


STANDARD ARTICLE

Influence of canine donor plasma hemostatic protein concentration on quality of cryoprecipitate

Macy Drinkhouse¹ | Marjory B. Brooks² | Darko Stefanovski³ | Kimberly Marryott¹ |
Mary Beth Callan¹ 

¹Department of Clinical Sciences and Advanced Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

²Comparative Coagulation Section-Animal Health Diagnostic Center, Cornell University, Ithaca, New York

³Department of Clinical Studies-New Bolton Center, University of Pennsylvania, Kennett Square, Pennsylvania

Correspondence

Mary Beth Callan, University of Pennsylvania School of Veterinary Medicine, 3900 Delancey Street, Philadelphia, PA 10104.
Email: callan@vet.upenn.edu

Background: Cryoprecipitate (CRYO) is a plasma component containing high concentrations of factor VIII (FVIII), von Willebrand factor (VWF), and fibrinogen. Because Greyhounds are reported to have lower plasma VWF and fibrinogen concentrations, their plasma may not yield high potency CRYO.

Objectives: To determine if plasma hemostatic protein concentration is a good predictor of CRYO potency and if a difference exists in quality of CRYO prepared from Greyhounds versus non-Greyhounds.

Animals: Twenty Greyhounds and 20 non-Greyhounds.

Methods: A 450 mL unit of blood was collected from each donor, centrifuged to prepare fresh frozen plasma (FFP), and processed to CRYO. Aliquots of FFP and CRYO were analyzed for FVIII, VWF, and fibrinogen content and factor recovery.

Results: A positive correlation was found among donor plasma FVIII, VWF and fibrinogen concentration, and CRYO factor content ($P < .001$). Mean recovery was highest for VWF (67%), followed by fibrinogen (47%), and FVIII (37%). No breed difference was found in mean CRYO FVIII content, but CRYO VWF and fibrinogen were lower in Greyhounds ($P = .004$ and $P < .001$, respectively). No difference was found between Greyhounds and non-Greyhounds for the number of CRYO units meeting human blood banking standards.

Conclusions and Clinical Importance: Factor concentration in FFP is associated with CRYO potency, suggesting that prescreening of blood donors may enhance CRYO quality. Despite lower VWF and fibrinogen content, CRYO prepared from Greyhounds is acceptable based on blood banking standards for humans, indicating that Greyhound FFP does not need to be excluded from CRYO production.

KEYWORDS

factor VIII, fibrinogen, Greyhound, Von Willebrand factor

1 | INTRODUCTION

Cryoprecipitate (CRYO) is a plasma component that contains cold-insoluble proteins, namely factor VIII (FVIII), von Willebrand factor

(VWF), fibrinogen, factor XIII, and fibronectin, that precipitate when fresh frozen plasma (FFP) is partially thawed and centrifuged.¹ The predominant use of canine CRYO is prevention or management of bleeding caused by von Willebrand disease (VWD) and hemophilia A. Studies comparing the efficacy of FFP and CRYO determined that CRYO is more effective than FFP in increasing VWF concentration² and shortening buccal mucosal bleeding time³ in dogs with type 1 VWD and at least as effective as FFP in increasing FVIII coagulant

Abbreviations: AABB, American Association of Blood Banks; COE, Council of Europe; CI, confidence interval; CPP, cryo-poor plasma; CRYO, cryoprecipitate; FFP, fresh frozen plasma; FVIII, factor VIII; FVIII:C, factor VIII coagulant activity; Hb, hemoglobin; VWD, von Willebrand disease; VWF, von Willebrand factor.

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activity (FVIII:C) in dogs with hemophilia A.² The main advantage of CRYO over FFP is a higher concentration of hemostatic proteins in a smaller volume, with a CRYO transfusion thereby resulting in therapeutic concentrations of deficient factors without the risk of volume overloading the recipient.³

With the availability of recombinant human FVIII and VWF proteins, CRYO now primarily is used in humans for management of acquired hypofibrinogenemia and dysfibrinogenemia.^{4,5} However, studies in the 1960s and 1970s focused on FVIII because CRYO was used for management of hemophilia A at that time. A study evaluating variables that influenced the potency of human CRYO, defined by factor content (IU/unit or mg/unit), concluded that donor plasma FVIII activity was significantly associated with the potency of CRYO, but the plasma FVIII activity varied as much as 6-fold on repeat visits of the same donor.⁶ Furthermore, high donor plasma FVIII activity did not always correspond to good recovery of FVIII in CRYO, suggesting that “cryoprecipitability” of FVIII varies from donor to donor.⁷

Because of wide interindividual variations in plasma FVIII and VWF concentrations among healthy dogs, concern exists about uniformity and standard potency of CRYO to treat dogs with hemophilia A or VWD.⁸ It has been proposed that the target for transfusion therapy in dogs with VWD or hemophilia A is to increase the recipient's plasma VWF concentration above 35 U/dL or FVIII activity above 30 U/dL, respectively (comparable to 35% or 30%, respectively, of a canine standard assigned a value of 100%).² Although Greyhounds are commonly used as blood donors, a previous study has identified lower plasma VWF and fibrinogen concentrations in Greyhounds compared to non-Greyhounds.⁸ Greyhound plasma, therefore, may not yield high potency CRYO.

