Original Research

Crossmatching Maternal and Fetal Blood in Sheep

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We investigated the incidence of *ex vivo* incompatibility between ovine maternal RBC and fetal plasma. Time-mated singleton pregnant ewes ($n = 8$) underwent cesarean delivery of the fetus; at the time of delivery, paired maternal and fetal blood samples were collected and subsequently separated for storage as packed RBC and fresh frozen plasma. Gel column crossmatching was performed 3 to 4 wk later. All fetus–dam crossmatches were considered major crossmatches, combining fetal (recipient) plasma with dam (donor) RBC. The plasma of 8 fetuses was cross-matched with RBC from 5 dams; all autologous controls were negative, and all but one crossmatch (1 of 40, 2.5%) were considered compatible. In addition, the plasma of 3 dams was crossmatched with RBC from 5 dams; all autologous controls were negative; however, significant incompatibility was noted. In total, 4 of 13 (30.8%) dam–dam crossmatches were considered incompatible. The results of this initial study suggest that when a single animal receives multiple blood-product transfusions, the risk of an immunologic transfusion reaction can be reduced by ensuring that the blood products are obtained from a single donor, performing a crossmatch prior to transfusion, and the use of synthetic products to increase the oxygen carrying capacity of fetal blood.

Abbreviation: EVE, *ex vivo* uterine environment

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A preterm lamb model of an ‘artificial placenta,’ or *ex vivo* uterine environment (EVE) therapy, has recently been developed.^{7,8,11,17} This model is an artificial version of extracorporeal support of the fetus, which is ordinarily provided by the placenta in utero.¹¹ Priming of the extracorporeal circuit in these models requires whole blood, and fresh maternal blood is used initially.^{7,8,17} Subsequent blood transfusions comprise either stored whole blood¹¹ or separated blood products (packed RBC and plasma).^{7,8,17}

Given the ongoing development of the EVE model, multiple blood transfusions may be required to sustain fetal life for days to weeks. In this context and other clinical scenarios, transfusion of blood or blood products aims to replace blood components and to increase the oxygen-carrying capacity of the blood.⁵ Although blood transfusion is a life-saving therapy, it is not without risk, and transfusion reactions can be associated with acute or delayed immunologic reactions, circulatory overload, transmission of infectious agents, metabolic alterations, and embolism.⁴

In a clinical environment, crossmatching of donor and recipient blood in sheep is not considered necessary if the animal has not been transfused previously.⁶ This practice is justified on the basis that crossmatching is time- and cost-prohibitive and that the incidence of transfusion reactions in small ruminants is low (16%).⁶ One retrospective study reported that the administration of non crossmatched blood to small ruminants represents minimal risk for the recipient.⁶ Other investigators emphasize

the importance of pretransfusion compatibility testing in sheep, given the documentation of 17% incompatibility in a prospective study using saline and albumin agglutination methods.¹⁶ To our knowledge, preterm and neonatal offspring of small ruminants that have received single or multiple blood transfusions have not been reported previously in the literature. Furthermore, clinical practices may not represent an appropriate benchmark for application to a complex and resource-intensive research model such as the EVE platform.

In the context of the EVE model, in which preterm lambs are supported for days to weeks, the administration of whole blood or blood products is required. Given this essential component of the model, the paucity of data in the literature describing the risks of maternal donor and fetal recipient reactions and the lack of a commercially available reagent or kit to type sheep blood, we investigated the incidence of *ex vivo* incompatibility between maternal RBC and fetal plasma.

Materials and Methods

This study was part of a group of studies approved by the Animal Ethics Committee at the University of Western Australia in accordance with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes*.⁹ Timed-mated singleton-pregnant Merino cross ewes ($n = 8$) underwent anesthesia for cesarean delivery of the preterm fetus at 121 d of gestation for a separate study. The ewes weighed 58.1 ± 6.2 kg and were approximately 4 y of age.

Prior to transport to the university farm, the sheep were inspected by a veterinarian on the farm of origin in the southwest of Western Australia to ensure that the animals were healthy and in good body condition (2.5 to 3.5 [maximum, 5]). The ewes had been treated (abamectin, oxfendazole, and levamisole) for

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intestinal parasites twice during pregnancy and had been vaccinated against ovine Johne disease (*Mycobacterium paratuberculosis*) as lambs. After arrival at the university farm, the ewes were monitored twice weekly for body weight, body condition, food and water intake, and general wellbeing. The ewes had been introduced to the university farm paddocks at least 1 mo prior to the study and moved from the paddock to the indoor shed environment 24 h prior to anesthesia. The ewes were fed a diet of pasture supplemented with oaten hay and received approximately 40 g of pellets (Macco Feeds 707 pellets) twice daily. Food was not withheld prior to anesthesia.

