Complementary therapy for severe Rh-alloimmunization

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Summary

Purpose of investigation: This report describes successful treatment, using invasive and noninvasive techniques, of a 36-year-old woman (gravida 10, para 0) referred to our center at 13 weeks' gestation for severe Rh alloimmunization. Pre-pregnancy indirect Coombs titers ranged from 1:1024-2048. All nine past pregnancies (conceived with three different partners) had ended in abortion, intrauterine death or neonatal death

Methods: The patient was treated with a single session of plasmapheresis (week 14) immediately followed by five days of immunoglobulin therapy and immunosuppressive therapy based on azathioprine and prednisone (weeks 15-22). Seven fetal transfusions (one intraperitoneal, six intravascular) were performed beginning at 16 weeks.

Results: The pregnancy, which was characterized by insulin-dependent gestational diabetes, spontaneously resolving polyhydramnios and peak indirect Coombs titers of 1:65536, ended at 27 weeks with cesarean section delivery of a viable female weighing 1,000 g. In spite of numerous neonatal complications, the child is physically well at age 3, with normal intellectual and psychomotor development.

Conclusion: In light of the negative outcomes of the patient's nine past pregnancies, our experience suggests that the early initiation of an integrated approach based on noninvasive and invasive techniques can play a potentially decisive role in the management of severe Rh-alloimmunization.

Key words: Pregnancy; Rh-alloimmunization; Fetal transfusions; Invasive tecniques; Indirect Coombs titer.

Introduction

Rh-alloimmunization has played a central role in the history of invasive fetal therapy.

Studies conducted on this disease laid the foundations for our current knowledge of maternal-fetal immunobiology, and the treatment approach developed by Liley paved the way for truly invasive fetal therapy [1]. Since the 1960s, when anti-D prophylaxis became widespread, the problem of Rh-alloimmunization has been significantly reduced in all parts of the world. There are still, however, cases in which the preventive approach proves to be unsuccessful, and these failures represent situations of high risk during the perinatal period [2]. The technique of intrauterine intravascular transfusion was introduced in the 1980s as a second line of defense in those cases where prophylaxis had failed. As transfusion volumes and protocols have been refined, the survival rate of severely affected fetuses has risen steadily, and women with decidedly unfortunate reproductive histories have been given new hope [3-7].

Today antenatal transfusion therapy is widely recognized as the most effective therapeutic approach to the fetus with anemia and/or hydrops [8]. The experience of numerous groups throughout the world reveals considerable improvement in survival rates for these fetuses [5, 6, 9], and the need for postnatal transfusions has clearly diminished [5, 10-14]. Delivery of blood or blood components directly to the fetal vascular compartment allows for more precise supplementation and more rapid utilization of the transfused products by the fetus. In many cases, this approach is associated with the disappearance of ultrasonographic signs of cardiac failure, ascites, and pericardial effusion, which are typical of hydrops fetalis [15, 16]. The advent of intrauterine intravascular transfusion has thus radically altered the natural history of hemolytic disease in the newborn [17]. Nonetheless, when performed prior to 20 weeks of gestation, invasive measures of this type carry a high risk of both technical failure and/or abortion. Consequently, in some cases the fetus has already suffered considerable hypoxic damage by the time transfusions are begun.

This paper describes an extremely severe case of Rh-alloimmunization that was successfully treated with a combination of transplacental immunosuppressive/immunomodulating therapy and early intraperitoneal/intravascular fetal transfusions.

Material and Methods

At 13 weeks' gestation, a 36-year-old woman (gravida 10, para 0) was referred to the Center for Perinatal Medicine, Department of Obstetrics and Gynecology, of the Catholic University in Rome, Italy. All nine past pregnancies had ended in abortion, intrauterine death or neonatal death (one). The details of the patient's obstetric history are shown in Table 1. The blood type of the patient's partner was Group 0 Rh positive.