The American Association of Blood Banks (AABB) and the Council of Europe (COE) have established requirements for minimum concentrations of hemostatic proteins in CRYO, but given that CRYO now is administered to humans almost exclusively for fibrinogen disorders, the need to regulate the FVIII concentration has been questioned.⁹ Nevertheless, AABB and COE standards require that CRYO contain ≥ 80 and ≥ 70 IU/unit of FVIII, respectively. Only the COE has set a minimum requirement for VWF of 100 IU/unit. The AABB and COE minimum standards for fibrinogen are 150 mg/unit and 140 mg/unit, respectively. Similar standards have not been established for CRYO production in dogs.

Our objectives were to determine if: (1) canine donor plasma hemostatic protein concentration is a good predictor of CRYO potency; (2) there is a difference in quality of CRYO prepared from Greyhounds versus non-Greyhounds; and (3) canine CRYO produced by our institution's protocol meets blood banking standards used for humans.

2 | MATERIALS AND METHODS

2.1 | Canine blood donors

Blood used in the study was obtained from 40 dogs, 20 Greyhounds and 20 non-Greyhounds, enrolled in our institution's volunteer blood donor program as part of a standard blood donation (ie, blood

components were prepared for patient use, with small aliquots obtained for the study). Canine donor requirements include body weight ≥ 25 kg, age 1-8 years, and good health, based on history, physical examination, and annual screening that includes a CBC, biochemistry profile, and testing for blood-borne pathogens, as outlined in the updated consensus statement of American College of Veterinary Internal Medicine.¹⁰ In addition, dogs had not been transfused previously and were not receiving any medications, other than heartworm preventative and flea and tick control. Dogs donated blood approximately 4 times per year (average of every 3 months).

2.2 | Blood collection and processing

Blood (450 ± 15 mL) was collected from the jugular vein using a standard triple blood pack unit containing anticoagulant citrate-phosphate-dextrose solution and RBC preservative solution (Teruflex blood bag system, CPD with Optisol, Terumo Corporation, Tokyo, Japan). Blood was processed (5000g for 15 minutes at 4°C) within 4 hours of collection, and the supernatant plasma was expressed into a satellite bag, leaving a small volume of plasma (approximately 2 mL) in the transfer tubing of the satellite bag. Any plasma units with evidence of lipemia or hemolysis were excluded. The tubing was removed using a dielectric sealer (Rapid Seal II, SE370, Genesis BPS, Ramsey, New Jersey), and for each donor the aliquot of plasma was transferred to 2 Eppendorf tubes, labeled as FFP, and stored at -80°C . Within 30 minutes of processing, the plasma units (labeled as FFP) were placed in a -80°C freezer for a rapid freeze (approximately 3-4 hours) and then transferred to an alarmed temperature-controlled freezer (-30°C) and stored for up to 3 months before preparation of CRYO.

Cryoprecipitate was prepared by thawing FFP units in a refrigerator (4°C) overnight (approximately 8-10 hours) until the plasma reached a slushy consistency and centrifuging at 5000g for 7 minutes at 4°C. The supernatant plasma (cryo-poor plasma [CPP]) was transferred to a satellite bag, with an aliquot of CPP obtained from the sealed tubing removed from the unit and stored at -80°C . Approximately 50 mL of plasma was left in the CRYO units, which were placed in a temperature-controlled (37°C) plasma thawing bath (Model 2032, Forma Scientific, Marietta, Ohio) for 3-5 minutes, until completely thawed and the cold-insoluble proteins were in suspension (ie, no visible precipitates in unit). This warming step is not part of the standard CRYO production process but was performed to ensure that aliquots obtained for analysis were representative of a unit with all proteins in suspension because residual precipitates would result in spuriously decreased factor concentration. After mixing well, an aliquot of CRYO was expressed into satellite tubing, sealed, transferred to Eppendorf tubes, and stored at -80°C . All samples were analyzed within 6 months of blood collection.

All whole blood units and plasma components (FFP, CPP, and CRYO) were weighed using a gram scale to determine the volumes of each. Calculations were based on whole blood having a density of 1.053 g/mL and plasma components approximately 1.02 g/mL.¹ Throughout the study, statements made in reference to FFP volume referred to that volume obtained from a 450 mL whole blood donation.

