Reviewed By
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Video Case

Rhesus Isoimmunization

Objectives:

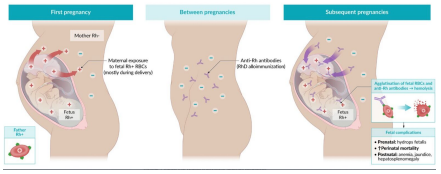
- → Define Rh Isoimmunization and determine its incidence.
- → Describe the indications, timing, and benefits for immunoglobulin administration / prophylaxis during pregnancy for the prevention of alloimmunization
- → Describe the pathophysiology and diagnosis of alloimmunization
- → Discuss the management of a patient with Rh-D sensitization in pregnancy

- → Slides
- → Important
- → Golden notes
- → Extra
- → 439 Doctor's notes
- → 441 Doctor's notes
- → 441 Female Presentation
- → Reference

Female Presentation

Video from Kaplan | Editing File

Rh Isoimmunization

Definition	When a pregnant woman develops **antibodies to foreign red blood cells (RBCs)**, most commonly against those of her **current or previous fetus(es)**. It is rarely caused by transfusion of mismatched blood.
Pathophysiology	• The initial response to exposure to Rh antigen is the **production of immunoglobulin M (IgM)** antibodies for a short period of time, followed by the **production of IgG antibodies** that are capable of crossing the placenta. If the fetus has the Rh antigen, these antibodies will coat the fetal red blood cells and cause hemolysis.  • The most common RBC antigens are of the Rh system (C, c, D, E, e) (**most common is big D**). • Antibodies to RBC antigens are detected by **indirect Coombs test** (atypical antibody test [AAT]). The concentration of antibodies is reported in dilutional titers with the lowest level being 1:1, and titers increasing by doubling (e.g., 1:1, 1:2, 1:4, 1:8, 1:16, 1:32…1:1,024, etc.).
Clinical features	• **Hemolytic disease of the newborn (HDN)** is a continuum ranging from hyperbilirubinemia to erythroblastosis fetalis. HDN is caused by maternal antibodies crossing into the fetal circulation and targeting antigen-positive fetal RBCs, resulting in hemolysis. When severe, this can result in anemia, fetal hydrops (parvovirus B19 is the most common cause of this condition), and even death. • **Prenatal** Hydrops fetalis (severe fetal anemia → hypoxia → ↓ hepatic and renal blood flow → activation of RAAS → ↑ central venous pressure and ↓ lymphatic flow → fetal edema) • **Postnatal** ○ Neonatal anemia ○ Hepatosplenomegaly (Increased destruction of erythrocytes in the spleen leads to a compensatory increase in hematopoiesis in the liver). ○ Neonatal jaundice - Usually present at birth or manifests within the first 24 hours of life - In Rh incompatibility, unconjugated bilirubin levels may be dangerously high, causing **kernicterus**. ○ Hypoxia (Via increased RBC destruction and acidosis) ○ Prematurity
Risk Factors	• Alloimmunization most commonly occurs when **fetal RBCs enter the mother's circulation** transplacentally at delivery. • It can also occur if a woman is **transfused** with mismatched RBCs. • Other pregnancy-related risk factors are: Amniocentesis, Ectopic pregnancy, D&C, Abruptio placentae & Placenta previa.
Protective Factors	**ABO incompatibility** decreases the risk of maternal alloimmunization from foreign RBCs. Naturally occurring anti-A and anti-B antibodies rapidly lyse foreign RBCs before maternal lymphocytes are stimulated to produce active antibodies.
Requirements (all must be present)	• **Mother** must be **antigen-negative.** • **Fetus** must be **antigen-positive**, which means the **father** must also be **antigen +ve.** • Adequate fetal RBCs must cross over into the maternal circulation to stimulate her lymphocytes to produce antibodies to the fetal RBC antigens. • Antibodies must be associated with Hemolytic disease of the newborn **(Erythroblastosis fetalis).** • Significant titer of maternal antibodies must be present to cross over into the fetal circulation and lead to fetal RBC hemolysis **(>1:16).**

- If the mother is antigen-**positive** and her husband is antigen-**negative**, there is no risk. Similarly, if both are positive or both are negative.
- If the mother is antigen-negative and her husband is antigen-negative the risk for Rh sensitization is 0%
- If the mother is Rh negative and the father is Rh negative the percentage of their child being Rh negative is 100%
- Fetal blood type may be determined by amniocentesis or percutaneous umbilical blood sampling (PUBS). If the fetus is RBC antigen-negative, there is no fetal risk.

Rh Isoimmunization

Detecting fetomaternal/transplacental hemorrhage:

- **Rosette test** (initial test of choice) is a **qualitative** screening test for detecting significant feto-maternal hemorrhage (≥10 mL). (blood is collected and placed on a slide, then the blood is exposed to an anti-D RhIg like RhoGAM. The blood is then observed for clumping. If clumping occurs, this means the blood sample contains Rh-positive HbF, and FMH is present. The clumping blood sometimes forms the shape of a rose, which is why the test is called the Rosette test. The reason why this clumping occurs is because the RhoGAM binds to Rh-positive fetal blood cells. When antibodies from the RhoGAM and antigens from the HbF meet, they stick together and clump up).
- If the rosette test is positive, conduct a Kleihauer-Betke test.
- **Kleihauer-Betke test** (acid elution test) **quantitates** the volume of fetal RBCs in the maternal circulation by differential staining of fetal and maternal RBCs on a peripheral smear. (If blood cells are from the fetus, exposure to the acid will leave them **red** because fetal blood cells are acid-resistant. If blood cells are from an adult, exposure to the acid will make them lose their color and turn **pale white.**) This can assess whether more than one vial of RhoGAM needs to be given when large volumes of fetal–maternal bleed may occur (e.g., abruptio placentae).
- It is dependent on the fact that adult hemoglobin is more readily eluted through the cell membrane in the presence of acid than is fetal hemoglobin (**HbF**). A maternal blood smear is exposed to acid and then stained. Adult hemoglobin is removed by the acid whereas fetal hemoglobin (HbF) is not, thus, cells appear pink on a positive test for fetal hemoglobin.
- # of fetal cells counted/ # of maternal cells counted = Estimated fetal blood volume (mL)/ Estimated maternal blood volume (mL).

