Rh iso-immunisation

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Introduction

- Rhesus **(Rh) negative** women who deliver an **Rh positive** baby or who are otherwise exposed to Rh positive red blood cells are at risk of developing **anti-Rh antibodies.**
- **Rh positive** fetuses/neonates of these mothers are at risk of developing hemolytic disease of the fetus and newborn, which can be lethal or associated with serious morbidity.
- **Anti-D** is the most commonly encountered antibody during pregnancy.

Introduction

- Before routine antenatal **anti-D prophylaxis**, late immunization during a first pregnancy was responsible for **18–27%** of cases.
- This rate fell to **2%** with routine postpartum administration of a single dose of anti-D immune globulin and was further reduced to as low as **0.1 %** with the addition of routine antenatal administration in the third trimester.
- Immunisation during a second or subsequent pregnancy probably accounts for a similar proportion of cases, although it is often impossible to distinguish late sensitisation from failure of prophylaxis at the end of the preceding pregnancy.

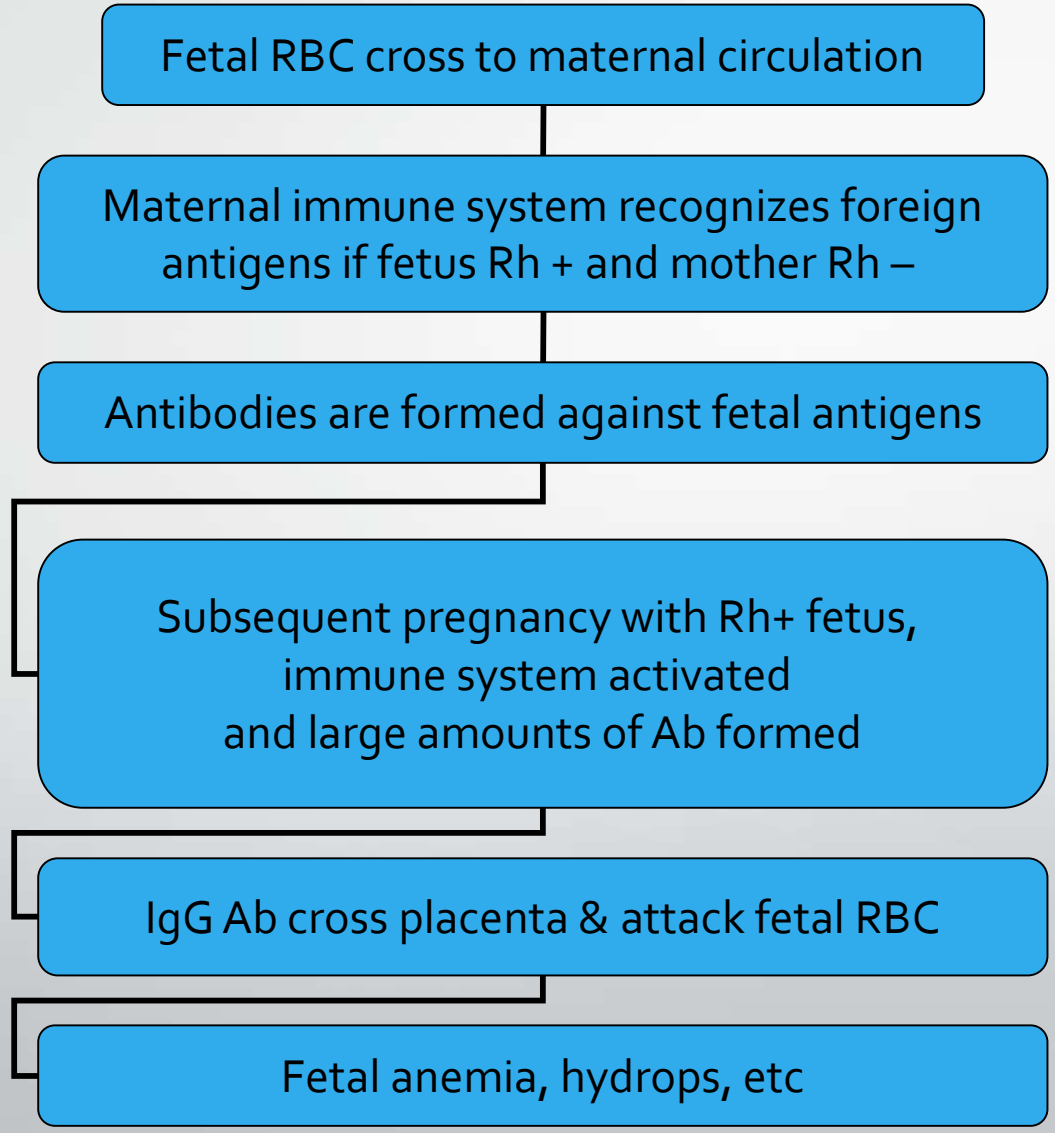
Rh system

- Rh factor is a Protein (antigens) occurring only on surface of RBC's.
- Rh + if proteins present.
- Rh – if proteins absent.
- Possible blood groups (A+, A-, B+, B-, AB+, AB-, O+, O-)
- Most important for pregnancy.
- Inheritance is Autosomal Dominant.
- 15% Caucasian population are Rh negative.

Rh system

- Rh blood system has other antigens: **C, c, D, E, e.**
- Weak **D** (Du) also exists.
- **RhD, C/c, and E/e** antigens are the product **two** genes localized to the short arm of chromosome 1 .
- D antigen is the most common, the only preventable one, and associated most commonly with **severe haemolytic disease.**
- **40%** of Rh-positive individuals are homozygous at the D locus (DD); the remainder is heterozygous (Dd).

Why studying Rh Status is important?



