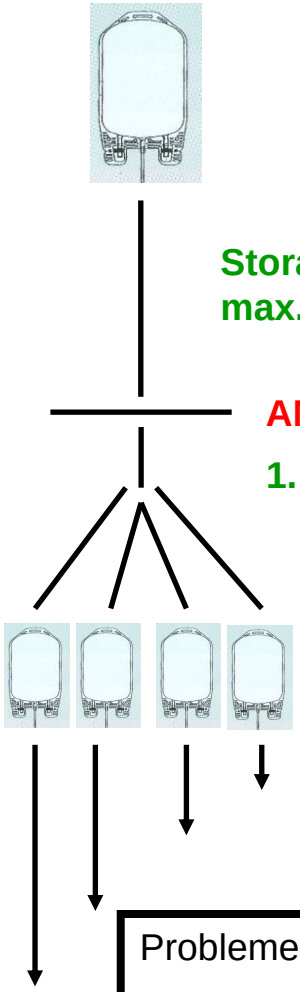
CMV-save , i.e. Leuko depleted and CMV-AK neg. (?)

Requirements for Red blood cell concentrates in prenatal and newborns

- Fresh red blood cells (≤ 7 Tage)
  - Intrauterine
  - exchange transfusion
  - Extrakorporaler circulation

- preferable same donor when repeated transfusion
  - split red blood cell concentrates

Rezeptur: bestrahlte geteilte-EK (DRK-BSD)



Storage/Age
max. 7 days

ANFORDERUNG

1. Irradiation

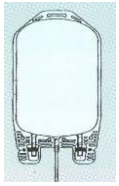
2. PORTIONIERUNG
(als Rezeptur)

Probleme:

- mögl. Laufzeit der Satelliten nicht ausgenutzt
- K^+ Anstieg nach Bestrahlung

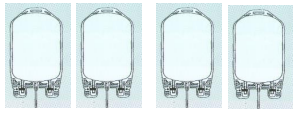
Lagerung max. Tag 14 nach Bestrahlung

Rezeptur: geteilte, bestrahlte-EK (DRK-BSD)



