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# ALLOIMMUNIZATION IN PREGNANCY: A COMPREHENSIVE REVIEW OF IMMUNE MECHANISMS AND FETAL OUTCOMES

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**ABSTRACT**

Alloimmunization is an immunological response that occurs when an individual is exposed to foreign antigens from another human, most commonly during pregnancy or blood transfusions. These antigens, typically cell surface proteins, are recognized as non-self by the recipient's immune system, leading to the production of specific antibodies by white blood cells. These antibodies signal the immune system to target and eliminate antigen-bearing cells. Although this process is a natural defense mechanism against pathogens, its occurrence in response to allogeneic blood products or fetal antigens can have severe clinical consequences, including hemolytic transfusion reactions and hemolytic disease of the fetus and newborn (HDFN). The pathophysiology of alloimmunization involves complex immune pathways, including the activation of B cells, cytokine signaling, and the generation of memory cells, which amplify the response upon repeated exposure. Understanding the factors that predispose individuals to alloimmunization, such as genetic polymorphisms, immunogenicity of antigens, and prior sensitization events, is critical for predicting and mitigating its effects. This review article provides an in-depth exploration of alloimmunization, covering its immunological underpinnings, clinical implications, and current preventive and therapeutic strategies. Additionally, a case-based scenario is included to contextualize the theoretical aspects and emphasize the real-world impact of alloimmunization, making this review a comprehensive resource for clinicians and researchers in the field.

## Rh ANTIGEN

Individuals have various blood types that are classified based on the antigens present on the red blood cells (RBCs). These blood types are classified in accordance with the ABO and Rhesus (Rh) blood group systems. The four blood groups in the ABO system are A, B, AB, and O, which indicate the presence or absence of the A and B antigens on the RBCs. Meanwhile, the Rh system includes approximately 50 antigens that cross the membrane of RBCs. The D antigen is the most frequently tested antigen. Therefore, Rh negative (Rh-) usually refers to RBCs lacking the D antigen, and Rh positive (Rh+) refers to those having the D antigen. A blood type may take both ABO and Rh systems into account. For example, AB+ would indicate the presence of A, B and D antigens on a person's RBCs.

The immune system responds to the presence of foreign antigens, or any antigens that are not present on their own RBCs, by producing antibodies. Antibodies have several important functions in the immune system, including inactivating the target antigen or marking the antigen for destruction. There are several types of antibodies, which have different shapes and functions. The most common antibodies in blood are Immunoglobulin M (IgM), which is the largest, and Immunoglobulin G (IgG), the smallest. Antibodies to A and B antigens are often produced naturally in the plasma of adults who lack the specific antigen on their own RBCs. This occurs because humans are often exposed to bacterial antigens that are similar to that of A and B antigens. In contrast, Rh antigen exposure generally occurs only during pregnancy or blood transfusions, which can then lead to alloimmunization.

The Rh blood group is one of the most complex blood groups known in humans. It was named after the Rhesus monkey; it has become second in importance only to the ABO blood group in the field of transfusion medicine. It has remained of primary importance in obstetrics, being the main cause of hemolytic disease of the newborn (HDN).

The complexity of the Rh blood group antigens begins with the highly polymorphic genes that encode them. There are two genes, RHD and RHCE, that are closely

linked. Numerous genetic rearrangements between them have produced hybrid Rh genes that encode a myriad of distinct Rh antigens. To date, 49 Rh antigens are known.

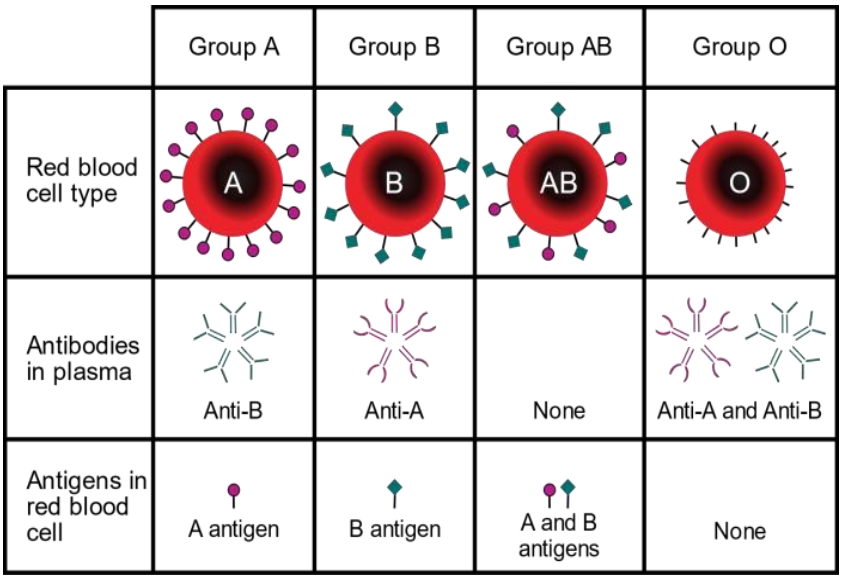


**Figure 1: Different types of blood group.**

The significance of the Rh blood group is related to the fact that the Rh antigens are highly immunogenic. In the case of the D antigen, individuals who do not produce the D antigen will produce anti-D if they encounter the D antigen on transfused RBCs (causing a hemolytic transfusion reaction, HTR) or on fetal RBCs (causing HDN). For this reason, the Rh status is routinely determined in blood donors, transfusion recipients, and in mothers-to be.[3]

**Pathophysiology**[2]

Spontaneous fetomaternal hemorrhage (FMH) refers to the transplacental passage of fetal erythrocytes into the maternal circulation, an event that occurs with increasing frequency and volume as gestation progresses. Multiple studies have reported a gestational age-dependent rise in the incidence and volume of FMH. Bowman et al., using the sensitive Kleihauer-Betke assay, demonstrated the presence of 0.01 mL or more fetal erythrocytes in the maternal circulation in 3%, 12%, and 46% of women during the first, second, and third trimesters, respectively. This progressive increase suggests that placental microtraumas or physiological changes in the uteroplacental interface predispose to FMH as pregnancy advances.