Pathogenesis

- By **30** days of gestation, the Rh(D) antigen is expressed as part of the RBC membrane.
- In most cases, the antigenic load of a putative antigen on the fetal RBCs is insufficient to stimulate the maternal immune system.
- Transplacental fetomaternal bleeding accounts for virtually all cases of maternal Rh(D) alloimmunization. Other possible causes:
  - ✓ Injection with needles contaminated by Rh(D)-positive blood
  - ✓ Inadvertent transfusion of Rh(D)-positive blood
- Tiny (0.1 mL) quantities of fetal RBCs gain access to the maternal circulation in nearly all pregnancies.

Pathogenesis

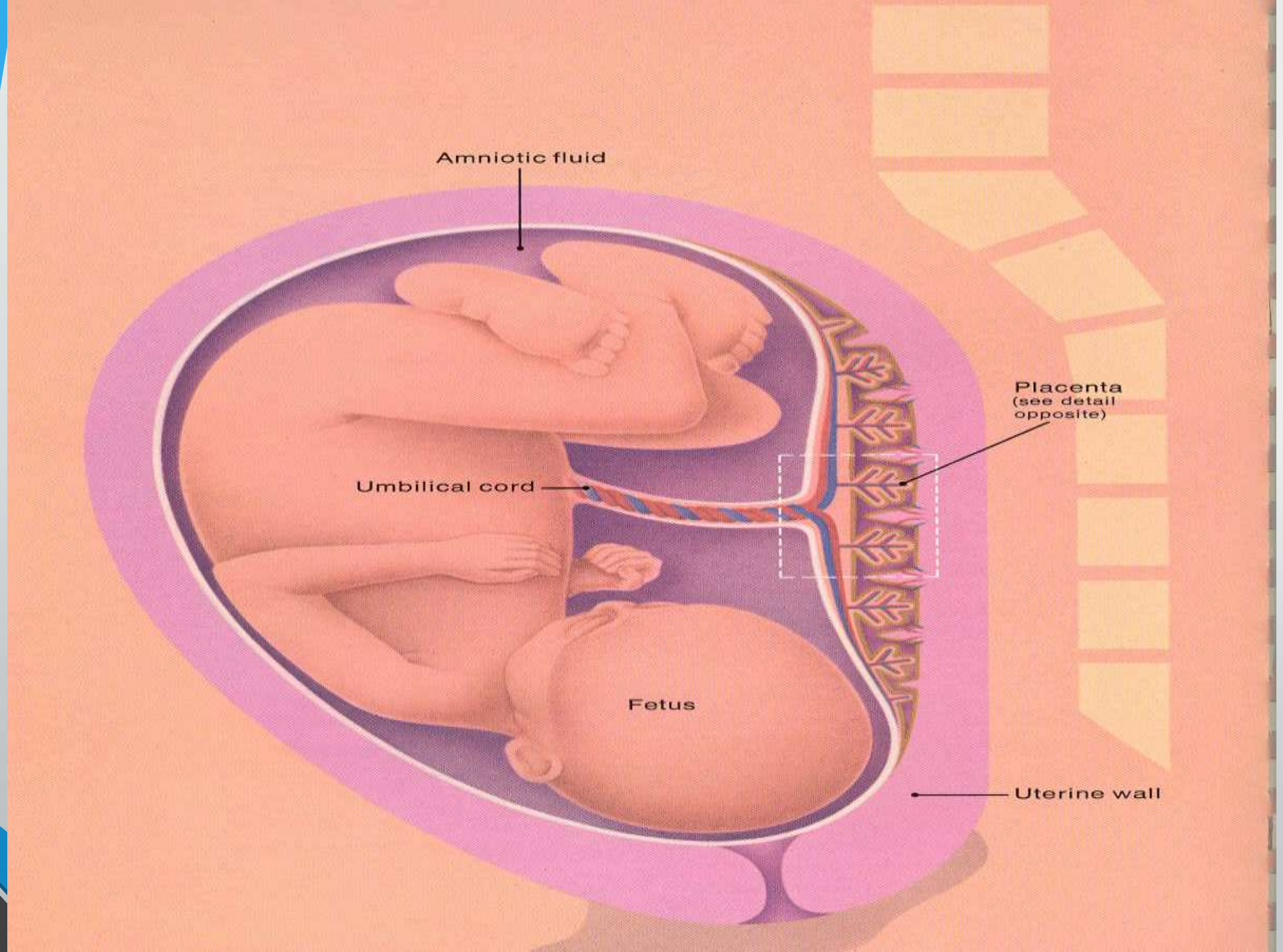
- The frequency and volume of spontaneous fetomaternal hemorrhage **increase with advancing gestational age and are highest at delivery.**
- Fetomaternal hemorrhage can also be associated with:
  - ✓ Miscarriage
  - ✓ Pregnancy termination
  - ✓ Ectopic pregnancy
  - ✓ Invasive in-utero procedures
  - ✓ Fetal death
  - ✓ Maternal abdominal trauma
  - ✓ Antepartum maternal hemorrhage
  - ✓ External cephalic version.

Pathogenesis

- In the case of a large fetomaternal hemorrhage before birth or at delivery, B lymphocyte clones that recognize the foreign red cell antigen are established.
- The initial maternal production of IgM is short-lived and is followed by a rapid change to an IgG response. (A human antiglobulin titer can usually be detected by 5 to 16 weeks after the sensitizing event).
- In a subsequent pregnancy, B lymphocyte stimulated by fetal erythrocytes, and differentiate into plasma cells that can rapidly proliferate and produce IgG antibodies and an increase in the maternal titer.
- Maternal IgG crosses the placenta and attaches to fetal erythrocytes that have expressed the paternal antigen.

