Journal of the American Association for Laboratory Animal Science
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Vol 45, No 6
November 2006
Pages 49-53

Repeated Automated Plasmapheresis in Goats (*Capra hircus*): A Clinically Safe and Long-term Refinement to Current Antibody Recovery Techniques

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Automated plasmapheresis is an optimal method of plasma collection because the donor is a part of a closed loop where whole blood is withdrawn and separated and packed cells are returned in a serial fashion until the desired amount of plasma is obtained. The typical approach to collection of antibody-rich plasma involves withdrawal of whole blood from vaccinated animals, yielding approximately 1000 ml plasma from each animal, which is euthanized after this process. In the present study, 32 goats (*Capra hircus*) were vaccinated and conditioned for restraint in a modified Panepinto sling. Each animal was monitored clinically, including complete and differential blood counts and serum chemistries 24 h before and 24 to 48 h after each procedure. A jugular vein was surgically prepped, a 16-gauge needle catheter was placed, and the animal was attached to an automated plasmapheresis machine. After plasma removal, return of the resuspended packed blood cells, and infusion of 500 ml 0.9% NaCl, the animal was disconnected from the machine, the catheter removed, and the animal returned to the barn. There were no clinically significant changes in either the complete blood counts or the clinical chemistries during the course of this study. These 32 animals produced 240,000 ml of immunoglobulin-rich plasma over the course of this project and more than 949,000 ml of plasma to date. This study identifies a refinement in current antibody-recovery techniques and potentially reduces the number of animals necessary to produce bioreagents on a long-term and continual basis.

Traditionally, plasmapheresis is performed manually by removing whole blood from the donor, allowing the plasma to separate from the cellular mass, extracting the plasma component, and returning the resuspended red blood cells to the donor.[2] Numerous plasma recovery studies use horses because of their rapid erythrocyte sedimentation rate.[3,4] Whole blood is harvested from the animal in bottles or bags containing acid-citrate-dextrose (ACD) anticoagulant and held at room temperature for 1 to 2 h. This technique is simple but has many drawbacks, including the potential for bacterial contamination and large numbers of cellular components in the plasma, which may create hypersensitivities in recipients.

The introduction of automated inline blood cell separators nearly 3 decades ago has revolutionized the plasmapheresis process. These in-line automated plasmapheresis machines were initially developed for plasma collection in human healthcare operations and are now used in the research setting to collect immunoglobulin-rich plasma for use as bio-reagents. In-line separators are safe, easy to use, efficient, and use microprocessor technology to monitor both machine and patient. Many automated plasmapheresis machines use disposable sterile equipment for each donor, thereby removing the risk of cross-contamination. Continuous-flow plasmapheresis allows for rapid and sterile separation of blood into its component parts, collection of the desired amount of plasma, and return of the cellular components to the donor.

The automated plasmapheresis machine used in the current study (Autophresis-C, Baxter Healthcare, Deerfield, IL) is a continuous system used in various human clinical and medical applications since 1986. This set-up is commonplace in many human plasma donor centers and is well-suited for automated plasma recovery in animals, especially goats.[1-3,6,8] The automated plasmapheresis machine removes blood from a peripheral vein through a single-lumen catheter and includes a spinning membrane filtration device, which rapidly and gently separates whole blood into plasma (which is diverted into a bag for collection) and concentrated cellular components (for reinfusion).

Current commercial and laboratory antibody production and plasma recovery protocols involve the use of mice, rats, guinea pigs, rabbits, goats, and horses.[2,8,9] Antibody recovery protocols using goats typically last approximately 120 d, with approximately 1 L of plasma recovered per goat per protocol;[7-9] the animal(s) are euthanized at the end of the study to maximize antibody and plasma recovery. Goats (*Capra hircus*) are useful in antibody-recovery protocols because they are easy to train and handle, have relatively large quantities of plasma, and are relatively inexpensive to procure and maintain.