LAGERUNG
max. 7 Tage

ANFORDERUNG



1. PORTIONIERUNG
(als Rezeptur)

2a. BESTRAHLUNG auf ABRUF vor AUSGABE

2b. BESTRAHLUNG und ABRUF vor AUSGABE

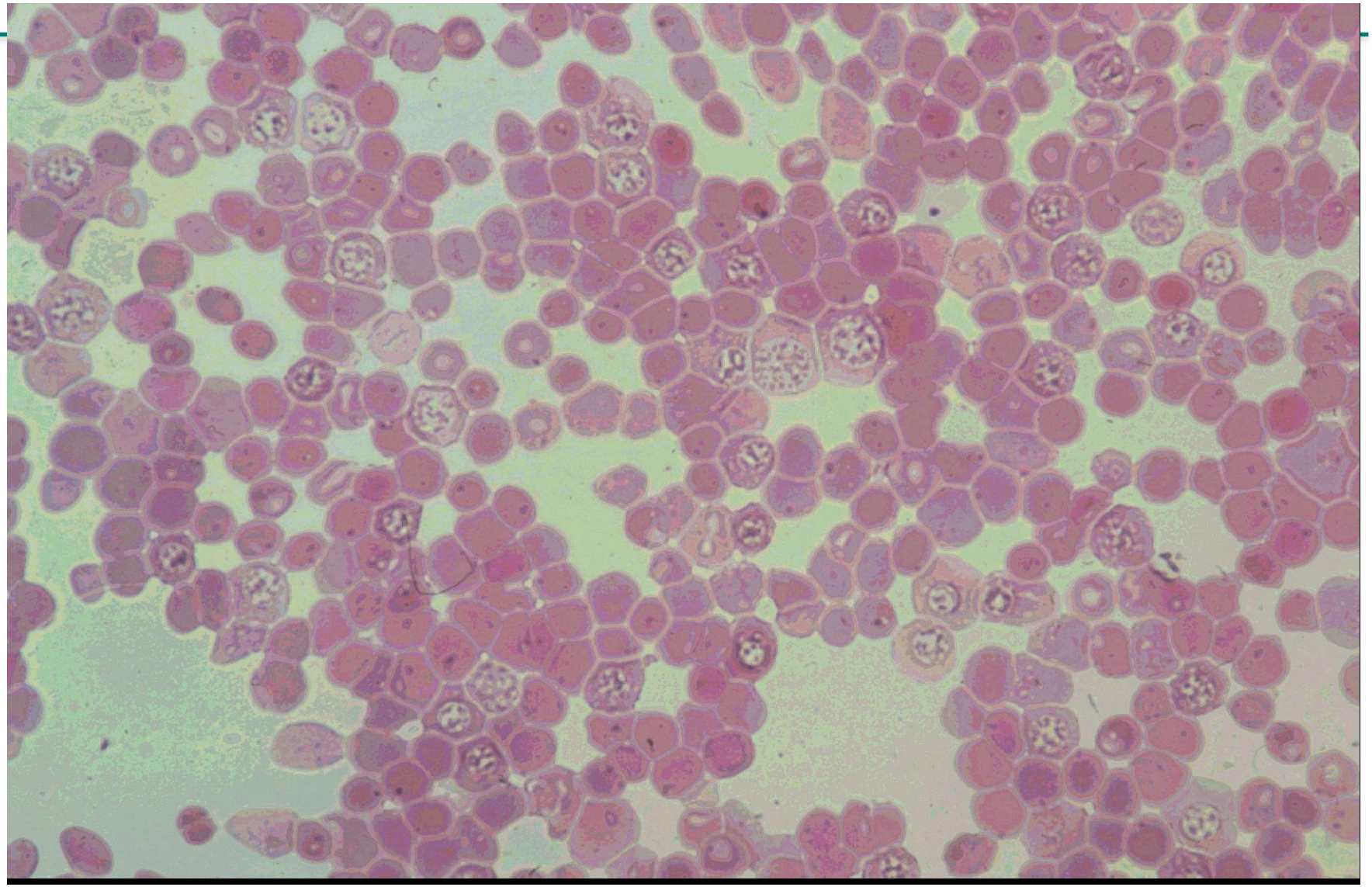
2c. BESTRAHLUNG auf ABRUF vor AUSGABE

2d. BESTRAHLUNG auf ABRUF vor AUSGABE

Lagerung nach Teilung max:
21 Tage

Max. Laufzeit nach Bestrahlung:
24 Stunden

Fetale Erythroblastose
