Haemolytic Disease of the Fetus and Newborn

Rh Isoimmunization

- **Isoimmunization** - one of the clinical forms imunopatology of pregnancy, provided that there is incompatibility of the mother and fetus to various antigens and leads to severe abnormalities of the fetus and newborn.

The most frequent:

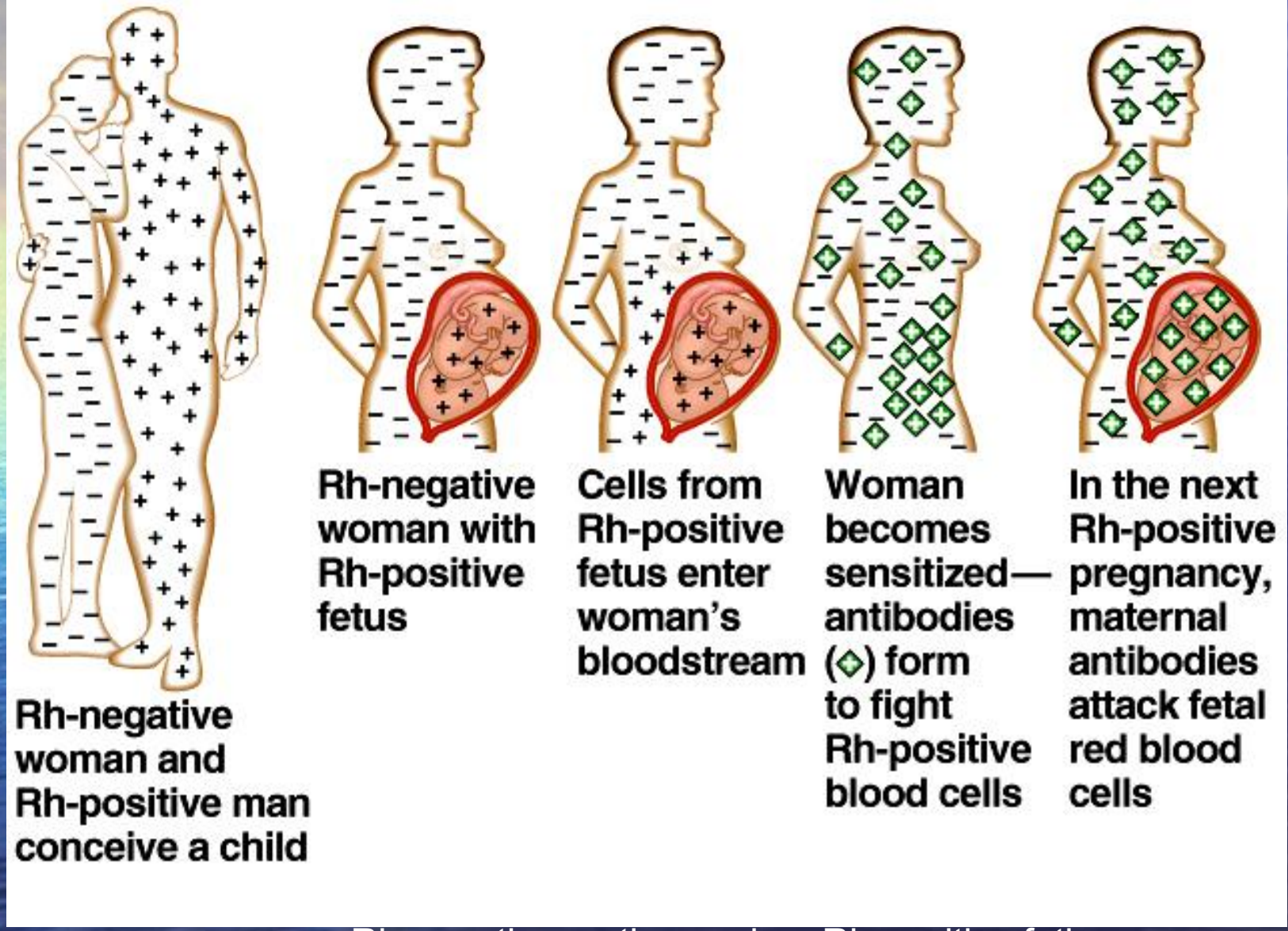
- **Isoimmunization** of Rh-factor;
- **Isoimmunization** AB0- system.

Alloimmune Hemolytic Disease Of The Fetus / Newborn:

Definition:

Rh-izoimunization - humoral immune response to erythrocytic antigens (Ag) fetal Rh-group, including Cc, Dd and Ee (coded Rh-allele). Antibodies (AB), which formed, penetrating through the placenta, causing hemolysis ekstravaskulyary (opsonization fetal erythrocytes AB women and phagocytosis of red blood cells) and anemia leading to:

- **fetal erythroblastosis.**
- **The mother become Isoimmunized.**
- **In The Newborn: HDN.**



Rh-negative mother and an Rh-positive father

Antibodies That May Be Detected During Pregnancy:

Innocuous Antibodies:

- Most Of These Antibody Are IgM Therefore Cannot Cross The Placental Barrier

Antibodies Capable Of Causing Significant Hemolytic Transfusion Reactions:

- IgG antibodies, Their Corresponding Antigens Are Not Well Developed At Birth Lu (b), Yt (a)

Antibodies That Are Responsible For HDN :

- Anti-c, Anti-d, Anti-e, And Anti-k (Kell)

Distribution of Rh negative Blood Group

Rh D negativity primarily occurs among Caucasians; the average incidence is 15 percent in this group.

Examples of the blood group distribution in various populations are illustrated below:

- Basques — 30 to 35 percent
- Finland — 10 to 12 percent
- American blacks — 8 percent
- Indo-Eurasians — 2 percent
- Native Americans and Inuit Eskimos — 1 to 2 percent.
- among the Indian population ± 8%

The main sections of our lectures

The RH Antigen – Biochemical and Genetic Aspects

Mechanism of Development of Maternal Rh Isoimmunization

Natural History of Maternal isoimmunization /HD of the Newborn

Pathogenesis of Fetal Erythroblastosis Fetalis

Diagnosis of Rh isoimmunization

The RH Antigen – Biochemical and Genetic Aspects

The Rh Antigen- Biochemical Aspects:

- The Rh Antigen Is A Complex Lipoprotein. Distributed Throughout The Erythrocyte Membrane In A Nonrandom Fashion
- It Can Not Be Seen By Routine Microscopy, But Can Be Identified By Specific Antisera

Function of the Rh antigen:

- Its Precise Function Is Unknown.
- Rh Null Erythrocytes Have Increased Osmotic Fragility And Abnormal Shapes.

The RH Antigen- Genetic Aspect

- The Rh gene complex is located on the distal end of the short arm of chromosome one.
- A given Rh antigen complex is determined by a specific gene sequence inherited in a Mendelian fashion from the parents. one haploid from the mother and one from the father.
- Three genetic loci, determine the Rh antigen (i.e. Rh blood group).
- Each chromosome will be either D positive or D negative (there is no "d" antigen), C or c positive, and E or e positive.

Genetic Expression (Rh Surface Protein Antigenicity):

- Grades Of "Positively" Due To Variation In The Degree Genetic Expression Of The D Antigen.
- Incomplete Expression May Result In A Weakly Positive Patient e.g. **Du** Variant Of Weakly Rh Positive Patient (They May Even Be Determined As Rh Negative).
- A Mother With Du Rh Blood Group (Although Genetically Positive) May Become Sensitized From A D-positive Fetus Or The Other Way Around May Take Place.

Factors Affect The Expression Of The Rh Antigen

- The Number Of Specific Rh-antigen Sites:
  - The Gene Dose,
  - The Relative Position Of The Alleles,
  - The Presence Or Absence Of Regulator Genes.

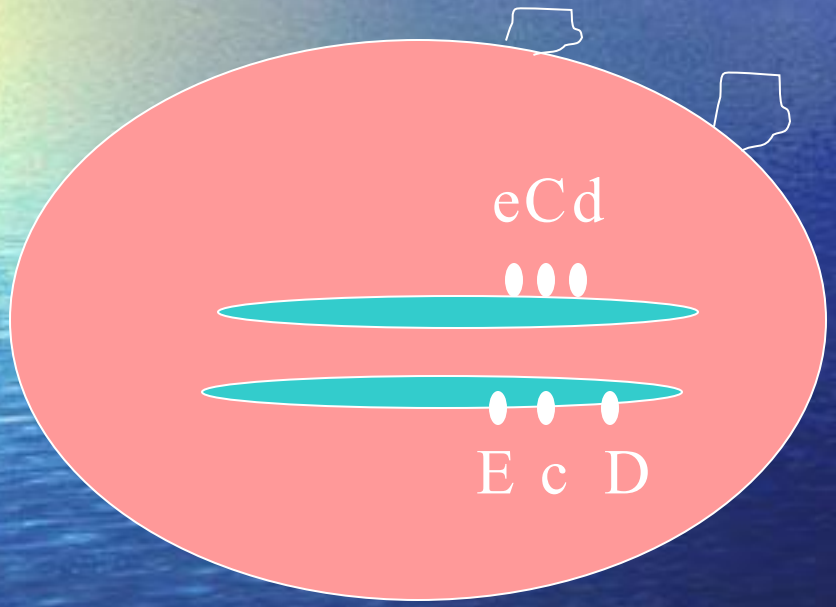
- Interaction Of Other Components Of The Rh Blood Group. Erythrocytes Of Individuals Of Genotype Cde/cde Express Less D Antigen Than Do The Erythrocytes Of Individuals Of Genotype cDE/cde.