Techniques to evaluate fetal Rh status:

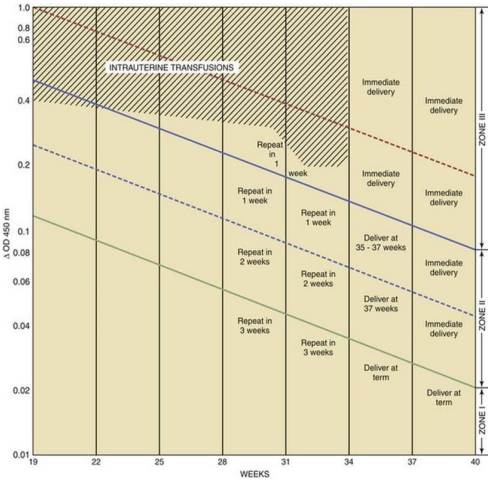
- Amniocentesis: The incidence of fetomaternal hemorrhage with amniocentesis → 8.4-11% per procedure.
- Free fetal DNA in maternal serum
- U/S (we may see hydrops*)
- **MCA doppler** (most valuable to detect fetal anemia)
- Amniotic fluid spectrophotometry (best to estimate fetal bilirubin concentration)** (Reference: not used anymore and replaced with MCA doppler due to high complication rate)
- Liley chart or modified Liley chart (Queenan chart)
- Percutaneous umbilical blood sampling (PUBS) → we can measure fetal Hb, Hct, blood gases, pH, and bilirubin levels.
  The incidence of fetomaternal hemorrhage with PUBS → as high as 40%.

*Both the placenta and the fetal liver are enlarged with hydrops. Fetal hydrops is easily diagnosed by the characteristic appearance of one or more of the following: ascites, pleural effusion, pericardial effusion, or skin edema.

**There is an excellent correlation between the amount of biliary pigment in the amniotic fluid and the fetal hematocrit, beginning at 27 weeks' gestation.

Rh Isoimmunization

Management:

1- Determine if the fetus is at risk for anemia	• **Fetal risk is present** only if - Atypical antibodies are detected in the mother 's circulation. - Antibodies are associated with HDN. - Antibodies are present at a significant titer (>1:8). - The father of the baby (FOB) is RBC antigen-positive. • **No fetal risk is present** if - The AAT is negative. - Antibodies are present but are NOT associated with HDN. - Antibody titer is ≤1:8. - The FOB is RBC antigen-negative. • **If the atypical antibody titer** is ≤1:8, management is conservative. Repeat the titer monthly as long as it remains ≤1:8.
2- Assess if the fetus is anemic using Doppler ultrasound	• Doppler ultrasound measures peak flow velocity of blood through the fetal middle cerebral artery (MCA). As fetal anemia worsens, the peak systolic velocity rises. Doppler MCA ultrasound is the **procedure of choice** since it is **non-invasive and has a high correlation with fetal anemia.** • Increased flow rate in the middle cerebral artery (MCA) should raise suspicion for fetal anemia and may require further tests (e.g., cordocentesis) to confirm the diagnosis. • It is the first step if the pregnant lady presents to the clinic with an increasing anti-D titer
3- Intervene if the anemia is severe	This is diagnosed when amniotic fluid bilirubin is in Liley zone III or PUBS shows fetal hematocrit to be ≤25% or MCA flow is elevated. • If gestational age <34 weeks, perform intrauterine intravascular transfusion. (150ml of Rh -ve blood/ kg) • If gestational age ≥34 weeks, perform delivery.
How to Manage the Baby?	➔ Intrauterine transfusion (fresh O Rh- blood and packed RBCs, repeat transfusions are scheduled at 1 to 3 week intervals, last transfusion should be between 32 and 34 weeks). ➔ Intraperitoneal transfusion* (RBCs are absorbed via the diaphragmatic lymphatics, non-hydropic fetuses absorption should occur in 7 to 9 days, in hydroptic ones it's variable) ➔ Maternal Plasmapheresis ➔ Phenobarbital (Has been shown to induce fetal liver enzyme activity and maturation, this is used 2-3 weeks before delivery) *Formula for intraperitoneal transfusion: Volume = [GA (wks) – 20] x10 

Rh Isoimmunization

Timing of delivery:

- Fetuses are evaluated at least twice weekly from **24 to 28 weeks** for fetal well-being (NST, modified biophysical profile) and fetal growth.
- Delivery is performed if gestational age is >34 week.
- If delivery is expected to occur before 34 weeks' gestation (or if amniocentesis suggests an immature lung profile), **betamethasone** should be given at least 48 hours before delivery to enhance fetal pulmonary maturation.

Prevention:

- RhoGAM is pooled anti-D IgG passive antibodies that are given IM to a pregnant woman when there is significant risk of fetal RBCs passing into her circulation. The passive IgG antibodies attach to the foreign RBC antigens, causing lysis to occur before the maternal lymphocytes become stimulated.
- **RhoGAM** is routinely given to Rh(D)-negative mothers **at 28 weeks,** and within 72 h of chorionic villus sampling (CVS), **amniocentesis**, or D&C. It is also given **within 72 h of delivery** of an Rh(D)-positive infant. About 300 mcg of RhoGAM will neutralize **15** ml of fetal RBCs or **30 mL** of fetal whole blood. (Dr:The numbers important) (We should give the mother RhoGAM before the immune system of the mother recognizes the antigen; if we give it to her after 72 hours, the immune system of the mother will develop the same antibody.)
- All pregnant women who are RhD -ve and Anti D -ve and experience→ (spontaneous or induced abortion, ectopic pregnancy, significant vaginal bleeding, abdominal trauma, or external cephalic version) should receive 50 to 100 µg before 12 week of gestation and 300 µg after 12 week.
- RhO-GAM is probably not necessary for "complete" molar pregnancy, but necessary for "partial" molar pregnancy.