The ewes were anesthetized with midazolam (0.5 mg/kg IV; Midazolam Apotex solution for injection 5 mg/mL, Apotex, New South Wales, Australia) and ketamine (10 mg/kg IV; Ilium Ketamil Injection 100 mg/mL, Troy Laboratories, New South Wales, Australia) administered as a bolus into a catheter in a cephalic vein. Subarachnoid injection of lidocaine (3 mL, Ilium Lignocaine 20 mg/mL, Troy Laboratories) at L6–L7 or at the lumbosacral junction by using a spinal needle (0.9 × 90 mm, Becton Dickinson, Madrid, Spain) then was performed before ventral midline laparotomy and hysterotomy for delivery of the preterm lamb fetus. The fetus was delivered and managed according to the ventilation study protocol for a maximum of 1 h.

Immediately after delivery of the fetus, paired maternal (4 mL) and fetal (2 mL) blood samples were collected from the jugular vein and umbilical artery, respectively, into vacuum phlebotomy tubes containing EDTA (Becton Dickinson). The blood was separated by centrifugation within 2 h for storage of packed RBC at 4 °C and plasma at –80 °C. The ewes were euthanized by using intravenous pentobarbitone (160 mg/kg, Lethobarb 325 mg/mL, Virbac, New South Wales, Australia) within 12 min of induction of anesthesia. Death of the ewe was confirmed by ensuring the lack of spontaneous ventilation, heartbeat, and corneal reflex.

Gel column crossmatching was performed 3 to 4 wk later. All fetus–dam crossmatches were considered major crossmatches, combining fetal (recipient) plasma with dam (donor) RBC. In addition, allogenic dam–dam crossmatches were performed for comparison. Autologous negative controls (recipient plasma with its own RBC) were performed also. All gel column crossmatches were performed according to the manufacturer's instructions (Gel: ID Cards, BioRad, Hercules, CA; NaCl, Enzyme Test, and Cold Agglutinins: DiaMed, Cressier, Switzerland). Briefly, 10 µL of packed RBC from each donor was added to a tube containing 1 mL of low-ionic-strength solution (LISS, ID-Diluent 2: DiaMed) and mixed gently, to prepare a 1% RBC suspension. Then, for each donor–recipient combination, 50 µL of the 1% donor RBC suspension and 25 µL of recipient plasma were added on top of the gel in successive card microtubes (6 microtubes per card) and incubated at 37 °C for 15 min in an incubator (Refrigerated Incubator, Thermoline Scientific, Smithfield, New South Wales, Australia). Thereafter, the gel column cards were centrifuged for 10 min at 85 × g, in a centrifuge provided by the manufacturer (model 12 S II, DiaMed). The magnitude of agglutination was graded from 0 (negative) to 4+ (strongly positive). An incompatibility score of 0 was given when all RBC were at the bottom of the gel, ruling out agglutination. A score of 1+ was assigned when RBC were in the bottom half of the gel; 2+ when RBC were throughout the entire gel, 3+ when RBC were seen in the top half of the gel, and 4+ when all RBC were in a tight disc at the top of the gel. Consistent with literature in dogs,² crossmatches were interpreted as compatible or negative when scores were 1+ or lower, and incompatible or positive when 2

or greater. Compatibility reactions were evaluated by a single experienced investigator (CRS).

Results

The plasma of 8 fetuses was crossmatched with RBC from 5 dams; all autologous controls were negative (representative example, Figure 1), and all but one crossmatch (1 of 40, 2.5%) were considered compatible (Table 1). In addition, the plasma of 3 dams was crossmatched with RBC from 5 dams; all autologous controls were negative, but marked incompatibility was noted (Table 2). In total, 4 of 13 (30.8%) dam–dam crossmatches were considered incompatible due to 2+ agglutination reactions.

Discussion

We performed this study to investigate the incidence of reactions between maternal RBC and fetal plasma in sheep. The results of this initial study demonstrated that the vast majority (97.5%) of maternal donors and fetal recipients were compatible. In addition, the incidence of incompatible crossmatching between adult ewes was 30.8%. Taken together, these data suggest that lambs may not be born with antibodies against other blood types but rather may acquire such antibodies at some time during early life.

Outside of a research setting, intensive care of individual sheep, including the administration of blood transfusions, is not common. One recent retrospective study in sheep underemphasizes the incidence of transfusion reactions,⁶ whereas another prospective study considers the risk of a reaction to be clinically important¹⁶ despite reporting a similar incidence of incompatibility (16% to 17%).¹ These differences of opinion, in combination with our current results, reflect the need for comprehensive and detailed knowledge in this field.