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Table 1. — *Obstetric history.*

Year	Pathology diagnosed	Outcome	Weeks at delivery	Method	Fetal birth weight (g)	Fetal outcome
1984		Abortion	11	D&C	–	–
1984		Abortion	12	D&C	–	–
1985		Preterm Delivery	26	VD	1650	Neonatal death
1987	Rh alloimmunization	Abortion	14	D&C	–	–
1991	Rh alloimmunization	Abortion	13	D&C	–	–
1992	Rh alloimmunization	Abortion	15	D&C	–	–
1993	Rh alloimmunization	Preterm delivery	26	VD	500	Intrauterine death
1994	Rh alloimmunization	Abortion	23	D&C	–	–
1995	Rh alloimmunization	Abortion	25	D&C	–	Intracardiac IUFT

Legend:
Preterm Delivery (≤ 37 weeks); D&C: Dilatation and Curettage; VD: Vaginal Delivery; IUFT: Intrauterine Fetal Transfusion

The patient presented with an indirect Coombs titer of 1:16, with pre-pregnancy titers ranging from 1:1024 to 1:2048 (all based on testing performed in the laboratory of our University Medical Center). At 14 weeks cervical cerclage was performed for cervical incompetence, and the same week the patient was also subjected to a single session of plasma exchange (volume: 1800 ml) followed by five days of immunoglobulin therapy (0.4 g/kg/day). The following week, immunosuppressive-immunomodulating therapy was begun with prednisone and azothioprine in an attempt to stabilize the situation until invasive fetal therapy could be attempted with less risk (Table 2). Given the severity of the case, however, intrauterine intraperitoneal fetal transfusion was begun at 16 weeks with infusion of 6 cc of Group 0, Rh-negative packed red cells (filtered, washed and concentrated) obtained from a donor. The fetus later received eight additional intrauterine intravascular transfusions of packed red cells at weekly intervals, as shown in Table 3.

Table 2. — *Non-invasive fetal therapy.*

Treatment	Begun (Gestational week)	Endend (Gestational week)	Daily dosage (route)
- Cervical cerclage	14.0	–	–
- Plasmapheresis	14.3	14.3	1800 cc exchanged
- Immunoglobulins	14.2	15.0	0.4 g/kg (IV)
- Prednisone	15.0	21.5	40 mg x 2 (IV) (Weeks: 15.0-16.2) 30+25+20 mg/day (PO) (Weeks: 16.3-20.0) 20+20 mg/day (Weeks: 20.1-20.2) 10+10 mg/day (Weeks: 20.3-20.6) 5+5 mg/day (Weeks: 21.0-21.2) 5 mg/day (Weeks: 21.3-21.5)
- Azathioprine	15.0	20.6	50 mg b.i.d. (PO)
- Regular Insulin	19.1	27.2	5+5+5 IU/day (SC) (Weeks: 19.1-20.0) 5+10+10 IU/day (Weeks: 20.1) 5+12+12 IU/day (Weeks: 20.2-21.0) 7+12+12 IU/day (Weeks: 21.1-21.2) 10+14+15 IU/day (Weeks: 21.3-21.6) 12+16+17 IU/day (Weeks: 22.0-22.4) 10+14+15 IU/day (Weeks: 22.5-23.0) 12+17+15 IU/day (Weeks: 23.1-27.2)