In most instances, the fetal antigenic load—specifically of the rhesus D (RhD) antigen on erythrocytes and their precursors—is insufficient to stimulate a maternal immune response. However, significant antenatal FMH, such as those caused by trauma, invasive procedures (e.g., amniocentesis or chorionic villus sampling), or FMH at delivery, can introduce a higher volume of RhD-positive erythrocytes into the maternal bloodstream. This antigen exposure activates naïve maternal B lymphocytes that recognize the RhD antigen. These B cells undergo clonal expansion and initially produce immunoglobulin M (IgM) anti-D antibodies, which are short-lived and do not cross the placenta.

The immunological process then transitions to a more robust adaptive response. The activated B cells undergo class switching, resulting in the production of immunoglobulin G (IgG) antiD antibodies. These IgG antibodies persist in the maternal circulation and are associated with immunological memory. Memory B lymphocytes formed during the initial sensitization phase remain dormant but are primed to respond rapidly upon subsequent exposure to the RhD antigen. This heightened secondary immune response occurs in subsequent pregnancies when fetal erythrocytes bearing the RhD antigen re-enter the maternal circulation.

During subsequent exposures, memory B lymphocytes differentiate into plasma cells, leading to a rapid and amplified production of IgG anti-D antibodies. These antibodies readily cross the placenta, where they opsonize RhD-positive fetal erythrocytes. The destruction of these erythrocytes by fetal macrophages in the reticuloendothelial system leads to fetal anemia, the hallmark of hemolytic disease of the fetus and newborn (HDFN). In severe cases, the resulting anemia can lead to hydrops fetalis, fetal heart failure, and intrauterine demise if left untreated.

Understanding the immunological dynamics of FMH and maternal alloimmunization is critical for developing effective prevention and management strategies. Prophylactic administration of anti-D immunoglobulin to RhD-negative mothers at key gestational time points and after sensitizing events has dramatically reduced the incidence of maternal RhD alloimmunization and its associated complications. Despite these advances, challenges remain in identifying high-risk cases,

optimizing antibody titration techniques, and managing severe cases of HDFN effectively. This underscores the need for continued research into the mechanisms of FMH, maternal immune tolerance, and therapeutic interventions.

**Prevalance of rbc alloimmunization in pregnancy**

The reported rate of alloimmunization in the general population ranges from 0.46% to 2.4%.[3] Besides pregnancy, patients who received transfusions are among the most affected by alloimmunization. Individuals with conditions such as SCD, and thalassemia, and those who undergo haemotherapy are exposed to multiple antigens from donor sources, leading to the development of alloimmunization. In these populations, female patients commonly exhibited a higher percentage of alloimmunization compared to males due to pregnancy and delivery exposure as an important independent risk factor (Table1).[38]

**Table 1: Frequency of RBC alloimmunization between genders among transfused patients.**

| Patient group | Total patient (N) | Total alloimmunized patient (n) | Male, n (%) | Female, n (%) |
|---|---|---|---|---|
| SCD | 50 | 31 | 14 (45.2) | 17 (54.8) |
| Transfusion-dependent β-thalassemia | 268 | 25 | 11 (44.0) | 14 (56.0) |
| Multi-Transfused, Oncology | 8115 | 18 | 1 (5.5) | 17 (94.5) |
| Chronic kidney disease | 249 | 31 | 12 (38.7) | 19 (61.3) |
| Haemotheraphy | 11253 | 179 | 35 (19.5) | 144 (80.5) |
| SCD | 556 | 107 | 46 (43.0) | 61 (57.0) |
| Total | 20491 | 391 | 109 (28.6) | 272 (71.4) |

The underlying disease, frequency of transfusions, and sample size also play crucial roles in contributing to the prevalence of alloimmunization in the population. RBC alloimmunization rates among pregnant women have been extensively studied in many areas around the world, with the frequency being found to range from 0.4% to 8.74% worldwide.

A common complication of RBC alloimmunization in pregnant women is FMH, which may have devastating consequences to the fetus such as fetal demise, neurologic injury, hydrops, delivery of a severely anaemic infant and stillbirth.[4-6] There are more than 60 different RBC antigens, which are commonly referred to as alloimmunization, especially the Rhesus, Kell, Duffy, Kidd, and MNS blood group systems.[7]

A prevalence study from 1995 until 2022 showed that anti-D is the most common alloantibody that causes alloimmunization in pregnant women even when RhIg prophylaxis is available as prevention indicates that rhesus antigen is the most clinically significant RBC antigen that frequency engages with RBC alloimmunization during pregnancy (Figure 1).[38]