- The Exposure Of The D Antigen On The Surface Of The Red Cell Membrane.

Genotype

Phenotype

eCd/EcD → **D positive**



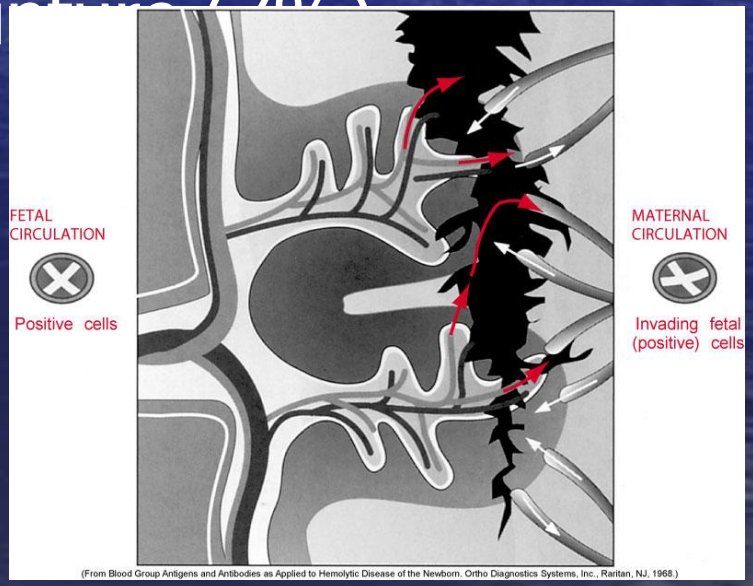
Antigenicity of the Rh surface protein:

- ✔ genetic expression of the D allele.
- ✔ Number of specific Rh antigen sites.
- ✔ Interaction of components of the Rh gene complex.
- ✔ Exposure of the D antigen on the surface of the red cell

Mechanism of Development of Maternal Rh Isoimmunization

FetoMaternal Hemorrhage

- Sensitization occurs as a result of seepage of fetal cells into maternal circulation as a result of a fetomaternal hemorrhage
  - Placental membrane rupture (7%)
  - Trauma to abdomen
  - Delivery (>50%)
  - Amniocentesis
  - Abortion



The Mechanism of Development of the Rh Immune Response:

Fetal RBC with Rh +ve antigen

↓

Maternal circulation of an Rh –ve mother

↓

The Rh +ve antigen will be cleared by macrophages; processed and transferred to plasma stem cell precursors (Develop an almost permanent immunologic memory)

↓

(Primary immune response)

↓

With subsequent exposure the plasma cell line proliferate to produce humeral antibodies

↓

(Secondary immune response).

The Primary Response:

- Is a slow response (6 weeks to 6 months).
- IgM antibodies
- a molecular weight of 900,000 that does not cross the placenta.

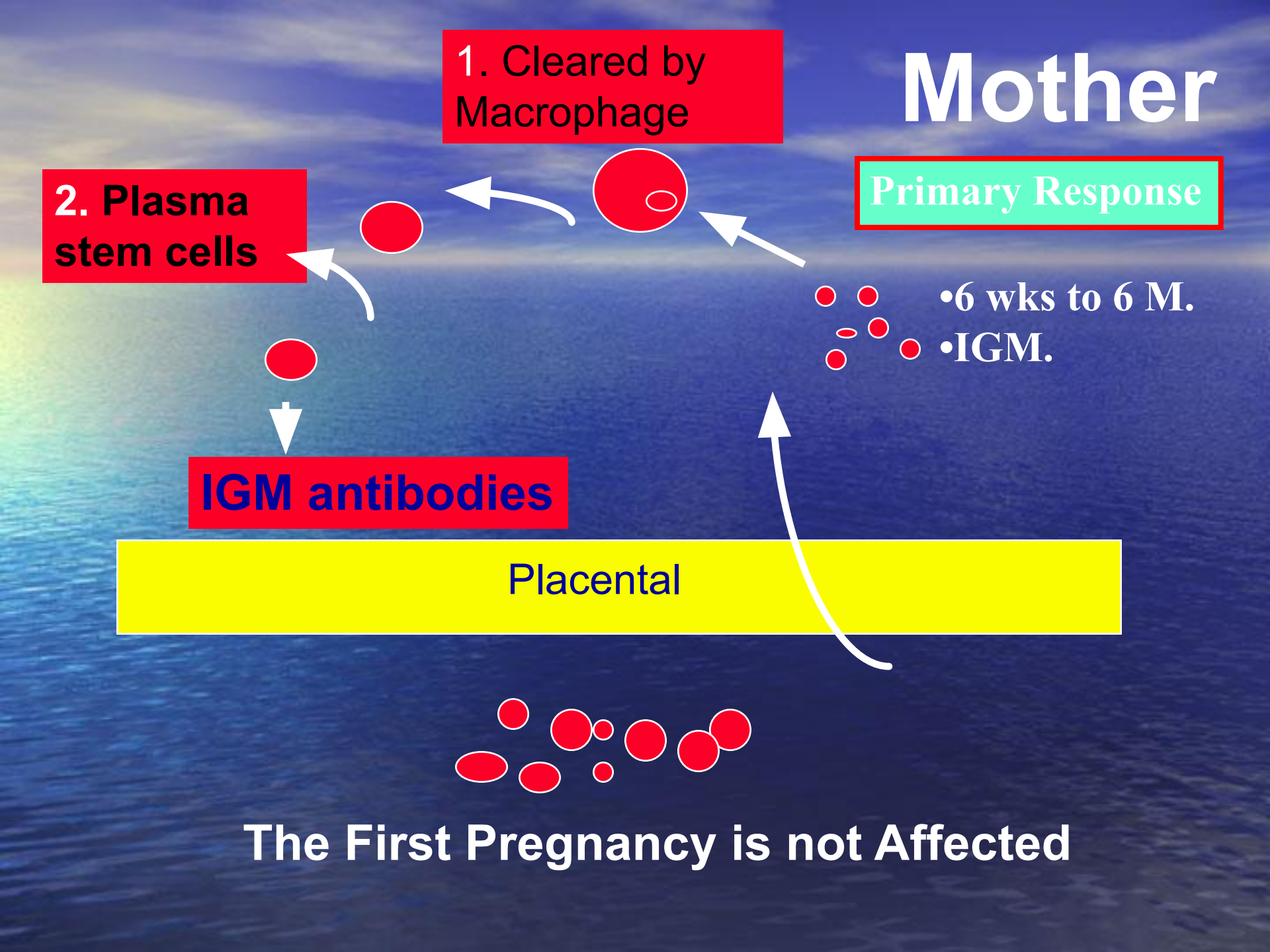
The Secondary Response:

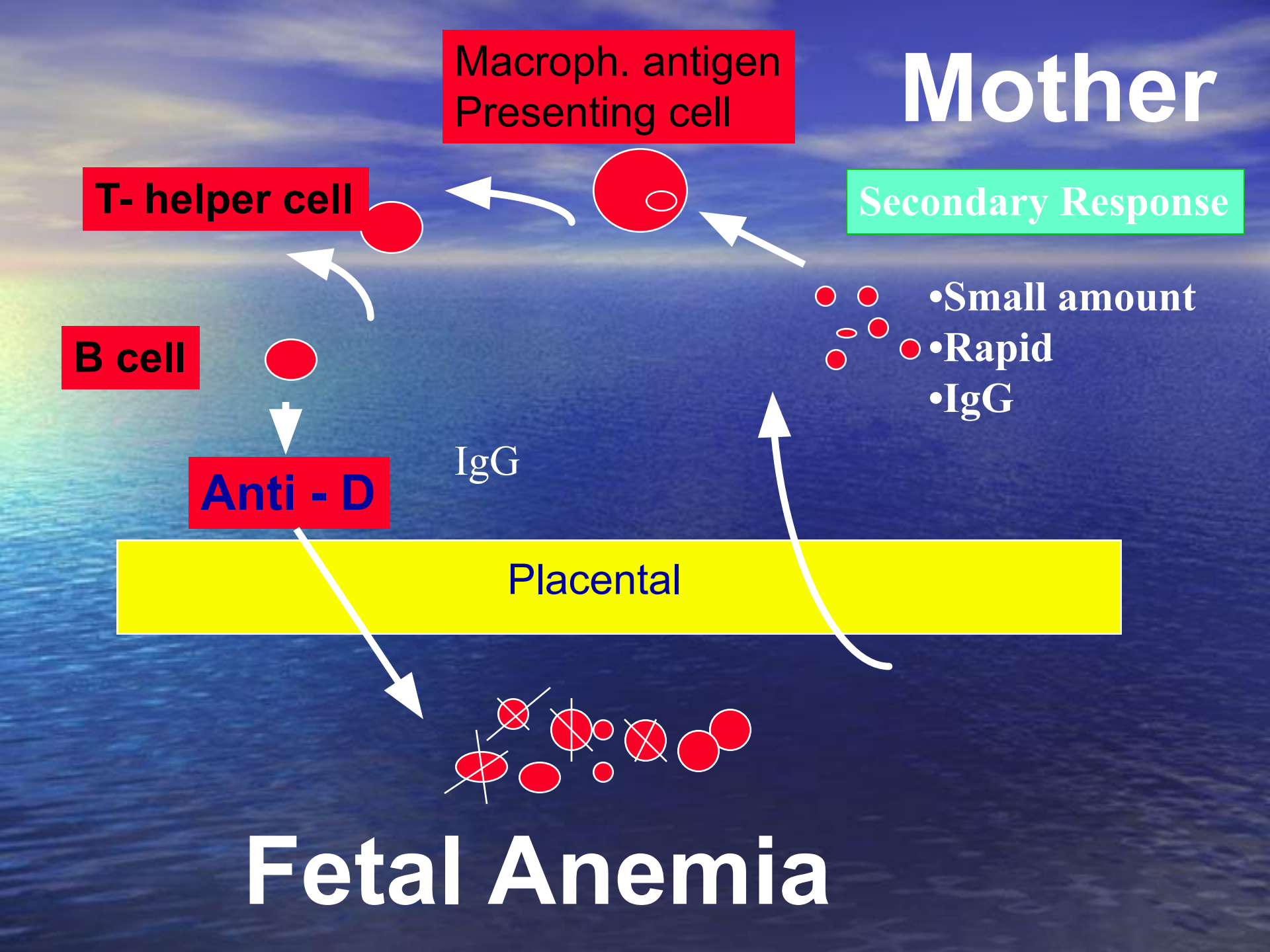
- Is a Rapid response
- IgG antibodies
- a molecular weight of 160,000 that cross the placenta.