Legend:
IV: Intravenous; PO: Per Os; SC: Subcutaneous

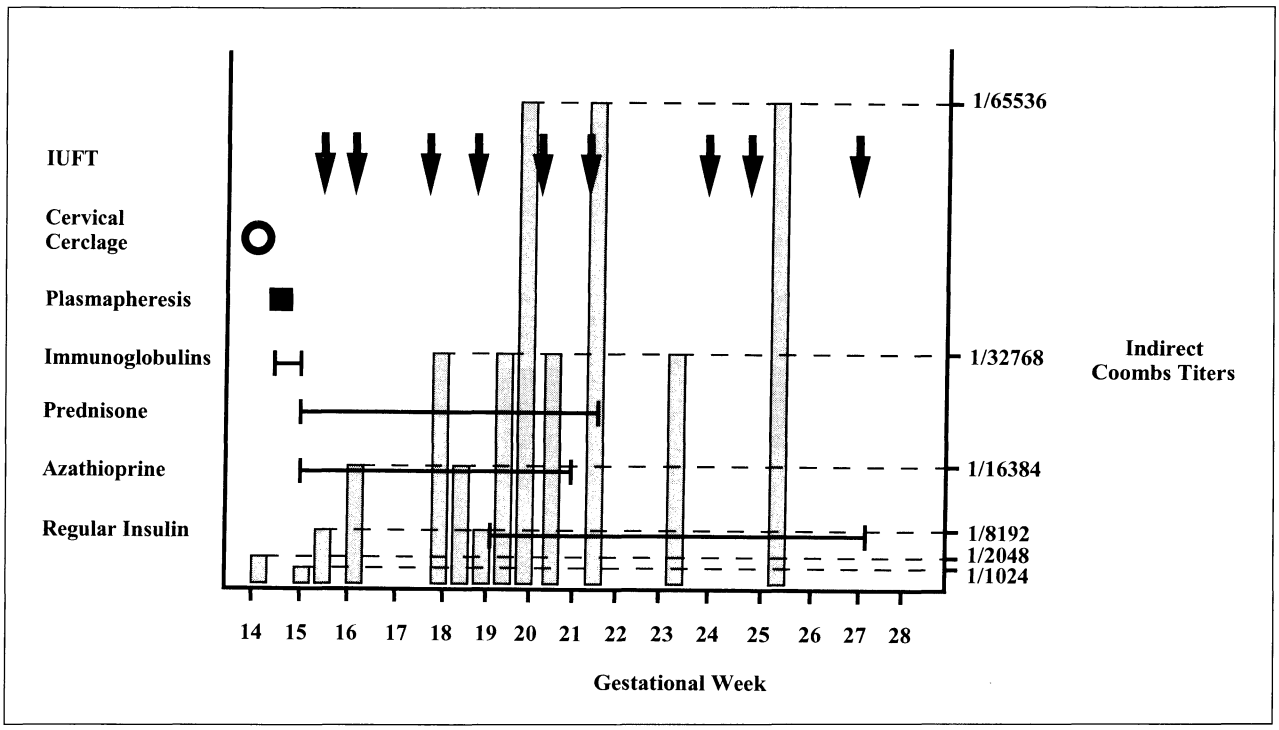
A total of 32 ultrasonographic examinations were performed between the 13th and 27th week of gestation to verify fetal viability and well being. Polyhydramnios was diagnosed during the 17th week but fortunately regressed spontaneously by week 18, and the indirect Coombs titer dropped from 1:32768 (17 weeks) to 1:8192. During the 19th week, however, the titer rose to a peak of 1:65536 (the highest value ever observed in our medical center), and the pregnancy was further complicated by the development of gestational diabetes, which was successfully managed with fractioned doses of regular insulin (maximum 38 IU/day). By the 23rd week, the titers had once again dropped to 1:32768, but the ultrasonogram performed at 24 weeks revealed moderate fetal ascites. Diagnostic cordocentesis revealed a fetal hemoglobin of 5.6/100 ml. During this procedure the seventh intrauterine intravascular transfusion was performed with 40 cc of packed red cells. The post-transfusion hemoglobin was 12.8 g/100 ml, and there was a clear decrease in the ascites on the sonogram performed the following week. Despite our efforts, peak titers were once again documented during the 25th week. At 27 weeks, during the ninth transfusion, intravascular thrombosis of the cord occurred with persistent fetal bradycardia necessitating an emergency cesarean section.

Table 3. — *Intrauterine fetal tranfusion (IUFT).*

Tranfusion	Gestational Week	IUFT (Type) (cc)	Packed red cells transfused (g)	Hb pre-IUFT (g)	Hb post-IUFT
I	15.2	IP	6	–	–
II	16.1	IV	4	–	–
III	17.5	IV	7	3.4	13.4
IV	18.5	IV	10	8.6	13.1
V	20.2	IV	13	9.1	11.8
VI	21.3	IV	16	9.3	12.4
VII	24.1	IV	40	5.4	12.8
VIII	25.0	IV	8	–	11
IX	27.0	IV	38	8	9.3

Legend:
Hb: Hemoglobin; IUFT: Intrauterine fetal transfusion
IP: Intraperitoneal; IV: Intravascular

Table 4. — *Correlation between indirect Coombs titers and invasive and non-invasive treatment of the patient's 10th pregnancy.*



Legend:
IUFT: Intrauterine fetal transfusion

Results

A female infant weighing 1,000 g, was delivered by emergency C-section at the 27th week of gestation. The newborn's blood type was group 0, Rh positive. The hemoglobin was 10.7 g/dl and the hematocrit was 30.4%. The newborn was immediately intubated and placed on assisted ventilation. In the postnatal period, she underwent one complete exchange transfusion and three weeks of treatment with human recombinant erythropoietin (rHuEPO) at a dose of 200 U/kg/day. A total of six postnatal blood transfusions were required, three prior to the rHuEPO therapy and three thereafter. The perinatal course was complicated by bronchopulmonary dysplasia, sepsis, retinopathy of prematurity, and an atrial septal defect with patent ductus arteriosus.