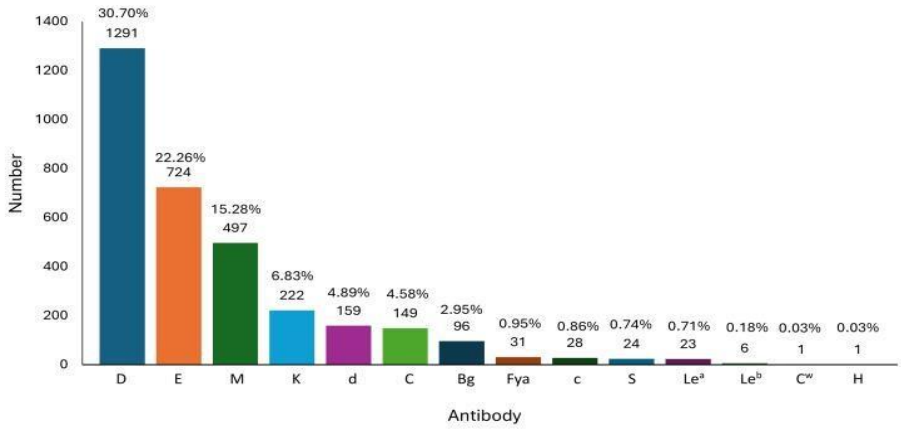


**Figure 2: Frequency of RBC alloimmunization between genders among transfused patients.**

**Screening for rbc alloimmunization in pregnancy**

Laboratory methods for the detection of RBC alloimmunization are often part of a comprehensive approach. The detection of RBC alloimmunization is important for determining the risk of HDFN, which is a rare but potentially severe event. The specific methods applied may vary based on the individual case and available resources in the healthcare setting.

**Abo/Rhd Typing and Antibody Screening**

ABO and RhD typing of the mother are routinely performed at the first prenatal visit to identify potential incompatibilities that may lead to alloimmunization. The presence or absence of ABO and RhD antigens on RBCs will rely on an agglutination reaction due to the interaction of known antibodies and parent's antigens. This test may be repeated in the late second trimester if needed.[10] Besides ABO and RhD typing, antibody screening by indirect antiglobulin test (IAT) is also a routine screening that will perform after blood typing to identify maternal antibodies against RBC antigens that can cause a risk to the fetus. This test also may be repeated more frequently if have a risk of alloimmunization.[7] Antibody screening also may be repeated at 28 weeks to detect new alloimmunization.

**Antibody Identification and Titration**

Following the presence of antibodies for antibody screening tests during pregnancy, the antibody identification test plays a crucial role in further elucidating the specific antibody involved and its potential impact on the fetus. By performing the antibody identification test, specific antigens targeted by the antibodies will be identified; thus, the clinical significance (anti-D, anti-K, anti-E) of the involved antibody and risk of HDFN can be assisted.[9] After a specific antibody is identified, antibody titration should be tested. This testing is crucial to assess the levels of antibodies present and their potential impact on the fetus, particularly in cases of HDFN due to maternal-fetal blood group incompatibility.[7] While a high antibody titre is often associated with severe HFDN, it's not the major factor. The specific type of antibody involved also impacts the severity of the condition. As reported from a previous study, anti-K is the type of antibody that has a lower titre but can contribute to the critical impact compared to anti-D.[8] The antibody titration test is typically repeated every two to four weeks when the pregnancy is identified with clinically significant antibodies.[10] For clinically nonsignificant antibodies (anti-M, anti-Jka, anti-Le), a general precaution might involve a repeat screening around second to third trimester of pregnancy.[10]

**Fetal Blood Sampling (FBS)**

In the case of the alloimmunized mother, if the case needs a comprehensive analysis of fetal health and blood parameters, including Rh status, anaemia severity, and potential infections, FBS will be performed. This test is performed via cordocentesis, where a needle is inserted through the mother's abdomen and into the umbilical cord under ultrasound guidance. This test will be conducted for high-risk situations with evidence of potential fetal compromise, where information from other tests such as ultrasound monitoring is insufficient.[14] Figure 2 shows the flowchart of screening and detection of alloimmunization in pregnancy.[38]

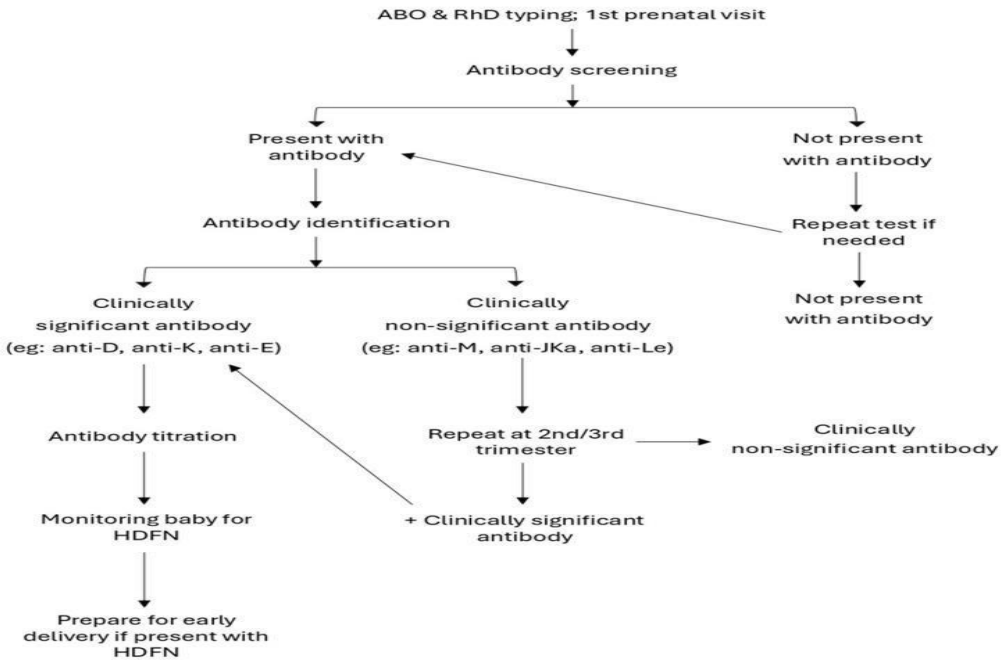


**Figure 3: The flowchart provides a simplified overview of screening and detection of alloimmunization in pregnancy starting from the first prenatal visit until delivery.**

