Comparison of hematologic values and transforming growth factor-β and insulin-like growth factor concentrations in platelet concentrates obtained by use of buffy coat and apheresis methods from equine blood

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Objective—To evaluate the buffy coat and apheresis methods for preparation of platelet concentrates from equine blood by comparing platelet and growth factor concentrations.

Animals—15 mature mixed-breed geldings.

Procedure—Whole blood samples were collected and processed by use of a buffy coat or apheresis method to obtain platelet poor and platelet concentrated fractions. The PCV, WBC count, and platelet count were compared among whole blood samples, platelet poor fractions, concentrates obtained by use of the apheresis method (ie, apheresis platelet concentrates), and concentrates obtained by use of the buffy coat method (ie, buffy coat platelet concentrates). Concentrations of transforming growth factor-β (ie, TGF-β1 and TGF-β2) and insulin-like growth factor were compared between buffy coat and apheresis platelet concentrates.

Results—Platelet concentrations were 8.9-fold and 5.2-fold greater in buffy coat and apheresis platelet concentrates, respectively, compared with whole blood. Platelet concentrations were 13.1-fold greater in filtered apheresis platelet concentrates, compared with whole blood. TGF-β1 concentrations were 2.8-fold and 3.1-fold greater in buffy coat and apheresis platelet concentrates, respectively, and TGF-β1 concentrations were 10.5-fold greater in filtered apheresis platelet concentrates, compared with whole blood. TGF-β2 concentrations were 3.6-fold greater in apheresis platelet concentrates, compared with whole blood. Platelet concentrations correlated with growth factor concentrations across all blood and platelet fractions. White blood cell counts had a significant positive correlation with TGF-β1 concentration in buffy coat platelet concentrates.

Conclusions and Clinical Relevance—Platelets and TGF-β1 can be concentrated reliably from equine blood by use of buffy coat or apheresis methods, without modification of the protocols used for humans. (*Am J Vet Res* 2004;65:924–930)

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Wound healing, whether in bone or soft tissue, involves a complex orchestrated chain of events. Growth factors are the conductors or modulators of wound healing, controlling each step of the wound healing process through induction of chemotaxis, stimulation of mitosis, and upregulation of protein production. In turn, growth factors control extracellular matrix synthesis, matrix turnover, and cell death.[1] Clinical treatment of wounds with single growth factors has been, for the most part, disappointing.[2,3] Given the complexity of growth factor regulation and interaction in wound healing, it is unlikely that administration of a single growth factor is optimal for treating nonhealing wounds.[4] Additionally, in this type of study, the vehicles used do not provide sustained release and it is likely that most of the growth factors are quickly consumed by wound proteases.[5] Potential methods for sustained release of multiple growth factors to a wound include, but are not limited to, gene therapy, inclusion of growth factors in a collagen matrix, hydrogels, calcium alginate beads, microspheres, and administration of platelet concentrates (ie, fibrin clot).[6-10]

Platelets contain a number of growth factors within their α-granules that are released upon activation.[11] Of particular importance to wound healing are the large amounts of **transforming growth factor (TGF)-β** and platelet derived growth factor, as well as smaller amounts of **insulin-like growth factor (IGF)**, epidermal growth factor, and TGF-α that are released upon activation from human blood.[12] These growth factors and others act synergistically to enhance neutrophil and macrophage infiltration, angiogenesis, fibroplasia, matrix deposition, scarring, and re-epithelialization.[1]

Several studies have attempted to take advantage of the growth factors that are readily available in platelet concentrates or platelet-rich plasma for clinical use. In oral and maxillofacial surgery, the results of 3 controlled studies[10,13,14] indicate that early bone healing of defects and around implants is improved when platelet-rich plasma is used alone, added to bone grafts, or added to plaster of Paris. Platelet concentrates have also been reported to improve soft tissue healing in chronic nonhealing wounds in humans and in wounds on the lower limbs of horses.[15,16] Platelet concentrates have been used as a preparation of fibrin glue to control bleeding and to adhere tissues together, seal tissue defects, and potentially act as a drug delivery system.[17-19]

Several commercial kits are available for collection of platelet concentrates. In humans, studies have been published comparing these methods with regard to volume of blood necessary for preparation, volume of platelet concentrate obtained, platelet count, WBC count, and growth factor concentrations.[20-30] Findings in these studies reveal that TGF-β1, TGF-β2, and platelet-derived growth factor are concentrated in human platelet concentrates.