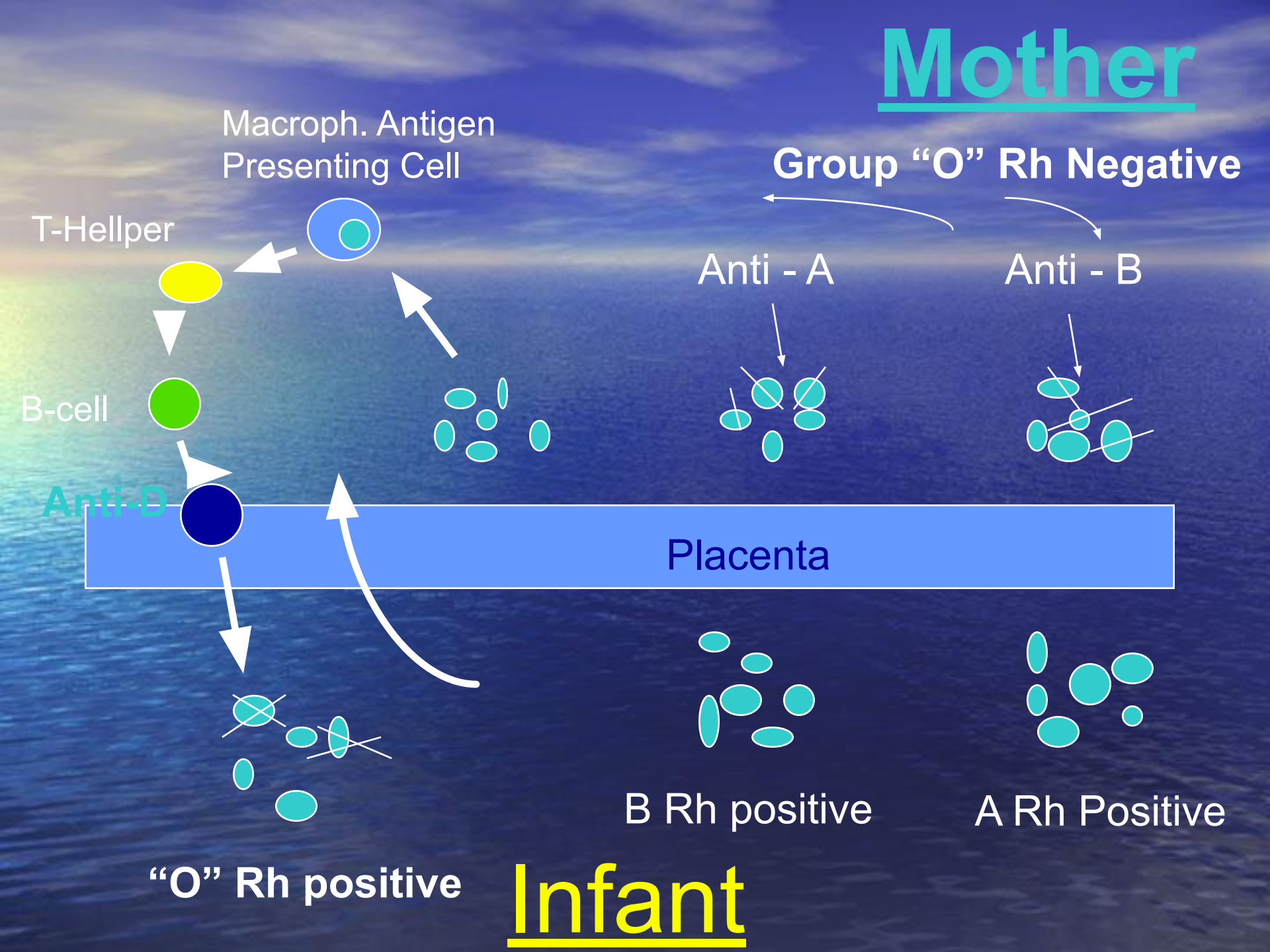
Exposure to maternal antigen in utero "the grandmother theory":

Explains the development of fetal isoimmunization in a primigravida, who has no history of exposure to incompatible Rh blood.

- Rh negative Fetus and the mother is Rh positive
- The Fetus is exposed to the maternal Rh antigen through maternal-fetal transplacental bleed.
- The fetus immune system develop a permanent template (memory) for the Rh-positive antigen.
- When the fetus becomes a mother herself and exposed to a new load of D antigen from her fetus (hence the grandmother connection) the immune memory is recalled and a secondary immune response occur.


1. Cleared by Macrophage
Mother
Primary Response
2. Plasma stem cells
•6 wks to 6 M.
•IGM.
IGM antibodies
Placental
The First Pregnancy is not Affected


Macroph. antigen
Presenting cell
Mother
T- helper cell
Secondary Response
•Small amount
•Rapid
•IgG
B cell
IgG
Anti - D
Placental
Fetal Anemia


Mother
Macroph. Antigen
Presenting Cell
Group “O” Rh Negative
T-Hellper
Anti - A
Anti - B
B-cell
Anti-D
Placenta
B Rh positive
A Rh Positive
“O” Rh positive
Infant

Natural History of Maternal isoimmunization /HD of the Newborn

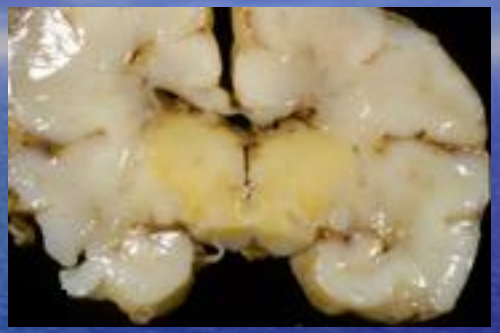
Natural History of Rh Isoimmunization And HD Fetus and Newborn

- Without treatment:
  - less than 20% of Rh D incompatible pregnancies actually lead to maternal isoimmunization
  - 25-30% of the offspring will have some degree of hemolytic anemia and hyperbilirubinemia.
  - 20-25% will be hydropic and often will die either in utero or in the neonatal period.
  - Cases of hemolysis in the newborn that do not result in fetal hydrops still can lead to kernicterus.

Kernicterus

- **Kernicterus** (bilirubin encephalopathy) results from high levels of indirect bilirubin (**>20 mg/dL in a term infant with HDN**).
- Kernicterus occurs at lower levels of bilirubin in the presence of **acidosis, hypoalbuminemia, prematurity and certain drugs** (*e.g.*, sulfonamides)**.**

Kernicturus



- Affected structures have a bright yellow color.
- **Unbound unconjugated bilirubin crosses the blood-brain barrier** and, because it is lipid soluble, it penetrates neuronal and glial membranes.
- Bilirubin is thought to be toxic to nerve cells
- The mechanism of neurotoxicity and the reason for the topography of the lesions are not known.
- Patients surviving kernicterus have severe permanent neurologic symptoms **(choreoathetosis, spasticity, muscular rigidity, ataxia,** deafness, **mental retardation)**.

The Risk of development of Fetal Rh-disease is affected by:

Less than 20% of Rh D incompatible pregnancies actually lead to maternal alloimmunization

- The Husband Phenotype And Genotype (40 % Of Rh Positive Men Are Homozygous And 60% Are Heterozygous).
- The Antigen Load And Frequency Of Exposure.
- ABO Incompatibility

Why Not All the Fetuses of Isoimmunized Women Develop the Same Degree of Disease?