Ausstrich des peripheren Blutes

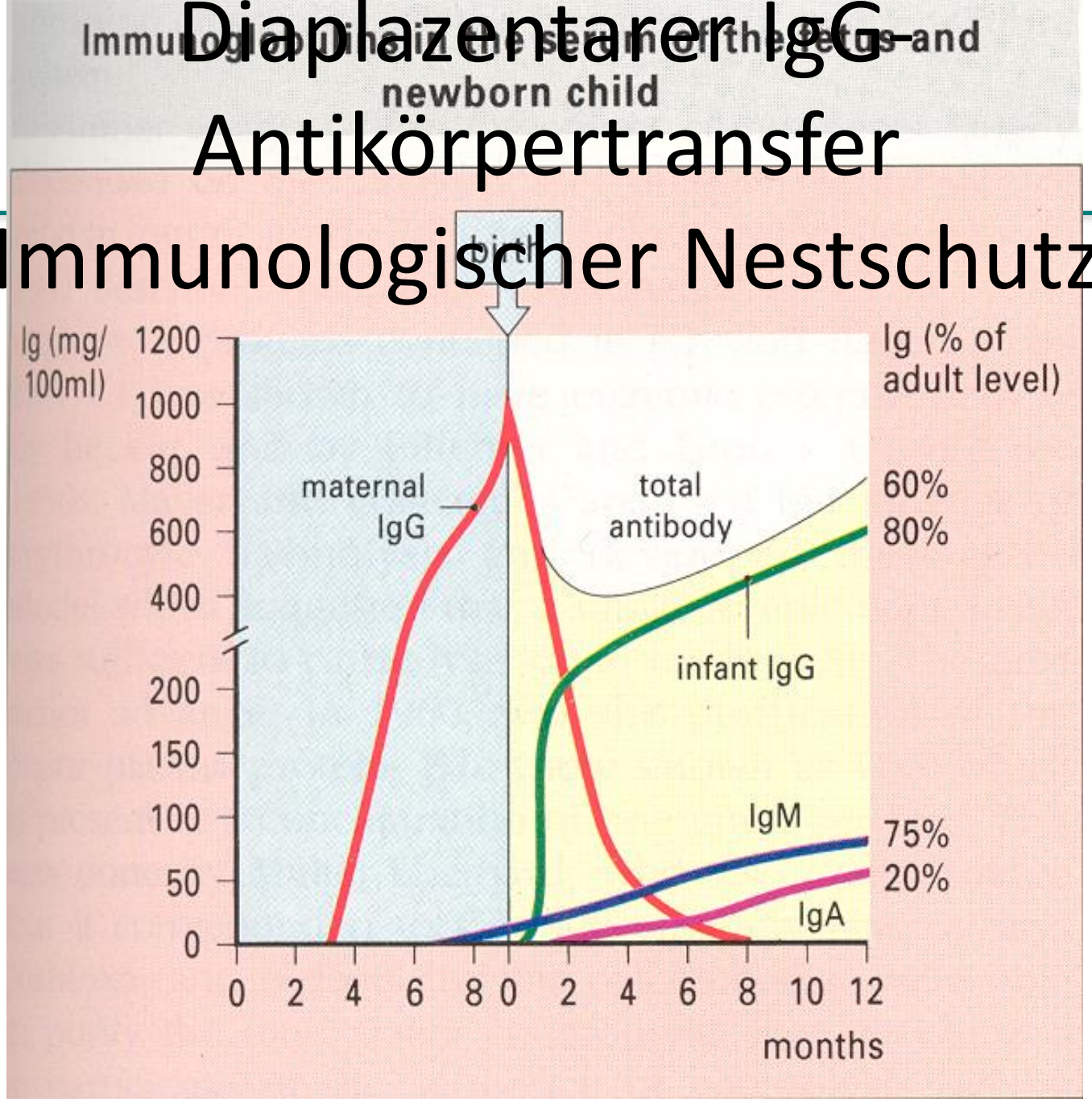


Rhesus-D-Inkompatibilität 1992, Hb bei 1. FBS 1,9 g/ dl

Blutgruppeninkompatibilität - andere Blutgruppenantigene

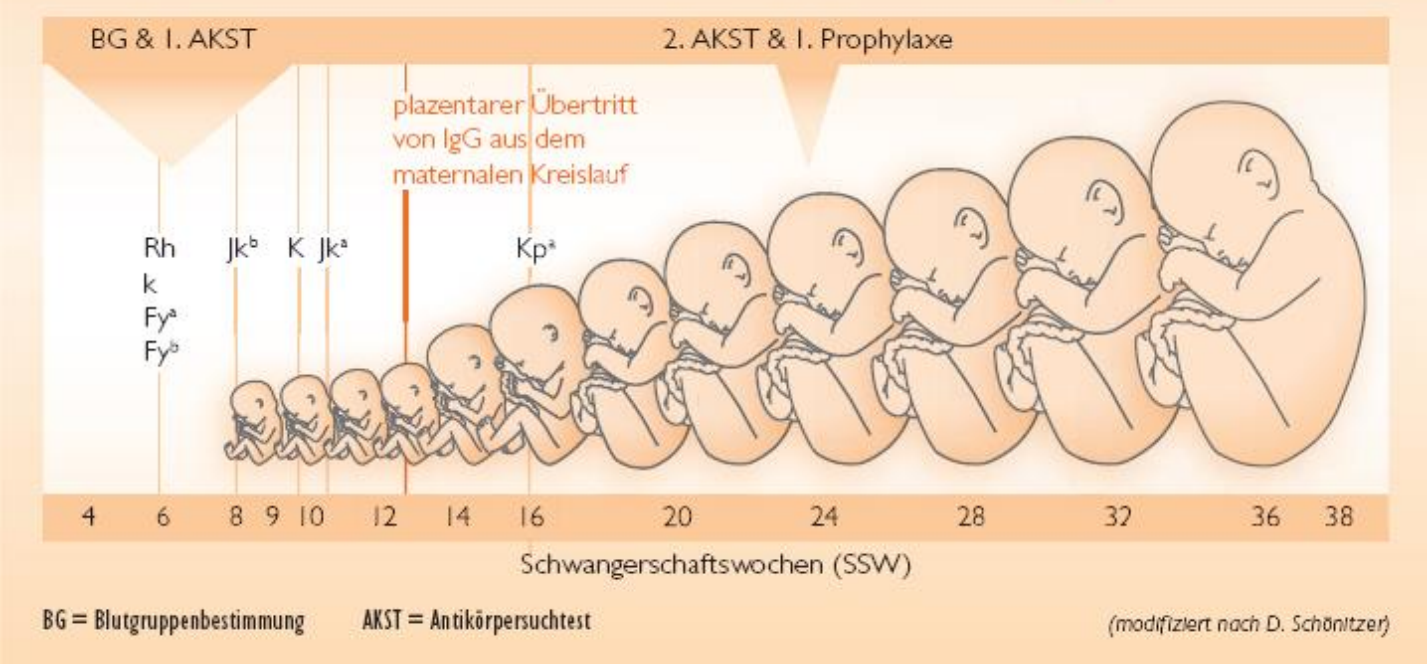
- Grundvoraussetzungen für klin. Relevanz
  - IgG-Antikörper der Mutter
  - korrespondierende Antigene auf den Erythrozyten des Fetus bzw. Früh- oder Neugeborenen

