Alloimmune Disorders in Pregnancy

(RBC & Thrombocytopenia in the Fetus)

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Discloses no relevant financial relationships with commercial interests.

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Objectives

Following this lecture, the participant will be able to:

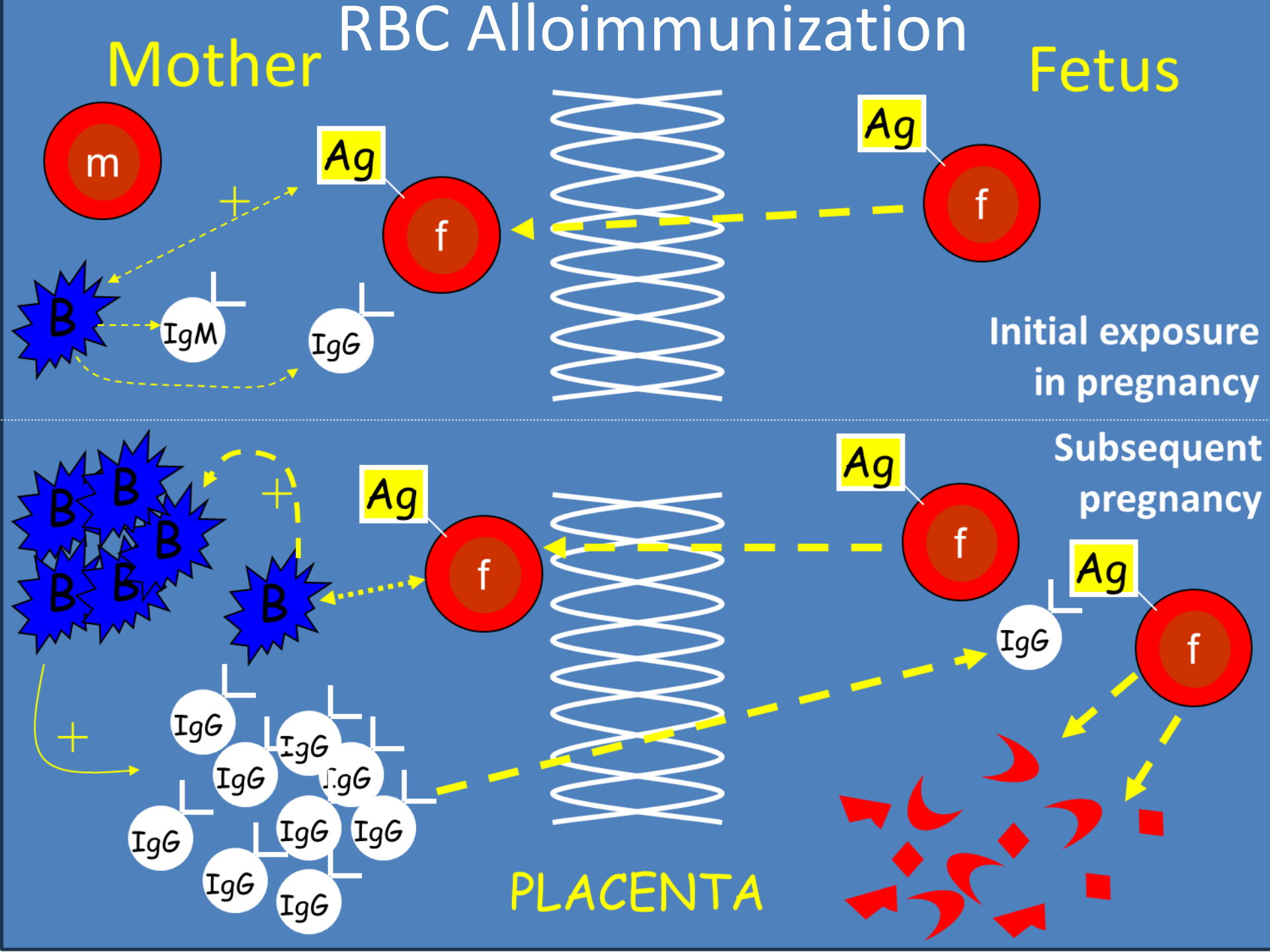
1. Identify which patients are at risk for RBC and platelet alloimmunization through screening processes
2. Discuss and use prevention strategies for Rh disease
3. Recognize when workup for NAIT should be performed.
4. Discuss referral and management (including delivery timing) approaches for RBC and platelet alloimmunization.
5. Discuss the implications for future pregnancies.

Alloimmune Diseases in Pregnancy

- Similar to graft-vs-host reaction

  "Mother develops an Ab directed against a fetal Ag that crosses the placental barrier to cause fetal disease."

- Alloimmune conditions in pregnancy:
  - RBC: Hemolytic disease of the Fetus and Newborn (HDFN)
    - In U.S.: 1-2% pregnancies (10-15% women are Rh-)
  - Plt: Fetal Neonatal Alloimmune thrombocytopenia (FNAIT)
    - 0.1-0.3% incidence in pregnancy
  - Liver: Gestational Alloimmune Liver Disease (GALD)
    - 4/10,000 live births


RBC Alloimmunization
Mother
Fetus
m
Ag
f
Ag
f
B
IgM
IgG
Initial exposure
in pregnancy
Subsequent
pregnancy
B
B
B
B
B
B
Ag
f
Ag
f
Ag
IgG
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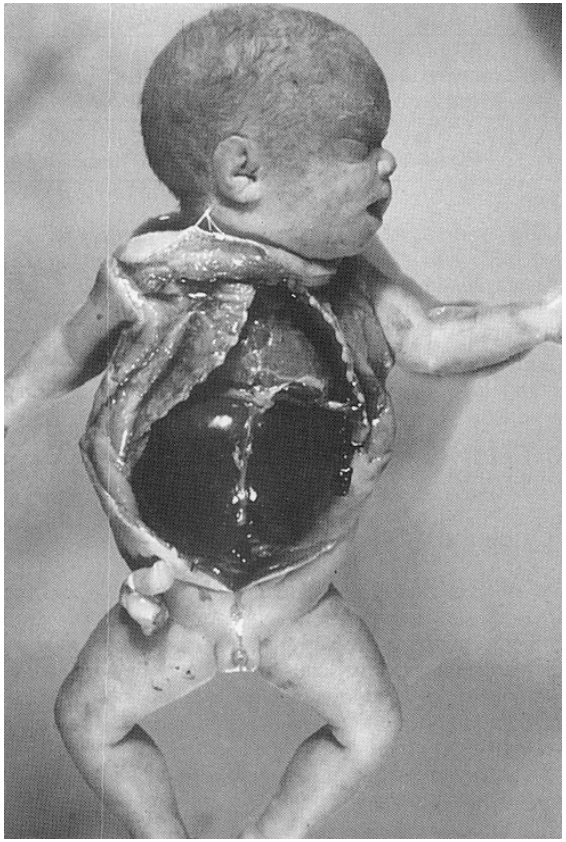
PLACENTA

Extramedullary Hematopoesis

Hydrops Fetalis & Death



Hepatosplenomegaly

Red Cell Alloimmunization

- >400 red cell antigens
- Mother Lacks Ag $\rightarrow$ Produce Ab
- Maybe harmful to the fetus or patient given a blood tx
- Isoimmunization uncommon. Why?
  - variable antigenicity
  - maternal immune response to Ag is variable
  - insufficient transplacental passage of Ag or Ab
  - protection by ABO incompatibility

ABO Incompatibility

- Most common cause of HDN
- 20% of all infants
  - 5% are clinically affected
  - Mild disease
    - Neonatal Jaundice or anemia
    - No erythroblastosis fetalis
  - Affects future offspring – “not progressive”

ABO Incompatibility

Why no concern antenatally?

- Milder than D-isoimmunization
- IgM isoantibodies- don't cross the placenta
- Fewer A and B Ag sites on fetal RBCs
- Offers some protection against D isoimm.
  - Fetal RBCs that cross - rapidly destroyed

Bottomline....

- Pediatric concern – not an OB concern

Rh Alloimmunization in Pregnancy

How common is it?