- Expression Of The Rh Antigen
- Classes Of IgG Family
- The Non-responders
- ABO Incompatibility

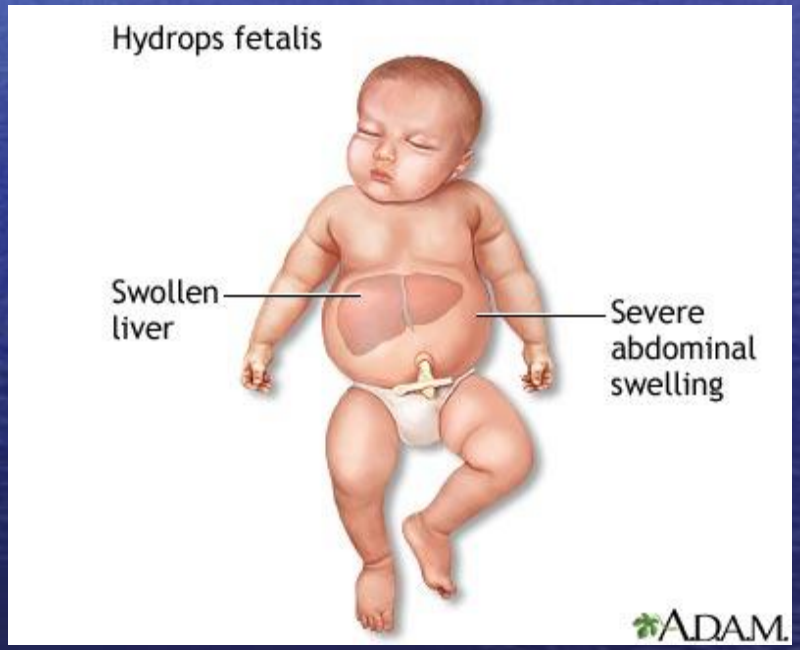
Risk factors:

- a history of artificial abortion;
- a history of spontaneous abortions;
- transfusion of Rh-positive blood in history;
- ectopic pregnancy;
-lack of Rh-specific prevention of conflict after the previous pregnancy;
- the presence of Rh-conflict in previous pregnancies.

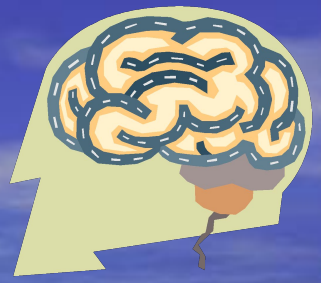
Pathogenesis of Fetal Erythroblastosis Fetalis

Pathogenesis

- When erythroblasts are used up in the bone marrow, erythropoiesis in the spleen and liver are increased
  - Hepatosplenomegaly (enlarged liver & spleen)
  - Hypoproteinemia (from decreased liver function) leads to cardiac failure edema, etc called "**Hydrops fetalis**"



Bilirubin



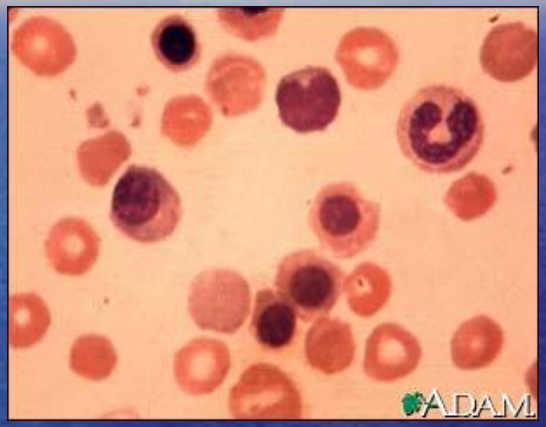
- Hemoglobin is metabolized to bilirubin
  - Before birth, "indirect" bilirubin is transported across placenta and conjugated in maternal liver ("direct") where it is excreted
  - After birth, the newborn liver is unable to conjugate the bilirubin
    - Unconjugated ("indirect") bilirubin can reach toxic levels (18-20 mg/dL)
    - This is called **kernicterus** and can lead to permanent brain damage

Laboratory Findings

Vary with severity of HDN and include:

- **Anemia**
- **Hyperbilirubinemia**
- **Reticulocytosis** (6 to 40%)
- ↑ **nucleated RBC count** (>10/100 WBCs)
- **Thrombocytopenia**
- **Leukopenia**
- **Positive Direct Antiglobulin Test**
- **Hypoalbuminemia**
- **Rh negative blood type or ABO incompatibility**
- **Smear:** polychromasia, anisocytosis, no spherocytes

Blood Smear



- Polychromasia
- Anisocytosis
- Increase NRBCs
- no spherocytes

Rh Antibodies

↓

Antibodies Coated Red Cells

↓

Destruction of Fetal Cells by Fetal RES

↓

Fetal Anemia

↓

Fetal Hypoxia and Stimulate of Erythropoietin

↓

Extra Medullary Red Cells Synthesis

↓

Hepatomegaly

↓

Hepatic Cell Failure

↓

Hypoproteinemia, Increased Intrahepatic Pressure, Portal hypertension

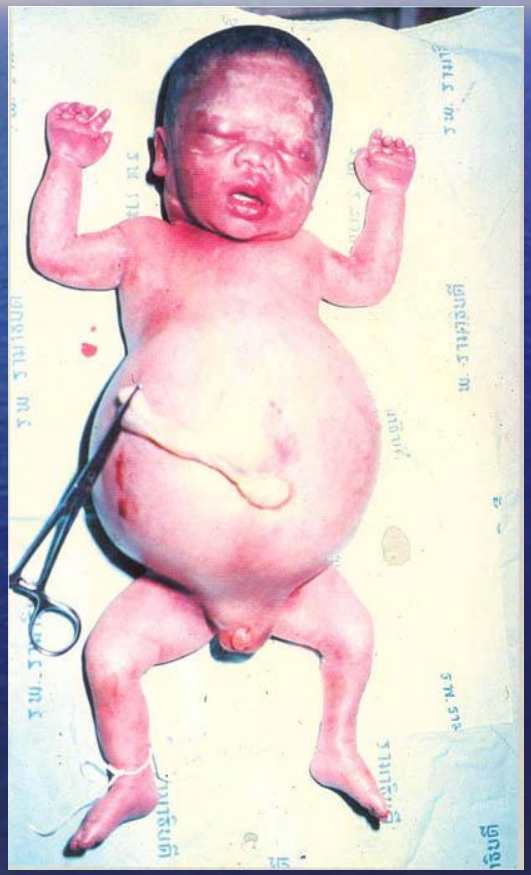
↓

Ascites, Edema, hypoxia, Placental Thickness, Polyhydramnios, Pericardial effusion

Complications of Fetal-Neonatal Anemia:

- Fetal Hydrops And IUFD
- Hepatosplenomegaly
- Neonatal Jaundice
- Compilations Of Neonatal Kernicterus (Lethargy, Hypertonicity, Hearing Loss, Cerebral Palsy And Learning Disability)
- Neonatal Anemia

Hydrops Fetalis



Management

- **Prevention**
- **Treatment:**

Prevention of Rh Isoimmunization

Prevention of Rh Isoimmunization

Prophylaxis during pregnancy in the absence of immunization of pregnant.

A by intramuscular administration of 1 dose (300 micrograms) of anti-Rho (D) immunoglobulin, which is allowed to use during pregnancy:

- at 28-32 weeks of gestation;
- if symptoms of miscarriages before 28 weeks;
- after amniocentesis or chorion biopsy;
- after ectopic pregnancy;
- after termination of pregnancy (within 48 hours after abortion);
- after accidental transfusion of blood Rh-positive Rh-negative women;
- after transfusion of platelet mass;
- in clinical situations accompanied by hit cells in the fetal blood stream of mother:

1. placental abruption, or uterine bleeding (etiology unclear);
2. Mother's trauma.

Dose of prophylactic Anti-D Ig:

- In term pregnancy before 13 weeks dose of anti-Rho (D) antibody is 75 micrograms, at the term of pregnancy over 13 weeks - 300 mcg.

Prevention of Rh Isoimmunization

Prevention of postpartum birth Rh-positive child: during the first 72 hours by intramuscular put 1 dose (300 micrograms) of anti-Rho (D) immunoglobulin.

Contraindications to the introduction of anti-Rho (D) immunoglobulin:
Known anaphylactic or severe systemic reaction to human globulin.

Prevention of Rh Isoimmunization

Prevention of hypertension in the system AB0 during pregnancy is not performed.

Pseudoreaction drug prevention and treatment of pregnant Rh-conflict does not take place.

Management of cases of Rh isoimmunization

- Diagnosis Of RH Isoimmunization
- Evaluation of Fetal Condition

Diagnosis of Rh isoimmunization

- **Family history**: a blood transfusion without regard to Rh-ownership, abortion, stillbirth or birth of children with hypertension, information about specific prevention izoimunizatsiyi in previous pregnancies.
- **Determination of titer of Rh-AB** in the dynamics of early pregnancy. Growth and instability titer Rh-AB show Rh-conflict. When titer of 1:32 or higher hypertension occurs more frequently, the risk of fetal death is high.
- **Definition group AB** conducted in pregnant women with 0 (I) blood group, who have a history of spontaneous abortion, stillbirth, infant death from hypertension.
- Diagnosis of hypertension fetus.

Diagnosis Maternal Isoimmunization

Antibody Titre in Saline: RhD-positive cells suspended in saline solution are agglutinated by IgM anti-RhD antibody, but not IgG anti-RhD antibody. Thus, this test measure IgM, or recent antibody production.

Antibody Titre in Albumin: Reflects the presence of any anti-RhD IgM or IgG antibody in the maternal serum.