Irregular antibodies:

- Kell Antibodies can elicit a strong IgG reaction similar to Rh isoimmunization.
- In Kell isoimmunization, the anemia is due to more of suppression of hematopoiesis rather than hemolysis.
- The predictor of anemia in this case is still the MCA PSV. (Like in Rh)

- Fetus can compensate for mild anemia hat is caused by hemolysis. So we only intervene if severe.
- If a woman has a previous pregnancy with fetal hydrops, there is a 90% chance of it occurring again in the next pregnancy (at the same time or earlier in the pregnancy).
- Only direct measure of fetal anemia is PUBS = Cordocentesis.
- Increasing levels of bilirubin will lead to fetal kernicterus which leads to cerebral palsy.
- In Intrauterine transfusions, the goal is to transfuse fresh group O, Rh- negative packed red blood cells/ Overall survival rate following intrauterine transfusion is about 85%.

Teaching case

A 32 year-old P1101 woman and her new husband present for prenatal care at 20 weeks gestation. Her past obstetric history is significant for a first child delivered at term following an abruption. Her second child died of complications of prematurity following in utero transfusions for Rh alloimmunization. Her initial prenatal labs this pregnancy indicate her blood type as A negative and an antibody screen positive for anti-D with a titer of 1:256[1]. You discuss any additional evaluation needed, her risks in this pregnancy, and the plan of management with her and her husband[2].

1. What is Rh alloimmunization and what are the red cell antigens involved?

- Occurs when any fetal blood group factor (in this case the Rh antigens) inherited from the father is not possessed by the mother. Antepartum or intrapartum fetal-maternal bleeding may stimulate an immune reaction in the mother.
- Most cases of Rh alloimmunization causing significant hemolytic disease (Anemia, Anemic heart failure, Hydrops fetalis) in the fetus or newborn are the result of D antigen incompatibility.

- why we don't look for ABO types in Maternal-fetal Blood mixing? Bc antibodies against ABO antigens are IgM, which can't cross placenta.

2. What are the risk factors for Rh alloimmunization?

- Any clinical situation that could lead to fetal-maternal hemorrhage.
- **Obstetric procedure**: pregnancy termination, chorionic villus sampling, amniocentesis, external cephalic version.
- Threatened abortion, ectopic pregnancy, abortion.
- Delivery of an Rh+ neonate to an Rh- mother (cesarean or vaginal delivery)— most common cause of alloimmunization.
- Multifetal gestation/ Abdominal trauma.
- Bleeding placenta previa or abruption / Manual removal of placenta.
- Spontaneous fetal-maternal hemorrhage has been detected to 10% of cases of alloimmunization.

1. Past situation was not managed appropriately, Either they didn't give her Anti D or she did not receive enough dose.
2. If the new husband is Rh -ve there is no need to follow up regarding Rh Alloimmunization, you might have a Q with same scenario and than ask you what you should do? Do nothing, or what's the chance that fetus will be effected by antibodies? 0%.

Teaching case

3. What is the mechanism for RhoGAM prophylaxis against Rh disease? What is the dose of RhoGAM? What is the recommended schedule for RhoGAM administration?

- Exogenous IgG (Rho(D) immune globulin) suppresses the maternal immune response through central inhibition, peripherally destroy feted RBCs. The Rh D IgG coated fetal RBCs are sequestered in the maternal spleen and these antigen antibody complexes inhibit the primary immune response (B cell transformation to plasma cells) and antigen specific B cell proliferation.
- 300 micrograms of anti-D immune globulin can prevent Rh D alloimmunization after an exposure to up to 30 mL of Rh D-positive blood or 14 mL of fetal cells.
- In the U.S. for Rh-mothers, the recommended immunoprophylaxis regimen using anti-D immunoglobulin is:

- 300 mcg dose at 28 week EGA.
- Second 300 mcg dose should be given if delivery has not occurred within 12 weeks of the initial dose.
- Within 72 hours after delivery of an Rh+ neonate.
- After first trimester pregnancy loss, threatened abortion, or elective termination.
- After invasive antepartum procedures or external cephalic version or trauma.
- After second or third trimester bleeding.

4. Could this patient's Rh alloimmunization have been prevented? What are the ways in which alloimmunization might be diagnosed? Is there any further blood work that should be obtained before you counsel this patient on her risks in this pregnancy? What are some ultrasound findings that may suggest Rh disease?

- Administration of an adequate dose of RhoGAM within approximately 72 hours prevents an active maternal antibody response to the fetal antigens. The extent of fetal to maternal hemorrhage can be estimated using the Kleihauer-Betke test.
- Maternal antibody screen is recommended at the first prenatal visit (measure the titer), at 28 weeks gestation, at the time of any event in pregnancy associated with possible fetal-maternal hemorrhage, and postpartum. Positive antibody screens should be evaluated for strength of antibody response (titer) and type of antibody. A critical titer that may be associated with fetal hemolytic disease is most often between 1:16 and 1:32.
- The paternal antigen status for the specific maternal antibody should be assessed to determine if the fetus is at risk. This assessment is accomplished by performing direct genotype testing of the father (test the new husband Rh type). If paternal testing is not possible, fetal antigen assessment can be accomplished through genetic analysis of fetal cells obtained through amniocentesis.
- Ultrasound findings consistent with severe fetal anemia include elevated peak velocity of the middle cerebral artery and evidence of hydrops fetalis (fetal subcutaneous edema, pleural and/or pericardial effusions, and ascites).

- It's important to know when to give Anti D and fetal complication.