2.3 | Hemostatic protein analyses

Plasma samples were shipped overnight on dry ice to the Comparative Coagulation Laboratory at Cornell University for measurement of FVIII:C, VWF concentration, and clottable (Clauss) fibrinogen concentration, as previously described.⁸ Samples were submitted in 5 batches over 9 months, with paired samples (FFP, CPP, and CRYO) from each collection from individual donors analyzed at the same time to avoid inter-assay variation. The results of FVIII activity and VWF concentration were reported as Units/mL (U/mL) compared with a pooled canine plasma standard prepared at the Comparative Coagulation Laboratory using healthy dogs. The standard has an assigned value of 1 U/mL (or 100%). The factor content in each CRYO unit was calculated by multiplying the total volume of the CRYO unit by the factor potency in U/mL (FVIII and VWF) or mg/dL (fibrinogen). Factor recovery in each CRYO unit was calculated by dividing the factor content in the CRYO unit by the factor content in the FFP unit and multiplying by 100%.

2.4 | Reproducibility of CRYO potency

To determine if CRYO quality was consistent for an individual canine blood donor, 3 dogs that produced CRYO with acceptable FVIII content (according to AABB standards) and 3 dogs that produced CRYO with substandard FVIII content were identified, and CRYO was prepared from 2 subsequent blood donations for each of the 6 dogs. Aliquots of plasma components were analyzed for FVIII activity, VWF concentration, and fibrinogen concentration, as described above, with the repeat samples for each donor assayed at the same time to minimize inter-assay variation, although initial samples had been analyzed previously to identify donors for the reproducibility study.

2.5 | Statistical analysis

All statistical analyses were performed by standard statistical software (Stata 15MP, StataCorp, College Station, Texas), with 2-sided tests of hypotheses and a *P* value <.05 as the criterion for statistical significance. Tests of normality were performed to determine extent of skewness, and transformation methods (eg, logarithmic) were used to normalize the distribution of markedly skewed variables. Descriptive analyses included computation of means (with 95% confidence intervals [95% CI]), SDs, medians, interquartile ranges of continuous variables, and tabulation of categorical variables. Exploratory statistical analysis was conducted by Spearman correlation to determine if an

association existed between independent variables and the outcome of interest. A *P* value <.2 was considered significant for data to be included in subsequent inference statistical analysis. For normally distributed dependent variables, a 2-sample *t* test was performed. Kruskal-Wallis equality-of-populations rank test was used for outcomes that were non-normally distributed.

3 | RESULTS

3.1 | Canine blood donor and baseline component characteristics

Routine whole blood donations were obtained from 40 dogs, 20 Greyhounds and 20 non-Greyhounds, the latter comprised of mixed breed dogs (*n* = 8), Doberman Pinschers (*n* = 4), Boxers (*n* = 2), and 1 of each of the following: Belgian Malinois, Cane Corso, Great Pyrenees, Weimaraner, German Shepherd, and Mastiff. There were 22 castrated males, 2 intact males, 14 spayed females, and 2 intact females. The Greyhounds included 12 castrated males and 8 spayed females. The mean pre-donation hemoglobin (Hb) concentration of all donors was 19.1 g/dL, with Greyhounds having a significantly higher Hb concentration than non-Greyhounds (Table 1). No difference was found in mean volume of blood collected, but mean FFP and CPP volumes were significantly lower for Greyhounds compared to those of non-Greyhounds (Table 1). The mean volume of CRYO was 53.2 mL, with no difference between groups.

3.2 | Hemostatic protein content of plasma components

3.2.1 | Von Willebrand factor

Two VWF-deficient Doberman Pinschers were identified (plasma VWF concentrations of 19% and 27%). The mean plasma VWF concentration of Greyhounds was 145.4% (95% CI 125.2-165.5), and was not significantly different than that of non-Greyhounds (mean 152.6%; 95% CI 124.0-181.2), even when the 2 VWF-deficient dogs were excluded (mean 167%; 95% CI 144.9-189.1). However, the mean VWF content of FFP, CPP, and CRYO units was less for Greyhounds compared to non-Greyhounds when the 2 VWF-deficient Doberman Pinschers were excluded from data analysis (Table 2). Three of 40 CRYO units (prepared from 2 VWF-deficient dogs and 1 Greyhound) did not meet the COE standard for VWF (≥ 100 IU/unit); the 25th and 50th percentiles for CRYO VWF content were

TABLE 1 Canine blood donor and baseline component characteristics

	Greyhounds (n = 20)	Non-Greyhounds (n = 20)	P value
Age (years)	5.6 (4.7-6.6)	4.0 (3.1-4.9)	.01
Body weight (kg)	32.8 (31-34.5)	36.4 (32.4-40.3)	.08
Hemoglobin (g/dL)	20.8 (20.1-21.5)	17.5 (16.7-18.2)	<.001
Whole blood volume donated (mL)	450.1 (446.8-453.4)	448.5 (444.9-452)	.47
Fresh frozen plasma volume (mL)	220.1 (211.3-228.9)	255.1 (248.9-261.2)	<.001
Cryo-poor plasma volume (mL)	165.4 (156.5-174.2)	199.8 (192.7-206.9)	<.001
Cryoprecipitate volume (mL)	52.2 (49.8-54.6)	54.1 (52.1-56.1)	.21

Variables are presented as mean and 95% confidence interval.