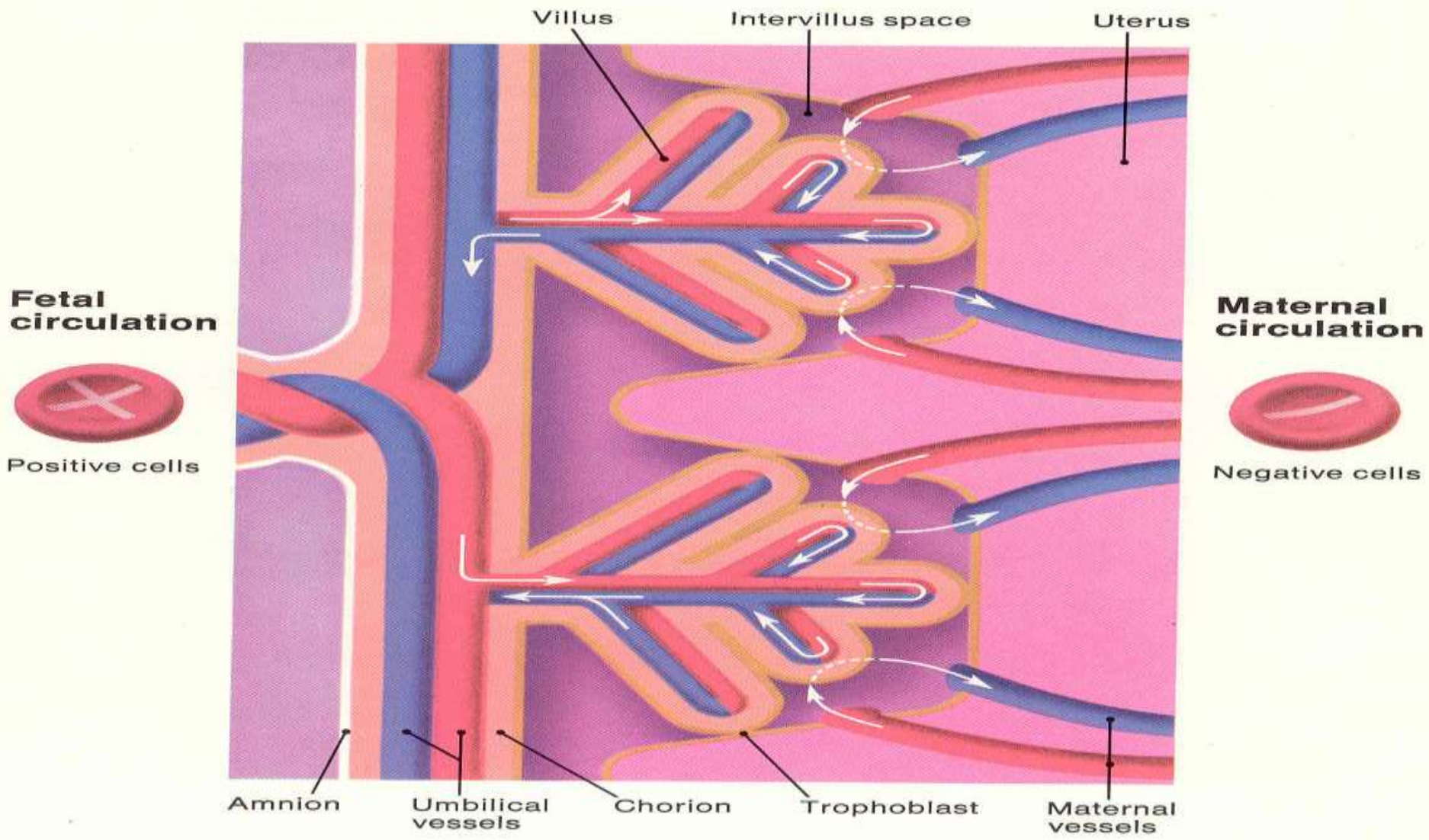
Pathogenesis

- These cells are then sequestered by macrophages in the fetal spleen, where they undergo extravascular hemolysis, producing fetal anemia.
- Enhanced bone marrow and fetal liver production of reticulocytes and erythroblasts, cardiac output increases.
- **Hydrops fetalis** (collection of fluid in at least two serous compartments) typically heralds end-stage disease, and it occurs with hemoglobin deficits of 7 g/dL or greater.
- The initial ultrasound finding of hydrops is usually fetal ascites, followed later by pleural effusion and finally scalp edema.


Amniotic fluid
Placenta
(see detail
opposite)
Umbilical cord
Fetus
Uterine wall

Scheme of Placental Circulations

White arrows depict separate routes of fetal and maternal circulations within the placenta. Dotted lines represent oxygen, nutrient and waste exchange through the placental barrier.