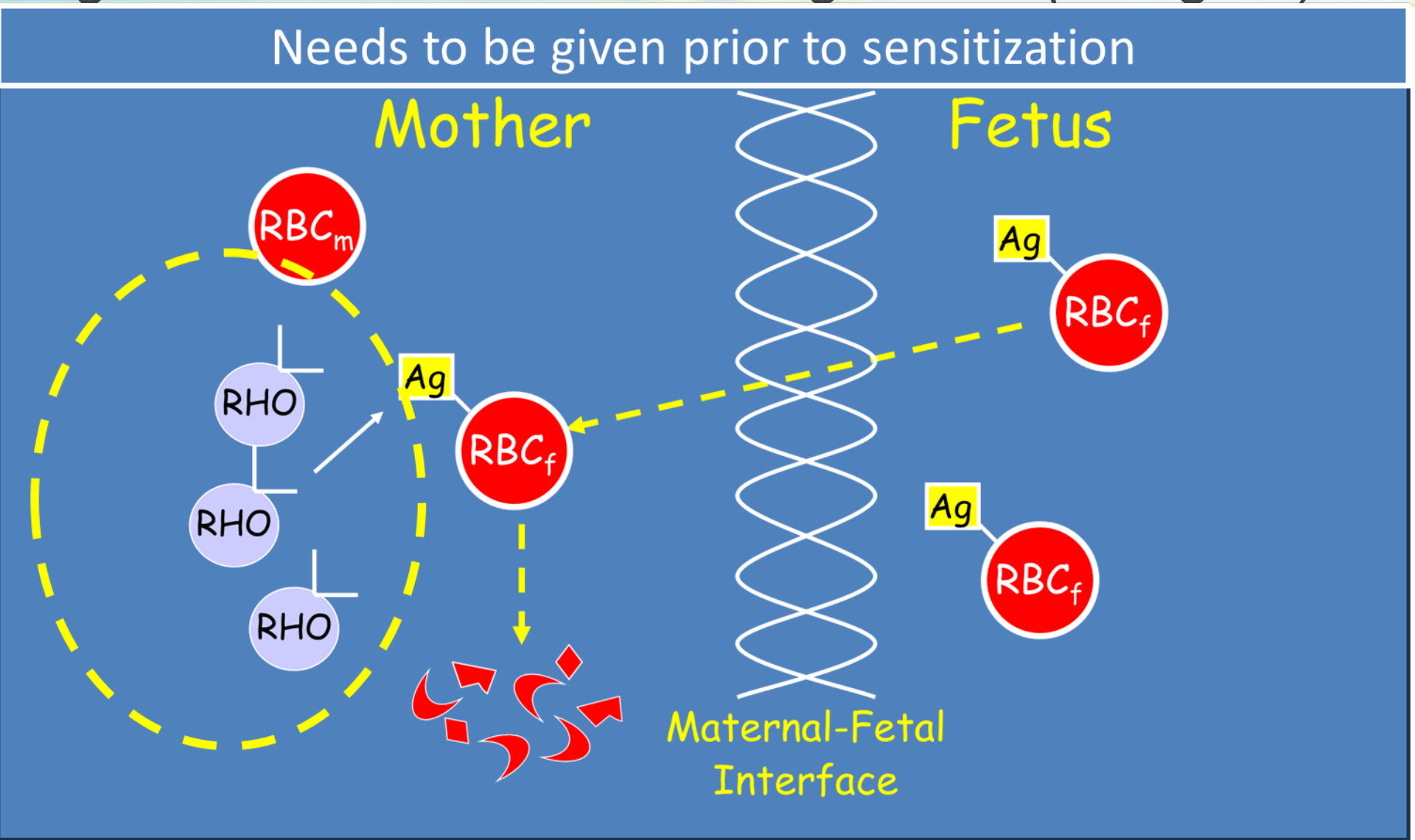
- In U.S.: 15% incidence of Rh- status; varies by race & ethnicity:
- Rh negative status:
  - Whites 15%
  - African Americans 5-8%
  - Asians & Native American 1-2%

- Basques – 30 to 35 percent
- White North Americans or Europeans – 15 percent
- Black or African Americans – 8 percent
- Africans – 4 to 6 percent
- Indians – 5 percent
- Native Americans and Inuit people – 1 to 2 percent
- Japanese – 0.5 percent
- Thais – 0.3 percent
- Chinese – 0.3 percent

Zipursky & Paul, Global burden of Rh Dz, 2011

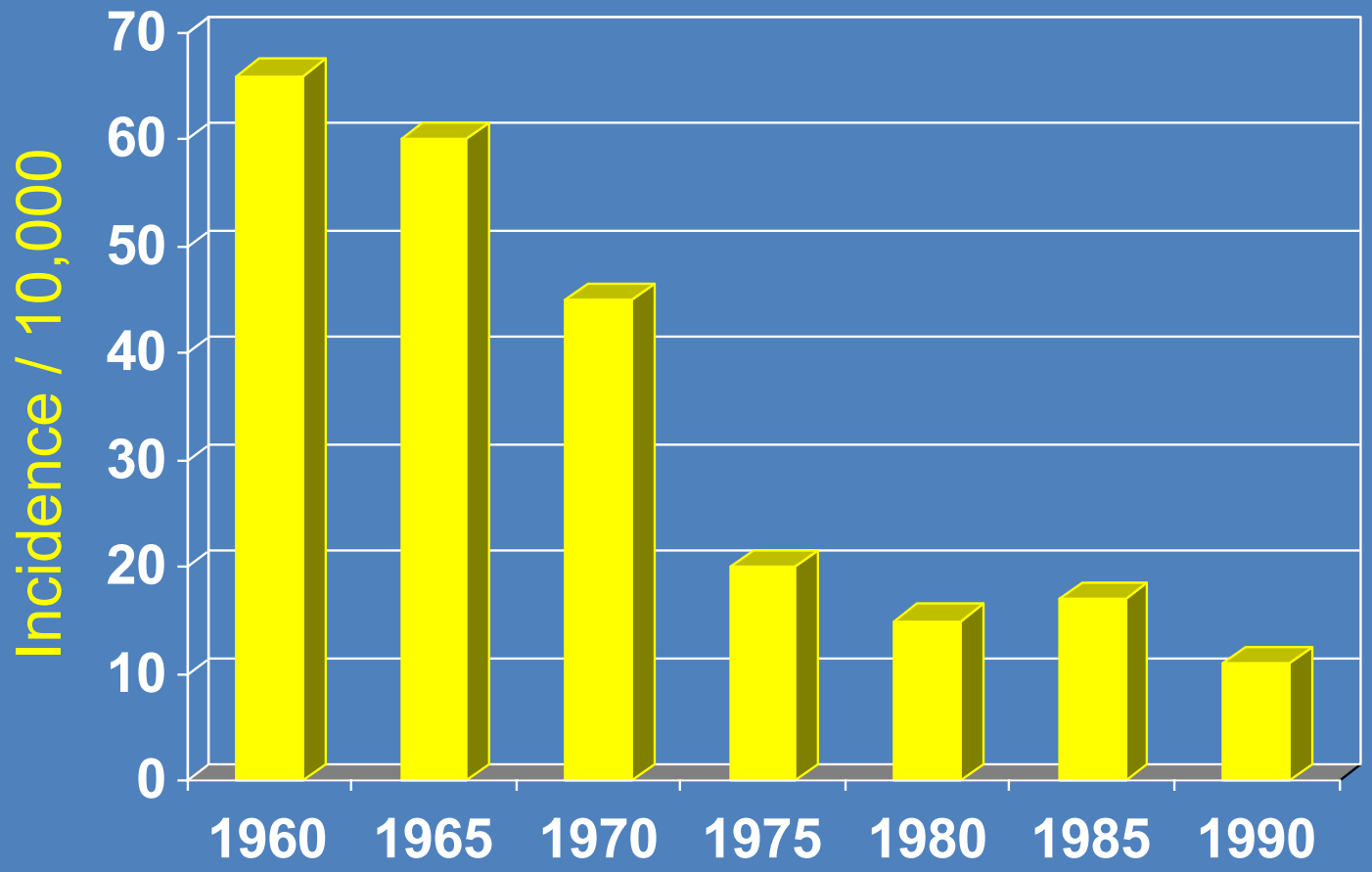
- Among whites:
  - Rh- woman has an 85% chance of reproducing with a Rh + male
  - 60% are heterozygous and 40% are homozygous at the D locus

The immune globulin used specifically to bind the Rh D antigen is referred to as Rh D immune globulin or anti-D immune globulin (Rhogam).



