Case Report

A case of assisted reproductive technology–induced maternal alloimmunization: an emerging sensitizing factor to consider?

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Red blood cell (RBC) alloimmunization can occur because of exposure to various sensitizing factors and poses a constant threat in transfusion. Assisted reproductive technology (ART) involves manipulation of sperm, ova, or embryos *in vitro* with the goal of producing a pregnancy. We present an interesting case of ART-induced maternal alloimmunization (AIMA) due to anti-c in a woman carrying a twin pregnancy. A 35-year-old primigravida, whose blood sample typed as group B, D+ and showed anti-c in her plasma, delivered twins by cesarean section. The spouse's blood group was also B, D+. The blood groups of twins I and II were confirmed to be B, D+ and AB, D+, respectively. The RBCs of twin I were c+, but those of twin II and the spouse were c–. On enquiry, history of ART with donor sperm insemination was noted. Because there were no previous sensitizations, antigenic inheritance from the sperm donor to twin I could be the possible sensitizing factor for maternal alloimmunization. To the best of our knowledge, this case is the first report of ART-induced maternal RBC alloimmunization in the literature. History of ART exposures should be documented, and appropriate RBC phenotyping of the parent as well as potential ART donors will help in timely detection or prevention of hemolytic disease of the fetus and newborn or other AIMA-related complications. ***Immunohematology* 2024;40:149–152. DOI: 10.2478/immunohematology-2024-020.**

Key Words: assisted reproductive technology (ART), ART-induced maternal alloimmunization (AIMA), ABO discrepancy, red cell sensitization, *in vitro* fertilization, infertility

Red blood cell (RBC) alloimmunization can occur after exposure of various sensitizing factors (e.g., pregnancy, blood transfusion, transplantation) and poses a constant threat in transfusion.[1] Assisted reproductive technology (ART) involves manipulation of sperm, ova, or embryos *in vitro* with the goal of producing a pregnancy. With the increasing need and practice of ART as an infertility treatment, ART may expose the mother to one or more sperm donors apart from her spouse, which can theoretically cause ART-induced maternal alloimmunization (AIMA) and a consequential dilemma in the serologic workup. We present an interesting case of AIMA due to anti-c in a primigravida carrying twins.

Case Report

A 35-year-old primigravida was admitted because of premature preterm rupture of membrane (PPROM) at 26 weeks of gestation. There was no history of previous pregnancies, abortions, or transfusions, and there was no report of antibody screening during her antenatal period. Lower segment cesarean section was performed because of PPROM, and she delivered two live healthy babies with Apgar scores of 9/10 for both babies.

Total bilirubin and hemoglobin were reported as 9.92 mg/dL and 17.2 g/dL (twin I) and 10.45 mg/dL and 12.6 g/dL (twin II), respectively, (bilirubin normal 0.3–1.0 mg/dL; hemoglobin normal 14–24 g/L). There was no evidence of hemolytic disease of the fetus and newborn (HDFN) in either twin.

During the type and screen testing in our blood center, the mother's blood group/type was recorded as B, D+ by column agglutination technology (CAT) (IH-Cards; Bio-Rad, Diamed, Basel, Switzerland). The antibody detection test was also performed using CAT (Diacell I-II-III; Bio-Rad, Diamed), and the specificity was identified as anti-c (ID-Diapanel; Bio-Rad, Diamed) (Table 1). Cord blood samples were received for blood typing. On initial testing, twin I was group B, D+, and the ABO results of twin II were inconclusive, showing very weak reactivity with anti-B by CAT (Fig. 1). Twin II was confirmed to be group AB, D+ by tube method; these results were reconfirmed after 3 weeks. (Table 2) Antibody screening of twin I was negative, and twin II was positive due to maternal anti-c. Direct antiglobulin tests of both twins were negative. Spouse sample was requested as a routine protocol