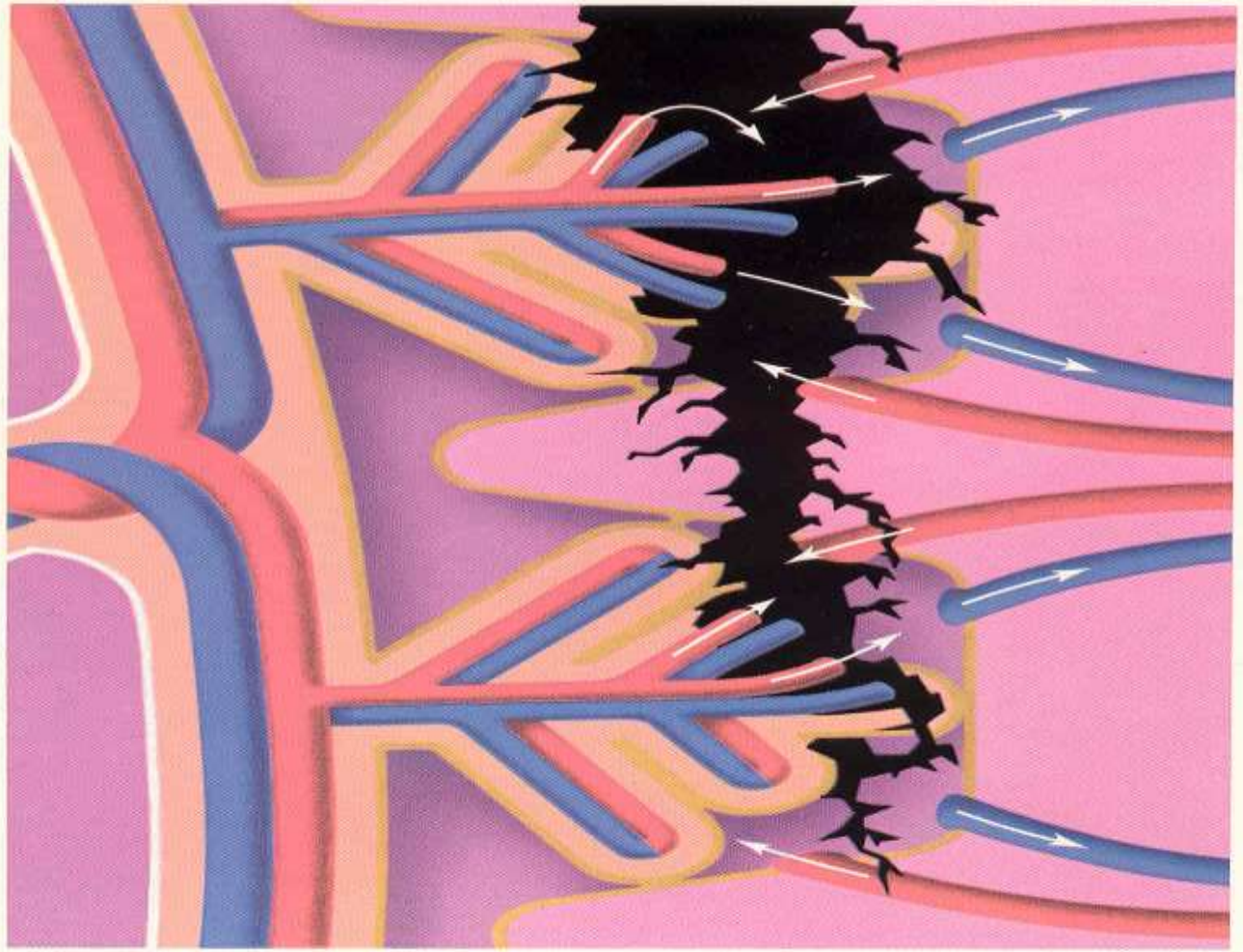


Separation of Placenta Following Delivery

Diagram portrays the rupture of placental vessels (villi) and connective tissue allowing escape of fetal blood cells. Prior to complete constriction of open-end maternal vessels, some fetal blood may enter maternal circulation.