Incidence of RH HDN in USA

1960-1990

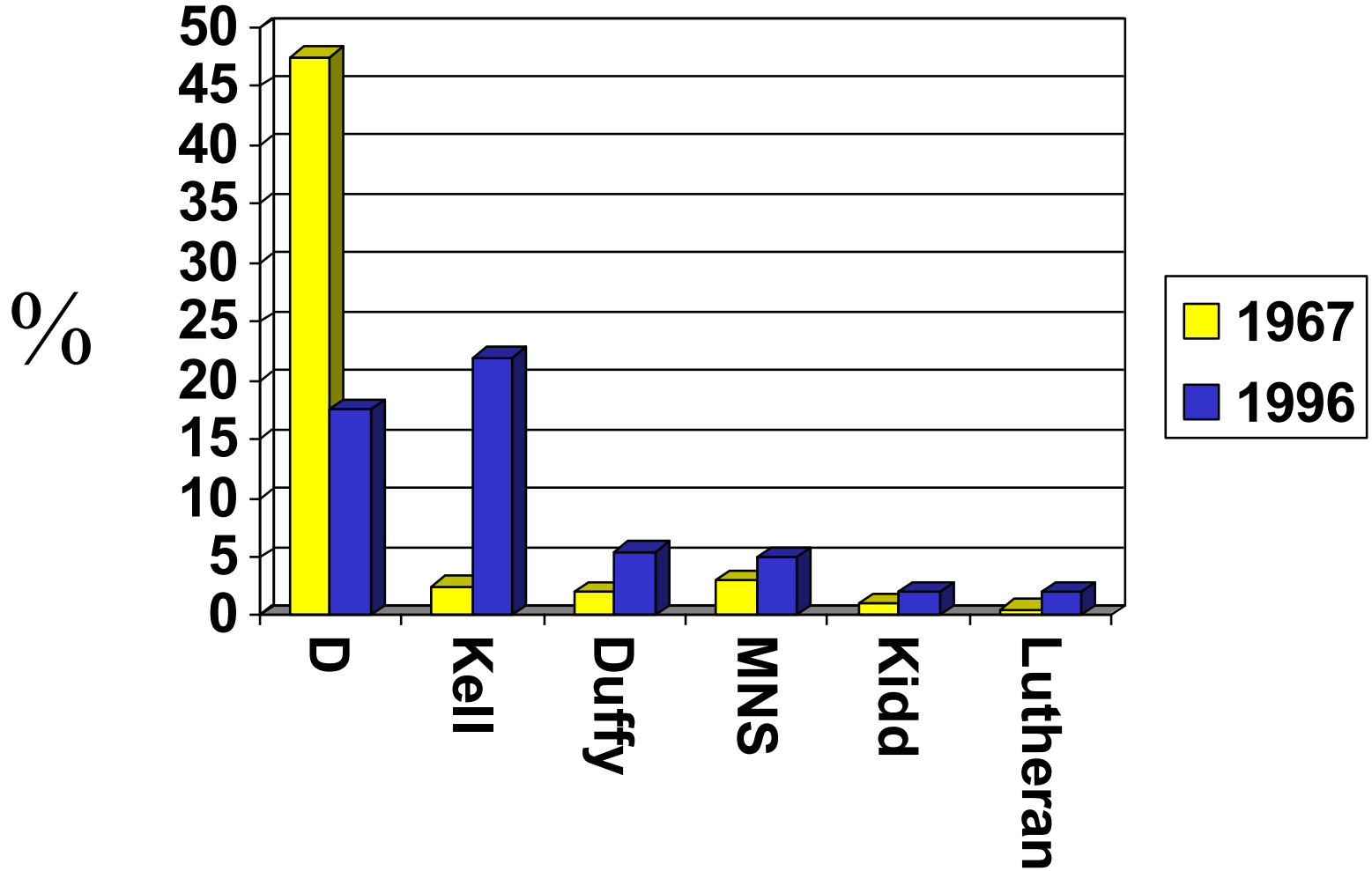


- **Alloimmunization from “irregular or atypical” (e.g. non-Rh) antigens *cannot* be prevented by prophylactic administration of immune globulin**

Leads to isoimmunization by other multiple antibodies

Frequency of Fetal Isoimmunization in USA

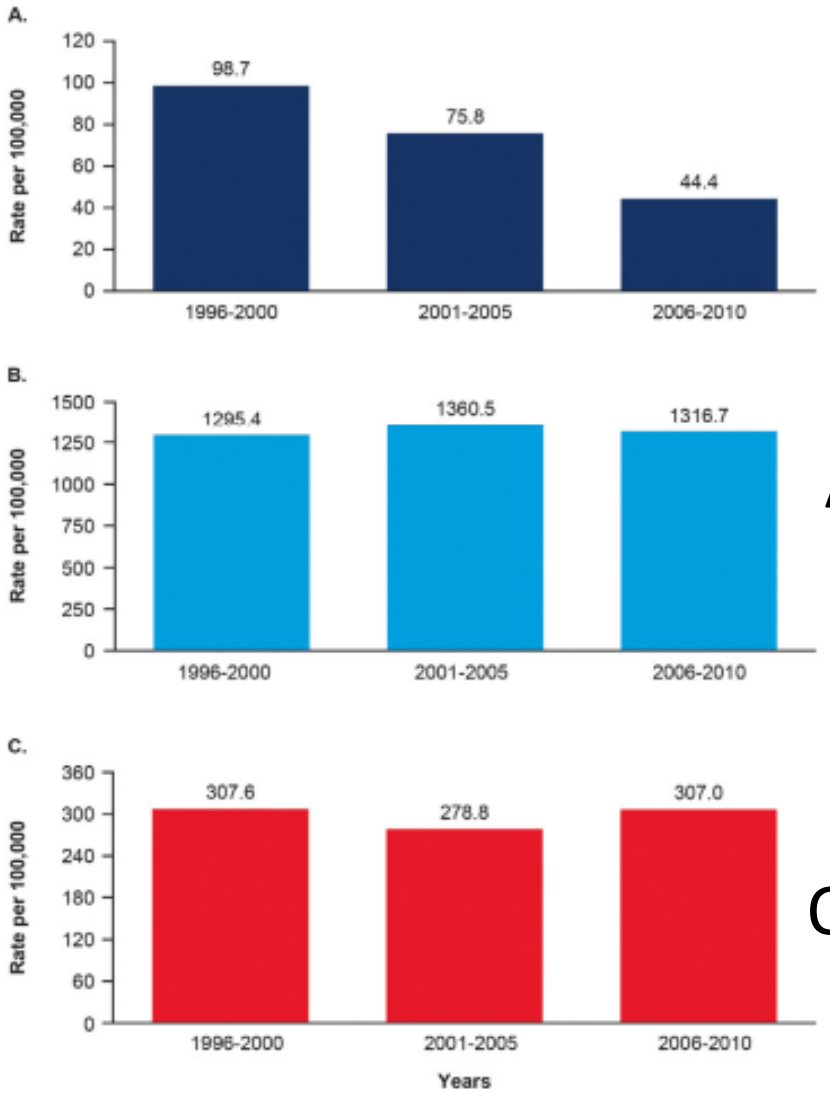
1967 vs 1996



Queenan et al. Ob-Gyn, 1969; Geifman-Holtzman et al. Ob-Gyn 1997

Rate of HDFN Alloimmunization by Year in US 1996-2010

- National Hospital Discharge Survey
  - 1996-2010
  - 480,245 livebirths (1700 annual cases)
  - HDFN by ICD-9 diagnosis
- Prevalence of HDFN:
  - 1695 cases / 100,000 LB (about 1-2% LB)
  - Among newborns with HDFN, 0.6% of cases were severe.



Yu. Live birth prevalence HDFN in US 1996-2010. Am J Glob Rep 2023;3:100203

“Irregular” red blood cell antigens

Table 1. Atypical Antibodies and Their Relationship to Fetal Hemolytic Disease

Blood Group System	Antigens Related to Hemolytic Disease	Hemolytic Disease Severity	Proposed Management
Lewis	*		
I	*		
Kell	K	Mild to severe†	Fetal assessment
	k	Mild	Routine obstetric care
	Ko	Mild	Routine obstetric care
	Kp^a	Mild	Routine obstetric care
	Kp^b	Mild	Routine obstetric care
	Js^a	Mild	Routine obstetric care
	Js^b	Mild	Routine obstetric care
Rh (non-D)	E	Mild to severe†	Fetal assessment
	C	Mild to severe†	Fetal assessment
	c	Mild to severe†	Fetal assessment
Duffy	Fy^a	Mild to severe†	Fetal assessment
	Fy^b	‡	Routine obstetric care
	By^3	Mild	Routine obstetric care
Kidd	Jk^a	Mild to severe	Fetal assessment
	Jk^b	Mild	Routine obstetric care
	Jk^3	Mild	Routine obstetric care
MNSs	M	Mild to severe	Fetal assessment
	N	Mild	Routine obstetric care
	S	Mild to severe	Fetal assessment
	s	Mild to severe	Fetal assessment
	U	Mild to severe	Fetal assessment
	M^g	Moderate	Fetal assessment
MSSs	Mt^a	Moderate	Fetal assessment
	Vw	Mild	Routine obstetric care
	Mur	Mild	Routine obstetric care
	Hil	Mild	Routine obstetric care
	Hut	Mild	Routine obstetric care
Lutheran	Lu^a	Mild	Routine obstetric care
	Lu^b	Mild	Routine obstetric care
Diego	Di^a	Mild to severe	Fetal assessment
	Di^b	Mild to severe	Fetal assessment
Xg	Xg^a	Mild	Routine obstetric care
P	$PP_{1}P^k$ (Tj^a)	Mild to severe	Fetal assessment
Public antigens	Yt^a	Moderate to severe	Fetal assessment
	Yt^b	Mild	Routine obstetric care
	Lan	Mild	Routine obstetric care
	En^a	Moderate	Fetal assessment
	Ge	Mild	Routine obstetric care
	Jr^a	Mild	Routine obstetric care
	Co^a	Severe	Fetal assessment
	Co^{1-3}	Mild	Routine obstetric care
Private antigens	Batty	Mild	Routine obstetric care
	Becker	Mild	Routine obstetric care
	Berrens	Mild	Routine obstetric care