TABLE 2 Hemostatic protein content of canine plasma components

	Greyhounds (n = 20)	Non-Greyhounds (n = 20)	P value
Fresh frozen plasma			
FVIII (U/unit)	253.1 (194.8-311.3)	276.8 (232.7-320.9)	.50
VWF (U/unit) ^a	318.3 (275.7-360.9)	425.3 (374.1-476.5)	.002
Fibrinogen (mg/unit)	472.4 (423.8-521)	706.4 (638-774.9)	<.001
Cryo-poor plasma			
FVIII (U/unit)	127.7 (92-163.3)	152.6 (120.7-184.5)	.28
VWF (U/unit) ^a	48.5 ^b (26.0-128.8)	63 ^b (33-229)	.03
Fibrinogen (mg/unit)	223.3 (188.1-258.5)	331.7 (283.6-379.8)	<.001
Cryoprecipitate			
FVIII (U/unit)	95 (65.6-124.3)	93.9 (78.9-108.8)	.94
FVIII recovery (%)	38.4 (33.1-43.7)	35.9 (30.5-41.4)	.50
VWF (U/unit) ^a	210.5 (174.4-246.6)	289.7 (248.5-330.9)	.004
VWF recovery (%)	67 (57.7-76.3)	67.2 (61.5-72.9)	.96
Fibrinogen (mg/unit)	221 (188.5-253.5)	332 (294.3-369.8)	<.001
Fibrinogen recovery (%)	46.6 (41.8-51.5)	47.6 (43-52.2)	.75

Abbreviations: CRYO, cryoprecipitate; FVIII, factor VIII; VWF, von Willebrand factor.

^a Data represents 18 non-Greyhounds, after exclusion of 2 VWF-deficient Doberman Pinschers. Including the 2 VWF-deficient dogs (n = 20 non-Greyhounds) decreased the mean VWF content of FFP (388.6 U/unit, $P = .08$) and CRYO (263.9 U/unit, $P = .08$).

^b Variables are presented as mean and 95% confidence interval, except for cryo-poor VWF (U/unit), which was not normally distributed and is presented as median and range.

180 and 232.5 U/unit, respectively (Figure 1A). The mean VWF recovery for all CRYO units was 67.1% (95% CI 61.9-72.3), with no difference between Greyhounds and non-Greyhounds (Table 2). No association was found between FFP VWF concentration and VWF recovery. A positive correlation was found between donor FFP VWF concentration and CRYO VWF content (Figure 2A).

In a linear regression model with CRYO potency as the outcome, Greyhound breed was identified as a confounder, an independent variable that influenced VWF content of CRYO units beyond FFP VWF concentration, being associated with a 42.4 U/unit decrease ($P = .01$) in CRYO VWF content when compared to all 20 non-Greyhounds and a 49.6 U/unit decrease ($P = .009$) when the 2 VWF-deficient

non-Greyhounds were excluded (Table 3). The FFP volume was not associated with CRYO VWF potency.

3.2.2 | Factor VIII

The mean plasma FVIII activity of Greyhounds (115.9%; 95% CI, 89.7-142) was not significantly different than that of non-Greyhounds (108.9%; 95% CI, 91.7-126.1). No significant difference was found in the mean FVIII activity of FFP, CPP, or CRYO units when comparing Greyhounds and non-Greyhounds (Table 2). Nineteen (8 Greyhounds and 11 non-Greyhounds) of 40 CRYO units met the AABB standard for FVIII (≥ 80 IU/unit), and 30 (15 Greyhounds and 15 non-Greyhounds) of 40 CRYO units met the COE standard for FVIII (≥ 70 IU/unit), with no significant difference between CRYO prepared from Greyhounds and non-Greyhounds; the 25th and 50th percentiles for CRYO FVIII were 67.4 and 78 U/unit, respectively (Figure 1B).

Mean FVIII recovery for all CRYO units was 37.1% (95% CI 33.5-40.8), with no significant difference between Greyhounds and non-Greyhounds (Table 2). An association existed between FFP FVIII activity and FVIII recovery ($P = .01$). A positive correlation was found between donor FFP FVIII activity and CRYO FVIII content (Figure 2B). In a linear regression analysis, Greyhound breed and FFP volume did not have any effect on prediction of CRYO FVIII potency beyond FFP FVIII activity (Table 3).

Two VWF-deficient dogs with plasma VWF concentrations of 19% and 27% had plasma FVIII activity of 84% and 108%, respectively, both within the reference interval (50%-200%). The FVIII activity of the CRYO prepared from the dog with the lower results (VWF 19%, FVIII 84%) was 61 U/unit, below the AABB and COE standards, whereas the CRYO FVIII for the second dog was 87 U/unit, acceptable by AABB and COE standards. No association was found between plasma VWF concentration and FVIII activity or FFP VWF and FVIII content when evaluating all 40 dogs.