The child has been followed for three years and the results are quite satisfactory. She has suffered recurrent bronchial infections, none of which have been severe. Her visual acuity is normal although she has mild strabismus. The cardiac anomalies are no longer evident, and her neuromotor and intellectual development appears to be within normal limits.

Discussion

Treatment of severe cases of Rh-alloimmunization is an important problem for physicians involved in the management of maternal-fetal blood incompatibility. The onset of hydrops prior to the 20th week of gestation is an obstacle that can be overcome only by a few highly experienced teams, and even then, success is rare [15]. Voto and Margulies have recently proposed an integrated approach to cases of this type, which is based on the IV administration of high doses of immunoglobulins to the mother prior to the 20th week. At this point, transfusion therapy can be undertaken with a much greater possibility of success [18]. Other investigators have confirmed the validity of this approach and the added contribution of immunoglobulin therapy in attenuating the hemolytic phenomena that characterize these cases [19]. Immunotherapy can be extremely useful in the post-natal period as well, during which it has been shown to exert a positive influence on the rate of elimination of anti-D antibodies by the newborn [20].

In the case described here, intrauterine transfusion was attempted quite early, during the 16th week of gestation, and this in itself is noteworthy considering the high risk associated with any invasive procedure prior to 20 weeks [16]. Indeed, we generally try to postpone transfusions by relying on plasmapheresis and immunotherapy until the 20th week. In this case, however, the severity of the case (later confirmed by the documentation of indirect Coombs titers of 1:65.536) and the rapid and early development of ascites and intrauterine death that had characterized the patient's last pregnancy prompted us to attempt transfusion early, but only after the initiation of agressive immunosuppressive therapy in the mother based on prednisone, azothioprine and immunoglobulins (Table 4).

Our management of this case was, in fact, strongly influenced by the negative outcome of the patient's ninth pregnancy, which ended in abortion at 25 weeks. A number of changes were therefore made in the timing of treatment and the doses used in this pregnancy (Tables 4 and 5).

Cervical cerclage was performed two weeks earlier (14 weeks vs 16 weeks in the previous pregnancy).

The use of plasmapheresis was reduced from seven treatments in pregnancy 9 to one in pregnancy 10 (during the 14th week). Our experience indicates, in fact, that the latter procedure can be associated with a rebound antibody response. In all probability, the avoidance of this effect can also be attributed in part to the prompt initiation of immunoglobulin therapy – two days of plasmapheresis, i.e., roughly two weeks earlier than in the ninth pregnancy.

Immunosuppressive therapy (based on prednisone and azothioprine), which was not used in the previous pregnancy, was begun during the 15th week of gestation and continued through the 22nd week in an attempt to limit the hemolytic damage to the fetus.

Finally, our ability to monitor fetal growth and well being and signs of anemia with ultrasound allowed us to continue transfusion therapy (a total of 9) until the fetus had reached the age of viability [21]. Although intrauterine intravascular transfusions were also used early in the ninth pregnancy (17 weeks vs week 16 through 27 in the present case), this approach was not sufficient to prevent intrauterine death.

Table 5. — *Management of the patient's previous (9th) pregnancy with indirect Coombs titers and ultrasound findings.*

Treatment	Gestational Week	Indirect Coombs Titers	Ultrasonography
	13.4	1/16	Normal
	14.2	1/512	
	15.0	1/16384	
– Cervical cerclage	15.1		
– Plasmapheresis (No. 7)	15.1		
	15.3		Normal
	15.4		
	15.5	1/32678 (post)	
	16.0	1/32678 (post)	
	16.2		Normal
	16.4	1/32678 (post)	
– Immunoglobulins (IV) 2g/Kg/day	16.2		
	16.5	1/4016	
	17.1	1/4016	
– 1st IUFT (IP, 7 cc packed red cells)	17.2		Abdominal fold pre-IUFT: 2 mm
			Abdominal fold post-IUFT: 5 mm
	17.3	1/8192	Abdominal ascites: 9 mm
			Scalp edema: 4 mm
– 2nd IUFT IV, 8 cc packed red cells)	17.5		Abdominal ascites: 8 mm

Legend:
IUFT: Intrauterine fetal transfusion; IP: Intraperitoneal
IV: Intravascular

The fetal birth weight, 1,000 g (which corresponds to the 60th percentile) confirms Roberts' observation in 1993 that intrauterine transfusions can have positive repercussions on fetal growth [22]. Significantly, none of the nine transfusions produced more than a fourfold increase in the fetal hematocrit with respect to pre-transfusion values, the threshold that carries the risk of potentially fatal increases in the fetal cardiac after-load [23]. Invasive transfusion therapy is undoubtedly effective in correcting anemia and increasing the possibility of fetal survival. However, considering the precocious development of fetal hypoxia, it is doubtful that the successful outcome of the present case would have been possible without the use of transplacental drug therapy.