My colleagues and I hypothesized the clinical chemistry and hematologic parameters of goats undergoing repeated automated plasmapheresis at 2 different plasma recovery volumes would not differ significantly from baseline clinical values. The objectives of this study were to improve the antibody-recovery process by using an automated plasmapheresis system, decrease the time and manpower required to produce large volumes of immunoglobulin-rich plasma, and monitor hematology and serum clinical chemistries for long-term use of the same animals beyond current 120-d protocols.

Materials and Methods

Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating

Received: 17 Apr 2006. Revision requested: 22 June 2006. Accepted: 22 June 2006.
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to animals and experiments involving animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals*[10] The United States Army Medical Research Institute of Infectious Diseases (USAMRIID) is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International.

Animals. Our antibody production protocol used 32 goats (age, 2 to 7 y; weight, 47.7 to 127.3 kg; 26 wethers and 6 does). The goats were purebred Boer, Nubian, Saanen, and Alpine animals and intercrosses. All animals underwent a conditioning regime at the vendor source, were serum-negative for brucellosis, Q fever, and caseous lymphadenitis, and were vaccinated against rabies (RabVac 3, Fort Dodge Animal Health, Fort Dodge, IA) and clostridial diseases (BarVac 7, Boehringer Ingelheim, St Joseph, MO). During a 14-d quarantine period at the Fort Detrick Large Animal Research Facility, baseline clinical chemistry and blood count values were established for each animal. Goats were maintained on mixed-grass pastures with access to sheds for protection against inclement weather. In addition, approximately 0.5 kg of flaked grain per animal (14% protein [minimum], 9% crude fiber [maximum], 2% fat [minimum], Farmers Co-op, Frederick, MD) was provided daily to supplement the goats' nutritional status.

Vaccinations. The initial injection for antibody production included complete Freund adjuvant, followed by subsequent monthly boosts with incomplete Freund adjuvant. Injections were given subcutaneously, alternating along the cranial half of the withers, every 2 wk for 6 wk. At 10 days after the third dose, a serum sample was drawn to verify antibody levels, and the animal was placed on the plasmapheresis schedule. Animals then were injected monthly to maintain high levels of circulating immunoglobulins.

Conditioning. All animals were conditioned to the Panepinto sling (BH, Denver, CO) during five 10-min training sessions before the first plasmapheresis procedure. Panepinto slings were chosen to decrease the manpower necessary to restrain the animals for plasma recovery. A single in-house modification was made to the Panepinto sling to position the animals for the plasmapheresis procedure: a small-ruminant head harness (Sydell, Burbank, SD) was attached to one end of the sling. This head harness allowed the animal to maintain a more normal position when undergoing plasmapheresis. In addition, links of chain wrapped in foam padding were attached to the head harness to prevent movement of the animal's head during the procedure. Head restraint was necessary to prevent movement of the needle catheter. Because none of several approaches to securing the catheters (including suturing, tissue glue, and tape) successfully kept them in place throughout plasma collection, an animal technician monitored the catheter and goat continuously during each procedure.

Each animal was led gently into the sling, its legs were placed in the holes of the sling, and the sling was wound to lift the goat from the ground to the level of the plasmapheresis machine. Once the animal was positioned in the sling and head harness, a single person could maintain and continuously monitor both the animal and the plasmapheresis machine. Each animal spent approximately 50 min in the sling over the 5 conditioning training sessions. While in the sling, goats were given various food rewards (carrots, slices of apple, sweet feed mixture, and alfalfa hay). Only 2 of the 32 animals required a dose of xylazine (10 mg, Sigma-Aldrich, St Louis, MO) during the first 2 conditioning sessions; these animals required no additional tranquilization during subsequent conditioning and plasmapheresis sessions.

Plasmapheresis. The day before plasmapheresis, goats were herded to the barn area, examined, and weighed, and blood was drawn for serum chemistries and hematology (preplasmapheresis samples). On the day of plasmapheresis, each animal was placed in the Panepinto sling and transported to the plasmapheresis room. Hair was clipped from the selected jugular groove, which was aseptically prepared with iodine scrub and isopropyl alcohol, a 16-gauge butterfly catheter was placed, and the animal was connected to the automated plasmapheresis machine. Jugular vein use was alternated for each plasmapheresis procedure to reduce trauma to the jugular veins.