The Indirect Coombs Test:

- **First Step:**
  RhD-positive RBCs are incubated with maternal serum
  Any anti-RhD antibody present will adhere to the RBCs.

- **Second Step**:
  The RBCs are then washed and suspended in serum containing antihuman globulin (Coombs serum).
  Red cells coated with maternal anti-RhD will be agglutinated by the antihuman globulin (positive indirect Coombs test).

The Direct Coombs Test

- Is Done After Birth To Detect The Presence Of Maternal Antibody On The Neonate's RBCs.
- The Infant's RBCs Are Placed In Coombs Serum.
- If The Cells Are Agglutinated This Indicate The Presence Of Maternal Antibody

Fetal Rhesus Determination

- RHD Type And Zygosity (If RHD-positive) Of The Father
- Amniocentesis To Determine The Fetal Blood Type Using The Polymerase Chain Reaction (PCR)
- Detection Of Free Fetal RHD DNA (FDNA) Sequences In Maternal Plasma Or Serum Using PCR
- Flow Cytometry Of Maternal Blood For Fetal Cells

Management of cases of Rh isoimmunization

- Diagnosis Of RH Isoimmunization
- Evaluation of Fetal Condition

Goals of managing Fetal Alloimmunization:

- Initially detecting fetal anemia prior to the occurrence of fetal compromise.

- Minimize fetal morbidity and mortality by correcting this anemia until fetal lung maturity and delivery can be achieved.

Evaluation of Fetal Condition

- Past Obstetric History
- Measurements Of Antibodies in Maternal Serum
- Determination of Fetal Rh Blood Group
- Ultrasonography
- Amniocentesis
- Fetal Blood Sampling

Past Obstetric History:

Although not reliably accurate in predicting severity of fetal disease, past obstetrical history can be somewhat prognostic

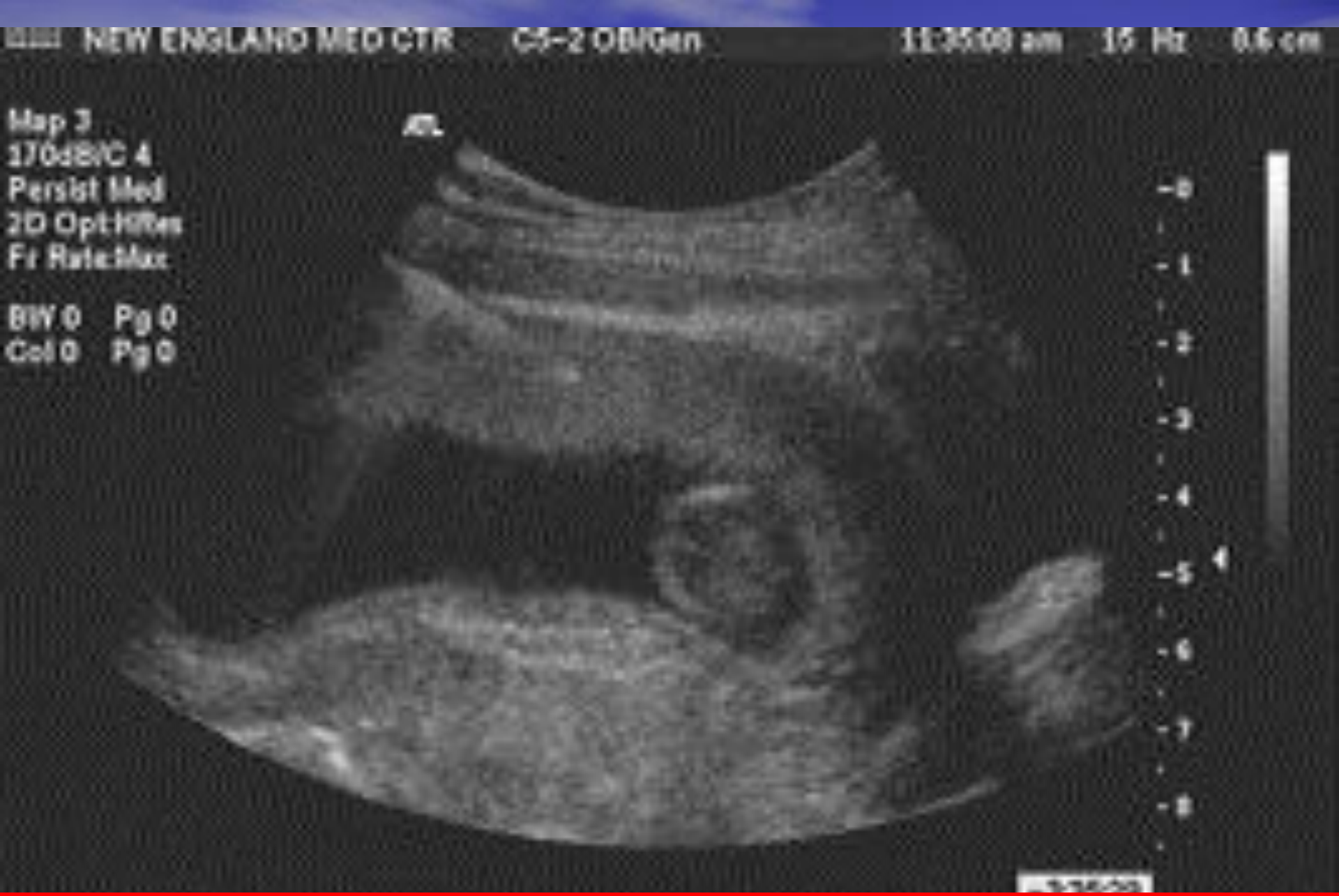
Maternal Anti-D Titer

- **Antibody Titer Is A Screening Test.**

  A Positive Anti-d Titer Means That The Fetus Is At Risk For Hemolytic Disease, Not That It Has Occurred Or Will Develop.

- Variation In Titer Results Between Laboratories And Intra Laboratory Is Common.

- A Truly Stable Titer Should Not Vary By More Than One Dilution When Repeated In A Given Laboratory.



Ultrasound Image of Transabdominal Chorion Villus Sampling

Ultrasonography

- To Establish The Correct Gestational Age.
- In Guiding Invasive Procedures And Monitoring Fetal Growth And Well-being.
- Ultrasonographic Parameters To Determine Fetal Anemia:
  - Placental Thickness.
  - Umbilical Vein Diameter
  - Hepatic Size.
  - Splenic Size.
  - Polyhydramnios.
  - Fetal Hydrops (e.g. Ascites, Pleural Effusions, Skin Edema).

Ultrasound scanning enables to establish the early signs of dropsy fetal dropsy fetal and that developed

Signs of early fetal dropsy:

- polihidramnion;
- hepatosplenomehaliya.

Symptoms of dropsy of the fetus that has developed:

- increased echogenicity of fetus colon;
- cardiomegaly and pericardial effusion;
- hidrothoraxis and ascites;
- swelling of the scalp and extremities;
- unusual outside of the fetus;
- reduction of physical activity;
- thickening of the placenta.

In pregnant women at risk for the emergence of Rh conflict ultrasound transmitting:

- Up to 30 weeks of pregnancy 1 per month;
- after 30 weeks, 2 times a month;
- when there are signs of fetal every day to delivery.

Doppler Velocimetry Of The Fetal Middle Cerebral Artery (MCA)

- For Predicting Fetal Anemia

- Cardiotocography is showing signs of chronic hypoxia and reduced compensatory ability of the fetoplacental complex.

Invasive Techniques

Amniocentesis

Fetal Blood Sampling

Transabdominal amniocentesis performed in the period after 26 weeks of pregnancy.

Questions about the need to solve the amniocentesis, depending on the titer of AB and data history. If there are indications for amniocentesis woman is sent to the highly Clinic.

Indications for amniocentesis:

- AB titer equal to or exceeding 1:64;
- increase in titer 4 times during the second study after 2 weeks;
- AB increase titer and ultrasound signs of fetal hypertension;
- stillbirth, birth of children with a history of hypertension and ultrasound signs of fetal hypertension.