3.2.3 | Fibrinogen

Greyhounds had a lower mean plasma fibrinogen concentration (214.3 mg/dL; 95% CI, 194.1-234.5) compared to non-Greyhounds (275.9 mg/dL; 95% CI, 252.5-299.3; $P < .001$). The fibrinogen content in FFP, CPP, and CRYO was significantly less in units prepared from Greyhounds compared to non-Greyhounds (Table 2). All 20 CRYO units prepared from non-Greyhounds met both the AABB and COE standards for fibrinogen content (≥ 150 and ≥ 140 mg/unit,

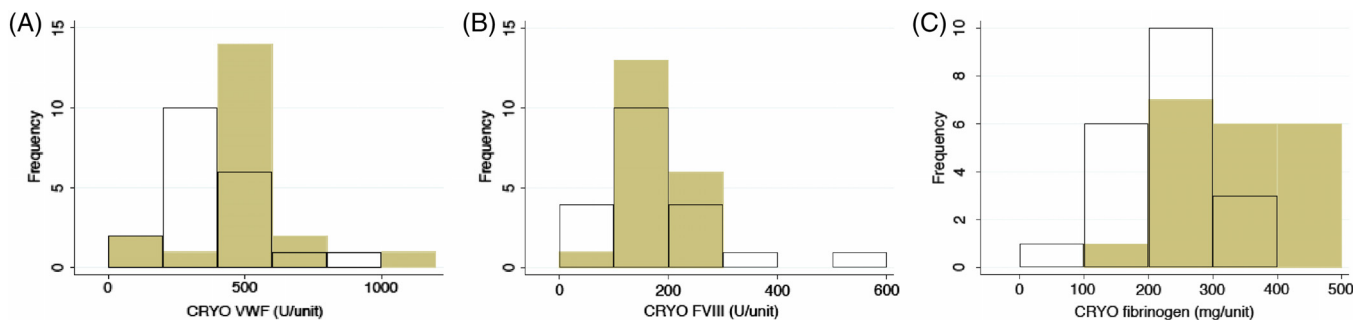


FIGURE 1 Histograms of distribution of cryoprecipitate (CRYO) hemostatic protein content for: A, von Willebrand factor (VWF); B, factor VIII (FVIII); and C, fibrinogen. Open bars represent Greyhounds (n = 20), and filled bars represent non-Greyhounds (n = 20). The width of each bar represents 200 U/unit for VWF, 100 U/unit for FVIII, and 100 mg/unit for fibrinogen

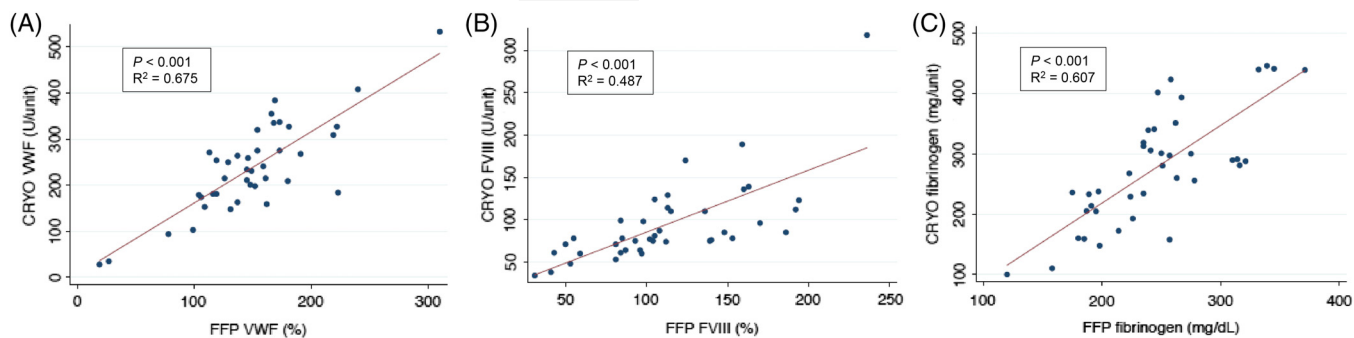


FIGURE 2 Spearman correlation between canine donor plasma (FFP) hemostatic protein concentration and cryoprecipitate (CRYO) hemostatic protein content for: A, von Willebrand factor (VWF); B, factor VIII (FVIII); and C, fibrinogen

respectively), whereas 17 and 18 CRYO units from Greyhounds met the AABB and COE standards, respectively; the 25th and 50th percentiles for CRYO fibrinogen content were 209.5 and 280.8 mg/unit, respectively (Figure 1C). The mean fibrinogen recovery for all CRYO units was 47.1% (95% CI 43.9-50.3), with no difference between Greyhounds and non-Greyhounds (Table 2). No association was found between FFP fibrinogen concentration and fibrinogen recovery. A positive correlation existed between donor fibrinogen concentration in FFP and fibrinogen content in CRYO units (Figure 2C). In a linear regression analysis of variables that could influence CRYO fibrinogen potency beyond FFP fibrinogen concentration, Greyhound breed, but not FFP volume, was determined to be a confounder, a variable independently associated with a decrease in CRYO fibrinogen content of 46.1 mg/unit ($P = .04$; Table 3).