Various historical descriptions of the blood groups of domestic sheep are available. Early descriptions from the 1960s refer to 7 sheep blood groups: A, B, C, D, M, R-O, and X-Z.^{13,14} Another source from 1982 reports 8 blood group systems (A, C, D, M, F30, B, R, and F41), with 1 to 12 factors within each system, yielding a total of 29 sheep blood-group factors.^{1,10} Without commercially available reagents and shared samples, integrating such data and replicate these typing experiments is difficult. Various authors have provided their interpretation, including one text that reports 7 major groups with the addition of the B and R groups to be considered clinically significant.¹⁵ More relevant to the EVE model and our current study is that these historic studies suggest that newborn lambs may not have antibodies against other blood types nor immunogenic antigens on their RBC surface.¹³

Information regarding lambs includes reference to R antigen and antiR antibodies, in addition to well as O antigen and antiO antibodies.¹³ AntiR antibodies are acquired passively from colostrum but disappear before the lamb begins to produce antiR antibodies, which may not be evident in their serum until 5 to 7 mo of age.¹³ Another feature in lambs is that those with R antigen in their sera had RBC that did not become R-positive until 16.4 d of age, whereas lambs with O antigen in their serum had RBC that only become O-positive at 28.4 d of age.¹³ These data suggest that preterm lamb fetuses can accept donor blood with minimal to negligible risk of an immunologic transfusion reaction, because they do not ingest colostrum and are too young to have produced antibodies against foreign RBC antigens. The results of the current study support this theory but do not address whether lambs receiving multiple transfusions of donor plasma develop antibodies to RBC antigens over time.

Figure 1. (A) Gel column crossmatches performed between fetal RBC (7002F) and plasma from 5 dams (7002, 7003, 7007, 7009, and 7010) revealed no evidence of incompatibility, because all of the RBC are at the base of the gel. (B) Gel column crossmatches performed between fetal RBC (7009F) and plasma from 5 dams (7002, 7003, 7007, 7009, and 7010) revealed incompatibility between maternal dam 7010 and fetus 7009.

Table 1. Compatibility results of gel column crossmatches performed between fetal RBC and dam plasma

Fetus	Autologous control	Dam number				
		7002	7003	7007	7009	7010
7002F	0	0	0	0	0	0
7003F	1+	0	0	0	0	0
7007F	0	0	0	0	0	0
7009F	0	1+	0	0	0	0
7010F	0	0	1+	1+	0	0
7012F	0	0	0	0	0	2+
7019F	0	0	0	1+	0	1+
7026F	0	0	0	0	0	1+

Note that 7002F is the fetus of dam 7002, 7003F is the fetus of 7003, and so on.

Scale: 0, no evidence of agglutination, with all RBC at the bottom of the gel; 1+, RBC in the bottom half of the gel; 2+, RBC throughout the entire gel.

In species in which blood transfusions are commonly performed, typing of blood from donors and recipients is recommended prior to the administration of blood or packed RBC, to facilitate the administration of type-specific blood products and thus avoid transfusion-associated reactions. Because commercially available ovine blood typing kits are unavailable, we recommend donor–recipient crossmatching to facilitate the identification of compatible blood products for transfusion. A major crossmatch is sufficient to demonstrate whether the recipient has been sensitized previously or has naturally occurring isoantibodies to the donor’s RBC that might cause an acute hemolytic transfusion reaction.⁴ The gel card crossmatching we performed in the current study is suitable to predict the compatibility of donor and recipient, as is the saline and albumin tube compatibility testing that has been reported recently.¹⁶

The results of this preliminary study can only be interpreted in view of the limitations, which include a small sample size, prolonged sample storage prior to crossmatching, and evaluation of

Table 2. Compatibility results of gel column crossmatches performed between dam RBC and dam plasma, demonstrating frequent incompatibility

Dam number	Autologous control	Dam				
		7002	7003	7007	7009	7010
7009	0	0	0	0	0 ^a	2+
7010	0	1+	0	2+	2+	0*
7012	0	0	1+	0	0	2+

Scale: 0, no evidence of agglutination, with all RBC tightly packed at the bottom of the gel; 1+, RBC in the bottom half of the gel; 2+, RBC throughout the entire gel; 3+, RBC in the top half of the gel; 4+, all RBC in a tight disc at the top of the gel.

^aAutologous

compatibility within a single breed. This study was small because it was intended as a preliminary investigation to determine whether a more comprehensive set of testing was justified. Crossmatching is ideally performed on fresh samples, and sample storage of 4 wk effects crossmatch results in horses.³ That being said, the aforementioned equine study suggested a worsening of compatibility with protracted storage, thus compatible crossmatches, despite storage, are likely to be truly compatible. In the current study, whether the single episode of maternal–fetal crossmatch incompatibility was due to an aged sample, or true incompatibility is unknown. Future prospective studies should use freshly collected blood samples for crossmatching, or at least use RBC stored with a preservative solution such as saline–adenine–glucose–mannitol. A single breed of sheep was used in this study and care should be taken when extrapolating these results to other breeds.

Further work is required to determine whether multiple blood-product transfusions will alter the compatibility of pre-term fetal sheep recipients over time and to determine whether the initial findings of a low rate of maternal–fetal crossmatch incompatibility holds true in a larger sample size. In the interim, donor ewes that are destined for euthanasia after cesarean delivery of the preterm fetus to the EVE platform should provide as much blood as possible. If multiple units can be collected and stored appropriately, it may be possible to ensure that a fetus receives blood from a single donor. When the blood product supply is exhausted or the shelf life is exceeded (especially for RBC) 2 suggestions are made: crossmatch prior to transfusion and, when available, use synthetic products to increase the oxygen-carrying capacity of fetal blood.¹²

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