Table 1. Atypical Antibodies and Their Relationship to Fetal Hemolytic Disease *(continued)*

Blood Group System	Antigens Related to Hemolytic Disease	Hemolytic Disease Severity	Proposed Management
Private antigens	Bles	Moderate	Fetal assessment
	Evans	Mild	Routine obstetric care
	Gonzales	Mild	Routine obstetric care
	Good	Severe	Fetal assessment
	Heibel	Moderate	Fetal assessment
	Hunt	Mild	Routine obstetric care
	Jobbins	Mild	Routine obstetric care
	Radin	Moderate	Fetal assessment
	Rm	Mild	Routine obstetric care
	Ven	Mild	Routine obstetric care
	Wright^a	Severe	Fetal assessment
	Wright^b	Mild	Routine obstetric care
	Zd	Moderate	Fetal assessment

*Not a proven cause of hemolytic disease of the newborn

†With hydrops fetalis

‡Not a cause of hemolytic disease of the newborn

Modified from Weinstein L. Irregular antibodies causing hemolytic disease of the newborn: a continuing problem. Clin Obstet Gynecol 1982;25:321.

ACOG Practice Bulletin, 2018

Antigen system	Specific antigen	Antigen system	Specific antigen	Antigen system	Specific antigen
Frequently associated with severe disease					
Kell	-K (K1)				
Rhesus	-c				
Infrequently associated with severe disease					
Colton	-Coa	MNS	-Mta	Rhesus	-HOFM
	-Co3		-MUT		-LOCR
Diego	-ELO		-Mur		-Riv
	-Dia		-Mv		-Rh29
	-Dib		-s		-Rh32
	-Wra		-sD		-Rh42
	-Wrb		-S		-Rh46
Duffy	-Fya		-U		-STEM
Kell	-Jsa		-Vw		-Tar
	-Jsb	Rhesus	-Bea	Other antigens	-HJK
	-k (K2)		-C		-JFV
	-Kpa		-Ce		-JONES
	-Kpb		-Cw		-Kg
	-K11		-Cx		-MAM
	-K22		-ce		-REIT
	-Ku		-Dw		-Rd
	-Ula		-E		
Kidd	-Jka		-Ew		
MNS	-Ena		-Evans		
	-Far		-e		
	-Hil		-G		
	-Hut		-Goa7		
	-M		-Hr		
	-Mia		-Hro		
	-Mit		-JAL		
Associated with mild disease					
Dombrock	-Doa	Gerbich	-Ge2	Scianna	-Sc2
	-Gya		-Ge3	Other	-Vel
	-Hy		-Ge4		-Lan
	-Joa		-Lsa		-Ata
Duffy	-Fyb	Kidd	-Jkb		-Jra
	-Fy3		-Jk3		

Non-Rhesus-D antibodies associated with hemolytic disease of the fetus and newborn

Moise K. Semin Fetal Neonatal Med. 2008 Aug;13(4):207-14

Screening for RBC Alloimmunzation

ACOG & Am Assoc of Blood Banks

All pregnant women, at first prenatal visit of each pregnancy should be tested for…

ABO blood group, RH-D type & RBC Ab screen (ABS)

- Repeat ABS before Rhogam administration:

  28 weeks

  Postpartum

  At time of any event…

ACOG PB 192, 2018

Volume of M-F hemorrhage leading to Rh D alloimmunization can be as small as 0.1ml

Box 1. Potential Sensitizing Events in Rh D-Negative Women in Pregnancy	**M-F Hemorrhage Risk**
Chorionic villus sampling, amniocentesis, cordocentesis	CVS: 14%; Amnio:2-6%
Threatened miscarriage or miscarriage	1st trimester: 3-11%
Ectopic pregnancy	ruptured: 24%
Evacuation of molar pregnancy	
Therapeutic termination of pregnancy	< 8 wks: 3-11%
Antepartum hemorrhage	
Abdominal trauma	up to 40%
Intrauterine fetal death	
External cephalic version	2-6%
Delivery	3rd trimester: 45%

ACOG PB 181, 2017

Management of Alloimmunization

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Once RBC Alloimmunization is established (positive maternal ABS)….

Determine if fetus is at risk

- FOR Non-RH+ ABS…Determine FOC Ag status / NIPT
- FOR RH+ ABS…Determine FOC Rh status (Ag status)
  - FOC Rh Negative → done—no further testing (Paternity?)
  - FOC Rh Positive → Zygosity Testing
    - homozygous (40%) → No further FOC testing (all fetuses Rh+)
    - heterozygous (60%) → ***fetal*** genotyping (cf fDNA over amnio)

If FOC Rh homozygous *OR* carries the non-Rh RBC Ag *OR* if unknown paternal status, *OR* Ag+ fetus by cf fDNA or amnio

Serial antibody titers

Serial Ab Screens

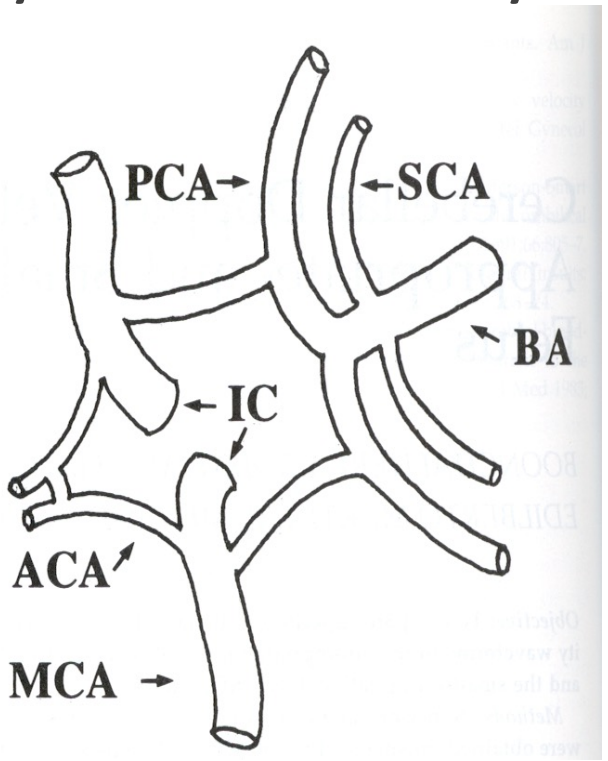
- Serial Ab titers until a “Critical Threshold Titer” is reached (monthly, q 2 wks if rising).
  - Critical titer varies by hospital
    - Typically: 1:16 or 1:32 (most are 1:16)
    - Check with your hospital blood bank
  - Exception is anti-Kell Ab which is 1:8