Methods for producing platelet concentrates traditionally are categorized by the use of the buffy coat, platelet-rich plasma, or apheresis. However, as the result of the development of multiple commercial methods, it is difficult to fit every system into these categories. Methods for procurement of platelet concentrates are now more commonly separated into either automated or manual methods. In the manual systems, the user has control of the plasma-RBC interface and can adjust for differences in PCV.

The purpose of the study reported here was to evaluate 2 manual methods (ie, the buffy coat and apheresis methods) for the preparation of platelet concentrates from horses. Specific objectives were to compare growth factor concentrations (TGF-β1, TGF-β2, and IGF-1) in platelet concentrates obtained by both methods and look for associations between growth factor concentrations and clinical laboratory data (ie, platelet count and WBC count) in an attempt to estimate growth factor concentrations in the perioperative period. Our hypotheses were that both systems would concentrate platelets, WBCs, TGF-β1, and TGF-β2, compared with whole blood values, and that growth factor concentrations of TGF-β1 and TGF-β2 would be positively correlated with platelet counts.

Materials and Methods

Blood collection—Fifteen geldings between 10 to 20 years of age were selected for blood donation. Blood was collected from 5 horses twice, resulting in 20 samples. Blood was collected in commercially available 2-L acid-citrate blood collection bags.[a] All blood was collected atraumatically through a single jugular venipuncture with a 12-gauge needle and stored at room temperature (approx 24°C) with gentle agitation until obtaining platelet concentrates later that day. The Institutional Animal Care and Use Committee at The Ohio State University approved the animal use protocol.

Buffy coat method derived platelet concentrates—Individual platelet concentrating kits[b] were used for each horse. Four commercially designed platelet sequestration tubes[b] were filled with 50 mL of anticoagulated blood via the labeled blood port (**Figure 1**). The tubes were centrifuged at 2,100 × *g* for 9 minutes in an adapted centrifuge tube provided by the supplier of the kits. After the first spin, the RBC layer was drawn off the bottom of the tube through the blood port, leaving exactly 3 mL of packed RBCs and platelets in the tube. The remaining contents in the centrifuge tube were then placed back into the centrifuge and spun at 2,100 × *g* for 3 minutes. Subsequently, the plasma was drawn off through the plasma port, which was calibrated to leave the bottom 5 mL that contained the final product. After discarding the plasma, the remaining platelet concentrate was withdrawn through the blood port. Platelet concentrates from the 4 centrifuge tubes were pooled, gently mixed, and placed in aliquots for hematologic examination and storage at –80°C.

Apheresis method derived platelet concentrates—An apheresis unit[c] was used to obtain platelet concentrate by a process of discontinuous centrifugation. The protocol for obtaining a platelet concentrate was followed according to the instructions of the manufacturer. In summary, a basic low volume (125 mL) bowl set[d] was installed into the apheresis unit, and a sequestering pack with burette assembly[e] was attached to the bowl. Three bags were included in the sequestering line: 1 for collection of plasma, 1 for collection of buffy coat, and 1 for air. The bowl inlet line was attached to the donor blood bag. By the use of a provided template, a marker was used to inscribe a line on the bowl for the optimum fill level. The apheresis unit was initially programmed at 5,650 rpm[d] with a pump speed of 60 mL/min. The donor bag was continuously and gently agitated to prevent RBC sedimentation. The program was started with the line leading to the plasma collection bag open. The bowl was filled until the RBC layer reached the inscribed line. At this point platelet poor plasma had entered the plasma bag and the blood pump was paused. The centrifuge speed was decreased to 2,400 rpm,[d] and the clamps were opened to the bag labeled platelet concentrate and closed to the bag labeled plasma. The next steps describe manual extraction of platelet concentrate or milking. This 2-step procedure allows blood to recir-