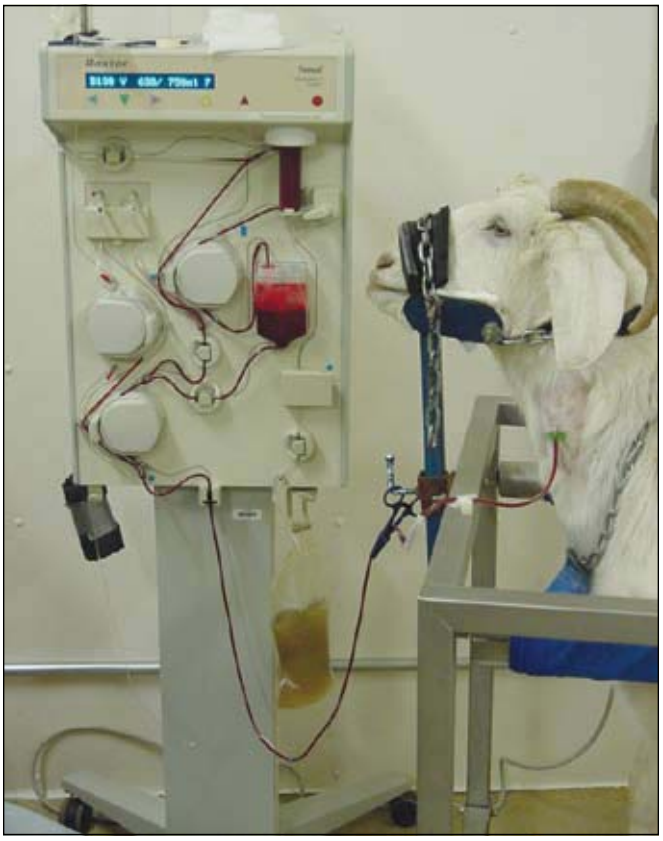

Figure 1. Automated plasmapheresis machine, modified Panepinto sling, and goat undergoing automated plasmapheresis.

Plasma was recovered via an automated plasmapheresis machine (Figure 1) and collected in 1-l plasma bags (Baxter Health Care, Deerfield, IL). The plasmapheresis machine continually removed whole blood in a collection cycle, separated the components into acellular (plasma) and cellular blood components, temporarily stored the cellular components until approximately 300 ml was collected, and then returned the resuspended cellular components to the animal before beginning another collection cycle. The plasma was directed continually into the 1-L plasma bag. The collection and reinfusion cycles continued until the preprogrammed amount of plasma was collected. The plasma was labeled and then stored in a –30 °C freezer pending antibody purification. When plasma collection was complete, the plasmapheresis machine infused 500 ml 0.9% saline to offset the plasma volume removed. After saline infusion, the needle catheter was removed, and a sterile 4 × 4 pad was placed over the venipuncture site and held in place until hemostasis was achieved. The animal then was moved to an outdoor corral for postprocedural monitoring.

All animals were continuously monitored during the procedure for signs of distress. If noted, the procedure was paused to allow a veterinarian to assess the animal (auscultation of