Critical Threshold Ab Screens

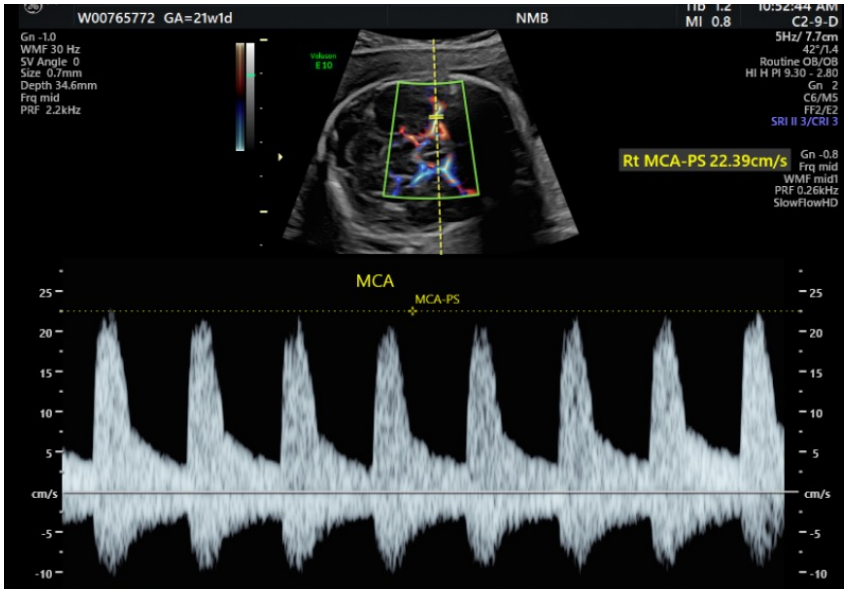
- Once met → Evaluate for fetal anemia.
- MCA PSV Doppler replaced ΔOD450
- Fetal anemia ↓s blood viscosity → ↑s velocity

PW Doppler of MCA

Circle of Willis



MCA Doppler Predicts Fetal Anemia



- >1.50 MoM MCA peak velocity for the detection of moderate/severe anemia
  - Sensitivity: 100%
  - False positive: 12%
  - Positive predictive rate: 65%
  - Negative predictive rate: 100%

Mari et al. NEJM 2000;342:9-14

Fetal Blood Sampling & Transfusion

RBC Alloimmunization

Delivery Timing

- Controversial
- Sensitized but critical titer not reached: 39w
- Mild disease (critical titer reached; normal MCA): 38-39w
- Moderate-Severe disease (e.g. IUTs):
  - 32-34w (historically)
  - If last transfusion 35-36w, delivery 37-38w
  - Phenobarbitol 30mg/d 1 week prior to delivery (?)

RBC Alloimmunization

Next Pregnancy

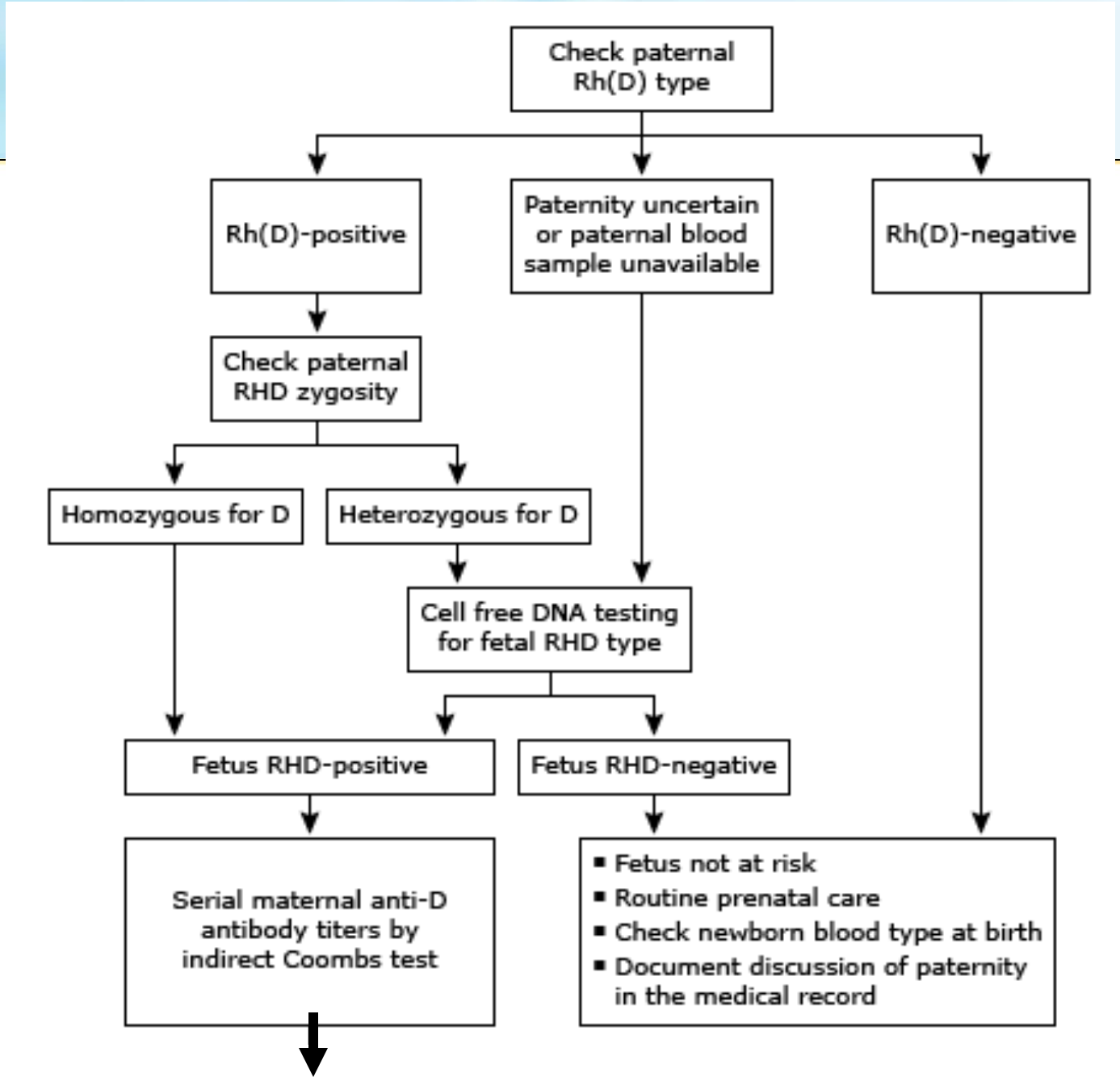
- Prior IUT, hydrops, HDFN PTB or NN exchange tx can expect development of severe fetal anemia if next fetus is Ag+ for offending Ab (e.g. FOC status: repeat zygosity testing. Same FOC?)
- Determine fetal Ag status early & begin MCA PSV at 16-18 weeks.
- Increasingly severe HDFN
- *Coming Soon*: mAb against IgG