Fetal circulation

Positive cells



Maternal circulation

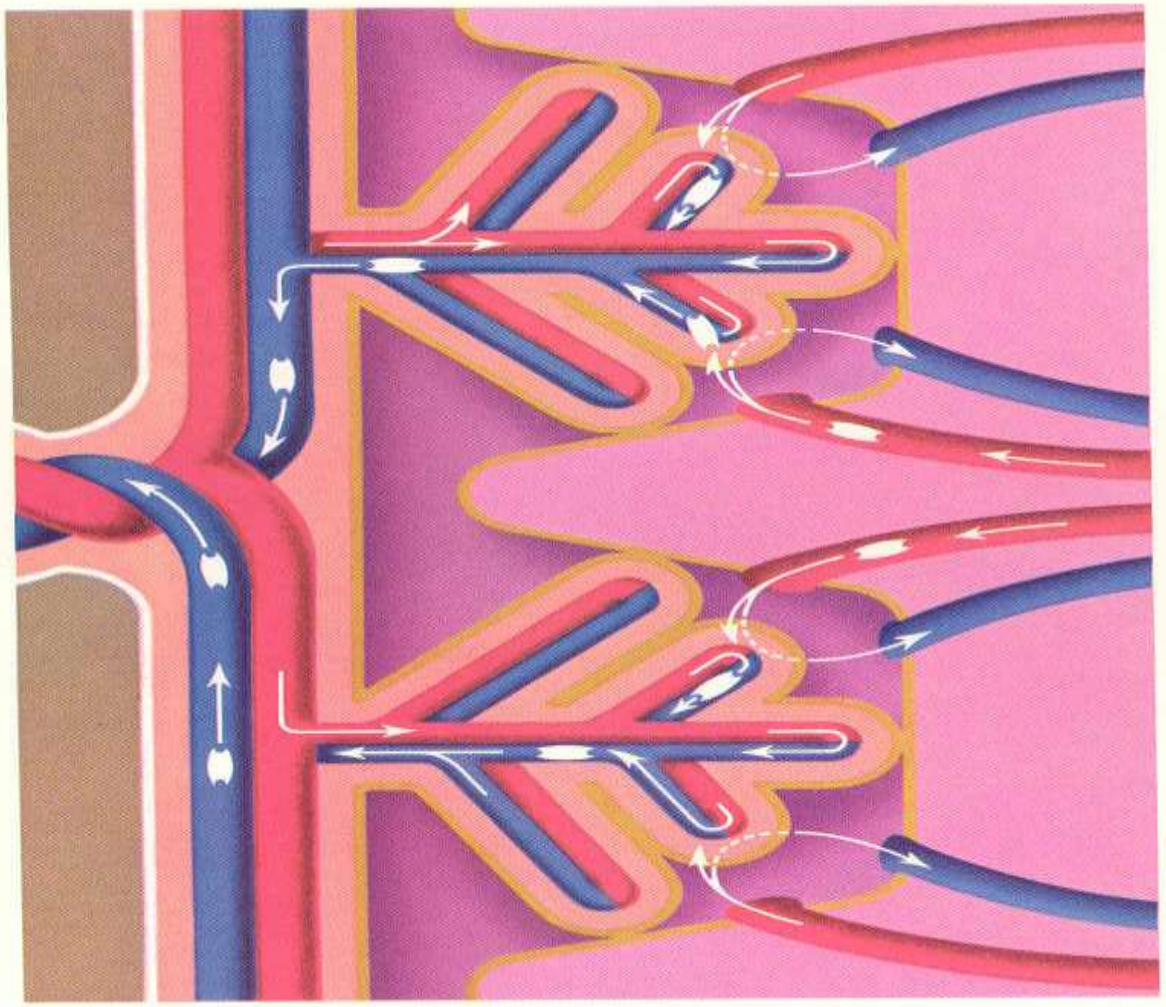
Invading fetal (positive) cells

Subsequent Incompatible Pregnancy

Residual antibodies produced as a response to red cells of a previous incompatible fetus or donor are transported through the placental barrier. They attach to the specific red cell antigen sites of the incompatible fetus of the current pregnancy. Sensitized cells do not have a normal life span; the baby suffers from anemia and its consequences.

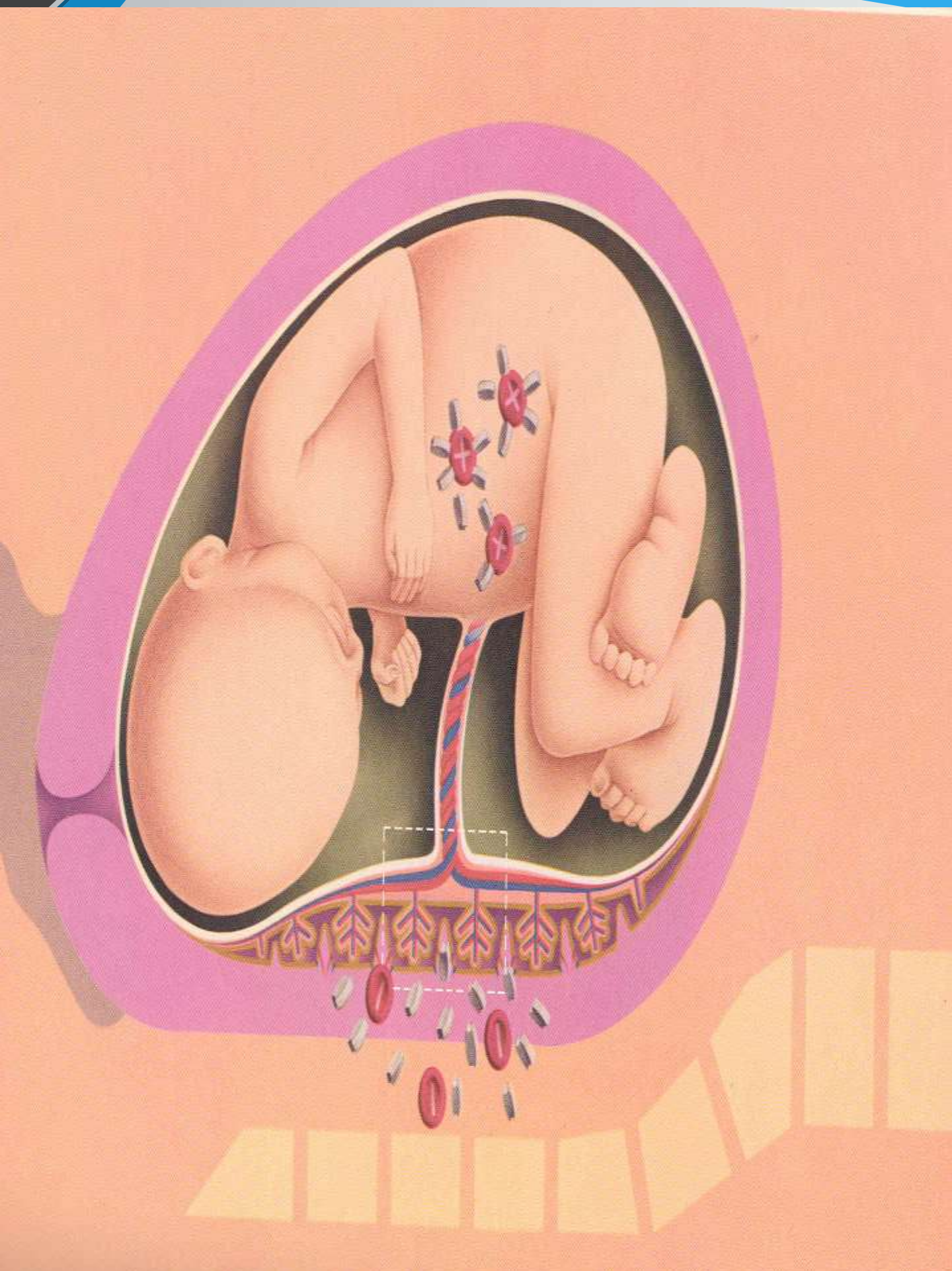
Fetal circulation

Maternal antibodies attach to incompatible fetal cells



Maternal circulation

Circulating maternal antibodies produced by previous isoimmunization enter placenta