439 Dr's Notes

- 80-85% of population are Rh positive, our concerns are the 15-20% of the population.
- Bleeding is fatal complication, blood tests are very important.
- Rh-Antigen is autosomal dominant gene, if pts is Rh +ve, Pt could have Homozygous (DD) or Heterozygous(Dd) genes, But Rh-Ve pt does not have the gene (dd).
- If the parents both are Rh-ve (dd), the chance of the fetus to be Rh-ve is 100%, but if the mother is Rh-ve (dd) and the father is Rh +ve hetero (Dd) there is 50% fetus to be Rh +ve, and 100% chance if the father is Homo (DD), Here we don't do genetic testing, We deal with the case as if the fetus is Rh +ve is the father is Rh +ve.
- In western centuries, you must conform the paternity of the husband.
- We calculate the needed dose of Anti D immunoglobulin by (Kleihauer-Betke test) where we take maternal blood and apply the test to know how many RBCs from fetal blood entered maternal circulation, and we give Anti-D immunoglobulin to destroy these cells.
- **What is Kleihauer-Betke test?** it's test to detect fetal cells in maternal circulation, Here we don't do it, we just give the maximum dose, (300 mcg = 1500 IU)
- To give anti-D immunoglobulin 72h after delivery mother must be Rh-ve and antibodies negative, while fetus is Rh+ve, The goal is to prevent maternal immune system from developing antibodies against Rh antigen.
- The **most common** cause of Rh alloimmunization is when you fail to give Anti D immunoglobulin within 72h after delivery,.
- Other causes are antenatal immunization due to antenatal bleeding: Abortion, ectopic, placenta Abruption, trauma, procedures (amniocentesis cordocentesis, chorionic villus sampling), external cephalic version, risk increase with multi-gestation.
- While fetus in uterus, must of bilirubin is conjugated and taken up by placenta, but increasing levels of bilirubin will lead to fetal kernicterus which will lead to Cerebral palsy.
- After delivery there will be Hyperbilirubinemia Unconjugated bilirubin will lead to jaundice .

441 Dr's Notes

- Senario: mother -ve and father -ve —> nothing
- Senario: if mother +ve —> we ask for antibody bc maybe she did blood transfusion and get the antibody
- Always we ask for blood type A,B,O,AB and + or - and for antibody
- ليش أول طفل مايصير له شي؟
  لأن أول يتكون IgM و هذه كبيرة ماتقدر cross placenta وتاخذ وقت عشان تصير IgG الي تقدر تعبر الplacenta
- RhoGAM give cover for 12 week
- In case fetus become **anemic** —> management blood transfusion (o-)
- If father DD(homozygot) —> fetus will be +ve no need for تحليل للطفل
- If father Dd(heterozygot) —> we check fetus +ve or -ve , if -ve nothing do, if +ve monitor
- NIPT costly تحليل الدم

if mother -ve and father +ve

-ve antibody

Give **preventive RhoGAM 300mcg ثابته الجرعة ما يحتاج تحليل** :
-At 28 week
-At delivery
-And in case of amniocentesis, CVS, D&C or postpartum hemorrhage —> we take sample to adjust the dose (the window to give is 72h)

example we found **85ml** of fetal blood as result of severe bleeding, what will be the dose of RhoGAM? 850 mcg

+ve antibody

We **don't** give RhoGAM لانها ممكن تروح للطفل وتكسر الدم حقه لذلك
- so we measure the **titer** and once the titer reaches 1:16 the **critical titer** we **start** monitoring with MCA
- The titer maybe 1:4 or 1:8 or 1:254 طيب ايش المعنى؟ في المعمل ياخذ عينة الدم ويلعب فيها يسوي dilutiion يحاول يخفف ثم يقيس الantibody الرقم 8 و 4 يعني عدد مرات التخفيف ومع ذلك تظهر ال antibody و254 رقم كبير يعني مع كل هالمرات بالتخفيف لسى يشوف antibody
- if the titer 1:4 do we start monitoring? **No** just check the titer after 4-6 weeks
- It may start with low titer and progress with time or stabilize never come down so if pt reaches critical titer no need to measure it again we just monitor the fetus if anemia symptoms occurs

Monitoring:
1-**MCA every 2 weeks** See if there is hydrops fetalis, **2** of the following:
- ascites
- Pleural effusion
- Pericardial effusion
- Polyhydramnios
- Skin edema

2-**Baby growth every 4 weeks**

Reference

Rhesus Alloimmunization

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CLINICAL KEYS FOR THIS CHAPTER

- In this chapter, the term *hydrops fetalis* refers to immune (or rhesus [Rh] antibody-mediated) hydrops fetalis and is synonymous with the older term *erythroblastosis fetalis*. Hydrops fetalis is a form of in utero heart failure. In the setting of Rh alloimmunization, it is characterized by the presence of fetal ascites, pericardial effusion, pleural effusion, subcutaneous edema (best seen as scalp edema), and polyhydramnios. The Rh complex is made up of a number of antigens, including C, D, E, c, d, and e. The vast majority of cases of Rh alloimmunization are due to antibodies to the D antigen.
- Identifying the pregnancy at risk for RhD-mediated hydrops fetalis involves two steps: (1) identifying all RhD-negative pregnant women who have a positive anti-D antibody screen and (2) determining the RhD status of the fetus, either by inference if the father is homozygous for the RhD antigen or by direct assessment of the fetal RhD antigen status through fetal DNA testing. Only pregnancies involving an RhD-negative mother sensitized to the D antigen carrying an RhD-positive fetus are at risk for RhD antibody-mediated hydrops fetalis.
- Once a pregnancy is identified as being at risk for RhD antibody-mediated hydrops fetalis, serial maternal anti-D antibody titers should be obtained. Once titers reach a critical threshold (≥1:16), or if the mother has a history of a previously affected fetus, serial fetal ultrasonography should be performed to detect fetal anemia. These include Doppler studies of the middle cerebral artery (MCA) and fetal imaging for evidence of placentomegaly, hepatomegaly, and hydrops fetalis.
- Treatment and management of an affected fetus involves percutaneous umbilical cord blood sampling (PUBS) for measurement of fetal hemoglobin, intrauterine transfusions, betamethasone to enhance fetal lung maturity, antepartum testing, and assessment of the need for early delivery. These fetuses often require additional treatment for hyperbilirubinemia or anemia in the neonatal period.
- RhD isoimmunization is the only form of isoimmunization that can be prevented with passive immunization. This is done by routinely administering Rh immune globulin to all RhD-negative women who are anti-D–negative at 28 weeks' gestation and within 72 hours of delivery of an RhD-positive fetus. Rh immune globulin should also be given to these women after any episode of antepartum bleeding or trauma.

Rhesus (Rh) alloimmunization is an immunologic disorder that occurs in a pregnant, Rh-negative woman who is carrying an Rh-positive fetus. The immunologic system in the mother is stimulated by fetal cells that cross the placental barrier into the maternal circulation to produce antibodies to the Rh antigen, which then cross the placenta into the fetal circulation and opsonize fetal Rh-positive red cells, resulting in their destruction in the spleen.