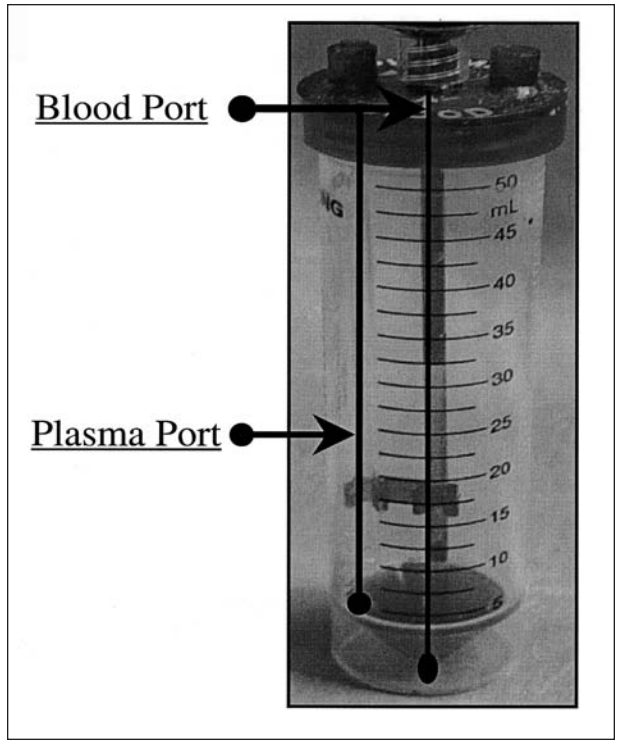

Figure 1—Photograph of the blood tube used to obtain platelet concentrates by use of the buffy coat method. Notice that the blood port extends to the bottom of the tube and the plasma port ends at the 5-mL mark.

culate in the bowl after emptying and increases the platelet output by keeping blood in the centrifuge chamber for a longer period. The apheresis unit pump was started again, and 3 mL was pumped into the buffy coat collection bag. The pump was paused again for 10 seconds before resuming and collecting another 3 mL of platelet concentrate into the collection bag. This milking process was continued until the first RBCs entered the collection bag and then was repeated 5 more times to collect an additional 15 mL. This resulted in collection of approximately 45 mL of platelet concentrate.

Filtration of apheresis method derived platelet concentrates—In 10 samples, platelet concentrate fractions obtained by use of the apheresis method were further concentrated with a commercial filter and gas piston chamber for multiple passes of the sample through the filter[f] (**Figure 2**). The filter has a hollow, cellulose, fiber-based membrane with an effective pore size of 30 kd that filters out water and low molecular weight solutes. Platelet concentrate (45 mL) was placed in a 60-mL syringe and attached to the filter with an additional 60 mL syringe attached at the other end of the filter. The sample was passed through the filter in both directions until the final volume of sample was decreased to 15 mL. The gas piston chamber automated this process, but it could be completed manually as well by alternating plunger depression through each syringe. Each filter was used just once, and the clear distillate was discarded. These samples were divided and frozen.

Equipment and disposable retail costs—Disposable retail costs for the buffy cost method were approximately 4-fold less than for the apheresis method. A standard swinging bucket centrifuge could be used for the buffy coat tubes. An apheresis unit[c-e] was necessary to acquire platelet concentrate by the apheresis method. The concentrating filters[f] had an approximately 1.7-fold greater cost than the apheresis disposables at the time of our study.

Sample collection and storage—Samples of whole blood (20 samples), platelet concentrate fractions (20 samples/method), and platelet poor fractions (5 samples/method) were submitted to The Ohio State University clinical pathology laboratory for platelet count, WBC count, and PCV determinations by use of an automated blood hematology unit.[g] Samples of whole blood (20 samples), platelet-concentrate fractions (20 samples/method), and platelet-poor fractions (5 samples/method) were divided and then transferred into 1-mL Eppendorf tubes and frozen at –80°C for subsequent growth factor analysis.