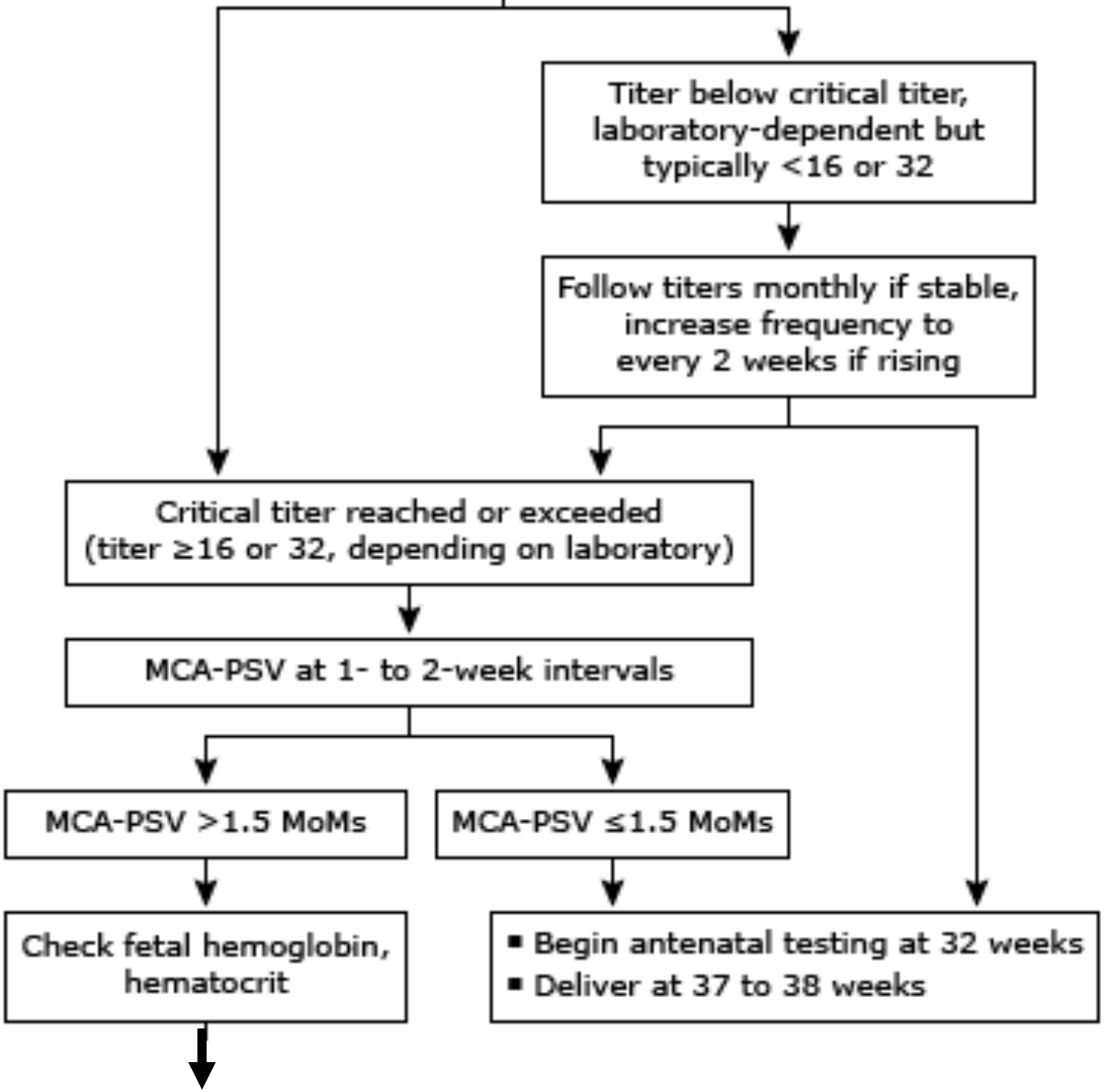
RBC Alloimmunization

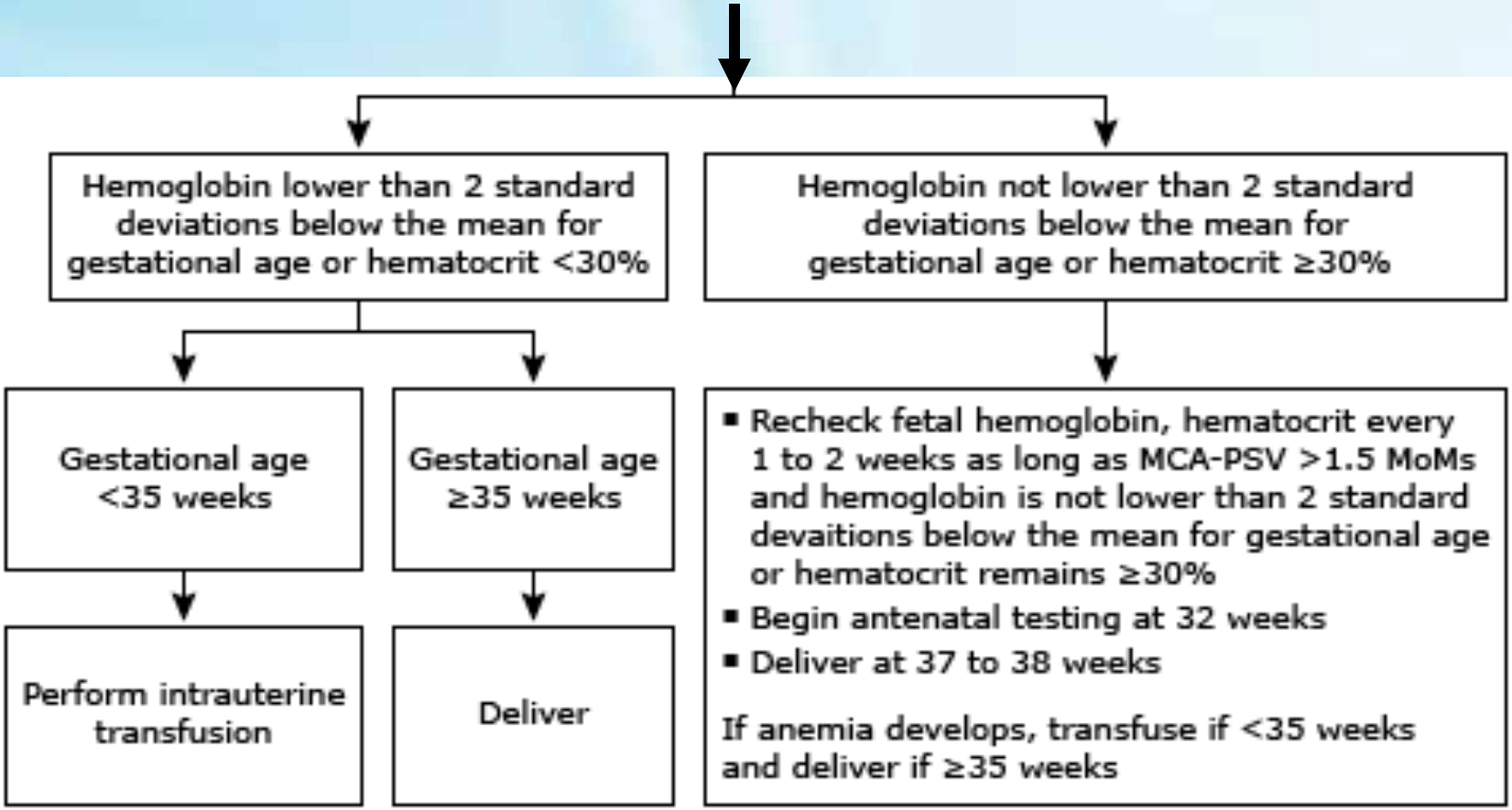
Next Pregnancy

- Nipocalamib treatment for severe HDFN
  - mAb blocks placental transfer of IgG and lowers maternal titers (FcRn receptor blockade)
- Phase II trial completed
  - 50% pts w prior early onset severe HDFN did not receive IUTs until after 32w.
- Phase III / RCT starting. Entry criteria:
  - Alloimmunization to D, c, E, Kell, Jka
  - Critical titer & positive cf fDNA for Ag at screening
  - 1 or more previous transfusions in prior pregnancy
  - <15w in current pregnancy
- Several U.S. fetal treatment centers participating
  - Study visits, travel & infusions covered.

Many Algorithms Published for RBC Alloimmunization




Titer below critical titer, laboratory-dependent but typically <16 or 32
Follow titers monthly if stable, increase frequency to every 2 weeks if rising
Critical titer reached or exceeded (titer ≥16 or 32, depending on laboratory)
MCA-PSV at 1- to 2-week intervals
MCA-PSV >1.5 MoMs
MCA-PSV ≤1.5 MoMs
Check fetal hemoglobin, hematocrit
Begin antenatal testing at 32 weeks
Deliver at 37 to 38 weeks


Hemoglobin lower than 2 standard deviations below the mean for gestational age or hematocrit <30%
Hemoglobin not lower than 2 standard deviations below the mean for gestational age or hematocrit ≥30%
Gestational age <35 weeks
Gestational age ≥35 weeks
Perform intrauterine transfusion
Deliver
Recheck fetal hemoglobin, hematocrit every 1 to 2 weeks as long as MCA-PSV >1.5 MoMs and hemoglobin is not lower than 2 standard devaitions below the mean for gestational age or hematocrit remains ≥30%
Begin antenatal testing at 32 weeks
Deliver at 37 to 38 weeks
If anemia develops, transfuse if <35 weeks and deliver if ≥35 weeks

Fetal & Neonatal Alloimmune Thrombocytopenia (FNAIT)

Colorado Fetal Care Center

FNAIT

- Fetal-Neonatal alloimmune thrombocytopenia (TCP) is the *platelet equivalent* of hemolytic disease of the fetus and newborn.
- Develops as a result of maternal alloimmunization to fetal platelet antigens with transplacental transfer of platelet specific antibody and subsequent platelet destruction.
- 15 plt specific antigens described. Most severe cases due to sensitization to HPA 1a
- Affects 1 in 1000-3000 live births

ACOG PB 207, 2019

Williamson et al. Blood, 1998

FNAIT: Management & Outcome of a Large International Retrospective Cohort (2017)

HPA type	Cases, n (%)	Mean PC $\times 10^9$/l	ICH, n
HPA-1a	544 (88)	105	19
HPA-5b	23 (3.6)	136	2
HPA-3a	7 (1.1)	147	
HPA-5a	4 (0.6)	184	
HPA-15a	5 (0.8)	200	
HPA-1a + -5b	18 (3)	94	2
HPA-1a + other	5 (0.8)		
Negative	2 (0.03)		
Unknown	7 (1.1)		
Total	615		

PC = Platelet count.