It is not possible to determine which of the three treatment modalities used had the most direct effect on maternal antibody production, but the early use of immunosuppressive therapy allowed us to reach our objective, which was to administer transfusion therapy to a fetus that was not already severely compromised.

The use of immunoglobulins, followed by treatment with prednisone and azothioprine, undoubtedly played a positive role in limiting the increases in IgG observed between the 15th and 19th week of gestation. During this interval, indirect Coombs titers rose from a low of 1:1024 (observed after plasmapheresis and administration of immunoglobulins) to a peak of 1:32768 during the 17th week, but by the end of week 18 (after four weeks of prednisone and azothioprine), titers had once again dropped to 1:8192.

At the 19th week, however, the titers reported were not only higher than they had ever been in any of the patient's previous pregnancies, but, to our knowledge, they were also higher than any that have been reported thus far in the literature. It is difficult to pin-point a specific cause for this dramatic rise, particularly since it occurred during the final stages of immunosuppressive therapy. A twofold increase in titers around the 20th week is not in itself surprising, however, and the extreme level reached is undoubtedly a result of the patient's multigravidity (although it may also be noteworthy that the ten fetuses were conceived with three different partners). Subsequent transfusions were followed by decreases in IgG titers to 1:32768, but these improvements were temporary, and peak titers were once again documented during the 25th week, shortly after two transfusions.

The indirect Coombs titers have been reported to document the severity of the patient's immune response rather than an index of fetal well being.

Conclusion

The case described here is undoubtedly peculiar in the severity of the mother's immune response, which was incompletely controlled by all of our efforts. In clinical terms, however, and in light of the negative outcomes of

previous attempts to treat this woman, the experience described here suggests that the early initiation of an integrated approach based on noninvasive and invasive techniques can play an important and potentially decisive role in the management of severe Rh alloimmunization.

References

[1] Freda V. J., Gorman J. G., Pollack W., Bowe E.: "Prevention of Rh hemolytic disease: Ten years clinical experience with Rh immunoglobulin". *N. Engl. J. Med.*, 1975, *292*, 1014.

[2] Noia G.: "Terapia dell'Isoimmunizzazione-Rh". Atti 70° Congresso S.I.G.O., 1994, S.E.U. Eds. 238.

[3] Barss V. A., Benacerraf B. R., Frigoletto F. D., Greene M. F., Penso C., Saltzman D. H. *et al.*: "Management of isoimmunized pregnancy by use of intravascular techniques". *Am. J. Obstet. Gynecol.*, 1988, *159*, 932.

[4] Bowman J. M.: "Hemolytic disease (erythroblastosis fetalis)". In: Creasy R. K., Resnik R. (eds.): "Maternal Fetal Medicine: Principles and practice". Philadelphia, WB Saunders, 1989, 613.

[5] Weiner C. P., Williamson R. A., Wenstrom K. D., Sipes S. L., Widness J. A., Grant S. S. *et al.*: "Management of fetal hemolytic disease by cordocentesis: I Prediction of fetal anemia". *Am. J. Obstet. Gynecol.*, 1991, *165*, 546.

[6] Bowell P. J., Selinger M., Ferguson J., Giles J., Mackenzie I. Z.: "Antenatal fetal blood sampling for the management of alloimmunized pregnancies: effect upon maternal anti-D potency levels". *Br. J. Obstet. Gynecol.*, 1988, *95*, 759.

[7] Azeem S. A., Samuels P., Rose R. L., Kennedy M., O'Shaughnessey R. W.: "The effect of the source of transfused blood on the rate of consumption of transfused red blood cells in pregnancies affected by red blood cell alloimmunization". *Am. J. Obstet-Gynecol.*, 1997, *177 (4)*, 753.