Growth factor analysis—All platelet-concentrate fractions, whole blood samples, and platelet-poor fractions from each method were thawed and mixed well prior to growth factor analysis by use of an ELISA. All samples were diluted 1:10 with a mammalian protein extraction reagent[h] and centrifuged at 15,000 × *g* for 10 minutes prior to assaying.[i] Concentrations of TGF-β1,[j] TGF-β2,[k] and IGF-1[l] were measured by use of commercially available ELISA kits on platelet concentrates (20 samples/method for TGF-β1 and TGF-β2 measurements, and 13 samples/method for IGF-1 measurements), platelet-poor fractions (5 samples/method for TGF-β1 and TGF-β2 measurements), and whole blood samples (20 samples/method for TGF-β1 and TGF-β2, and 13 samples/method for IGF-1 measurements). The sensitivities of the assays were < 7 pg/mL for TGF-β1 and TGF-β2, and < 30 pg/mL for IGF-1. Acid activation with 1N hydrochloric acid was used to activate TGF-β bound to latency associated peptide in TGF-β assays.[j,k] An acid-ethanol extraction was performed prior to IGF-1 assaying to detect IGF-1 bound to IGF binding proteins.[l] All analyses were performed in duplicate.

Statistical analysis—A commercial statistical software package was used to analyze all data.[m] Nonparametric tests were selected for all variables because they were either not normally distributed or failed the test for equal variance. All quantitative measurements were evaluated by use of descriptive statistics (median and range). The Kruskall-Wallis 1-way ANOVA on ranks was used to compare platelet counts, WBC counts, PCVs, and growth factor concentrations among whole blood samples, concentrates obtained by use of the apheresis method (ie, apheresis platelet concentrates), and concentrates obtained by use of the buffy coat method (ie, buffy coat platelet concentrates). A Tukey test for pairwise comparison was used to compare individual groups. A Mann-Whitney rank sum test was used to compare the TGF-β concentrations, platelet counts, and WBC counts between apheresis platelet concentrates and filtered apheresis platelet concentrates. The Spearman rank correlation coefficient (r_s) was used to evaluate associations between platelet counts, WBC counts, and growth factors between and within whole blood samples and platelet concentrates. Significance in all instances was set at a value of $P < 0.05$. All duplicate values were within 10% of each other, and the mean of duplicates was used for data analysis.

Results

Hematologic examination and processing—Setup and processing times for the buffy coat method were shorter (estimate of 15 minutes) than for the apheresis method (estimate of 45 minutes). The minimum vol-

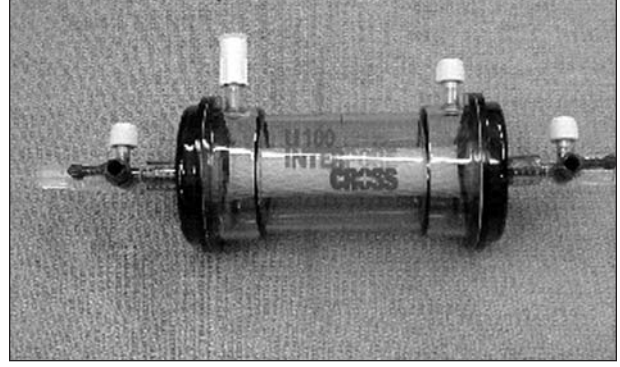

Figure 2—Photograph of the filter used to further concentrate platelets obtained by use of the apheresis method. Notice that the filter consists of a cellulose-based, hollow fiber membrane in a plastic cartridge. Platelet concentrate is passed back and forth through the filter, removing water and low molecular-weight solutes.

Table 1—Median (range) value of variables in blood fractions.

Variables	Whole blood	Platelet poor fractions		Platelet concentrate fractions		
		Buffy coat method	Apheresis method	Buffy coat method	Apheresis method	Apheresis followed by filtration
Platelet count (cells × 10^3/μL)	165.5 (96.4–237.0)	44.6 (40.4–71.7)	13.9 (10.8–21.7)	1,472.5 (571.0–4,409.0)	855.0 (318.0–1,319.0)	2,172.0 (1,211.0–3,855.0)
WBC count (cells × 10^3/μL)	6.3 (2.70–7.74)	NO	NO	32.5 (9.3–97.0)	33.7 (18.20–52.10)	61.2 (27.50–99.80)
PCV (%)	33.0 (19.0–59.0)	NO	NO	64.5 (36.9–72.4)	8.1 (5.0– 29.0)	NO
TGF-β1 (ng/μL)	5.5 (1.6–12.6)	1.7 (1.2–2.2)	1.2 (0.95–1.80)	15.3 (6.5–36.6)	23.6 (2.0–43.1)	57.9 (37.8–69.0)
TGF-β2 (ng/μL)	1.2 (0.36–8.90)	NO	NO	1.0 (0.11–8.20)	4.3 (2.3–10.5)	NO
IGF-1 (ng/μL)	171.5 (112.9–250.0)	NO	NO	107.4 (82.6–146.0)	183.4 (135.5–340.6)	NO

TGF-β = Transforming growth factor-β. IGF = Insulin-like growth factor. NO = Not obtained.