Table 1. Laboratory data from goats after plasmapheresis of 500 and 1000 ml

			500 ml			1000 ml			
	Normal range	Unit	Mean	1 SD	Range	Mean	1 SD	Range	*P*
Alkaline phosphatase	39–328	U/l	203.36	150.24	38–652	229.5	198.5	39–968	0.6557
Alanine transferase	19–38	U/l	32.17	4.71	23–49	33.4	6.7	21–58	0.1685
Asparagine transferase	55–144	U/l	98.86	48.66	45–438	111.0	90.4	50–734	0.2973
Ca^{2+}	7.9–9.8	mg/dl	8.77	0.57	7.2–10	8.7	0.6	7.4–10.4	1.0000
Cl^-	105–114	mmol/l	109.14	2.59	100–116	110.2	2.9	103–118	0.0020[a]
Creatinine	0.5–1.1	mg/dl	0.84	0.15	0.6–1.9	0.8	0.1	0.6–1.3	1.0000
Glucose	48–80	mg/dl	69.79	30.46	41–306	72.8	13.9	46–159	0.8299
Hematocrit	21.6–34.9	%	28.54	4.83	16.5–39.6	28.9	4.6	18.9–40	0.9965
Hemoglobin	8.1–13.8	g/dl	10.96	1.92	6.2–15.8	11.0	1.7	7.2–14.3	1.0000
K^+	4.1–5.4	mmol/l	4.75	0.47	3.4–6.1	4.7	0.5	3.1–6.1	0.6557
Mean corpuscular hemoglobin	5.7–7.8	pg	6.77	0.42	5.6–7.7	6.8	0.5	5.5–7.9	0.9999
Mean corpuscular hemoglobin concentration	35.9–41.8	g/dl	38.44	1.79	32.3–41.7	40.2	27.8	30.9–387.8	0.9999
Mean corpuscular volume	14.8–20.2	fl	17.62	1.00	15.1–19.5	17.9	1.5	12.9–22.3	0.6254
Na^+	142–151	mmol/l	146.78	2.20	141–155	146.3	2.3	138–154	0.9916
PO_4^-	4–9.1	mg/dl	6.34	1.53	2.5–11.3	5.9	1.4	2.9–9.8	0.0545
Red blood cells	11.94–20.88	$\times 10^6/\mu l$	16.21	2.78	8.85–22.7	16.3	2.5	9.9–22.2	1.0000
Red cell distribution width	31.2–42.7	%	37.38	3.61	27.8–50.9	37.2	3.3	28.1–47.1	0.9999
Total bilirubin	0.2–0.5	mg/dl	0.34	0.09	0.2–0.9	0.4	0.1	0.2–1.3	1.0000
Total protein	5.8–8.1	g/dl	6.68	0.58	5.3–8.1	6.3	0.6	4.8–7.9	0.0000[a]
White blood cells	3.8–15.4	$\times 1000/\mu l$	10.59	4.26	3.5–24.8	11.5	5.3	4.3–35.9	1.0000

SD, standard deviation.
[a]Value for post 500 ml plasma is significantly ($P < 0.05$) different from post 1000 ml.

the thorax; evaluation of heart rate, skin turgor, and mucous membranes), with subsequent continuation of the procedure as appropriate. After the procedure the animals were monitored in the corral for increases in respiratory rates or changes in behavior. Each animal was checked visually every hour for approximately 4 to 8 h and then was re-examined at 24 to 48 h (at the time of postprocedural blood collection).

At 24 to 48 h after plasmapheresis, an additional blood sample was collected for postprocedural serum chemistries and hematology. Each goat underwent plasmapheresis twice a month. The hematology and serum biochemical values analyzed in this study were the pre- and postprocedural data for the first 5 sessions for recovery of 500 ml plasma and those for the first 5 sessions for recovery of 1000 ml plasma during each session. There were 160 data points for each pre- and postprocedural plasma amount collected for a total of 320 pre-samples and 320 post-samples.

All serum chemistries were evaluated in an automated analyzer (Vitros 250, Ortho-Clinical Diagnostics, Raritan, NJ) that used disposable tips, ensuring no carryover between samples. The complete blood counts were performed in an automated hematology machine (Cell-Dyn 3700, Abbott Labs, Abbott Park, IL) with manual differential cell counts.

Data analysis. All dependent variables met assumptions of normality and homogeneity of variance. No outliers were identified. Laboratory values obtained before and after plasmapheresis were compared. Abnormal laboratory values were assessed using *z* tests to compare each value against the corresponding normal range for the specific lab test. Stepdown Bonferroni procedures were used to adjust for multiple comparisons within each group of analyses. Analyses were conducted using SAS statistical software (version 9.1, SAS Institute, Cary, NC). A *P* value of <0.05 was used to define statistical significance among the values tested.