One of the earliest signs of fetal anemia caused by Rh alloimmunization is an elevated fetal middle cerebral artery (MCA) Doppler peak systolic velocity. Other early ultrasonic signs are an increase in the size and thickness of the placenta and fetal hepatomegaly. If the hemolysis is allowed to progress untreated, it will result in severe extramedullary hematopoiesis, portal hypertension, hypoalbuminemia, and the progressive development of in utero heart failure or hydrops fetalis. Figure 15-1 shows a fetus severely affected by erythroblastosis fetalis, which should now be completely preventable.

Pathophysiology

The Rh complex is made up of a number of antigens, **including C, D, E, c, d, e, and other variants, such as**

Incidence

Although fetomaternal hemorrhage is very common, the incidence of RhD immunization within 6 months of the delivery of the first RhD-positive, ABO-compatible infant is only about 8%. In addition, the incidence of sensitization with the development of a secondary immune response before the next RhD-positive pregnancy is 8%. Therefore, the overall risk of immunization for the second full-term, RhD-positive, ABO-compatible pregnancy is about one in six pregnancies. The risk of RhD sensitization following an ABO-incompatible, RhD-positive pregnancy is only about 2%. The protection against immunization in ABO-incompatible pregnancies is due to the destruction of the ABO-incompatible cells in the maternal circulation and the removal of the red blood cell debris by the liver.

Fetomaternal hemorrhage may also occur before delivery. Establishment of the fetal circulation occurs at approximately 4 weeks' gestation, and the presence of the RhD antigen has been demonstrated as early as 38 days following conception. Consequently, RhD isoimmunization can occur at any time during pregnancy, from the early first trimester onward. In the first trimester, the most common causes of fetomaternal hemorrhage are spontaneous or induced abortions. **The incidence of immunization following spontaneous abortion is 3.5%, whereas that following induced abortion is 5.5%.** The risk is low in the first 8 weeks, but it rises to significant levels by 12 weeks' gestation. **The risk of immunization following ectopic pregnancy is about 1%. Fetomaternal hemorrhage can also occur in the setting of second- or third-trimester vaginal bleeding, after invasive procedures such as amniocentesis or chorionic villus sampling, after abdominal trauma, or after external cephalic version.** If necessary, the amount of fetal blood entering the maternal circulation after an episode associated with fetomaternal hemorrhage can be estimated using the Kleihauer-Betke test (described in the next section of this chapter). **All pregnant RhD-negative women who are not sensitized to the D antigen should routinely receive prophylactic Rh immune globulin at 28 weeks' gestation, within 72 hours of delivery of an RhD-positive fetus, and at the time of recognition of any of the problems cited above that are associated with fetomaternal hemorrhage.**

Detecting Fetomaternal Hemorrhage

The Kleihauer-Betke test is dependent on the fact that adult hemoglobin is more readily eluted through the cell membrane in the presence of acid than is fetal hemoglobin. The maternal blood is fixed on a slide with ethanol (80%) and treated with a citrate phosphate buffer to remove the adult hemoglobin. After staining with hematoxylin and eosin, the fetal cells can readily be distinguished from the maternal cells. All cells are then counted. The percentage of fetal cells present on the slide is determined and can be used to estimate the extent of the fetomaternal hemorrhage (measured in milliliters of whole blood) on the basis of the following equation:

$$\text{Percentage of fetal cells} \times 5000 \text{ (estimated maternal blood volume in milliliters)}$$

As an example, if the Kleihauer-Betke is reported as 0.2%, then the estimated volume of fetal blood in the maternal circulation would be 0.002 × 5000, or 10 mL of fetal whole blood. There are a number of different formulas available for estimating the degree of fetomaternal hemorrhage, and all should be viewed as estimates based on their underlying assumptions regarding maternal and fetal blood volume. However, they are of value in determining the amount of Rh immune globulin to administer to prevent sensitization of an RhD-negative woman who is suspected of having a fetomaternal hemorrhage (refer to the "Prevention of RhD Alloimmunization" section later in this chapter).

Recognition of the At-Risk Pregnancy

A blood sample from every pregnant woman should be sent at the first prenatal visit for determination of the blood group and RhD type and for antibody screening. **In RhD-negative patients whose anti-D antibody titers are positive (i.e., those who are RhD-sensitized), the RhD status of the father of the baby should be determined.**

PATERNAL RHD GENOTYPING

If the father is RhD-negative, the fetus will be RhD-negative and hemolytic disease will not occur, so further monitoring is unnecessary. If the father is RhD-positive, his Rh genotype should be determined using quantitative polymerase chain reaction. If he is homozygous for the D antigen, the fetus will be RhD-positive and potentially affected. In this case, the pregnancy must be monitored closely for hemolytic disease. If the father is heterozygous, the fetus has a 50% chance of being RhD-positive, indicating the need for fetal RhD genotyping. Approximately 56% of RhD-positive whites are heterozygous for the RhD antigen. If it is not possible to test the D antigen status and zygosity of the father, it must be assumed that he is D antigen–positive.

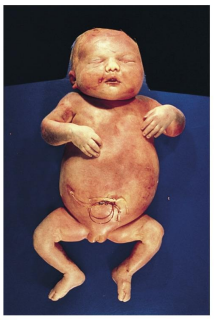

FIGURE 15-1 Fetal hydrops is the most serious condition associated with severe rhesus (Rh) incompatibility (erythroblastosis fetalis) between the mother and fetus. The fetal anemia caused by the blood incompatibility can lead to extramedullary hematopoiesis, portal hypertension, heart failure, and excessive fluid leakage into the extracellular space of the fetus. This can lead to subcutaneous edema, hepatomegaly, ascites, pericardial effusion, and pleural effusion. Note the increased abdominal circumference of the infant in this photograph. The placenta is also enlarged (not pictured) in this condition, and increased fetal renal output (in response to the edema) leads to an increase in amniotic fluid (polyhydramnios). Fetal death is common. This condition should be very rare with preventive measures and modern management of Rh incompatibility.

partial D antigens. More than 90% of cases of Rh alloimmunization are due to antibodies to the D antigen, and this is the only form of alloimmunization that can be prevented with Rh immune globulin prophylaxis. Therefore, this chapter is limited to a discussion of the D antigen, although the same principles apply to other antigen-antibody combinations. A person who lacks the D antigen on the surface of the red blood cells is regarded as being "RhD-negative," and an individual with the D antigen is considered to be "RhD-positive."