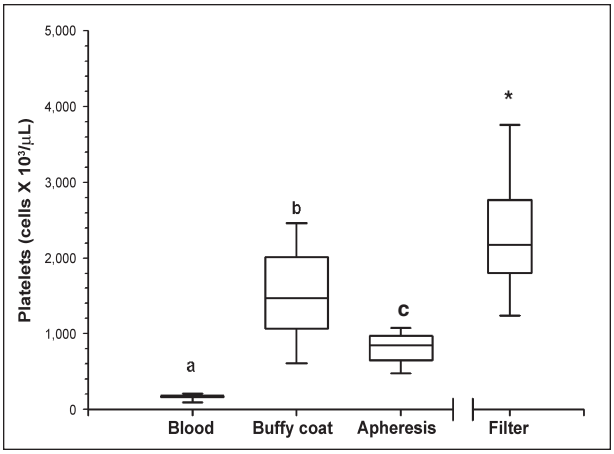

Figure 3—Box plots of the median values of automated platelet counts of whole blood samples, platelet concentrates (obtained by use of the buffy coat and apheresis methods), and filtered apheresis platelet concentrate. [a,b]Different letters designate significant ($P < 0.05$) differences among groups. *Significant ($P < 0.05$) differences between apheresis platelet concentrate and filtered apheresis platelet concentrate. Whiskers represent the 95% confidence interval.

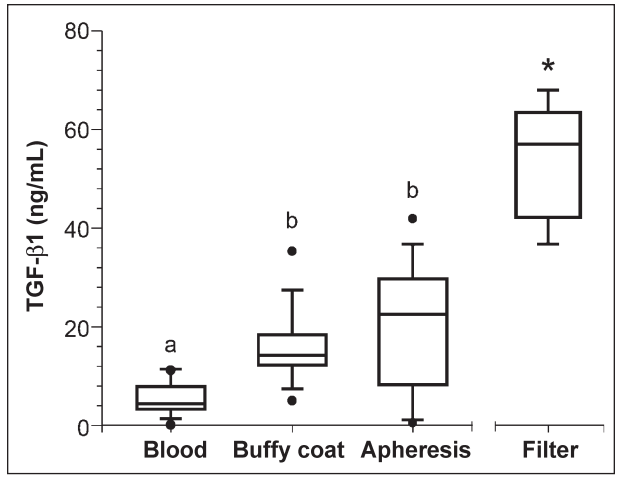

Figure 4—Box plots of the median values of transforming growth factor (TGF)-β1 concentrations of whole blood samples, platelet concentrates (obtained by use of the buffy coat and apheresis methods), and filtered apheresis platelet concentrate. *See* Figure 3 for key.

ume of blood required for each method was 50 mL for the buffy coat method and approximately 370 mL for the apheresis method.

Platelet counts, WBC counts, PCV, and growth factor concentrations were determined (**Table 1**). Platelet concentrates obtained by use of both methods had a significant ($P < 0.001$) increase in the platelet count, compared with whole blood samples. Buffy coat platelet concentrates had a significant ($P < 0.001$) increase in platelet counts, compared with apheresis platelet concentrates. Platelet concentrations were 8.9-fold and 5.2-fold greater in buffy coat platelet concentrates and apheresis platelet concentrates, respectively (**Figure 3**), compared with whole blood samples. Use of the apheresis method followed by filtration resulted in a 13.1-fold increase in the concentration of platelets, compared with that of whole blood samples. Filtered apheresis platelet concentrates had a significant ($P < 0.001$) increase in the concentration of platelets, compared with prefiltered apheresis platelet concentrates. Platelet numbers were significantly ($P < 0.001$) decreased in the platelet-poor fractions, compared with whole blood samples.