Results

Automated plasmapheresis according to the described schedule was well tolerated by all goats and produced highly concentrated immunoglobulin-rich plasma for the length of this study. No animals required removal from the plasmapheresis machine because of increased respiratory rate, signs of discomfort, or adverse cardiovascular effects. No weight loss was noted in these animals over the course of this study.

Large-volume plasmapheresis produced few abnormal differences in clinical laboratory data from established baseline values over 2 different plasma recovery amounts (500 and 1000 ml). All animals whose initial blood values were beyond the established normal values for our lab underwent a second blood draw to re-evaluate the abnormal value. All of the recheck values were within normal limits, and the animals remained clinically normal. Study goats tolerated recovery of as much as 1 L (1000 ml) of plasma twice monthly and were clinically normal (according to assessment of animal's pre- and postprocedural behavior and activity, weight, clinical pathology, and physical examination) throughout the duration of the study. The automated plasmapheresis machine collected and cycled an average of 1531 ml whole blood over 36.72 min to yield 500 ml plasma and an average of 2625 ml whole blood over 58.17 min for 1000 ml plasma (a range of approximately 15 to 60 ml plasma per min).

Only the mean values for Cl^- and total protein (TP) differed significantly ($P < 0.05$) between the postprocedural blood samples, but these means remained within the normal ranges for goats as posted by our in-house clinical laboratory. The plasma Cl^- concentration after collection of 1000 ml plasma (110.2 mmol/l) was slightly but significantly ($P = 0.002$) higher than that after 500 ml (109.14 mmol/l), and TP levels after 1000 ml was decreased compared with 500 ml (6.68 g/dl versus 6.3 g/dl, $P = 0.000$). Table 1 displays the serum chemistry profiles

Table 2. Dates of first and last plasmapheresis and total plasma volume donated for each animal while on protocol

Goat no.	Start date	End date	Total plasma (ml)
29	13 Nov 2003	01 Dec 2005	38,236
32	13 Nov 2003	01 Dec 2005	37,250
36	13 Nov 2003	01 Dec 2005	39,250
45	13 Nov 2003	01 Dec 2005	38,050
54	13 Nov 2003	01 Dec 2005	39,250
59	13 Nov 2003	01 Dec 2005	38,250
68	13 Nov 2003	01 Dec 2005	39,139
70	13 Nov 2003	01 Dec 2005	38,036
80	13 Nov 2003	01 Dec 2005	39,159
84	13 Nov 2003	01 Dec 2005	38,070
113	03 Jun 2004	01 Dec 2005	28,103
122	28 Jul 2004	01 Dec 2005	22,112
123	28 Jul 2004	20 Apr 2005	13,500
125	28 Jul 2004	01 Dec 2005	23,250
126	03 Jun 2004	not applicable	34,949
127	03 Jun 2004	not applicable	36,000
130	28 Jul 2004	21 Oct 2005	21,750
131	03 Jun 2004	not applicable	36,116
132	28 Jul 2004	01 Dec 2005	23,250
133	28 Jul 2004	01 Dec 2005	23,189
134	28 Jul 2004	01 Dec 2005	23,250
135	28 Jul 2004	01 Dec 2005	23,250
136	28 Jul 2004	01 Dec 2005	23,250
137	28 Jul 2004	01 Dec 2005	23,340
138	01 Jul 2004	not applicable	35,500
142	01 Jul 2004	not applicable	36,010
143	28 Jul 2004	21 Oct 2005	22,500
146	28 Jul 2004	21 Oct 2005	22,500
147	28 Jul 2004	01 Dec 2005	23,250
148	28 Jul 2004	01 Dec 2005	23,070
150	28 Jul 2004	01 Dec 2005	23,128
153	28 Jul 2004	01 Dec 2005	23,250
Total			949,207

End date denotes removal from the animal from the plasmaphresis schedule. Animals without a listed end date donate 750 ml every 3 wk.

and complete blood counts obtained after plasmapheresis for all animals in this study. No significant changes in differential blood cell counts occurred over the course of this study. The 32 goats in this study produced more than 240 L of immunoglobulin-rich plasma during the 320 plasma collection sessions.