3.3 | Reproducibility of CRYO potency

Three blood donors with CRYO FVIII meeting the AABB standard (≥ 80 IU/unit) and 3 blood donors with CRYO FVIII below the standard were identified to evaluate the reproducibility of CRYO potency by determining hemostatic protein content in plasma components prepared from 2 subsequent blood donations (Supporting Information Table S1). The 3 dogs with CRYO FVIII content < 80 U/unit on their first donation all had substandard FVIII in CRYO prepared from 2 subsequent blood donations. However, of the 3 dogs with CRYO FVIII content ≥ 80 U/unit on their first donation, only CRYO prepared from 1 dog yielded a FVIII content ≥ 80 U/unit on a single subsequent donation. The intraclass correlation coefficient, an estimate of correlation between individual CRYO FVIII measurements for each of 6 dogs, was 0.365 (95% CI -0.015 to 0.827), indicating poor reproducibility.

The CRYO fibrinogen content for all 6 dogs met the AABB standard (≥ 150 mg/unit) on all 3 blood donations, but the fibrinogen

content varied for an individual dog between donations (Supporting Information Table S1). Similarly, the CRYO VWF content met the COE standard (≥ 100 U/unit) for 5 dogs on all 3 donations and for 1 dog on 2 of 3 donations.

4 | DISCUSSION

Although recombinant human VWF and FVIII proteins have replaced CRYO for management of bleeding caused by VWD and hemophilia A, respectively, in humans, CRYO remains the blood component of choice for management of bleeding associated with these hemostatic disorders in dogs. Although the AABB has developed minimum standards for quality assurance that are compatible with Food and Drug Administration requirements, no similar oversight of blood component quality exists in veterinary medicine. Based on the method of CRYO preparation used by our institution's blood bank, the canine donor's plasma concentrations of VWF, FVIII, and fibrinogen were associated with their respective CRYO content, suggesting that prescreening of blood donors' plasma hemostatic proteins could help ensure CRYO quality. However, given the variability in plasma FVIII for an individual canine donor, the finding of a plasma FVIII at the upper end of the reference interval does not necessarily mean that particular donor will always produce CRYO with high FVIII potency, similar to the FVIII variability noted with human CRYO.^{6,7} Although blood donations from Greyhounds yield a smaller volume of plasma and Greyhounds have a lower plasma fibrinogen concentration compared to other breeds, CRYO prepared from Greyhounds did not differ from that of other breeds in meeting the minimum human blood banking standards for hemostatic protein content, suggesting that Greyhounds do not need to be excluded from the donor pool for CRYO production.

TABLE 3 Linear regression analysis of Greyhound breed and fresh frozen plasma (FFP) volume as confounders influencing hemostatic protein content of cryoprecipitate units beyond FFP protein concentration

Hemostatic protein	Predictor: FFP protein concentration		Greyhound breed		FFP volume	
	Coefficient	P value	Confounder coefficient	P value	Confounder coefficient	P value
VWF ^a	1.37	<.001	-49.6	.009	0.66	.09
FVIII	0.73	<.001	-4.01	.72	0.06	.80
Fibrinogen	1.29	<.001	-46.1	.04	0.31	.61

^a Data represents 18 non-Greyhounds, after exclusion of 2 von Willebrand factor (VWF)-deficient Doberman Pinschers.

Given that no standards are available for evaluating canine CRYO potency, we looked to blood banking standards used in humans. Reported reference intervals for relevant human plasma hemostatic proteins (FVIII 50%-150%; VWF 50%-150%; fibrinogen 150-400 mg/dL)^{11,12} are similar to those of canine proteins (FVIII 50%-200%; VWF 70%-180%; fibrinogen 150-490 mg/dL; Comparative Coagulation Laboratory at Cornell University), suggesting that a comparison to guidelines used in humans could be a reasonable starting point. The AABB does not mandate the volume of residual plasma in CRYO units, and thus wide variability is reported in the literature, ranging from 5 to 50 mL.⁵ Because the concentration of hemostatic proteins in CRYO varies with the volume of residual plasma, human CRYO potency is defined by factor content (IU/unit or mg/unit), which takes into account factor concentration and CRYO volume.