The WBC counts in the platelet concentrates obtained by both methods were significantly ($P < 0.001$) greater than in whole blood samples, but were not significantly different from each other. Use of the apheresis method resulted in concentrates with significantly ($P < 0.001$) more RBCs, compared with use of the buffy coat method. The apheresis method also resulted in concentrates with a significant ($P < 0.001$) decrease in the number of RBCs, compared with whole blood samples. The concentration of RBCs in buffy coat platelet concentrates was significantly ($P < 0.001$) greater than in whole blood samples.

Growth factor analysis—The concentration of TGF-β1 in buffy coat platelet concentrates or apheresis platelet concentrates were significantly ($P < 0.001$) increased, compared with the concentration of TGF-β1 in whole blood samples. The concentration of TGF-β1 in buffy coat platelet concentrates or apheresis platelet

concentrates was not significantly different (Table 1; **Figure 4**). The TGF-β1 concentrations in platelet-poor fractions were significantly ($P = 0.008$) lower than in whole blood samples. Both platelet counts and TGF-β1 concentrations were significantly ($P < 0.001$) increased in filtered apheresis platelet concentrates, compared with prefiltered apheresis platelet concentrates.

The concentration of TGF-β2 was significantly ($P = 0.018$) increased in apheresis platelet concentrates, compared with whole blood samples. No significant differences were found between the TGF-β2 concentrations in whole blood samples and buffy coat platelet concentrates (**Figure 5**). Buffy coat platelet concentrates had significantly ($P < 0.001$) decreased IGF-1 concentrations, compared with that of whole blood samples and apheresis platelet concentrates (**Figure 6**).

Platelet counts and TGF-β1 concentrations were positively correlated when evaluated across all blood and platelet fractions or between platelet concentrates and whole blood samples ($P < 0.001$; $r_s = 0.680$). In buffy coat platelet concentrates, a positive correlation was found between WBC counts and TGF-β1 concentrations ($P = 0.001$; $r_s = 0.680$). Within individual platelet-concentrate fractions, platelet count did not correlate with TGF-β1 concentrations. In apheresis platelet concentrates, a correlation was not found between the WBC count and the TGF-β1 concentration and between the WBC count and the TGF-β2 concentration.

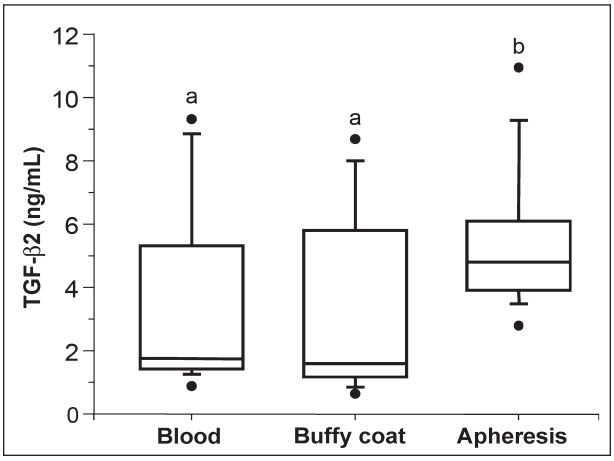

Figure 5—Box plots of the median values of TGF-β2 concentrations of whole blood samples, platelet concentrates (obtained by use of the buffy coat and apheresis methods), and filtered apheresis platelet concentrate. *See* Figure 3 for key.

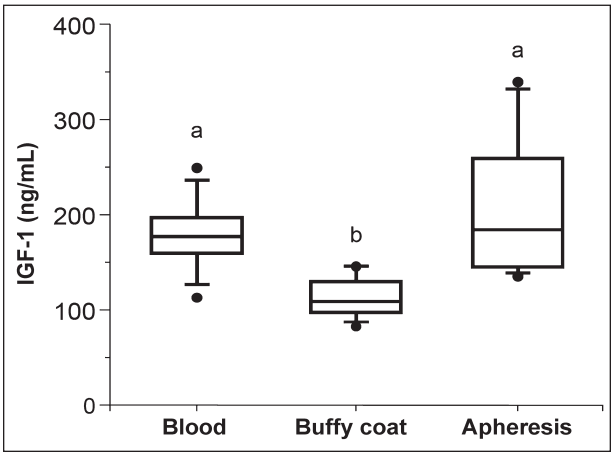

Figure 6—Box plots of the median values of insulin-like growth factor (IGF)-1 concentrations of whole blood samples, platelet concentrates (obtained by use of the buffy coat and apheresis methods), and filtered apheresis platelet concentrate. *See* Figure 3 for key.