Kamphuis MM, Tiller H et al. Fetal Diagn Ther 2017;41(4):251

Causes of Maternal TCP in Pregnancy

Maternal TCP & Fetal TCP Risk

Box 1. Causes of Thrombocytopenia in Pregnancy

- Gestational thrombocytopenia
- Hypertension in pregnancy
  - Preeclampsia
  - HELLP syndrome
- Primary immune thrombocytopenia
- Secondary immune thrombocytopenia
  - Antiphospholipid syndrome
  - Systemic lupus erythematosus
  - Infectious (such as HIV, hepatitis C virus, cytomegalovirus, *Helicobacter pylori*)
  - Drug-induced thrombocytopenia (use of drugs such as heparins, antimicrobials, anticonvulsants, analgesic agents)
- Association with systemic conditions
  - Disseminated intravascular coagulation
  - Thrombotic thrombocytopenia/hemolytic uremic syndrome
  - Splenic sequestration
  - Bone marrow disorders
  - Nutritional deficiencies
- Congenital thrombocytopenia

Abbreviations: HELLP, hemolysis, elevated liver enzymes, and low platelet count; HIV, human immunodeficiency virus.

ACOG PB 207, 2019

Neonatal Thrombocytopenia Risk

- Gestational thrombocytopenia → Low:0.1-2.3%
- Preeclampsia → Low: 0.0-1.8%
- Primary immune thrombocytopenia →
  - ITP
    - plt <150k, 25% risk (not severe)
    - No correlation b/w maternal & fetal plt counts
    - Although 8-15% newborns will be treated for TCP, severe complications are rare (<<1%)
  - Thus, these conditions of maternal TCP are low risk for fetal complications and more of a neonatal concern

FNAIT

General Features

- Uncomplicated pregnancy & maternal plt cts are normal
- 25% FNAIT occur in the first pregnancy
- Leading cause of severe TCP in fetus & neonate
- Majority of NAIT mild (petechiae, bruising or TCP on CBC)
- Leading cause of *intracranial hemorrhage* (ICH) in term NN
- 15% of infants with plt cts <50 x 10^9/L have an ICH
- ICH: 80% occur antenatally and ½ can be seen prenatally
- High recurrence risk (upwards of 100%) if subsequent sibling carries the offending plt antigen

ACOG PB 207, 2019; Peterson et al.Br J Haematol 2013;161:3
Kovanlikaya et al. Pediatr Blood Cancer, 2017

FNAIT

Screening & Diagnosis

- Screening
  - No blood screening test
  - History of an affected child
    - Unexpected thrombocytopenia
    - History of ICH
  - Direct relation to such a woman
  - Incidentally found to lack HPA-1a

Thus, reliant on the screening history at OB intake.

- Diagnosis: laboratory workup
  - Experienced reference laboratory
  - Flow cytometry: rapid method of detecting plt reactive Abs
  - Screen for Class I HLA Ab and for the specific glycoprotein the maternal Ab is targeting.

FNAIT

Antenatal Imaging

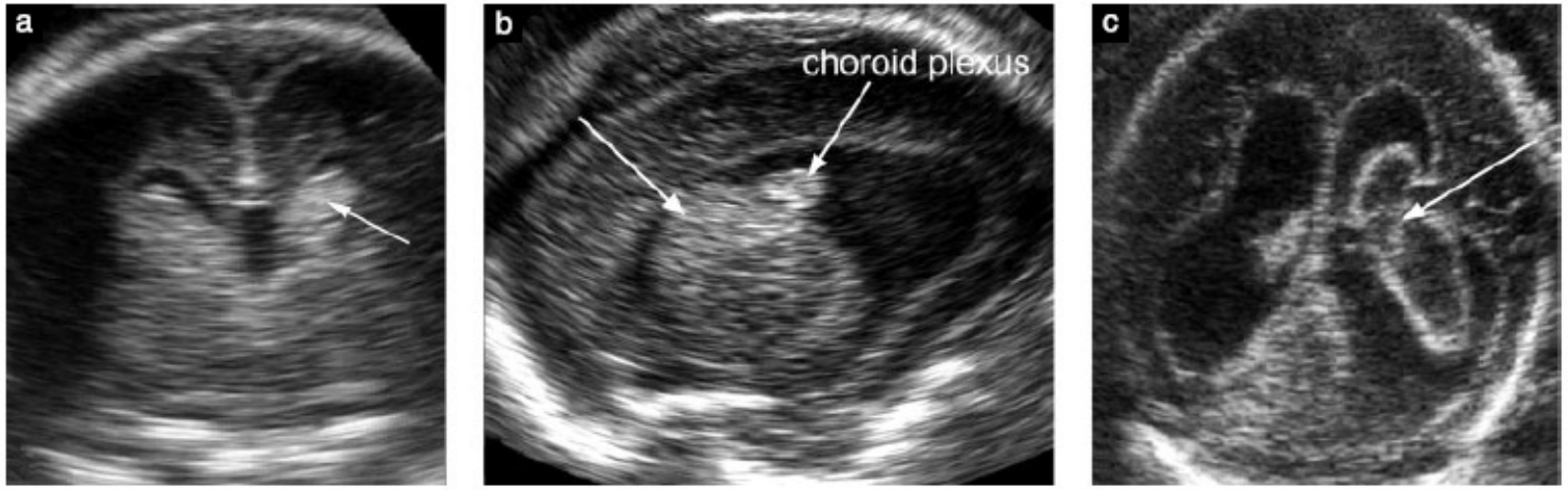
Prenatal Ultrasound

- ICH (acute vs chronic)
- Ventriculomegaly
- Porencephalic cysts
- ICH documented as early as 14 weeks

MRI

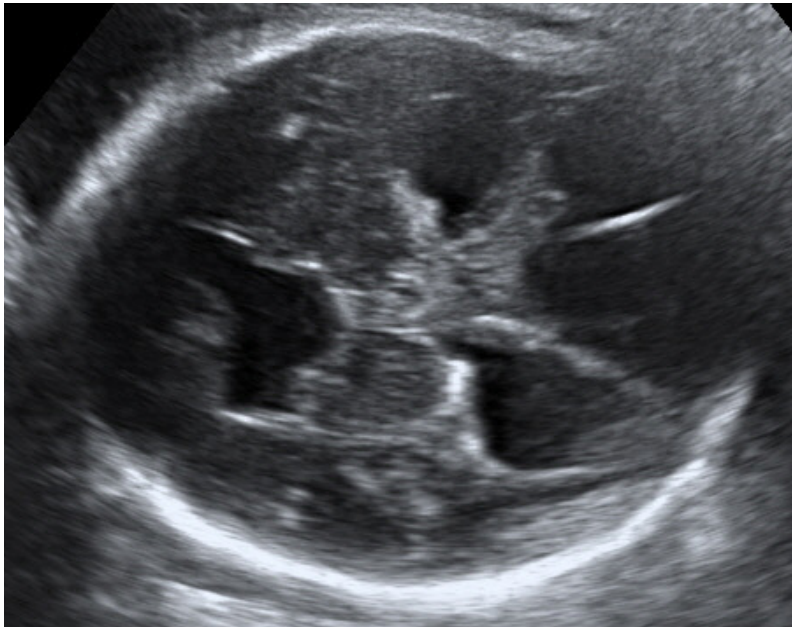
- T1 weighted images to identify blood
- May detect hemorrhage not seen on US
- Maybe useful in identifying old vs new hemorrhage