Similar to the first description of preparation of human CRYO in the mid-1960s,^{13,14} the production of CRYO today continues to start with thawing of FFP at 1°C-6°C, followed by centrifugation and removal of the supernatant to leave the cold insoluble precipitate in the bag with a variable amount of plasma.⁵ In an effort to maximize CRYO potency, early investigations evaluated steps in CRYO production, as well as influence of donor characteristics, that could impact FVIII recovery; VWF and fibrinogen content were not addressed.^{6,7} The presence of ice crystals in the bag while the supernatant plasma was removed resulted in increased FVIII recovery (51 ± 13%) compared to the absence of ice crystals in the bag (43 ± 18%).⁶ In our CRYO production technique, the FFP units were centrifuged when approximately 90% was thawed, resulting in a frozen component still present in the bag as the supernatant was expressed into a satellite bag. Two studies documented marked variability in FVIII content of human CRYO, with the only donor characteristic consistently related to CRYO potency being plasma FVIII concentration.^{6,7} One study⁷ found an association between donor plasma FVIII concentration and "cryoprecipitability" or recovery of FVIII, whereas the other study⁶ found no relationship between these 2 variables. In a study of 169 donors, recovery of plasma FVIII in CRYO ranged from 11.2% to 89.4% (mean 38 ± 18%), and FVIII content of CRYO ranged from 29 to 379 IU/unit (mean, 111 ± 77 IU/unit).⁶ In addition, plasma FVIII concentration varied as much as 6-fold on repeated visits of the same donor, suggesting that it is not possible to predict that an individual donor will consistently produce high potency CRYO with regard to FVIII content.⁶

Similar to these early investigations with human CRYO, results of our study indicate that a positive correlation exists between canine donor plasma FVIII activity and CRYO FVIII content, as well as donor plasma VWF and fibrinogen concentration and their respective CRYO content. Variability in canine donor coagulation factor activity and VWF concentration has been documented previously.¹⁵ In addition, of 9 coagulation factors and hemostatic proteins measured in plasma samples from 38 donor dogs, plasma FVIII has been noted to have the greatest variability among canine donors, ranging from 31% to 225%.⁸ Fresh plasma obtained from 4 monthly blood collections from 7 healthy Greyhounds had inter-assay coefficients of variation ranging from 11% to 34.9% for VWF and from 7.1% to 39% for FVIII.¹⁵ Variations in plasma FVIII, VWF, and fibrinogen content were noted for each of the 6 dogs in our study donating blood on 3 occasions (total

of 18 units), yet the variability had little to no effect on CRYO units meeting the minimum blood banking standards for fibrinogen (all 18 units met the AABB criteria of ≥150 mg/unit) and VWF (17 of 18 units met the COE criteria of ≥100 IU/unit) used in humans. However, the factor content of CRYO units not only depends on initial FFP factor concentration but also on factor recovery, which can vary with processing technique, as well as donor-specific attributes of the plasma.⁷ Of the 3 proteins evaluated in our study, the mean recovery was highest for VWF (67%), followed by fibrinogen (47%) and FVIII (37%). Variation in plasma factor concentration for an individual donor is likely offset by higher recovery of VWF and fibrinogen compared to FVIII, resulting in CRYO units meeting an arbitrary standard for VWF and fibrinogen, but not FVIII, on repeat donations.

Although Greyhounds frequently are used as blood donors because of their temperament, conformation, and high hematocrit, a previous report⁸ documented that Greyhounds have lower plasma VWF and fibrinogen concentration (and lower FX activity) compared to non-Greyhounds, suggesting that their plasma might not be ideal for preparation of CRYO. With their higher hematocrit, it follows that the mean FFP volume of Greyhounds was significantly less than that of non-Greyhounds in our study. Interestingly, FFP volume was determined not to be a confounder in predicting CRYO potency for any of the 3 hemostatic proteins evaluated in our study. Whether the 2 VWF-deficient Doberman Pinschers were included or excluded, no significant difference was found in mean FFP VWF concentration when comparing Greyhounds to non-Greyhounds. However, when the 2 VWF-deficient Doberman Pinschers were excluded, Greyhounds were found to have lower VWF content in their plasma components (FFP, CPP, and CRYO) compared to non-Greyhounds. Similar to the previous study,⁸ Greyhounds were found to have lower FFP fibrinogen concentration compared to non-Greyhounds, and the fibrinogen content of all plasma components (FFP, CPP, and CRYO) was significantly less for Greyhounds. Greyhound breed was identified as a confounder in predicting a decrease in CRYO potency for both VWF and fibrinogen, but not FVIII. Nevertheless, CRYO units contained sufficient VWF and fibrinogen to meet minimum blood banking standards used in humans for 19 and 17-18 of Greyhounds, respectively, indicating that there is not a good justification for excluding plasma from Greyhounds for CRYO production. The wide variability in plasma FVIII activity and relatively low FVIII recovery were issues for both Greyhounds and non-Greyhounds, with no differences between groups.