Diagnosis

- **Maternal Antibody Determination:**
  - ✓ Rh(D) typing and an antibody screen should be performed at the first prenatal visit.
  - ✓ Test husband for Rh(D) type and, if Rh(D)-positive, zygosity is determined by Quantitative polymerase chain reaction (PCR) DNA testing.
  - ✓ If an antibody is detected, it is first identified to determine its clinical significance. Then human antiglobulin titer (indirect Coombs ICT) is used to determine the degree of alloimmunization, because it measures the maternal IgG response.
  - ✓ If negative antibody screen and uncomplicated pregnancy, repeat at 28 weeks of gestation.
  - ✓ A critical titer is defined as the titer associated with a significant risk for fetal hydrops. This titer varies with the institution and the methodologies used; however, most centers use a critical value for anti-D, and most other antibodies, of **32 (value of 15 IU/mL).**

Diagnosis

- **Fetal Genotype Testing:**
  - ✓ Cell free fetal DNA (**cffDNA**) may be detected in the maternal circulation as early as 38 days of gestation.
  - ✓ the amount of DNA increases with advancing gestational age and disappears soon after delivery.
  - ✓ Fetal Rh(D) status can be determined by evaluation of **cffDNA** sequences in maternal plasma using a reverse transcriptase PCR.
  - ✓ **cffDNA** testing to determine the fetal antigen status is available in Europe for the C, c, E, and Kell (K1) antigens.
  - ✓ If **cffDNA** testing is not available, then amniocentesis can be performed at 15 weeks of gestation to obtain amniocytes.
  - ✓ Transplacental amniocentesis is avoided, if possible, as it may worsen alloimmunization.

Management

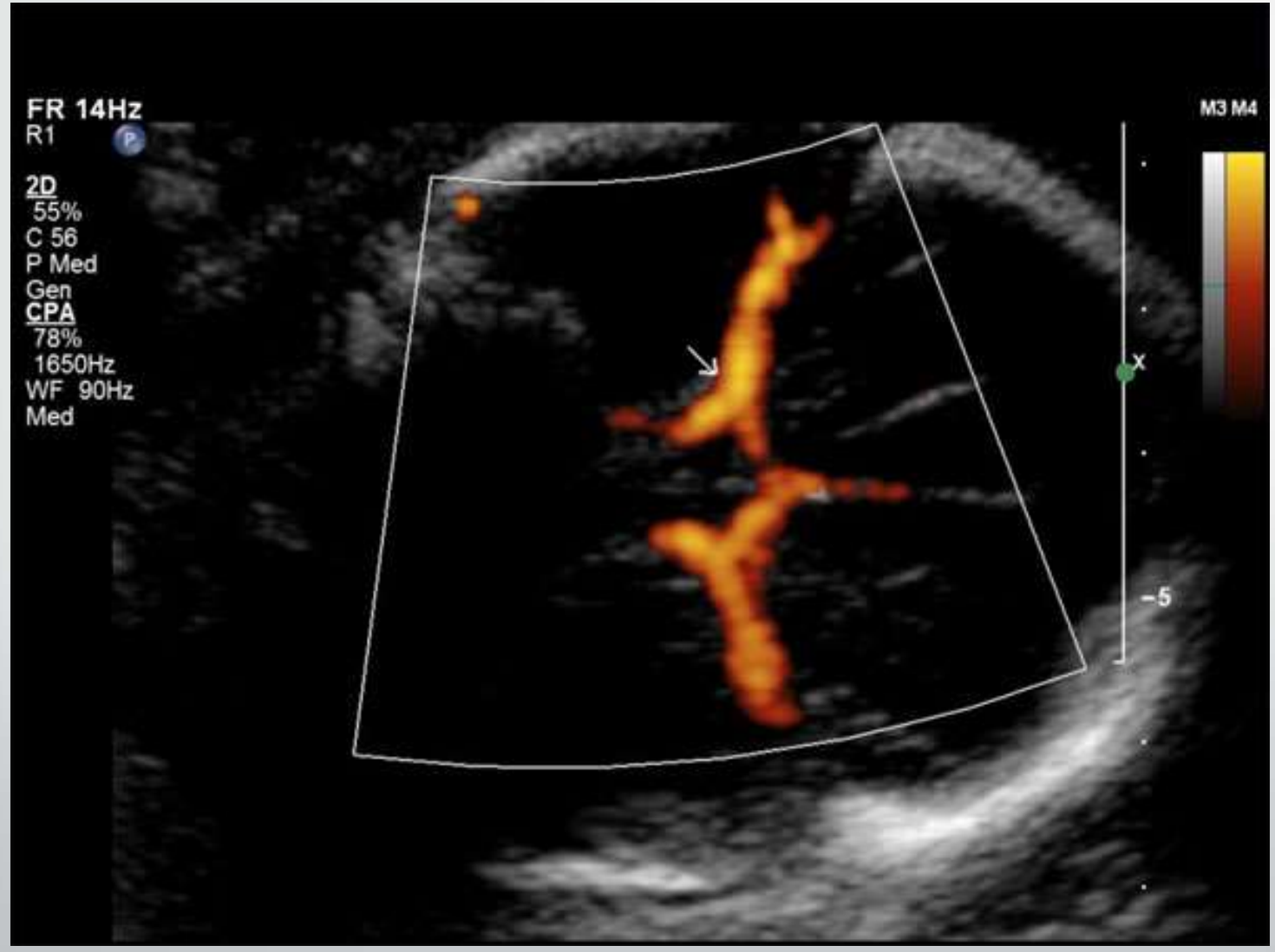
- **First alloimmunized pregnancy with Rh(D) positive fetus:**
  - ✓ fetal effects is **less sever** in the first affected pregnancy, and worsen with each subsequent affected pregnancy.
  - ✓ **serial antibody titers** are determined every **2-4 weeks after 20 weeks**.
  - ✓ If the critical titer is reached or exceeded, then further assessment to determine whether sever fetal anemia is present is required and checking maternal titers can be discontinued.