**Therapeutic approach**

**Intrauterine transfusion**

Historically, the intraperitoneal transfusion remained the mainstay of fetal therapy for almost 20 years after its introduction by Liley in 1963. With the advent of real-time ultrasonography for guidance, direct access to the fetal circulation by puncturing the umbilical cord at its placental insertion became common place. As a result,

the direct intravascular transfusion has replaced the intraperitoneal transfusion in most centers. Compared with the intraperitoneal transfusion, the intravascular transfusion is clearly advantageous to the hydropic fetus where absorption of cells from the peritoneal cavity is compromised. Some centers continue to incorporate the intraperitoneal transfusion in the form of a combined procedure in conjunction with the intravascular transfusion. This can result in a more stable fetal hematocrit and a more prolonged interval between procedures. Many European centers prefer to use the intrahepatic portion of the umbilical vein as the site of intravascular transfusion. One advantage cited is a lower incidence of fetal bradycardia because an umbilical artery cannot be inadvertently punctured in this location. In addition, extravasated blood can be absorbed from the peritoneal cavity.

The red cells for intrauterine transfusion are typically from a blood type O, RhD-negative, cytomegalovirus-negative unit collected in the previous 72 hours. Some centers undertake an extended crossmatch with the maternal blood type to prevent sensitization to new red cell antigens. Cells are packed to a hematocrit of 75–85% to prevent volume overload in the fetus. The unit undergoes 25 Gy of gamma irradiation to prevent fetal graft-versus-host reaction. Additionally, many centers will leukoreduce the unit using millipore filters. This has the theoretical advantage of decreasing cytomegalovirus transmission because this virus usually remains dormant in the polymorphonuclear leukocytes.

At the start of the intravascular transfusion procedure, an initial fetal hematocrit is determined by puncture and sampling of the umbilical vein. The optimal site is from the cord near the placental insertion to provide stabilization of the cord. This is not always possible, and sometimes a floating loop of cord must be targeted. A paralyzing agent is usually administered to cause cessation of fetal movement. Our center uses vecuronium at a dose of 0.1 mg/kg of estimated fetal weight. The total amount of red cells to transfuse will depend on the initial fetal hematocrit, fetoplacental blood volume, and hematocrit of the donor unit. If the donor unit has a hematocrit of approximately 75%, the estimated fetal weight in grams using ultrasonography can be multiplied by a factor of 0.02 to determine the volume of red cells to be transfused to achieve a hematocrit increment of 10%.[12] A final target hematocrit of 40–50% is used; a decline of approximately 1% per day can be anticipated between transfusions. In the extremely anemic fetus, the initial hematocrit should not be increased by more than fourfold to allow the fetal cardiovascular system to compensate for the acute change in viscosity.[12] In this circumstance, a repeat procedure is undertaken 48 hours later to normalize the fetal hematocrit. Hydrops will typically reverse after one or two intravascular transfusions; placentomegaly is the last feature of the hydropic state to reverse. After attainment of a fetal hematocrit of 40–50%, subsequent procedures are scheduled at 14-day intervals until suppression of fetal erythropoiesis is noted on Kleihauer-Betke stains. This usually occurs by the third intrauterine transfusion. Thereafter, the interval for repeat procedures can be determined based on the decline in hematocrit for the individual fetus, usually a 3-week to 4-week interval. An unanticipated rapid decline in fetal hematocrit may reflect bleeding from the umbilical cord puncture site of the previous procedure, transplacental hemorrhage, or maternal sensitization to new red cell antigens to which the fetus is susceptible. Detti et al[15] have proposed that the MCA peak systolic velocity may be used to time the second intrauterine transfusion. A modified threshold of 1.32 MoM (instead of 1.5 MoM) should be used to detect moderate-severe anemia. After the second transfusion, there are insufficient data to guide the clinician on the use of MCA Doppler to time subsequent procedures. A calculated decline in hemoglobin of 0.4 g/dL, 0.3 g/dL, and 0.2 g/dL for the first, second, and third transfusion intervals, respectively, can be used to decide when to perform the next intrauterine transfusion.[11]

During the era of intraperitoneal transfusions, fetuses were routinely delivered at 32 weeks of gestation and often suffered complications of prematurity such as hyaline membrane disease and the need for neonatal exchange transfusions for the treatment of hyperbilirubinemia. Most authorities now will perform the final intrauterine transfusion (widespread development) at up to 35 weeks of gestation, with delivery anticipated at 37–38 weeks. Such a practice allows maturation of both the pulmonary and hepatic systems, virtually eliminating the need for neonatal exchange transfusions. After a viable gestational age is attained, performing the transfusion in immediate proximity to the labor and delivery suite appears prudent so that operative delivery can be undertaken if fetal distress should occur. Finally, the administration of maternal oral phenobarbital may be considered in the 7–10 days before delivery. This has been proposed to induce hepatic maturity to allow for improved conjugation of bilirubin. One retrospective study has demonstrated a reduction in the need for neonatal exchange transfusions for hyperbilirubinemia.[13]