Diaplazentarer IgG-Antikörpertransfer "Immunologischer Nestschutz"



Expression von Blutgruppenmerkmalen auf Nabelschnurerythrozyten

- **vollständig**
  - Rh, Kell, Duffy, Kidd, MNSsU, Diego, Dombrock, Scianna, Gerbich,, Cartwright b
- **abgeschwächt**
  - ABH, P, Lutheran, Xg a
- **sehr schwach bzw. fehlend**



Relevanz der Antikörperspezifität

Blutgruppensystem	Merkmal	FMI-Ausprägungsgrad
Rhesus	D (RH1)	mittel-schwergradig
	C (RH2)	mittel-schwergradig
	E (RH3)	mittel-schwergradig
	c (RH4)	mittel-schwergradig
	e (RH5)	leicht-schwergradig
	f (ce;RH6)	schwergradig
	Ce (RH7)	schwergradig
	C^W (RH8)	leicht-mittelgradig
	G (RH12)	mittelgradig
	Rh;14	mittel-schwergradig

FMI-Grade

leicht =
keine Anämie, Hyperbilirubinämie (PT)

mittel =
Anämie, Hyperbilirubinämie (PT)

schwer =
Hydrops fetalis, Ikterus gravis (AT)

Quellen:

L. Weinstein, Clin.Obstet.Gynecol 1982;25:321ff

L.D. Petz, G. Garratty: Immune Hemolytic Anemias, 2004, Churchill Livingstone:519

ACOG Practice Bulletin, Obstet. Gynecol. 2006;108:457ff

Relevanz der Antikörperspezifität

Blutgruppensystem	Merkmal	FMI-Ausprägungsgrad
Kell	K	leicht-schwergradig
	k	leicht-mittelgradig
	Kp^a	leichtgradig
	Kp^b	leicht-schwergradig
	Js^a	mittelgradig
	Js^b	leicht-schwergradig
	Ku	leicht-schwergradig
	K11	mittel-schwergradig
	K22	leicht-schwergradig
Duffy	Fy^a	leicht-schwergradig

FMI-Grade

leicht =
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Relevanz der Antikörperspezifität

Blutgruppensystem	Merkmal	FMI-Ausprägungsgrad
Lutheran	Lu^a	leichtgradig
	Lu^b	leichtgradig
	Lu9	leichtgradig
MNSs	M	leicht-schwergradig
	N	leichtgradig
	S	schwergradig
	s	schwergradig
	U	schwergradig
	Mi^a	leicht-schwergradig
	Mi; U	schwergradig

FMI-Grade

leicht =
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Pathophysiologisch relevante Einflussgrößen

- Antikörperspezifität (Expression, Verteilung Gewebe/Flüssigkeiten)
- Antikörper-Quantität
- Antikörper-Immunglobulin-Klasse (IgG-Subklassen)
- Effizienz des diaplazentaren Antikörpertransfers
- Interaktion mit FcγR-tragenden Effektorzellen
- Inhibition der Hämatopoese

Ausblick
Diagnostik, Therapie und Prophylaxe der fetalen Erythroblastose

- freie fetale DNA im mütterlichen Plasma
  - nicht invasive molekulargenetische Diagnostik

CE van der Schoot, Transfusion Med Rev 2003;17:S1-14

- (supportive) Therapeutika

E Wiener et alt., *Eur J Haematol* 2003;70:67-71

  - monoklonale Antikörper gegen FcγR

- Prophylaxe

AM Hall et alt., Blood;2005:2175-2179

  - Immuntoleranzinduktion durch nasale Exposition mit alloreaktiven Rh-Peptiden