Table 1. Antibody identification panel results of mother

	Rh-hr						Kell				Duffy		Kidd		Lewis		P	MNS				Lutheran		AHG
Cell	D	C	E	c	e	C^w	K	k	Kp^a	Kp^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P1	M	N	S	s	Lu^a	Lu^b	Pt
1	+	+	0	0	+	+	0	+	0	+	+	0	+	+	0	+	+	+	0	0	+	0	+	0
2	+	+	0	0	+	0	+	+	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0
3	+	0	+	+	0	0	0	+	0	+	+	+	+	0	+	+	+	+	0	+	0	0	+	2+
4	0	+	0	+	+	0	0	+	0	+	+	+	+	0	0	+	+	+	0	+	0	0	+	2+
5	0	0	+	+	+	0	0	+	0	+	+	0	+	0	0	0	0	+	+	0	+	0	+	2+
6	0	0	0	+	+	0	+	+	0	+	0	+	+	+	0	+	0	+	+	+	+	0	+	2+
7	0	0	0	+	+	0	0	+	+	+	+	+	0	+	0	0	0	0	+	0	+	0	+	2+
8	+	0	0	+	+	0	0	+	0	+	0	0	+	+	0	+	+	+	+	+	+	0	+	2+
9	0	0	0	+	+	0	0	+	0	+	0	+	0	+	0	0	+	+	+	0	+	+	+	2+
10	0	0	0	+	+	0	0	+	0	+	0	+	0	+	+	0	0	0	+	+	0	0	+	2+
11	0	0	0	+	+	0	0	+	0	+	+	0	+	0	0	0	+	+	+	+	0	0	+	2+
Pt																								2+

AHG = antihuman globulin; Pt = patient.

for phenotype confirmation in an alloimmunized pregnancy. Spouse ABO/D blood group/type was B, D+. Twin I was c+, but twin II and the spouse were both c– as shown in Table 3.

On enquiry, we found this pregnancy to be a case of ART with donor sperm insemination. As there were no previous sensitizing events such as transfusion, pregnancy, abortion, or transplantation, antigenic inheritance from the sperm donor to twin I could be the possible sensitizing factor for maternal alloimmunization. Because of confidentiality, the sperm donor could not be tested for c.

Table 2. Results of ABO and D typing of twin II

Sample	Test	Anti-A	Anti-B	Anti-AB	Anti-D	Interpretation
Cord sample	CAT	3+/mf	+/–	3+/mf	4+	A, D+ (inconclusive)
	Tube method (after wash)	3+	2+	4+	4+	AB, D+ (inconclusive)
Repeat after 3 weeks	CAT	3+	4+/mf	4+	4+	AB, D+
	Tube method	3+	3+	4+	4+	AB, D+

CAT = column agglutination technology; mf = mixed field (a dual population of agglutinated and non-agglutinated red blood cells, observed in both ABO groupings by CAT).

Discussion

RBC alloimmunization is the formation of antibodies to non-self RBC antigens that occurs after exposure through transfusion, pregnancy, abortion, or transplantation.[1] Common blood group systems with clinically significant RBC antibodies include Rh, Kell, Kidd, and MNS. Women in the childbearing age-group are at higher risk of alloimmunization than men. Maternal alloimmunization can lead to HDFN and hemolytic transfusion reaction (HTR).

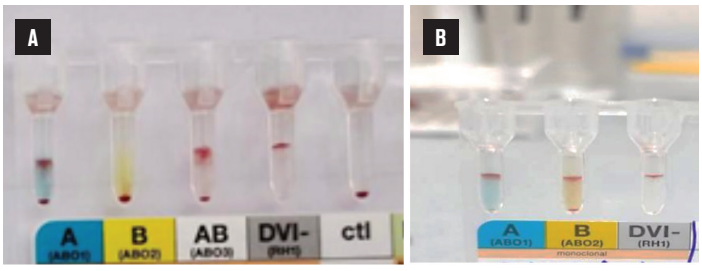

Fig. 1 Results of ABO and D testing by column agglutination technology for twin II: at birth (A) and at 3 weeks (B).

According to a survey, approximately 1 in 8 women in the reproductive age-group receives infertility treatment.[2] Commonly used infertility treatments include medical, surgical, or assisted conception. ART includes intrauterine insemination and *in vitro* fertilization. Intrauterine insemination is performed by injecting sperm directly into the uterus, whereas *in vitro* fertilization is performed via fertilization of eggs with sperm in a controlled laboratory setting; these fertilized eggs are later transferred into the woman's uterus.[3] Further new advancements in the scope of female infertility treatment include usage of donor sperm, donor egg, or frozen embryo.[2]

Table 3. Results of antibody screening and c typing of twins and parents

	Mother	Spouse	Twin I	Twin II	Remarks
ABO group/D type	B, D+	B, D+	B, D+	AB, D+	Unclear of origin of A in twin II
Antibody detection test	Positive	Negative	Negative	Positive	Anti-c in mother; spouse is c–
c Typing	Negative	Negative	Positive	Negative	Unclear of origin of c
DAT	NT	NT	Negative	Negative	No evidence of sensitization or hemolysis in the twins

DAT = direct antiglobulin test; NT = not tested.