The pregnant patient with a history of recurrent early second trimester pregnancy loss due to fetal hemolytic disease is especially challenging. Despite improved ultrasound resolution, targeting an umbilical vessel at less than 20 weeks of gestation can be technically challenging, often leading to iatrogenic fetal death. One approach is to use intraperitoneal intrauterine transfusions as early as 15 to 16 weeks of gestation as a bridging therapy until intravascular transfusions can be undertaken. Unfortunately, guidance on the amount of blood to transfuse is lacking. Alternatively, manipulation of the maternal immune system through a combination of serial plasmaphereses followed by weekly intravenous immune globulin also has been reported to delay the need for intrauterine transfusions until later in

gestation.[9,15] The author's protocol consisted of a single volume plasmapheresis every other day for three procedures in the 12th week of gestation, with 5% albumin used for volume replacement. The patient's immunoglobulin G pool was replaced after the third procedure by administering a 1 g/kg loading dose of intravenous immune globulin (IVIG) diluted in normal saline. The 10% IVIG infusion was started at a rate of 60 mL/h and increased by 30 mL/h every 30 minutes to a maximum rate of 240 mL/h. A second dose of 1 g/kg IVIG was given the following day. The patients then were treated with a weekly dose of 1 g/kg IVIG until 20 weeks of gestation.

**Short-Term Outcome**

Perinatal survival after intrauterine transfusion varies by center and the experience of the operator. Clearly, intervention before the appearance of hydrops fetalis is preferable. The largest experience at one center is reported from Leiden University in the Netherlands, a national referral center for the entire country. Overall survival in their series of 740 intrauterine transfusions in 254 fetuses was 89%.[16] Hydrops fetalis was associated with a decreased rate of survival to 78%. In cases of mild hydrops, 98% of fetuses survived; in cases of severe hydrops, only 55% survived. Suppression of erythropoiesis is not uncommon after several intravascular transfusions. These infants are born with a virtual absence of reticulocytes, with their red cell mass almost entirely comprised of donor red cells. Because exchange transfusion is rarely required, passively acquired maternal antibodies remain in the neonatal circulation for weeks. This results in a 1-month to 3-month period in which the infant may need several top-up red cell transfusions.[7] Weekly neonatal hematocrit and reticulocyte counts should be assessed. Threshold hematocrit values of less than 30% in the symptomatic infant or less than 20% in the asymptomatic infant have been suggested for transfusion. Iron therapy in these neonates is unnecessary because of elevated stores as a consequence of their in utero hemolytic disease and intrauterine transfusion therapy.

**Long-Term Outcome**

Advances in treatment techniques for the fetus with severe hemolytic disease now allow the more severely anemic fetus to survive. Standardized developmental assessments at 2 to 3 years of age in children born after successful intrauterine transfusions have failed to detect an association between poor scores and the degree of fetal anemia or the presence of hydrops.[4] In one recent series of 16 hydropic patients who survived to 10 years of age, two of the children were found to exhibit severe neurologic morbidity (12.5%).[5] Cerebral palsy and developmental delay seem therefore to be more common in fetuses with hemolytic disease of the fetus/newborn when compared with unaffected infants, although a normal outcome can be expected in more than 90% of cases. Sensorineural hearing loss is more frequent in these infants, probably because of their prolonged exposure to elevated levels of bilirubin and its toxic effect on the developing eighth cranial nerve.[4] Hearing tests should be conducted at birth and yearly for the first few years of life.

**Interpretation**

Significant advancements in the treatment of red cell sensitization have substantially reduced the incidence of perinatal loss associated with hemolytic disease of the fetus and newborn (HDFN). However, despite these strides, it is estimated that approximately 200 fetuses succumb annually in the United States due to HDFN or complications arising from its treatment. These statistics underscore the importance of ongoing improvements in diagnostic and therapeutic strategies to further mitigate fetal morbidity and mortality.

The management of pregnancies complicated by alloimmunization has evolved significantly over the years. Traditional approaches such as amniocentesis, which involves invasive sampling of amniotic fluid to assess the severity of fetal anemia, have largely been supplanted by non-invasive methodologies. One such advancement is the widespread adoption of free fetal DNA (ffDNA) testing, which enables the determination of fetal RhD status from a maternal blood sample. This method is highly sensitive, specific, and poses no risk to the fetus, making it an invaluable tool in stratifying pregnancies at risk of HDFN.

In addition to ffDNA testing, serial measurement of the middle cerebral artery (MCA) peak systolic velocity using Doppler ultrasound has become the cornerstone of fetal anemia assessment. Increased MCA velocities correlate with reduced blood viscosity due to anemia, allowing clinicians to accurately identify fetuses requiring intervention without resorting to invasive procedures. This approach has greatly enhanced the ability to monitor and manage affected pregnancies in a safer and more efficient manner.

Despite these diagnostic advancements, the treatment of severe fetal anemia continues to rely heavily on intrauterine transfusion (IUT), a highly specialized procedure that delivers donor red blood cells directly to the fetal circulation. While IUT has been instrumental in improving survival rates, it is associated with procedural risks, including preterm labor, infection, and, in rare cases, fetal demise. To address these limitations, the focus of research is shifting toward less invasive therapeutic modalities.