Discussion

The changes in Cl^- and total protein values were attributed to the postprocedural replacement of plasma with 0.09% NaCl. Although individual animals had biochemical values outside the laboratory normal ranges, none of the goats showed any adverse clinical effects from the procedure. All animals with values beyond the 95% confidence interval or beyond the normal ranges were rechecked 24 h after the last postprocedural blood draw. No animal demonstrated an abnormal value at the recheck blood draw, and all goats appeared clinically normal for the duration of the study and beyond.

Although some animals had values outside the laboratory normal range, only the data for the initial postprocedural blood samples were analyzed statistically, and not the 24 h rechecked laboratory values. However, the critical *P* value of 0.05 or less, the standard for defining statistical significance, does not necessarily indicate a difference of biologic significance affecting the health of the animal. What ultimately is important is whether the statistically significant difference causes (or is likely to cause) any harm. At present, the significantly different clinical chemistry values described are not likely to biologically affect the plasmapheresed goats.

The positive clinical results of this study allowed these 32 animals to continue to produce plasma beyond the five 500-ml and five 1000-ml sessions described. Table 2 shows the amounts of plasma recovered from each animal over the course of the antibody recovery protocol. These 32 goats have produced more than 949 L of immunoglobulin-rich plasma to date, and 5 animals continue to donate 750 ml every 3 wk.

A current literature search revealed no research papers describing automated plasmapheresis and its possible long-term biochemical effects on goats. Feige and colleagues published several studies that compared various plasma collection protocols with automated plasmapheresis and associated effects on clinical, hematological, and coagulation variables in horses.[4,5] These papers were informative in designing the present study and allowed the formulation of collection maximums for goats. This study is the first to address the effects of repeated long-term automated plasmapheresis in goats.

The automated plasma collection technique described here is cost-effective and may be suitable for smaller bioreagent collection operations. Including the initial purchase of 4 plasmapheresis machines, 4 Panepinto slings, and 320 sterile disposable plasmapheresis sets and catheters and excluding labor costs, animal costs, and per-diem fees, our equipment cost per ml of plasma collected was approximately $0.49 for this study. This cost per ml will decrease as larger amounts of plasma are recovered. For example, the initial purchase of this equipment has enabled plasma collection from these goats beyond the scope of this report, to the extent of 949 L of plasma to date at a cost of approximately $0.19 per ml.

As delineated here, automated plasmapheresis is a safe and reliable long-term method of removing plasma from goats and offers technical improvement and refinement to manual plasma recovery techniques. Animals can undergo multiple collection procedures with collection of as much as 1000 ml of plasma twice monthly without adverse clinical effects. This procedure also has the potential to dramatically reduce the number of animals required to produce the quantity and quality of immunoglobulin desired for biomedical research. The results of the present study support the long-term use of the same animals for production and recovery of antibody-rich plasma without adverse clinical and serum chemistry effects.

Acknowledgments

Special thanks are extended to Carol Eisenhauer, PJ Rico, Randal Schoepp (USAMRIID, Fort Detrick, MD) and Peter Emanuel (Critical Reagents Program, Aberdeen Proving Ground, Aberdeen, MD) for supporting and providing the resources needed for this project; Susan Goodwin, Alec Hail, Ken Jacobsen for their editing of the manuscript; Sarah Norris for her diligent statistical analysis; and Mike Davis, Edwin Picado, Reese Baker, Todd Williams, Heidi DiPerna, Eric Kline, Brenda O'Quinn, and the soldiers, technicians, and caretakers for providing the expert handling and care for these and all the animals of the Veterinary Medicine Division, USAMRIID, Fort Detrick, MD. The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the true views of the Department of Defense, the Department of the Army, or USAMRIID.

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