- **Subsequent pregnancies with Rh(D) positive fetus:**
  - ✓ If the patient has had a prior significantly affected pregnancy, then sever fetal anemia, in subsequent pregnancies with an Rh(D)-positive fetus is almost certain.
  - ✓ The severity of fetal anemia is assessed beginning at **16 -18 weeks** of gestation and maternal antibody titers are not routinely evaluated, as they do not reliably predict the severity of fetal anemia.

Assessment for severity of fetal anemia

- Measurement of the peak systolic velocity (**PSV**) in the fetal middle cerebral artery (**MCA**) has been shown to be an **accurate noninvasive** method for detecting fetal anemia.
- **MCA-PSV** increases with advancing gestation, and values should be reported in MoM to account for changes in gestational age.
- A threshold value of **1.5 MoM** was used to predict moderate to severe anemia **(<0.65 MoM** for fetal hemoglobin).
- The fetus should be in a quiescent state during examination, as accelerations of the fetal heart rate can result in a false depression in the **MCA-PSV**, especially late in the third trimester.
- Measurements can be initiated at as early as **16 to 18 weeks** gestation and should be performed **weekly.**

Middle Cerebral Artery Doppler



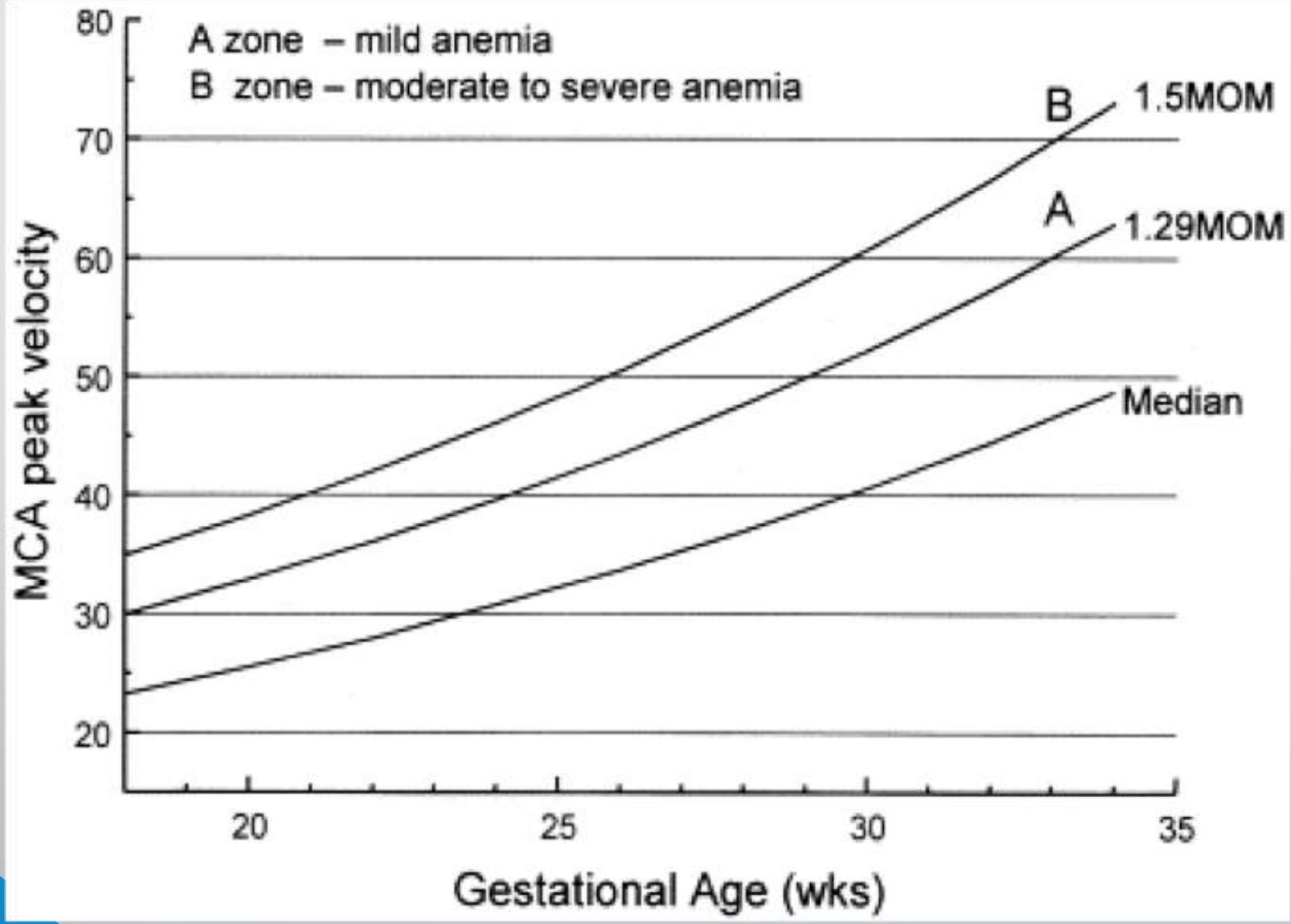
Middle Cerebral Artery Doppler

Table 1 Values for middle cerebral artery peak systolic velocity (cm/s) based on multiples of the median between the 23rd and 35th gestational weeks.

Gestational age (weeks)	Multiples of the median for MCA-PSV			
	1.0	1.29	1.50	1.55
23	35.44	45.72	53.16	54.93
24	35.48	45.77	53.22	55.00
25	35.81	46.20	53.72	55.51
26	36.45	47.03	54.68	56.50
27	37.43	48.29	56.15	58.02
28	38.77	50.01	58.15	60.09
29	40.49	52.23	60.73	62.75
30	42.61	54.97	63.91	66.04
31	45.16	58.26	67.74	70.00
32	48.17	62.13	72.25	74.66
33	51.65	66.62	77.47	80.05
34	55.63	71.76	83.44	86.22
35	60.13	77.56	90.19	93.20

MCA-PSV, middle cerebral artery peak systolic velocity.