Maternal immunotherapy represents a promising future direction in the treatment of red cell sensitization. This approach aims to modulate the maternal immune response, preventing the production of pathogenic antibodies that target fetal red blood cells. Early preclinical studies have demonstrated the potential of using monoclonal antibodies or other immunomodulatory agents to inhibit maternal

sensitization or neutralize existing antibodies. If successfully translated into clinical practice, maternal immunotherapy could reduce or eliminate the need for IUT, thereby lowering procedural risks and enhancing fetal outcomes.

In summation, the integration of non-invasive diagnostic tools such as ffDNA testing and MCA Doppler into routine clinical practice has revolutionized the management of red cell sensitization, minimizing risks and improving outcomes for affected pregnancies. Future innovations, particularly in maternal immunotherapy, hold the potential to further transform the landscape of HDFN treatment, offering safer and more effective options for both mothers and their unborn children. Continued research and clinical trials will be essential to bring these advances to fruition.

## ➢ Factors that influence alloimmunization in pregnancy

- **Prior antigen exposure**

The primary factor influencing alloimmunization is blood transfusion and pregnancy.[22] If a woman receives blood with incompatible antigens, her immune system will develop memory cells and antibodies against those antigens. Comparably, during miscarriage or delivery, even without complications, small amounts of fetal blood can enter the maternal circulation and trigger the immune system if the fetus possesses an incompatible antigen. This sensitization can lead to either single alloimmunization or multiple alloimmunization, depending on the exposure history, and significantly impacts future pregnancies. Once the immune system remembers antigens from previous exposure, the subsequent exposure will trigger a faster and stronger response. Multiple alloimmunizations are more significant compared to single alloimmunization due to the increased complexity and potential clinical implications associated with developing antibodies against multiple RBC antigens.[19] When an individual experiences multiple alloimmunizations, difficulty identifying compatible blood units, and higher transfusion are required for the fetus or newborn. For example, a woman with antiRhD and anti-Kell antibodies would require blood negative for both antigens, thus significantly narrowing compatible donor options.

- **Fetomaternal hemorrhage**

During pregnancy, a common obstetrical event called FMH occurs where a small amount of fetal blood cells enters to the maternal bloodstream. Usually, this event happens without any maternal or fetal consequences.[23] However, in some cases, larger amounts of fetal blood can cross the placental barrier and enter the maternal circulation due to trauma (maternal falls or motor vehicle accidents), invasive procedures during pregnancy (amniocentesis or chorionic villus sampling, abortion), miscarriage, and intrauterine infection.[24-26] Furthermore, around the time of delivery, pregnant women are exposed to fetal RBCs, which is also one of the factors that contribute to alloimmunization.[22] In developing countries, RhIg prophylaxis is given to Rh-negative mothers at 30 weeks of gestation and 48 hours of delivery or after an invasive prenatal procedure to prevent an alloimmunization reaction towards Rh-positive fetus. Despite adequate antenatal and postnatal RhIg prophylaxis, postnatal events such as caesarean section, manual removal of the placenta, excessive postpartum haemorrhage, delivering at or after 42 weeks of gestation, and perinatal death are still found as the risk factors to alloimmunization occur.[26,27] FMH volume testing and extra prophylaxis shots may be required after the postnatal procedure. As a modern approach, molecular determination of fetal RhD through maternal plasma known as noninvasive prenatal testing (NITP) can provide pregnancy safety as NIPT can be done earlier in pregnancy, allowing the detection of alloimmunization without the risk of FMH. This testing can determine the fetal RhD status by real-time polymerase chain reaction using cell-free DNA obtained from maternal plasma.[28] As the detection of fetal RhD phenotype is sensitive during the third trimester, the result of this test can be a guide to deliver the targeted anti-D prophylaxis during antenatal and postnatal, thus reducing the unnecessary treatment and leading to cost savings.[30] Mothers with a history of blood transfusions in the past may have produced antibodies against foreign blood group antigens. If the fetus inherits these antigens from the father, the mother may produce antibodies against them. These antibodies can enter the placenta and harm the red blood cells of the developing fetus, leading to HDFN, which can cause a variety of complications, such as anaemia, jaundice, and, in severe instances, brain injury and even death.[1]

- **RBC Antigen**

Besides procedures or events during prenatal or postnatal, the RBC antigen itself is also one of the risk factors that contribute to RBC alloimmunization in pregnant women. The ability to present the RBC antigen to the immune system is known as immunogenicity, which also depends on the type of antigen and the individual's genetic makeup.[22] The antigen copy number is also related to immunogenicity as a low number of antigen copy number reduces the ability to mount antigen to the immune system; thus, the immune system is unable to generate alloantibodies. In the context of pregnant alloimmunization, the immunogenicity and antigen copy number of the fetal RBC antigen can all play a role in determining whether the mother will produce antibodies against the antigen. Highly immunogenic antigens with high copy numbers are more likely to stimulate an immune response and lead to the production of antibodies. While the density of antigens on RBCs refers to the number of antigen molecules on the surface of each RBC, antigen density can vary depending on the antigen zygosity.[22] For example, individuals who are homozygous for the D antigen (R1R1, R2R2) express nearly twice the number of D

antigens compared to those who are hemizygous (R1r, R2r) for the D antigen. When exposed to incompatible blood group antigens, the body produces numerous alloantibodies, which can cause a significant clinical implication. The specific types of alloantibodies are determined by the antigens to which the individual has been exposed. Some of the common types of alloantibodies identified in pregnant women include anti-D, anti-C, anti-K, anti-M, and anti-E. These alloantibodies can lead to HDFN and other complications, emphasizing the importance of identifying and monitoring the presence of these antibodies during pregnancy. The prevalence and specificity of clinically significant red cell alloantibodies in pregnant women have been studied, with anti-D, anti-K, anti-M, anti-C, and anti-E being among the most frequently identified alloantibodies.[9] Alongside assessment of antigen expression on RBC using the phenotyping method, the prediction of the antigen expression using the genotyping method allows for more accurate prediction of alloimmunization risk, thus minimising the risk of complications and giving treatment to the needs. Table 3 summarises the molecular method used to predict RBC antigen expression by using the genotyping method.