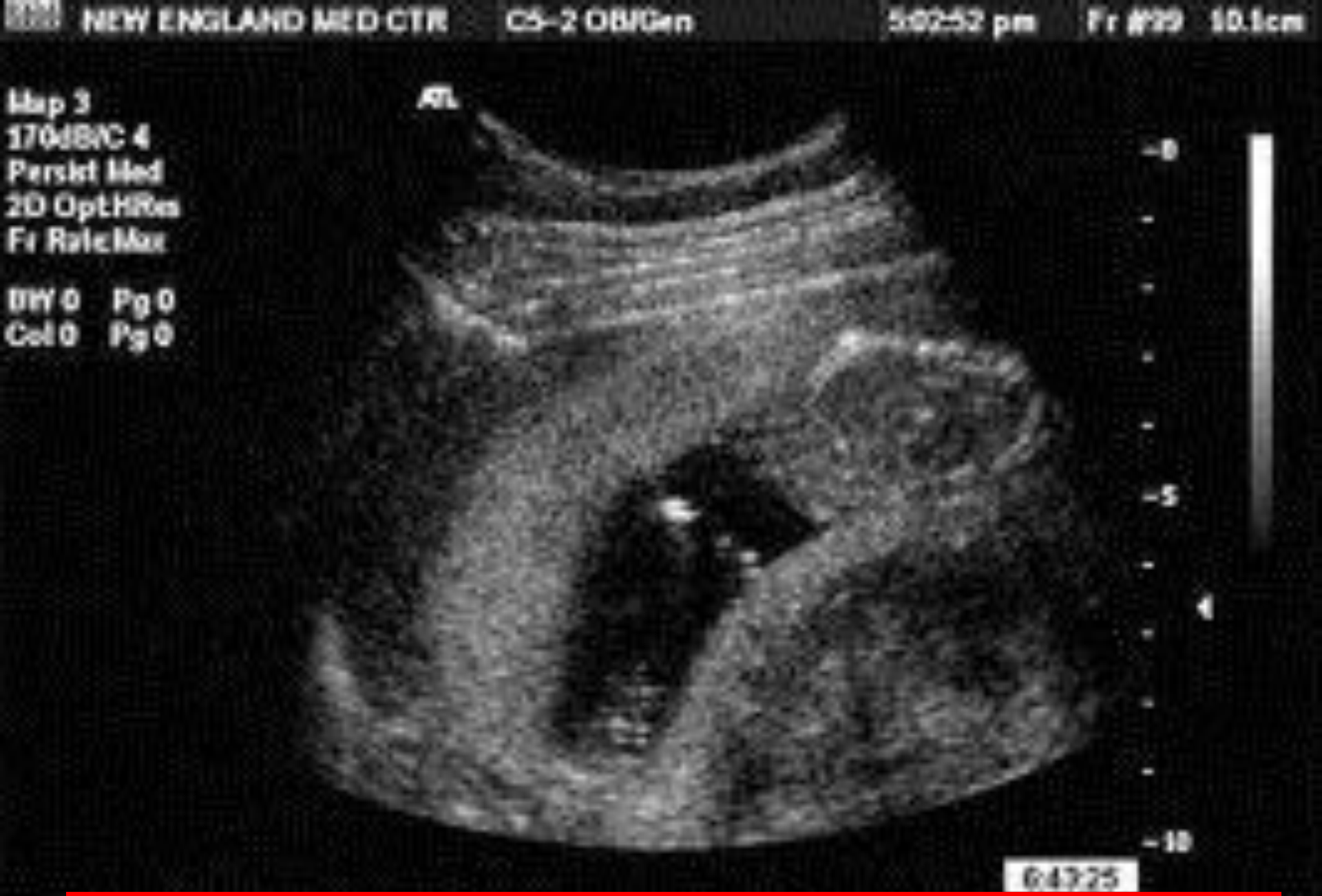
Contraindications:

- the threat of premature birth;
- fever.

Studies of amniotic fluid to assess the severity of fetal anemia.

In cases of fetal hypertension increase the concentration of bilirubin in fetal growth rate and water optical density of membranes (WODM) reflects the severity of hypertension.

- If WODM 0.1 and below, you can prolong the pregnancy to delivery on time.
- When WODM 0.15 and above begin preparations for delivery.



Ultrasound image of amniocentesis at 16 weeks of gestation

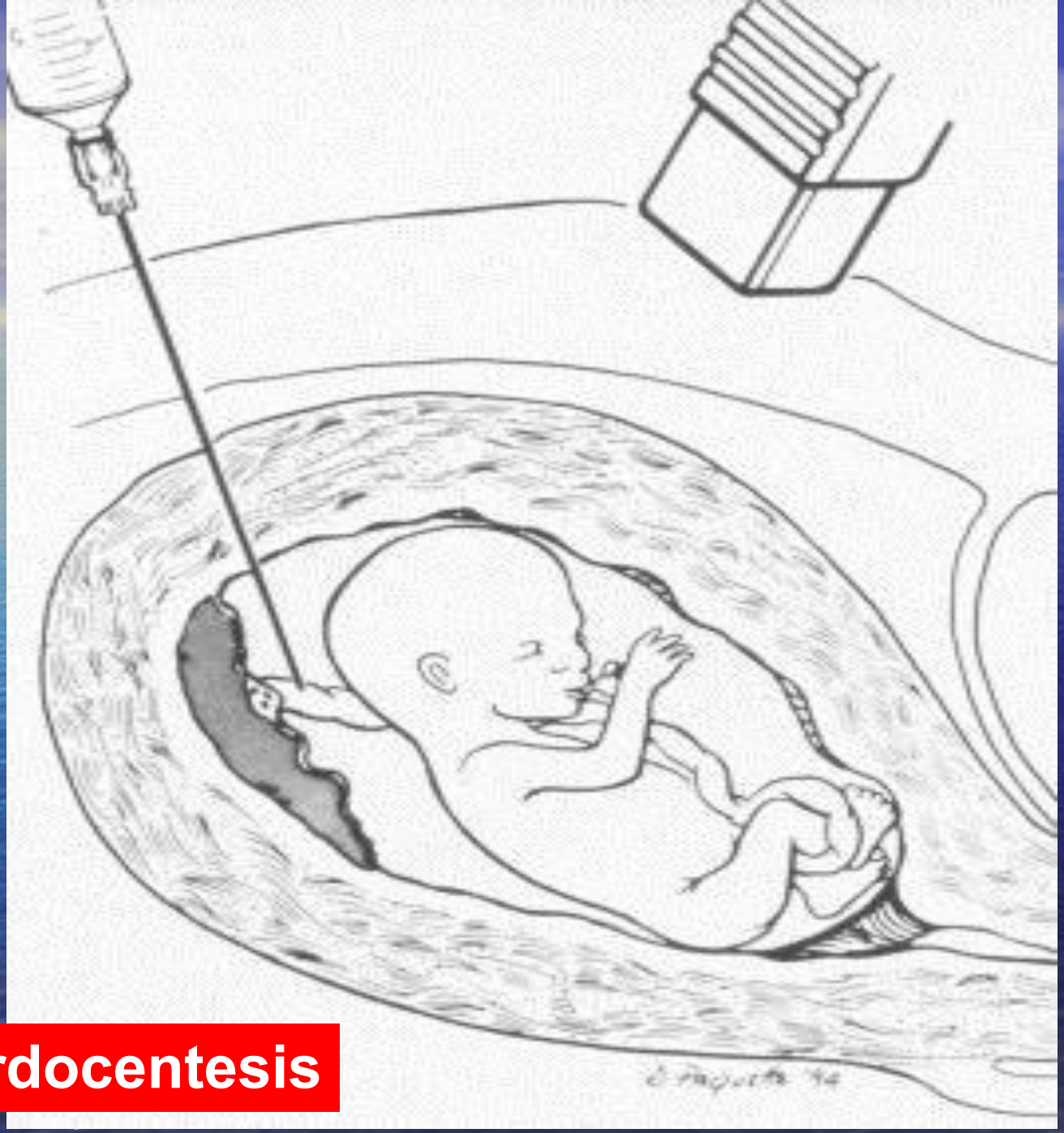
- **Cordocentesis** - taking blood from the umbilical cord through the anterior abdominal wall women.
  In determining fetal cord blood:
  - hemoglobin and hematocrit;
  - blood group and Rh-factor;
  - the level of bilirubin;
  - the number of reticulocytes;
  - protein;
  - AT, fixed on erythrocytes of the fetus.

Fetal blood sampling:

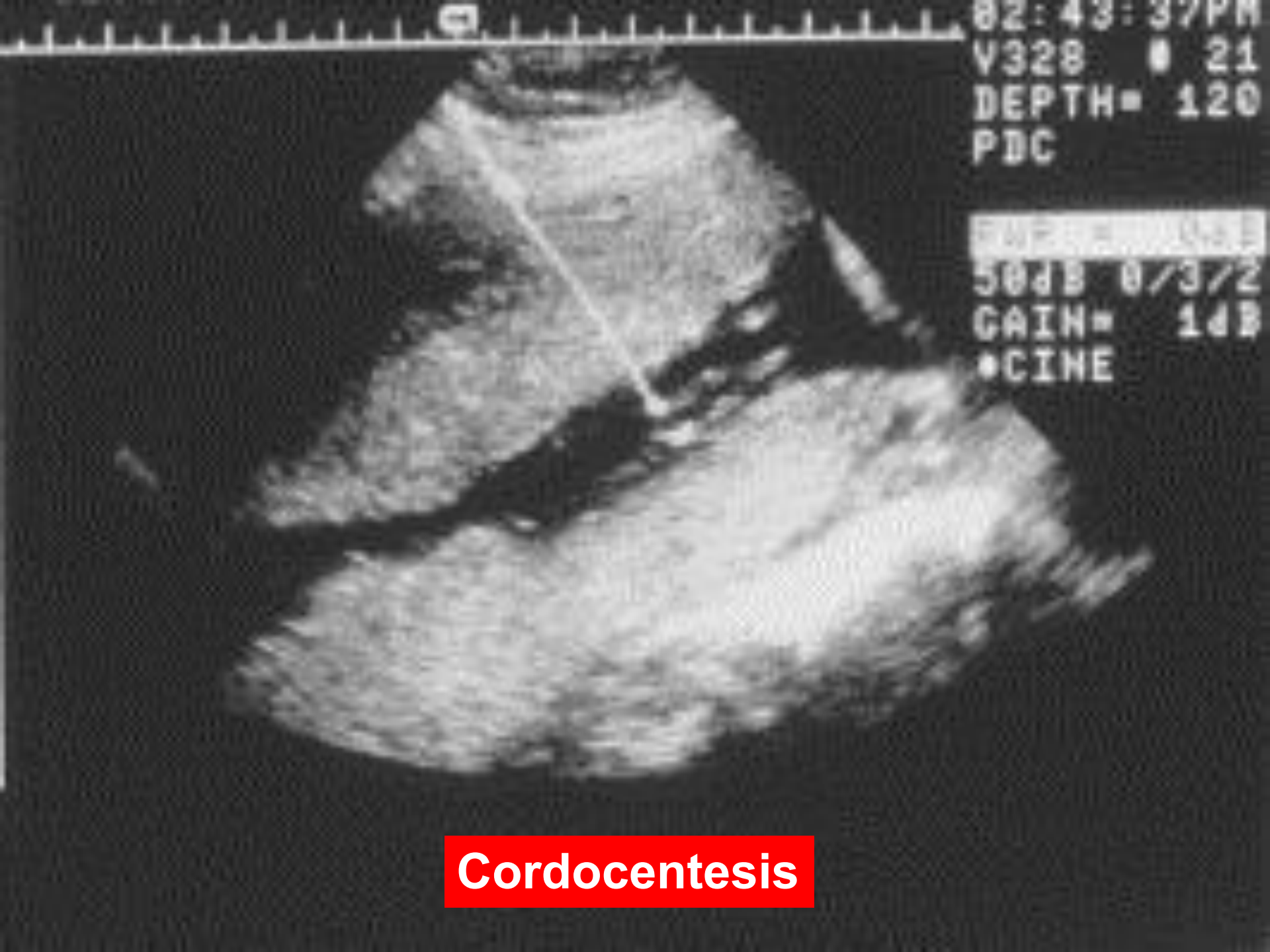
Is the gold standard for detection of fetal anemia.