These procedures pose extra antigenic exposure, leading to potential maternal alloimmunization.

Knowing the biological parent's blood group and RBC phenotype often helps in predicting the likelihood of the baby inheriting the specific blood group antigen and the risk for alloimmunization. Hence, in planned ART, particularly AIMA cases, donors should be screened for blood group and partial (C, c, E, e, and K) or extended RBC phenotyping.

In our case, for the anti-c, history of previous sensitizing events like pregnancy or abortion, transfusion, and transplantation were ruled out, narrowing the possibility of antigen from the sperm donor being the only sensitizing exposure to the mother. This antigenic exposure to the mother (c–) through twin I (c+) might have led to anti-c production. This presumption was further strengthened by the expression of A in twin II, which is lacking in the mother and her spouse.

This case report highlights the serologic difficulties that should be clarified whenever dealing with a pregnancy with blood group discordance or maternal alloimmunization. In ART, especially with the use of donor gametes or embryos, ABO discordance can arise if the blood group of the donor differs from the intended parent's blood group. The parent should be counseled and be made aware of possible blood group mismatch between child and parent.

During the ART procedure, already sensitized female patients exposed to sperm donors with corresponding blood group antigens can lead to severe HDFN and poor outcome. Theoretically, there is no clear evidence of higher risk of alloimmunization with regard to the ART procedure.[3] The risk of alloimmunization might be similar for any ART or non-ART pregnancy. However, women undergoing multiple ART procedures due to failed pregnancies might be at higher risk because of multiple donor exposures. AIMA can lead to HDFN and HTR in the mother. This risk emphasizes the importance of the history of ART (as an additional sensitizing factor) in alloimmunized pregnancies, in which there is an alloantibody, and the presumed father is negative for the respective antigen; in our case, the father is c–.

According to the Royal College of Obstetricians & Gynaecologists guidelines (in the management of women with RBC antibodies during pregnancy), there is no evidence of ART increasing the risk of RBC alloimmunization.[4] However, the guidelines have recommended performing fetal genotyping in a mother with alloantibody if donor eggs are used for conception. This recommendation warrants the careful selection of potential donors based on RBC genotype for alloimmunized female patients with AIMA. These aspects are crucial in ART to ensure safe and successful outcomes for both the mother and the child.

This case is the first report of AIMA with anti-c. With advancement and rising practice of these newer techniques, this case highlights the risk of maternal alloimmunization by ART. A history of ART should be emphasized as an important additional sensitizing factor (in addition to history of pregnancy, transfusion, and transplant) in cases of HDFN, antenatal workups, or alloimmunization in female individuals of childbearing age. This information would help prevent unnecessary testing and confusion.

Because of increases in advancement and practice of these newer techniques, we may encounter these challenges more often in the near future, which may lead to AIMA-induced HTR and HDFN. We recommend ABO, Rh (D, C, c, E, e), and K phenotyping for patient, spouse, and donor. In the case of AIMA, the obstetrician should be notified of the potential risk and antigen-matched donors should be preferred.

Conclusion

Because there is no available evidence of AIMA to date, this case underscores the importance of detailed documentation and guides appropriate clinical practices to manage such risk. History-taking with regard to sensitizing events in the form of

ART exposures should be documented, and appropriate RBC phenotyping of the parents as well as potential ART donors will prevent AIMA and related complications (such as HDFN and HTR).

References

1. Zalpuri S, Zwaginga JJ, van der Bom JG. Risk factors for alloimmunisation after red blood cell transfusions (R-FACT): a case cohort study. BMJ 2012;2:e001150. doi: 10.1136/bmjopen-2012-001150.
2. Carson SA, Kallen AN. Diagnosis and management of infertility: a review. JAMA 2021;326:65–76. doi: 10.1001/jama.2021.4788.
3. Jain M, Singh M. Assisted reproductive technology (ART) techniques [article online]. In: StatPearls [Internet]. Treasure Island, FL: StatPearls Publishing; 2024. Available from https://www.ncbi.nlm.nih.gov/books/NBK576409. Accessed 11 October 2024.
4. Royal College of Obstetricians & Gynaecologists. The management of women with red cell antibodies during pregnancy (green-top guideline no. 65). Available from https://www.rcog.org.uk/guidance/browse-all-guidance/green-top-guidelines/the-management-of-women-with-red-cell-antibodies-during-pregnancy-green-top-guideline-no-65/. Accessed 22 August 2024.

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