**Table 3: Alloimmune and Their advantages and disadvantages Summary of molecular methods used to identify RBC antigens of.**

| Method | Purpose | Advantages | Limitation |
|---|---|---|---|
| Polymerase chain reaction (PCR) real time and QF (quantitative fluorescence) | To amplifies specific DNA regions linked to RBC antigens | Highly sensitive and specific | More expensive than serological methods |
| Single-nucleotide polymorphism (SNP) genotyping | To identifying variations in genes that control RBC antigens | Highly specific and detects wider range of antigens | Expensive and requires specialized equipment |
| Sanger sequencing | Determine the exact sequence of nucleotides in a specific region RBC antigen | Provides highly accurate sequencing data for the targeted region | Only analyse one target region at a time |
| Next-generation sequencing (NGS) | To identify the targeted RBC antigen and uncover any unexpected variations in immune response genes | Most comprehensive method, detecting all known and potentially unknown antigens | Not widely available |

- **Immunomodulatory and Genetic**

During pregnancy, a pregnant woman's immune system may recognize fetal red RBC antigens as foreign if they differ from her own. This can trigger an immune response called alloimmunization, where the mother's body produces antibodies against the fetal RBC antigens. Factors such as immunomodulatory processes and a woman's genetics can significantly influence the likelihood and severity of alloimmunization. Immunomodulatory and genetics may influence alloimmunization in pregnant women. Human leukocyte antigen (HLA) plays an important role in presenting antigens to T-cells. Due to HLA restriction, some antigens cannot be presented to T-cells, which can impact the production of antibodies. The term ‘nonresponder’ can be used for the mother with HLA restriction since the mother fails to generate detectable alloantibodies to RBC antigens after exposure.[31] HLA restriction study may provide insight by preventing certain HLA, preventing the alloantibodies expression, and then promoting safe transfusion and pregnancy.[22,32] Specific HLA types, such as HLADQB1*0602, are associated with an increased risk of Rh D alloimmunization.[33] In pregnant women, dendritic cells (DCs) will encounter fetal RBCs that have crossed the placenta during pregnancy. DCs act as APC captures and processes foreign RBC antigens and present them to T cells in conjunction with specific HLA molecules. The specific HLA-antigen complex determines how the T cell recognizes the antigen.[19] Depending on the presented antigen and HLA context, different T cell subsets become activated. For example, Th1 cells will promote cellular immunity, while Th2 cells, activated by specific cytokines such as IL-4 and IL-6, will become central to antibody production. They secrete cytokines (IL-5, IL-10) that stimulate B cells to proliferate and differentiate into plasma cells.[19,18] B lymphocytes also act as APC recognizing the foreign fetal RBC antigen through their B cell receptors (BCRs), and some B lymphocytes will proliferate and become plasma cells.[20,21] These antibody factories produce large amounts of Ig, primarily IgG antibodies, specifically targeting the fetal foreign RBC antigens. These antibodies can cross the placenta and attack fetal RBCs, potentially leading to complications such as haemolytic anaemia in severe cases. Natural killer (NK) cells can eliminate cells that present fetal cells that present with foreign HLA molecules, thus influencing the overall

immune response.[34] The variations of killer cell immunoglobulin-like receptors (KIRs) have been linked to susceptibility or resistance to alloimmunization.[35] While cytokines become the chemical messengers that regulate the immune response, specific cytokines may influence T cell activation, B cell proliferation, and antibody production. For example, IL-4 secreted by Th2 is associated with type 2 immune responses and promotes B cell proliferation and IgE production.[36] IL-10, on the other hand, is an immunoregulatory cytokine produced by B cells that can have both anti-inflammatory and regulatory effects on T cells.[37] Figure 3 summarizes the factors that influence RBC alloimmunization in pregnant women.[38]