Complications:

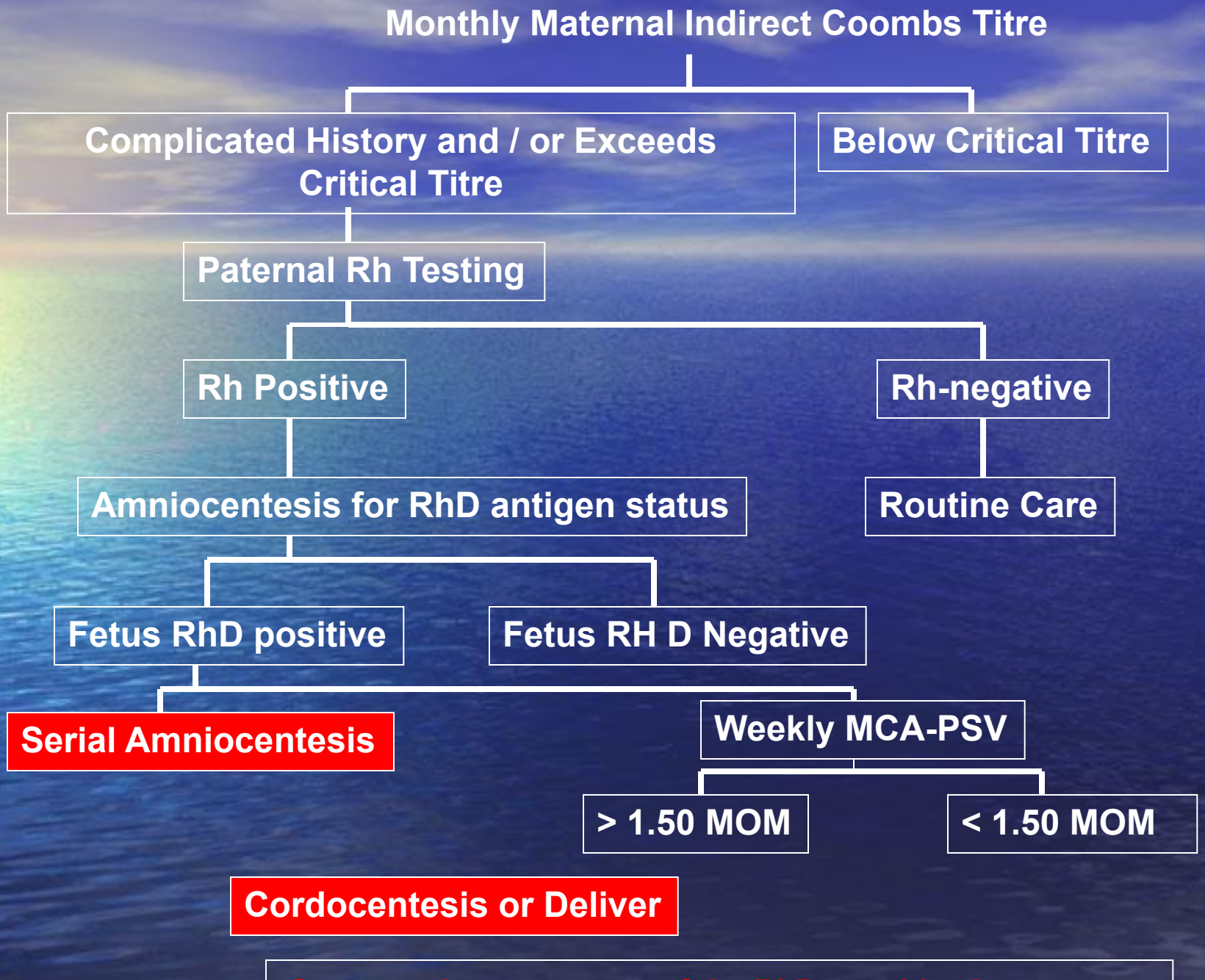
- Total Risk of Fetal Loss Rate 2.7% (Fetal death is 1.4% before 28 weeks and The perinatal death rate is 1.4% after 28 weeks).
- Bleeding from the puncture site in 23% to 53% of cases.
- Bradycardia in 3.1% to 12%.
- Fetal-maternal hemorrhage: occur in 65.5% if the placenta is anterior and 16.6% if the placenta is posterior.
- Infection and abruptio placentae are rare complications



Cordocentesis



Cordocentesis



Suggested management of the RhD-sensitized pregnancy

Antibody Titer in maternal blood

Titers greater than 1:4 should be considered Rh alloimmunized. However, the threshold for invasive fetal testing varies at different institutions and generally is 1:16 or greater because these titers have been associated with fetal hydrops

Indications for early obstetrical complications in Rh-conflict:

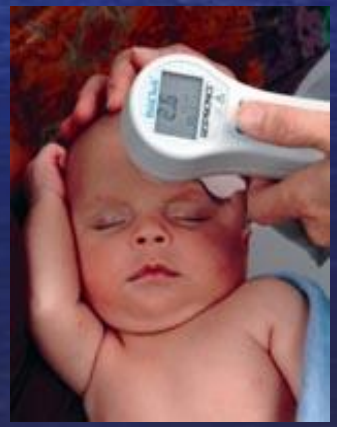
1. AB titre equal to or more than 1:64 (critical level);
2. Increase in titer re-analysis of 4 times;
3. The water optical density of membranes 0,35-70 or above the concentration of bilirubin in amniotic fluid 4,7-9,5 mg / l;
4. Ultrasonic signs of hypertension in the fetus;
5. Stillbirth and birth of children with a history of hypertension.

Spectrophotometric measurements of bilirubin in amniotic fluid

Because the wavelength at which bilirubin absorbs light is 420-460 nm, the amount of shift in optical density from linearity at 450 nm in serial amniotic fluid samples can be used to estimate the degree of fetal hemolysis.

Transcutaneous Monitoring

- Transcutaneous bilirubinometry can be adopted as the first-line screening tool for jaundice in well, full-term babies.
- This leads to about 50% decrease in blood testing.



❖TREATMENT

- Exchange transfusion
- Phototherapy

Intrauterine Transfusion (IUT)

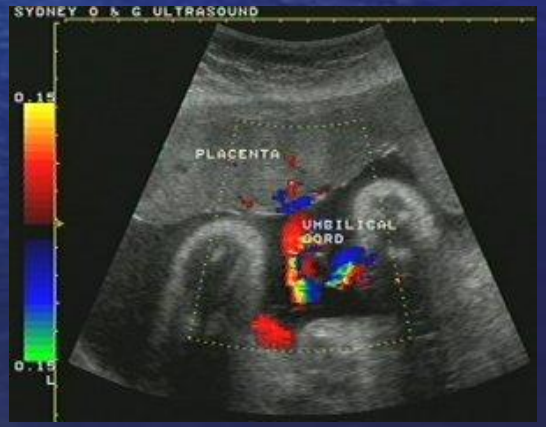
- Given to the fetus to prevent hydrops fetalis and fetal death.
- Can be done as early as 17 weeks, although preferable to wait until 20 weeks
- Severely affected fetus, transfusions done every 1 to 4 weeks until the fetus is mature enough to be delivered safely. Amniocentesis may be done to determine the maturity of the fetus's lungs before delivery is scheduled.
- After multiple IUTs, most of the baby's blood will be D negative donor blood, therefore, the Direct Antiglobulin test will be negative, but the Indirect Antiglobulin Test will be positive.
- After IUTs, the cord bilirubin is not an accurate indicator of rate of hemolysis or of the likelihood of the need for post-natal exchange transfusion.