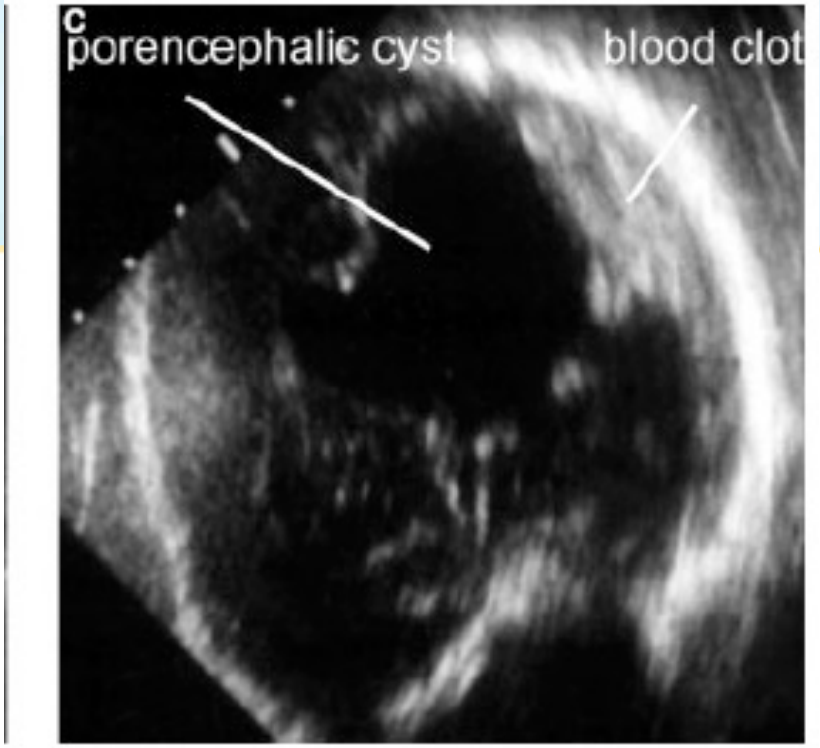
Recent Bleed



Older Bleeds

Porencephaly
& ventriculomegaly





Newborn Findings

generalized bruising, suffusions, petechiae

FNAIT

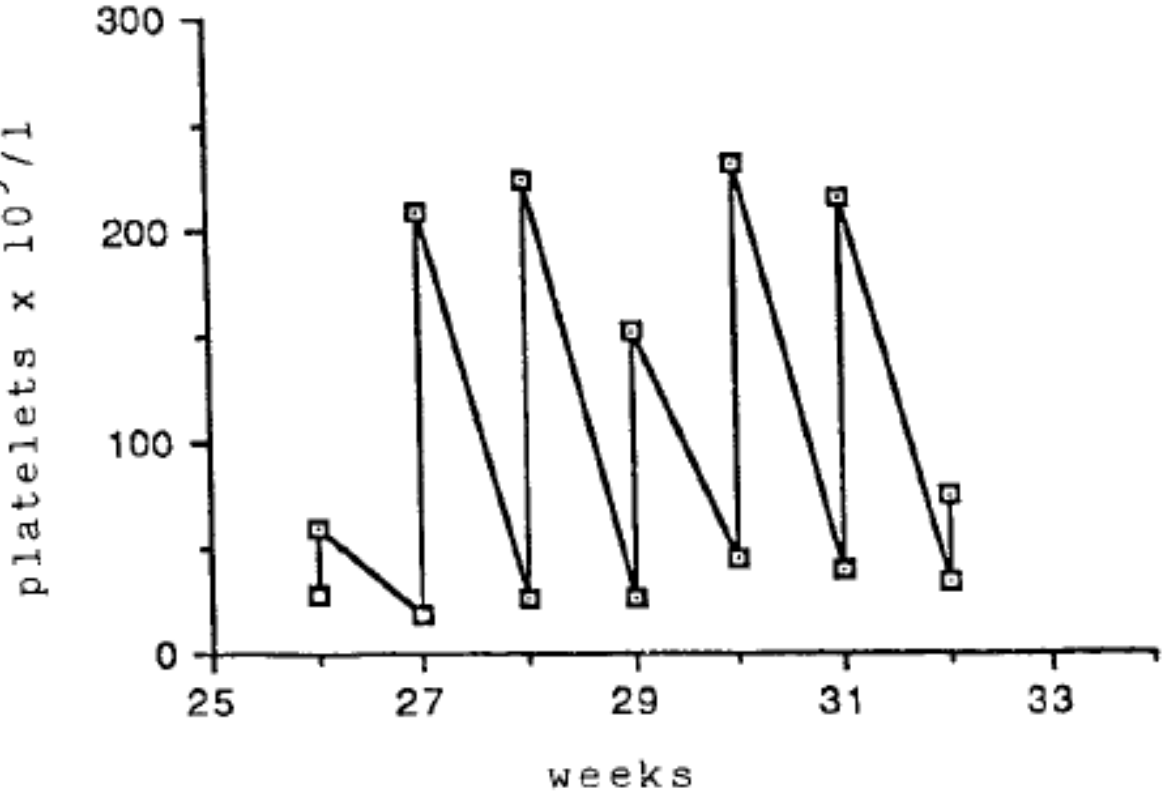
Management

Primary goal in OB mgmt of FNAIT: ICH prevention

Optimal treatment remains uncertain.

- Patients stratified based on + or – ICH and GA at manifestation of ICH in sibling(< or >28 weeks)…1996 RCT & 2017 MA
  - Several therapies & dosing strategies
    - Maternal IVIG at 12wks: 1mg/kg for ICH <28w; 2mg/kg if ICH >28w
      - At 20 wks dosing doubled; Prednisone added
    - No plt sampling for therapy monitoring (11% exsanguination risk).
  - None of these were effective in all cases in the RCT
    - IVIG generally improved fetal plt ct by 68K by delivery
    - 0/55 ICH w IVIgG (10 in sibs)
    - Platelet transfusions increase plt ct, but short ½-life & high IUFD complication rate in other studies (11%)

Weekly Platelet Transfusions for AIT



Platelet count (× 10^9/l) before and after weekly platelet transfusions between 26 and 32 weeks' gestation in fetus with alloimmune thrombocytopenia.

Nicolini et al. Lancet 1988;2:506

FNAIT

Management

- Historically, FBS included in FNAIT management to assess effectiveness of therapy
  - Prospective therapeutic studies – FBS not needed.
- IVIG w or w/o corticosteroids equally effective vs IUT platelet transfusion in raising plts w/o exsang risk.
- Consensus guidelines:
  - early empiric initiation of IVIG +/-steroids based on risk stratification.
  - FBS reserved for >32 wks to assess tx effect IF, SVD desired
  - CS at 37 weeks recommended.

Bussel et al. AJOG 2010;203:135.14; Berkowitz et al. Obstet Gynecol 2007;110:249
Winkelhorst et al. Blood 2017;129:1538–47. (Systematic Review)
Pacheco et al. Obstet Gynecol 2011;118:1157–63

Comparison of Anti-D HDFN & FNAIT

Factors that Differ	FNAIT caused by anti HPA-1a	HDFN caused by anti-D
At-Risk Pregnancies	~2% of women HPA-1a-negative; <1% also DRRB3*0101 positive	Approximately 10% of women (D-)
Occurrence of disease	First pregnancy, commonly	2nd & subsequent pregnancies (commonly anti-D)
Ab response after incompatible transfusion	Anti-HPA-1a is rarely formed	Anti-D is most frequent
Effect of alloantibodies	Thrombocytopenia	Anemia, hemolysis
Causes of fetal death	Intracranial hemorrhage	Heart failure, hydrops
Causes of neonatal death	Intracranial hemorrhage	Kernicterus/bilirub encephalopathy
HLA association with Ab response	>90% HLA DRB3*0101; >90% HLA DOB1*0201	Weak or no association
Routine screening	None	First prenatal visit; D phenotype
Antibody detection	Postnatally; usually after birth of baby with thrombocytopenia	1st & 3rd trimester by screening
Ab concentration in preg	Some correlation w TCP severity	Good correlation w anemia severity
Postnatal antibody	Remains for years	Declines after months
Noninvasive fetal dx	None	Doppler of MCA for fetal anemia
Tx to pregnant woman	IVIG ± steroids; dose/duration based on prior history	None; IVIG now for early disease
Tx of babies in severe cases	Fetal IUT & Neonatal transfusion	Fetal IUT, NN exchange Tx, phototx
Immunization prevention	None	Ante & post-natal RhIG

Key Points & Clinical Pearls

HDFN

Clinical Pearls

1. The major cause of HDF is D and kell sensitization, not ABO incompatibility.
2. Ab titers remains the mainstay for screening & detection of sensitized mothers.
3. Know the critical titer at your hospital (1:16 or 1:32).
4. Kell does not act like other RBC antigens and requires a lower threshold for FBS (1:8).
5. Ultrasound is useful for establishing dates, evaluating for hydrops, HSM, MCA PSV and for transfusions.
6. MCA peak systolic velocity has replaced amnio and serial ΔOD 450 testing for fetal anemia.

FNAIT
Clinical Pearls

- Screening **focus**: OB history & Newborn outcomes at initial PNV
- Suspect FNAIT: Unexplained fetal/NN TCP, hemorrhage or ICH
- What tests should be ordered?
  - HPA type & zygosity of both parents; confirm incompatible
  - Use an experienced regional reference laboratory
  - Plt typing helpful when FOC is heterozygous; can be done from amnio or from cf fDNA testing.
- Primary goal in OB management of FNAIT: ICH prevention
- Early detailed anatomic survey by ultrasound & serial ultrasound evaluations
- Early referral to MFM for IgG ± steroid therapy

Thank you!