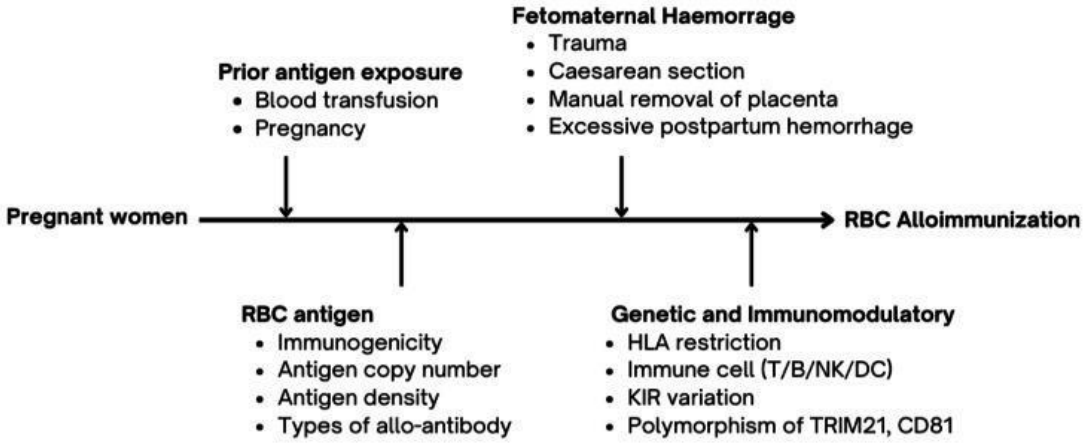


**Figure 4: Factors that influence RBC alloimmunization in pregnant women.**

**Case report based in india**

A few months ago, All India Institute of Medical Sciences (AIIMS) blood bank in New Delhi received a desperate call requesting for a unique blood group. The call was made by the blood bank of Lady Hardinge Hospital where a Haryana-based woman in her late 20s had suffered a miscarriage and needed an urgent blood transfusion to remove the foetus. Her haemoglobin was 6 grams per decilitre (g/dl) against the normal 12-15g/dl range. She was facing recurrent intra-uterine deaths. The blood group of the mother and unborn child was incompatible and in turn, the condition for the growth of the baby was difficult. Hence, she suffered the death of seven unborn children. A woman who previously suffered seven failed pregnancies has recently delivered a healthy child with the help of doctors at.

AIIMS Delhi after they successfully treated her foetus suffering from a rare blood disorder through the transfusion of O D phenotype.

The AIIMS team checked the donor data in India but there was no donor available. The case was referred to AIIMS, and the doctors prepared for another emergency procedure to take the baby out while the mother's haemoglobin was still low. The team had no option but ended up removing the baby, successfully.

Unfortunately, the woman had lost her seventh child too but she was saved despite acute anaemia. The team efforts reaped results when she came back to the hospital in her eighth pregnancy – again in a critical position. However, a team of over four doctors from the Department of Obstetrics and Gynaecology, successfully saved her eighth child and made history. The team performed a first-of-its-kind procedure in India and the eighth in the world.

**Mission to save the 8th unborn child**

The Obstetrics and Gynaecology Department of AIIMS, told that the incompatibility between the red blood cells of the mother and the baby in the womb led to problems for the unborn child due to the destruction of the red cells leading to anaemia, jaundice, heart failure and even death of the baby. The most common known incompatibility is due to the RhD antigen (commonly known as D+ or D-) and in severe cases of fetal anaemia RhD- blood is transfused to the baby inside the mother's womb through the umbilical cord.

However, in this case, the mother was negative for the Rh 17 antigen which is very rare to find. Due to this, the babies in her womb would suffer from incompatibility and develop anaemia and she had previous seven pregnancy losses.

When she came to AIIMS in her seventh pregnancy, she had already lost her child inside her womb but in that pregnancy, the blood group was identified by the blood bank team. In her eighth pregnancy, she came to us during the fifth month and it was found that the baby was already anaemic and required blood to be given urgently.

This time, the blood bank team moved aggressively as they had earlier sent the sample of the mother's rare blood group to the United Kingdom's International

Blood Group Reference Laboratory. The WHO-supported laboratory had sent the list of countries with donors of the same blood group.

They moved quickly as the list had gave names of around 10 countries. Zeroed in on Japan because the country was relatively nearer and the list of donors was longer with almost 30 registered donors. They reached out to the Japanese Red Cross for around six units of blood that was planned to be given to the baby through the umbilical cord in the next few months to sustain the pregnancy.

These specialised blood units were given as intrauterine transfusion and this procedure ensured the health and survival of an unborn child suffering from severe anaemia. The anaemia in child resulted from the destruction of red blood cells (hemolysis) caused by antibodies transferred from the mother. The condition is known as hemolytic disease of the fetus and newborn (HDFN).

The foetus received six blood transfusions inside the mother's womb and the state of hydrops (heart failure) reverted to normal. The pregnancy continued till eight months when the baby was delivered by a caesarean section and this became the first case of a successful pregnancy outcome in a case of alloimmunisation due to Rh 17 Ag in India and eighth case in the world.

According to studies, HDFN affects 3 out of every one lakh to 80 out of every one lakh patients every year. Haemolytic disease of the foetus and newborn is an immune-mediated red blood cell disorder. However, reasons and severity could vary from case to case.

## CONCLUSION

This review highlights the multifaceted aspects of alloimmunization, encompassing its pathophysiology, immunological mechanisms, and clinical implications, while integrating advancements in diagnostic and therapeutic approaches. The inclusion of a case report based in India underscores the global relevance of this condition and illustrates the challenges faced in resource-limited settings, where timely diagnosis and access to advanced care such as intrauterine transfusion may be constrained.

Advancements such as non-invasive free fetal DNA testing and Doppler ultrasound-based monitoring of middle cerebral artery peak systolic velocity have revolutionized the diagnosis and management of at-risk pregnancies. However, despite these innovations, red cell sensitization continues to contribute to perinatal morbidity and mortality, as evidenced by the case report. This underscores the need for enhanced awareness, early screening protocols, and equitable access to advanced therapies.

Looking ahead, the potential of maternal immunotherapy as a less invasive and safer alternative to intrauterine transfusion offers hope for improving outcomes. As demonstrated in the Indian case report, the integration of emerging technologies into routine obstetric care, even in low-resource settings, is critical to minimizing the burden of alloimmunizationrelated complications.

In conclusion, this review emphasizes the importance of a multidisciplinary approach that combines early identification, timely intervention, and ongoing research into novel therapeutic strategies to address alloimmunization and its consequences effectively. By drawing from global advancements and contextualizing them within local healthcare frameworks, we can move closer to achieving optimal outcomes for mothers and their unborn children worldwide.

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