Intrauterine Transfusion

- An intrauterine fetal blood transfusion is done in the hospital. The mother may have to stay overnight after the procedure.
- The mother is sedated, and an ultrasound image is obtained to determine the position of the fetus and placenta.
- After the mother's abdomen is cleaned with an antiseptic solution, she is given a local anesthetic injection to numb the abdominal area where the transfusion needle will be inserted.
- Medication may be given to the fetus to temporarily stop fetal movement.
- Ultrasound is used to guide the needle through the mother's abdomen into the fetus's abdomen or an umbilical cord vein.
- A compatible blood type (usually type O, Rh-negative) is delivered into the fetus's abdominal cavity or into an umbilical cord blood vessel.
- The mother is usually given antibiotics to prevent infection. She may also be given tocolytic medication to prevent labor from beginning, though this is unusual.

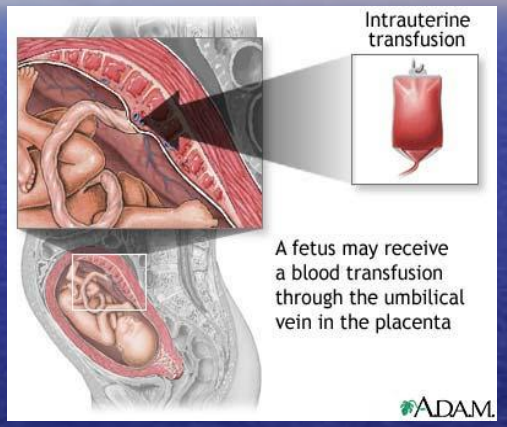
Intrauterine Transfusion

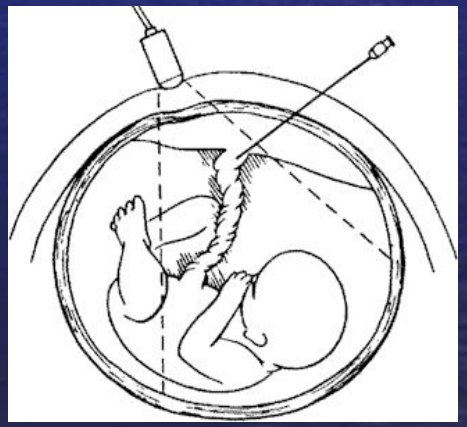
- Increasingly common and relatively safe procedure since the development of high resolution ultrasound particularly with colour Doppler capability.
- MCA Doppler velocity as a reliable non-invasive screening tool to detect fetal anemia.
  - The vessel can be easily visualized with color flow Doppler as early as 18 weeks' gestation.
  - In cases of fetal anemia, an increase in the fetal cardiac output and a decrease in blood viscosity contribute to an increased blood flow velocity



Intrauterine Transfusion

- The risk of these procedures is now largely dependent on the prior condition of the fetus and the gestational age at which transfusion is commenced.

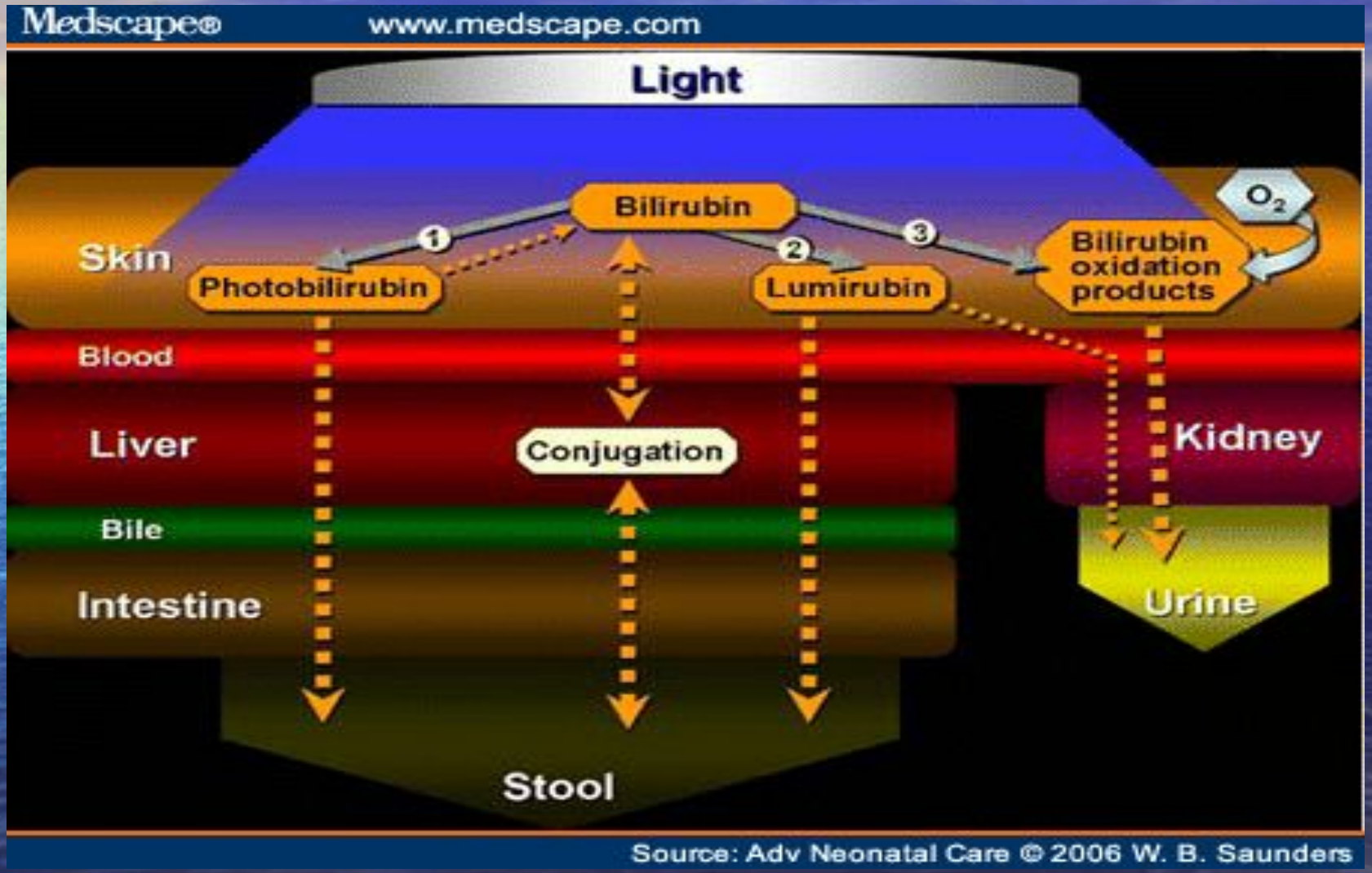




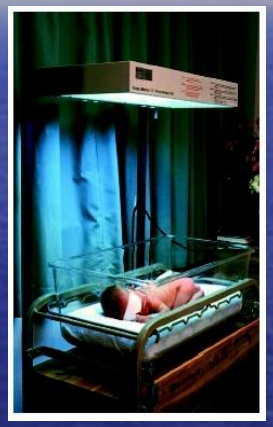
Treatment of Mild HDN

- Phototherapy is the treatment of choice.
- Phototherapy process slowly decomposes/converts bilirubin into a nontoxic isomer, ***photobilirubin,*** which is transported in the plasma to the liver.
- HDN is judged to be clinically significant (phototherapy treatment) if the peak bilirubin level reaches 12 mg/dL or more.

Bilirubin Degradation by Phototherapy

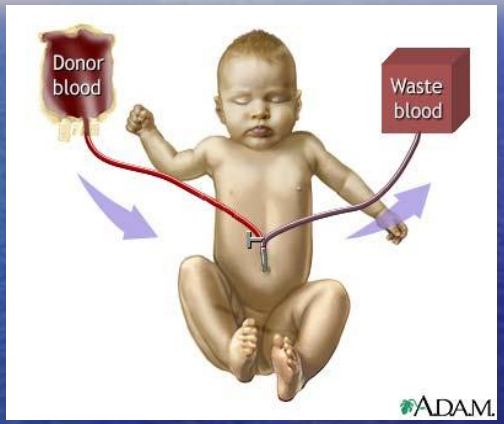


Phototherapy



- The therapy uses a blue light (420-470 nm) that converts bilirubin so that it can be excreted in the urine and feces.
- Soft eye shields are placed on the baby to protect their eyes from damage that may lead to retinopathy due to the bili lights.

Exchange Transfusion



- Full-term infants rarely require an exchange transfusion if intense phototherapy is initiated in a timely manner.
- It should be considered if the total serum bilirubin level is approaching 20 mg/dL and continues to rise despite intense in-hospital phototherapy.
- The procedure carries a *mortality rate of approximately 1%* and there may be substantial morbidity

Goals of Exchange Transfusion

- Remove sensitized cells.
- Reduce level of maternal antibody.
- Removes about 60 percent of bilirubin from the plasma, resulting in a clearance of about 30 percent to 40 percent of the total bilirubin.
- Correct anemia by providing blood that will have normal survival.
- Replacement with donor plasma restores albumin and any needed coagulation factors.
- Rebound – usually a 2 volume exchange is needed as bilirubin in tissues will return to blood stream.

Summary

- All types of HDN vary in severity.
- Laboratory testing key to diagnosing and monitoring- great care to be taken when interpreting ABO/D typing on affected infants.
- Therapy dependent on severity: phototherapy alone or with transfusion.