About 8% of African Americans are RhD-negative, whereas about 15% of white Americans are RhD-negative. **Only 1-2% of Asian and 1-2% of Native Americans are RhD-negative. When RhD-negative patients are exposed to the RhD antigen, they may become sensitized.** Most cases of sensitization are caused by a placental leak of fetal red blood cells into the maternal circulation (fetomaternal hemorrhage) during pregnancy. The fetal and maternal circulations are normally separated by the placental barrier. Small hemorrhages occur in either direction across the intact placenta throughout pregnancy. With advancing gestational age, the incidence and size of these transplacental (fetomaternal) hemorrhages increase, with the largest hemorrhages usually occurring at delivery. **Most immunizations occur at the time of delivery, and antibodies appear either during the postpartum period or following exposure to the antigen in the next pregnancy.**

Sensitization can also occur if an RhD-negative woman is exposed to RhD-positive blood via mismatched transfusion or hematopoietic stem cell transplantation or by injection with contaminated needles. In rare cases, the "grandmother" theory has been invoked. This theory suggests that an RhD-negative woman may have been sensitized from birth by receiving enough RhD-positive cells from her mother during her own delivery (i.e., a maternal-fetal hemorrhage) to produce an antibody response.

In general, two exposures to the RhD antigen are required to produce any significant sensitization, unless the first exposure is massive. The first exposure leads to primary sensitization, whereas the second causes an anamnestic response leading to the rapid production of immunoglobulins. **The initial response to exposure to the RhD antigen is the production of immunoglobulin M (IgM) antibodies (which cannot cross the placenta) for a short period of time, followed by the production of IgG antibodies that are capable of crossing the placenta. If the fetus has the RhD antigen, these antibodies will coat the fetal red blood cells,** causing them to be destroyed, or hemolyzed, in the spleen. If the hemolysis is mild, the fetus can compensate by increasing the rate of erythropoiesis. If the hemolysis is severe, it can lead to profound fetal anemia, resulting in extramedullary hematopoiesis, portal hypertension, hypoalbuminemia, hyperbilirubinemia, and heart failure (hydrops fetalis), as well as intrauterine fetal death. High bilirubin levels can damage the central nervous system and lead to **neonatal encephalopathy and kernicterus.** Before the widespread use of RhD immune globulin for prevention of RhD isoimmunization, kernicterus was one of the leading causes of cerebral palsy and sensorineural deafness.

If a pattern of mild, moderate, or severe disease has been established with two or more previous pregnancies, the disease tends either to be of the same severity or to become progressively more severe with subsequent pregnancies. **If a woman has a history of fetal hydrops with a previous pregnancy, the risk of hydrops with a subsequent pregnancy is about 90%.** Hydrops usually develops at the same time as or earlier than in the previous pregnancy.

TECHNIQUES FOR EVALUATING FETAL RHD STATUS

Fetal RhD status should be determined in RhD-sensitized pregnancies when the father is heterozygous for the RhD antigen or his RhD antigen status is unknown. This can be done noninvasively by testing cell-free fetal DNA in maternal plasma as early as the end of the first trimester. If this testing is inconclusive, amniocentesis can be performed in the second trimester and fetal RhD genotyping can be done using amniocytes. A risk of amniocentesis, as noted earlier, is fetomaternal hemorrhage and worsening of the hemolytic disease. Chorionic villus sampling carries an even greater risk of worsening hemolytic disease if the fetus is RhD-positive, and its use for determining fetal RhD status is discouraged.

MATERNAL RHD ANTIBODY TITER

Maternal anti-D antibody titers are used as a screening tool to estimate the severity of fetal hemolysis in Rh disease. At many centers, anti-D antibody titers are used to help guide decision making regarding the initiation of testing procedures (e.g., MCA Doppler studies and percutaneous umbilical blood sampling). **The American College of Obstetricians and Gynecologists and independent researchers have stated that a fetus in the first immunized pregnancy is not in serious jeopardy when the anti-D antibody titer remains below 1:16.** In patients with a positive titer less than 1:16, repeat titers should be obtained every 2 to 4 weeks. If the titer rises to 1:16 or greater, a detailed ultrasound to detect hydrops and Doppler studies of the MCA are indicated. Titers are not generally useful for following a patient with a history of a previous fetus or neonate with hemolytic disease. In this setting, even if the titers are below the critical threshold, the patient should be followed and evaluated as if her titers were high.

ULTRASONIC DETECTION OF FETAL HEMOLYTIC DISEASE

Ultrasonic examinations of a woman with a fetus at risk for hemolytic disease include MCA Doppler studies and a detailed examination looking for the advent of fetal hydrops. **Serial Doppler assessments of peak systolic velocity in the fetal MCA have proven to be the most valuable tools for detecting fetal anemia.** In at-risk pregnancies, this test should be performed every 1 to 2 weeks from 18 to 35 weeks' gestation. A fetal MCA peak systolic velocity value above 1.5 multiples of the median for gestational age is predictive of moderate to severe fetal anemia and is an indication for percutaneous umbilical blood sampling for precise determination of fetal hemoglobin concentration. Intrauterine fetal transfusion should follow if indicated. **After 35 weeks' gestation, this test may produce a higher false-positive rate** (Figures 15-2 and 15-3).

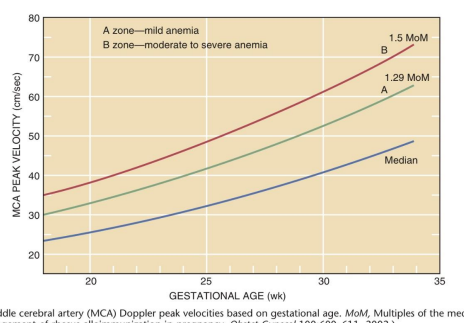

FIGURE 15-2 Middle cerebral artery (MCA) Doppler peak velocities based on gestational age. *MoM*, Multiples of the median. (Data from Moise KJ Jr: Management of rhesus alloimmunization in pregnancy. *Obstet Gynecol* 100:600–611, 2002.)