[8] Egberts J., van-Kamp I. L., Kanhai H. H., Meerman R. H., Giordano P. C., Gravenhorst J. B.: "The disappearance of fetal and donor red blood cells in alloimmunised pregnancies: a reappraisal". *Br. J. Obstet. Gynaecol.*, 1997, *104 (7)*, 818.

[9] Nicolaides K. H., Warenski J. C., Rodeck C. H.: "The relationship of fetal plasma protein concentration and hemoglobin level to the development of hydrops in rhesus isoimmunization". *Am. J. Obstet. Gynecol.*, 1985, *152*, 341.

[10] Rodeck C. H., Nicolaides K. H., Warsoff S. L., Fish W. J., Gamsu H. R., Kemp J. R.: "The management of severe rhesus isoimmunization by fetoscopic intavascular transfusions". *Am. J. Obstet. Gynecol.*, 1984, *150*, 769.

[11] Harman C. R., Bowman J. M., Manning F. A., Menticoglou S. M.: "Intrauterine transfusion-intraperitoneal versus intravascular approach: a case control comparison". *Am. J. Obstet. Gynecol.*, 1990, *162*, 1053.

[12] Noia G., De Santis M., Monni M. *et al.*: "Impact of cordocentesis in preventing fetal brain injury". *Int. Symp. on Perinatal Nutrition and Brain Development*, Troina, 1993, 21.

[13] Plecas D. V., Chitkara U., Berkowitz G. S., Lapinski R. H., Alvarez M., Berkowitz R. L.: "Intrauterine intravascular transfusion for severe erythroblastosis fetalis how much to transfuse?". *Obstet. Gynecol.*, 1990, *75*, 965.

[14] Noia G., Garzetti G. G., Serri F. G., Masini L., Vincenzoni M., Bellati U.: "Severe Rh disease: our experience with intraperitoneal transfusion of hydropic fetuses". *International Symposium on fetal diagnosis and therapy*, Rotterdam, 1987.

[15] Socol M. L., MacGregor S. N., Pielet B. W., Tamura R. K., Sabbagha R. E.: "Percutaneous umbilical transfusion in severe Rhesus isoimmunization: Resolution of fetal hydrops". *Am. J. Obstet. Gynecol.*, 1987, *69*, 268.

[16] Noia G., Romano D., De Santis M., Gozzo M. L., Colacicco L., Mariorenzi S. *et al.*: "Ontogeny of the fetal immune system (Study on Pregnancies with Rh-Isoimmunization and Nonimmune Fetal Hydrops)". *Clin. Immunol. Immunopathol.*, 1999, *90*, *1*, 115.

[17] Noia G., Caruso A., Mancuso S.: "Le Terapie Fetali Invasive". S.E.U, Eds., Roma, 1998.

[18] Voto L. S., Margulies M.: "Inutero intravascular transfusion of the fetus for the managements of severe Rhesus isoimmunization: a reappronisal". *Br. J. Obstet. Gynecol.*, 1998, *95 (7)*, 730.

[19] Voto L. S., Mathet E. R., Zapateiro J. L., Orti J., Lede R. L., Margulies M.: "High-dose gammaglobulin (IVIG) followed by intrauterine transfusions (IUTs): a new alternative for the treatment of severe fetal hemolitic disease". *J. Perinatol. Med.*, 1997, *25 (1)*, 85.

[20] Porter T. F., Silver R. M., Jeckson G. M., Branch D. W., Scott J. R.: "Intravenous immune globulin in the management of severe Rh D hemolitic disease". *Obstet. Gynecol. Surv.*, 1997, *52 (3)*, 193.

[21] Janssens H. M., de Haan M. J., van Kamp I. L., Brand R., Kanhai H. H., Veen S.: "Outcome for children treated with fetal intravascular transfusions because of severe blood group antagonism". *J. Pediatr.*, 1997, *131 (3)*, 373.

[22] Roberts A., Grannum P., Belanger K., Pattison N., Hobbins J.: "Fetal growth and birthweight in isoimmunized pregnancies after intravenous intrauterine transfusion". *Fet. Diagn. Ther.*, 1993, *8*, 407.

[23] Radunovic N., Lockwood C. J., Alvarez M., Plecas D., Chitkara U., Berkowitz R. L.: "The severely anemic and hydropic isoimmune fetus: change in fetal hematocrit associated with intrauterine death". *Obstet. Gynecol.*, 1992, *79*, 390.

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