Homozygous expression of fetal red cell antigen in donor oocyte pregnancy complicated by allo-immunisation: are current antibody thresholds to trigger increased monitoring relevant?

Dear Sir,

Haemolytic disease of the fetus and newborn (HDFN) is caused by clinically significant maternal red cell allo-antibodies that target fetal red cell antigens. When these immunoglobulin G (IgG) antibodies cross through the placental barrier they can cause destruction of the fetal red cells or their progenitors, resulting in fetal anaemia. Heterozygous expression of the cognate antigen on the fetal red cells is the norm in haemolytic disease in pregnancy due to maternal red cell allo-immunisation, as the mother can neither possess nor pass on the offending antigen.

We are aware of a case of protracted HDFN due to anti-c, managed with phototherapy initially, then intervention with red cell top-up transfusion at 4 weeks post-delivery. The baby's red cells were direct antiglobulin test positive (3+, scale 0–4), total bilirubin increased from 58 to 286 μmol/L and haemoglobin levels had dropped to 7·2 g dL. Anti-c was not detectable at booking bloods and was first detected at delivery. Antibody screening was not performed at 28 weeks gestation which was the policy for that institution, for RhD-positive women. Antibody quantification was performed at 30 days post-delivery and the anti-c had a relatively low quantification level of 5·97 iu mL. Anti-c levels <7·5 iu mL are associated with a low risk of HDFN and fetal assessment is not indicated (Gooch *et al.*, 2007), however, anti-c quantification was not performed antenatally in this case. This pregnancy involved a donor oocyte and the fetal red cells had homozygous expression of the c-antigen. Homozygous expression of the causal antigen was not previously possible in cases of haemolytic disease due to red cell allo-antibodies, prior to the advent of oocyte donation *in vitro* fertilisation (IVF). The case presented as unexpectedly severe HDFN and brought to our attention the potential for homozygosity in donor oocyte pregnancy. We believe that homozygous expression of the c antigen most likely contributed to the severity of HDFN.

Current British Committee for Standards in Haematology Guidelines (Gooch *et al.*, 2007) recommend fetal assessment when anti-c quantification levels are >7·5 iu mL. These guidelines are based on the findings of Kozlowski *et al.* (1995), a study of 120 patients with anti-c and 100 infants, all of whom would have had heterozygous expression of the c-antigen. It is possible that the risk of haemolytic disease may not be accurately predicted in cases of donor oocyte IVF where there is homozygous expression of the implicated red cell antigen. In addition, the risk of allo-immunisation may also be increased where there is double dose expression of antigen. Phenotyping of the partner to predict likelihood of inheritance of red cell antigens, in the absence of fetal genotype, remains to have some merit; however, it may not help predict the zygosity of the fetus in most cases especially if donor oocyte antigen status is not known. In future, a non-invasive fetal genotype could help ascertain zygosity in known donor oocyte pregnancies complicated by allo-immunisation.

The addition of a third person into the reproductive equation also permits the possibility of unexpected fetal blood groups; in this case the fetus was RhD-negative (cde/cde). An RhD-positive fetus is normally expected when a mother has anti-c as the mother is most likely R_1R_1 (CDe/CDe). While it is possible for a fetus to be RhD-negative when a mother has anti-c in a naturally conceived pregnancy, donor oocyte IVF increases the likelihood of such incompatibilities. This can lead to difficulties in sourcing suitable blood for transfusion.

Current risk thresholds for red cell allo-antibodies in pregnancy differ based on the specificity detectable and the method used to quantify them, however, the threshold levels are founded on heterozygous expression of the causal antigen on fetal red cells *in utero*. Donor oocyte IVF treatment opens up the possibility of homozygous antigen expression on fetal red cells. Risk of haemolytic disease and antibody level indicators for referral for fetal assessment may need to be evaluated in light of this, especially given the increase in use of assisted reproductive technology methods.

Unexpectedly severe haemolytic disease has previously been reported in cases of donor oocyte pregnancy (Mitchell & James, 1999; Mair & Scofield, 2003), with homozygous expression of the causal red cell antigen being a possible contributory factor in some reported cases (Mair & Scofield, 2003; Patel *et al.*, 2003). Notably, Mair and Scofield reported a case of HDFN requiring transfusion due to an anti-c which had a maximum titre of 4. A more severe form of neonatal allo-immune thrombocytopenia due to anti-HPA-1a has also been reported in cases of IVF with donor oocyte (Curtis, 2005), with homozygous expression of HPA-1a being a possible factor.

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There has been an increase in the use of assisted reproductive technologies in Europe with 13 609 cases involving oocyte donation in 2008 alone (Ferraretti *et al.*, 2012). We feel the clinical relevance of homozygous expression of antigen on fetal red cells, needs to be evaluated with regards to potential severity of HDFN. In the interim, current clinical advice may need to stipulate that referral indicator levels for specialist fetal assessment are based on heterozygous expression of fetal red cell antigens and not homozygous expression, the latter now being possible with the advent of donor oocyte implantation into a secondary uterus.

The lack of information available to clinicians regarding nature of conception is central to this issue, and is especially problematic in this jurisdiction where legal guardianship for prospective parents is ambiguous and is currently being tested in the courts. In future, the nature of conception could be queried with mothers when clinically significant red cell allo-antibodies have been detected, however, accurate information may not always be forthcoming and there remains a stigma attached to the use of donor oocyte IVF. If a pregnancy involving donor oocyte IVF is identified and red cell antibodies are detectable, closer monitoring may be appropriate. Lack of information regarding donor oocyte red cell antigen profile is also an issue with limited or no testing being performed by IVF clinics in this regard.

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B. D. analysed the case and drafted the letter. All authors contributed to the revision of the letter.

CONFLICT OF INTEREST

The authors have no competing interests.

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REFERENCES

Curtis, B.R. (2005) Fetal and neonatal alloimmune thrombocytopenia in pregnancies involving *in vitro* fertilisation: a report of four cases. *American Journal of Obstetrics and Gynecology*, **192**, 543–547.

Ferraretti, A.P., Goossens, V., de, Mouzon, J. *et al.* (2012) Assisted reproductive technology in Europe, 2008: results generated from European registers by ESHRE. *Human Reproduction*, **27**, 2571–2584.

Gooch, A., Parker, J., Wray, J. & Qureshi, H. (2007) Guideline for blood group and antibody testing in pregnancy. British Committee for Standards in Haematology Blood Transfusion Task Force. *Transfusion Medicine*, **17**, 252–262.

Kozlowski, C.L., Lee, D., Shwe, K.H. & Love E.M. (1995) Quantification of anti-c in haemolytic disease of the newborn. *Transfusion Medicine*, **5**, 37–42.

Mair, D.C. & Scofield, T.L. (2003) HDN in a mother undergoing in vitro fertilization with donor ova. *Transfusion*, **43**, 288–289.

Mitchell, S. & James, A. (1999) Severe haemolytic disease from rhesus anti-C antibodies in surrogate pregnancy after oocyte donation. *The Journal of Reproductive Medicine*, **44**, 388–390.

Patel, R.K., Nicolaides, K. & Mijovic, A. (2003) Severe haemolytic disease of the fetus following in vitro fertilization with anonymously donated oocytes. *Transfusion*, **43**, 119–120.