Reference

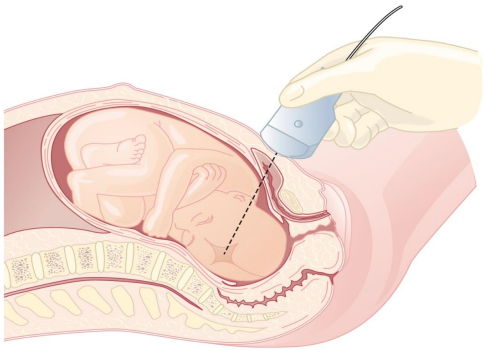

FIGURE 15-3 Obtaining a middle cerebral artery Doppler peak systolic velocity.

The ultrasonic examination should include a detailed fetal assessment for anatomy, growth, estimated fetal weight, and (if viable) biophysical profile, plus a determination of placental size and thickness and hepatic size. **Both the placenta and the fetal liver are enlarged with hydrops fetalis. Fetal hydrops is easily diagnosed on ultrasound by the characteristic appearance of two or more of the following: ascites, pleural effusion, pericardial effusion, skin edema, or polyhydramnios.** Appearance of any of these factors during an ultrasonic examination necessitates therapeutic intervention, depending on the fetal gestational age.

AMNIOTIC FLUID SPECTROPHOTOMETRY

Before widespread use of MCA Doppler studies, spectrophotometric analysis of amniotic fluid bilirubin concentration was the most frequently used method of gauging the severity of fetal hemolysis. The optical density deviation (ΔOD) at 450 μ from a baseline drawn between the OD values at 365 and 550 μ measures the amniotic fluid unconjugated bilirubin level, which in turn correlates with the cord blood hemoglobin of the newborn at birth.

Liley devised a graph based on the correlation of cord blood hemoglobin concentrations at birth and the amniotic fluid ΔOD at 450 μ. Using this method, he was able to establish the Liley graph or curve with predictive zones for mild, moderate, and severe disease. **The great drawback of using amniotic fluid spectrophotometry to determine the severity of fetal hemolytic disease is that amniocentesis, especially if transplacental, can increase the severity of fetomaternal transfusion and worsen the severity of the disease. For this reason and others, amniotic fluid spectrophotometry has been replaced by serial Doppler assessments of MCA peak systolic velocities and is discussed for historical purposes only.**

PERCUTANEOUS UMBILICAL BLOOD SAMPLING

If there is ultrasonic evidence of fetal hydrops, or if the MCA peak systolic velocity is greater than 1.5 multiples of the median for gestational age (see Figure 15-2), moderate to severe fetal anemia may be present and there is an indication for fetal blood sampling if the fetus is at less than 35 weeks' gestation. Advances in fetal interventional techniques and high-resolution ultrasonography have made direct fetal blood sampling the most accurate method for the diagnosis of fetal hemolytic disease. **Percutaneous umbilical blood sampling (PUBS) can allow measurement of fetal hemoglobin, hematocrit, blood gases, pH, and bilirubin levels.** If the fetal hematocrit is less than 30, or more than two standard deviations below the mean for gestational age, intrauterine transfusion is indicated.

Prevention of RhD Alloimmunization

Because RhD immunization occurs in response to exposure of an RhD-negative mother to the RhD antigen, the mainstay for prevention is the avoidance of maternal exposure to the antigen. Rh immune globulin diminishes the availability of the RhD antigen to the maternal immune system, although the exact mechanism by which it prevents RhD alloimmunization is not well understood.

Rh immune globulin is prepared from fractionated human plasma obtained from hyperreactive sensitized donors. The plasma is screened for hepatitis B surface antigen and anti-HIV-1. The globulin is available in several dosages for intramuscular injection. **Since the advent of its use in 1967, Rh immune globulin has dramatically reduced the incidence of Rh isoimmunization. Three hundred micrograms (or 1 U) of Rh immune globulin can neutralize 30 mL of fetal RhD-positive blood in the maternal circulation.**

Because the greatest risk for fetomaternal hemorrhage occurs during labor and delivery, Rh immune globulin was initially administered only during the immediate postpartum period. This resulted in a 1-2% failure rate, which is thought to be caused by exposure of the mother to fetal red blood cells during the antepartum period. **The indications for the use of Rh immune globulin have therefore been broadened to include any antepartum event (such as amniocentesis) that may increase the risk of transplacental hemorrhage. The routine prophylactic administration of Rh immune globulin at 28 weeks' gestation is now the standard of care.** Despite adherence to this suggested Rh immune globulin protocol, 0.27% of primiparous RhD-negative patients still become sensitized. Although this is a low rate, it is still unacceptable, given that it is preventable.

It is the responsibility of every health care practitioner who is involved in the care of pregnant women to prevent RhD alloimmunization by the appropriate administration of Rh immune globulin. Box 15-1 **lists the indications and dosing for Rh immune globulin.**

IRREGULAR ANTIBODIES

Although RhD alloimmunization is the most common cause of hemolytic disease in the newborn, **other antigens in the Rh system (C, c, E, e) and other blood group systems, such as Kell, Duffy, or Kidd, can also cause fetal hemolytic disease.** Kell antigen can elicit a strong IgG response similar to RhD alloimmunization.