Relatively little information on canine CRYO is available in the veterinary literature. An early report on the administration of CRYO to 2 German Shepherd puppies with hemophilia A indicated that the mean half-life of FVIII in CRYO, based on time for the partial thromboplastin time to return to pre-CRYO results after 6 separate administrations, was 13.2 hours, ranging from 7.7 to 32.3 hours.¹⁶ The mean CRYO FVIII recovery was 26.6%, ranging from 9.2% to 66%, exhibiting wide variability as noted in our study. In a subsequent study evaluating stability of VWF and FVIII in canine CRYO under various storage conditions, CRYO was prepared from 7 Greyhounds given desmopressin 30-60 minutes before blood collection in an effort to increase donor plasma VWF and FVIII concentrations.¹⁷ The CRYO production method involved thawing FFP units in a 4°C water bath (rather than

in a refrigerator as used in our study) and leaving 60 mL of plasma in the CRYO unit. Although results were not reported as hemostatic protein content per unit, based on our calculations (assuming a 60 mL CRYO volume), the mean FVIII and VWF contents were 87 and 169 U/unit, respectively.¹⁷ Results of this study indicated that thawed canine CRYO can be maintained for 24 hours at room temperature without substantial reduction in FVIII or VWF.¹⁷ In another study by the same investigators, whole blood again was obtained from Greyhounds pre-treated with desmopressin, and FFP was thawed in a 4°C water bath to produce CRYO.² The mean CRYO volume was 37 mL, and we calculated the mean FVIII and VWF contents to be 54 and 151 U/unit, respectively, with reported mean FVIII and VWF recoveries being 23% and 59%, respectively.² Results of this study indicated that CRYO was more effective than FFP in increasing VWF concentration in dogs with VWD and was at least as effective as FFP in increasing FVIII activity in dogs with hemophilia A.² Finally, in a study evaluating the effect of CRYO and FFP on plasma VWF multimers and bleeding time in Doberman Pinschers with type 1 VWD, it was shown that CRYO, particularly that prepared from desmopressin-treated donor dogs, is a concentrate of the most hemostatically active multimers of VWF and decreases buccal mucosal bleeding time in dogs with VWD.³ Mean VWF recovery in that study was reported to be 57.5%, similar to our median VWF recovery of 65.3%; CRYO VWF content could not be calculated from information available in the report.³ In a recent study comparing colloid osmotic pressure and concentrations of albumin, VWF and coagulation proteins in canine CPP, CRYO, and FFP, the authors confirmed that their CRYO production method (similar to ours) yielded CRYO units having higher concentrations of FVIII, VWF, and fibrinogen compared to FFP and CPP.¹⁸ However, we cannot directly compare results of this study to ours because the CRYO volume was not specified; CRYO was reportedly split into satellite bags containing 9-30 mL, so the total volume is unclear. Reporting canine CRYO hemostatic protein content as U/unit (for FVIII and VWF) or mg/unit (fibrinogen), as done in publications describing CRYO content in humans, would improve our ability to interpret and compare results of different studies.

Our study was not designed to set blood banking guidelines for minimum factor content for canine CRYO. However, in comparing our 25th percentiles for CRYO hemostatic protein content to blood banking standards used in humans, the COE minimum of ≥ 100 IU/unit for VWF appears low for canine CRYO, with 75% of our units having ≥ 180 U/unit. On the other hand, relatively poor FVIII recovery in canine CRYO resulted in 25% of our units having FVIII content ≤ 67 U/unit, less than both the AABB (≥ 80 IU/unit) and COE (≥ 70 IU/unit) minimum standards. Because we evaluated only a single method of CRYO production, it is unclear whether alterations to the process could improve yield of FVIII in CRYO units or whether a species difference will persist regardless of technique. Finally, 75% of our canine CRYO units had a fibrinogen content >209 mg/unit, similar to the AABB and COE minimum standards of 150 and 140 mg/unit, respectively.

The limitations of our study include comparison of the potency of our CRYO units to blood banking standards used in humans because similar standards have not yet been established for dogs. In addition, we evaluated only 1 method of CRYO production, which is used by our institution's blood bank, as 1 of our goals was to determine if our

method produces CRYO of acceptable quality. Furthermore, reproducibility of CRYO potency was evaluated in only 6 dogs with 3 donations each because of limited funding for the study. Finally, we did not evaluate the efficacy of the CRYO units in increasing hemostatic protein concentration and preventing or controlling bleeding in recipients with VWD, hemophilia A, or fibrinogen disorders, because doing so was beyond the scope of our study.

In conclusion, the factor concentration in donor FFP is associated with CRYO potency, suggesting that prescreening of blood donors may enhance CRYO quality. Cryoprecipitate prepared from Greyhounds is not inferior to that from other breeds in meeting minimum blood banking standards used in humans, justifying the use of their plasma for preparation of CRYO. Although most CRYO units met blood banking standards used in humans for VWF and fibrinogen, variable recovery of FVIII resulted in approximately 50% of the CRYO units having FVIII content below minimum standards used in humans. Control of bleeding in dogs with hemophilia A may require transfusion to effect because of nonuniform FVIII potency of single CRYO units.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

ORCID

Mary Beth Callan  <https://orcid.org/0000-0002-4508-9980>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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