Middle cerebral artery doppler



Other methods to assess fetal anemia

- **Spectral analysis of amniotic fluid** :
  - ✓ In the past, amniocentesis to determine amniotic fluid bilirubin levels was the usual method for indirectly estimating the severity of fetal anemia.
  - ✓ Bilirubin present in amniotic fluid derives from fetal pulmonary and tracheal effluents and correlates with the degree of fetal hemolysis.
  - ✓ Doppler velocimetry is as, or more, sensitive and specific for detection of severe fetal anemia and noninvasive.

- **Fetal blood sampling:**
  - ✓ Fetal blood sampling allows direct access to the fetal circulation to study hematocrit, direct Coombs, fetal blood type, reticulocyte count, and platelet count.
  - ✓ Reserved for patients with **increased MCA-PSV.**
  - ✓ Can be followed by intrauterine transfusion.

Intrauterine transfusion

- Severe fetal anemia can be defined as a hematocrit of 30 % or 2 SDs below the mean hematocrit for the gestational age.
- Intrauterine transfusions for severe fetal anemia are generally performed between **18 and 35** weeks of gestation.
- Intrauterine transfusions can be performed via 2 methods:
  - ✓ Intravascular transfusion(cord insertion into the placenta, intrahepatic vein, direct cardiac puncture).
  - ✓ Intraperitoneal transfusion.
- Red cells to be used for IUT should be:
  - ✓ CMV seronegative.
  - ✓ The unit should be packed to a final hematocrit of 75% to 85%.
  - ✓ The leukocyte number reduced using specialized micropore filters.
  - ✓ The unit irradiated with 25 Gy to prevent graft-versus-host reaction.

Time of delivery

- Until the introduction of the direct Intravascular transfusion, fetuses with hemolytic disease were routinely delivered by **32 weeks** gestation.
- Most experienced centers now perform the final IUT at **up to 35 weeks** gestation, with delivery anticipated at **37 to 38 weeks.**
- After **35 weeks**, the procedure is generally considered riskier than late preterm delivery for neonatal treatment of severe anemia.
- The administration of maternal oral phenobarbital may be considered **7 -10 days** before delivery.

(This has been proposed to induce hepatic maturity to allow improved conjugation of bilirubin)

Prevention

- **Anti-D immune globulin**:
  - ✓ Is a sterile solution containing IgG anti-D (anti-Rh) manufactured from human plasma.
  - ✓ A single **300 microgram dose** (**1 microgram = 5 IU**) contains sufficient anti-D to suppress the immune response to **15 mL** of Rh-positive red blood cells **30 mL** fetal whole blood.
  - ✓ A single **50 microgram** dose contains sufficient anti-D to suppress the immune response to **2.5 mL** of Rh-positive red blood cells.
  - ✓ Formulations (**RhoGAM, HyperRHO S/D**, **Rhophlac**, **WinRho-SDF**).
  - ✓ Anti-D immune globulin is not effective once alloimmunization to the Rh(D) antigen has occurred.

Prevention

- **Indications for administration of anti-(D) immune globulin:**
  - ✓ At 28 weeks of gestation
  - ✓ Spontaneous abortion, threatened abortion, induced abortion
  - ✓ Ectopic pregnancy
  - ✓ Invasive procedures: genetic amniocentesis; chorionic villus sampling; multi-fetal reduction; fetal blood sampling
  - ✓ Hydatidiform mole
  - ✓ Fetal death in the second or third trimester
  - ✓ Blunt trauma to the abdomen
  - ✓ Antepartum hemorrhage in the second or third trimester (eg, placenta previa or abruption)
  - ✓ External cephalic version

Prevention

- **Administration:**
  - ✓ All pregnant patients should undergo an antibody screen at the first prenatal visit.
  - ✓ If there is no evidence of alloimmunization in the RhD-negative woman, patients should receive 300 µg of RhIG at 28 weeks gestation.
  - ✓ A dose of 50 µg of RhIG is effective until 12 weeks gestation because of the small volume of red cells in the fetoplacental circulation.
  - ✓ 300 µg of RhIG should be administered within 72 hours of delivery, if umbilical cord blood typing reveals an RhD-positive infant.
  - ✓ If RhIG is inadvertently omitted after delivery, some protection has been proven with administration within 13 days; recommendations have been made to administer it as late as 28 days after delivery.
  - ✓ This dose of immune globulin is adequate to protect against maternal sensitization from as much as **15 mL** red blood cells (30 mL Rh(D)-positive fetal whole blood).

Screening for Feto-Maternal Hemorrhage(FMH)

- Approximately **3/1000** deliveries are associated with an excessive FMH.
- Up to **50%** of large FMHs occur after normal deliveries.
- The following clinical circumstances are more likely to be associated with large FMH:

  a- Traumatic deliveries &CS

  b-Manual removal of placenta

  c-Stillbirths & IUD

  d-Abdominal trauma during 3rd trimester

  e-Twin pregnancies

  f-Unexplained hydrops fetalis

Screening for Feto-Maternal Hemorrhage(FMH)

- **Rosette test :**
  - ✓ A qualitative test (usually performed first).
  - ✓ Results are reported as positive or negative.
  - ✓ Fetal RBCs suspension incubated with IgG anti-D , then washed to remove unbouned anti-D, agglutination will happened ,and according to the number of agglutinations the test designated as positive or negative.

- **Kleihauer-Betke test:**
  - ✓ Fetal RBC contain **Hb F**, which is resistant to acid elution than **Hb A.**
  - ✓ After exposure to acid, only fetal cells remain & can be identified with stain.
  - ✓ Evaluate the percentage of fetal cells in maternal circulation.