BOX 15-1

INDICATIONS AND DOSING FOR RH IMMUNE GLOBULIN

- Blood type and antibody screen are performed for all pregnant women at their first prenatal visit.
- Women who are RhD-negative with a negative initial screen should have a repeat screen at 28 weeks.
- Those women with a negative screen at 28 weeks should receive 300 μg of Rh immune globulin (prophylactically).
- Those women with a positive screen should have their antibodies identified. If RhD-negative, they should also receive 300 μg of Rh immune globulin.
- All pregnant women who are RhD-negative and who are not sensitized (anti-D–negative) and who experience (1) spontaneous or induced abortion, (2) ectopic pregnancy, (3) significant vaginal bleeding, (4) amniocentesis, (5) abdominal trauma, or (6) cephalic version should receive 50-100 μg of Rh immune globulin before 12 weeks' gestation and be administered 300 μg if later than 12 weeks.
- Rh immune globulin is not necessary for complete molar pregnancies, but it is necessary for partial molar pregnancies, where fetal tissue may be present. Because this is not always clear at the time of evacuation, 300 μg of the immune globulin should be given.
- The greatest risk of fetomaternal hemorrhage is at the time of delivery. Rh immune globulin (300 μg) should be given routinely within 72 hours of delivery to all Rh-negative, anti-D–negative women who deliver an Rh-positive child.
- Additional Rh immune globulin is indicated if the delivery is complicated by excessive hemorrhage (>30 mL of fetal blood suspected or documented by Kleihauer-Betke testing).

For this reason, any positive antibody screen in pregnancy, even in an RhD-positive woman, should be followed up with an antibody identification and titer. If the antibody screen is positive for one or more antibodies associated with hemolytic disease of the newborn, the pregnancy should be followed in a fashion similar to that advised for the RhD-sensitized pregnancy.

A potential exception to this is Kell sensitization. Antibody titers are not as reliable for the detection of fetal anemia in this situation, probably because the anemia is due more to suppression of hematopoiesis than to hemolysis. The MCA peak systolic velocity remains an excellent predictor of anemia in this setting. It is extremely important to test the Kell antigen status of the father before any invasive testing, because 90% of the population is Kell-negative.

The technique for fetal blood sampling is similar to that described for fetal intravenous transfusion. One drawback is that it requires expertise above and beyond that required for amniocentesis. **The major risk is fetal exsanguination from tears in placental vessels,** so in most cases blood will have been ordered before the procedure and will be on hand in case an intrauterine transfusion is needed (see below). If the procedure is performed by an experienced practitioner, the risk of this complication and fetal death is no more than 1-2%. **However, there is a greater risk of fetomaternal hemorrhage.** Percutaneous umbilical blood sampling should not be a first-line method of assessing fetal status unless clearly indicated.

Management of the At-Risk Pregnancy

INTRAUTERINE TRANSFUSION

Intrauterine transfusion, initially introduced in 1963 as an intraperitoneal transfusion and currently usually administered as an intravascular transfusion, has markedly changed the prognosis for severely affected fetuses. **The goal is to transfuse fresh group O, Rh-negative packed red blood cells.** In addition to routine blood bank screening for viruses such as hepatitis and HIV, the blood for transfusion is irradiated, washed, processed through a leukocyte-poor filter, and screened for cytomegalovirus. Transfusions are done under ultrasonic guidance using sterile technique, either in or near the operating room if the fetus is potentially viable so that delivery can be accomplished expeditiously should the fetal status deteriorate irreversibly.

Transfusions usually cannot be done until 18 to 20 weeks' gestation, because fetal size limits vascular access. Repeat transfusions are generally scheduled at 1- to 3-week intervals, and the final transfusion is typically performed at 32 to 35 weeks' gestation. In general, the fetus is delivered when the lungs are mature, when it reaches 37 weeks, or if antepartum testing indicates severe fetal compromise.

The overall survival rate following intrauterine transfusion is about 90%, but it is significantly lower for fetuses with hydrops before the transfusion. Approximately 90% of survivors are reported to have normal neurologic outcomes.

Fetal Intraperitoneal Transfusion

Red blood cells are absorbed via the subdiaphragmatic lymphatics and proceed via the right lymphatic duct into the fetal intravascular compartment. After transfusion, the absorption of blood may be monitored with serial transverse ultrasonic scans of the fetal abdomen. **In nonhydropic fetuses, the blood should be absorbed within 7 to 9 days.** In the presence of hydrops, absorption is variable and the survival rate of hydropic fetuses is lower than after intravascular transfusion. Because of this, intravascular transfusion is the method of choice for correcting fetal anemia, and intraperitoneal transfusion is reserved for cases in which intravascular transfusion is not possible, such as gestational age less than 20 weeks.

Intravascular Transfusion

In most cases, intravascular transfusion is the preferred method. Fetal survival is better with this technique than after intraperitoneal transfusions, especially if there is ascites or other evidence of hydrops. In addition, transfusion into the peritoneal cavity can result in fetal bradycardia or a pseudosinusoidal fetal heart rate pattern following the procedure because of compression at the site of insertion of the umbilical cord.

Under ultrasonic guidance, and using sterile technique, a 22-gauge spinal needle is inserted into the umbilical vein near the placental insertion. An initial fetal hematocrit is determined, and a paralyzing agent is injected. The volume of blood to be transfused is based on the estimated fetal body weight, as determined by ultrasonography, the initial fetal hematocrit, the target fetal hematocrit, and the hematocrit of the packed red cells to be transfused.

OTHER MODES OF THERAPY

Maternal plasmapheresis combined with administration of intravenous immunoglobulin may be helpful in cases of severe erythroblastosis when intrauterine transfusions have not been successful, but further research must be done before this can be recommended. **Phenobarbital** has been used to induce fetal hepatic enzyme maturation, thereby increasing uptake and excretion of bilirubin by the liver. Treatment with phenobarbital is initiated at least 1 week before delivery.

TIMING OF DELIVERY IN THE RH-SENSITIZED FETUS

In addition to serial MCA Doppler studies and detection of hydrops, these fetuses should be evaluated twice weekly from at least 32 weeks until delivery for fetal well-being (nonstress tests, modified biophysical profile) and every 3 weeks for fetal growth. **Although the goal is a term delivery, the risks of intrauterine demise, including that caused by procedure-related losses, must be balanced against the risks of prematurity.** There is no absolute gestational age cutoff for intrauterine transfusions, but after 35 weeks the risk of an intrauterine loss may be greater than the risk of a neonatal death. It may be prudent in this setting to deliver the fetus and transfuse the neonate, if necessary. **If delivery is expected to occur before 34 weeks' gestation (or if amniocentesis suggests an immature lung profile), betamethasone should be given at least 48 hours before delivery to enhance fetal pulmonary maturation.**

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