Discussion

Platelet counts in buffy coat platelet concentrates and apheresis platelet concentrates of horses in our study were similar to results in the human literature; both methods resulted in times the blood platelet concentration.[25,29] Setup and processing times for the apheresis method were longer than for the buffy coat method, and the retail unit cost and cost for necessary disposables were also greater for the apheresis method. Therefore, the buffy coat method has advantages for use in horses. The apheresis unit has more applications than platelet concentration, including surgical blood salvage and blood component collection that offers advantages in human medicine. The possibility of bacterial contamination of concentrates may be greater when using buffy coat methods rather than apheresis methods (0.32% vs none, respectively), which are performed in completely closed disposable systems.[27]

Both methods were successful in concentrating platelets from equine blood without adjustments to the human protocols. Buffy coat platelet concentrates had significantly higher platelet counts than apheresis platelet concentrates, but the 1 cycle of processing (two 50 mL tubes) resulted in a much lower overall final volume of platelet concentrate (8 to 12 mL vs 45 mL). Therefore, the total number of platelets available for treatment is less. Additionally, use of the apheresis method followed by filtration resulted in the greatest platelet concentration. To achieve a similar total volume of platelets as obtained with the apheresis method, 4 cycles of the buffy coat method with 400 mL of blood would need to be performed.

Apheresis platelet concentrates had a significantly lower PCV than buffy coat platelet concentrates. This is a function of how many packed RBCs remained in the tube (ie, buffy coat method) or how many packed RBCs are allowed to flash into the platelet concentrate collection bag (ie, apheresis method). The importance of increased RBC numbers with regards to treatment is not known, but it may be of particular concern if the platelet concentrate is used on a patient other than the donor. Use in horses is anticipated to be primarily autologous transfer. Optional modifications of the buffy coat method to deplete most RBCs in the platelet concentrate are included in the latest human protocols. The overall effect of RBC depletion on growth factor concentrations is yet to be determined and may be substantial because the more dense platelets cannot be effectively separated from less dense RBCs.

Characteristically, WBC counts in the platelet concentrate obtained by both methods were significantly higher than in whole blood samples. Most platelets are located within the buffy coat. Although high WBC

counts are unacceptable in blood preparations used for hemotherapy as the result of increased transfusion reactions, they are generally accepted in platelet concentrates used for autologous topical application or as an additive to a bone graft. It is a matter of speculation as to whether they are beneficial (ie, additional growth factors and antimicrobial effects) or deleterious to wound healing. Again, this may raise concerns if the platelet concentrate is used in a patient other than the donor.

Few studies have evaluated blood, serum, and plasma concentrations of TGF-β1 and TGF-β2 in horses. Studies on the use of the same ELISA kits as in our study reported circulating equine serum concentrations of latent TGF-β1 of 5 to 18 ng/mL[31] and TGF-β2 concentrations of 1.75 ng/mL.[32] These data were similar to our findings in whole blood samples in which the TGF-β1 concentration had a median value of 5.5 ng/mL and TGF-β2 concentration had a median value of 1.19 ng/mL.

The TGF-β1 was concentrated by use of both methods. Although not significantly different, apheresis platelet concentrates had higher TGF-β1 concentrations, compared with buffy coat platelet concentrates. The difference in TGF-β2 concentrations was significant between apheresis platelet concentrates and buffy coat platelet concentrates. The concentration of TGF-β2 in buffy coat platelet concentrates was not significantly different from that of whole blood samples. We do not have an explanation as to why TGF-β2 was not concentrated in buffy coat platelet concentrates because TGF-β1 and platelets were reliably concentrated in these samples.

Within individual platelet concentrate fractions and whole blood samples, platelet counts were not significantly correlated with TGF-β1 and TGF-β2 concentrations in our study. Other studies[23-25,30] in humans have had similar findings. In contrast, in at least 1 other study, TGF-β1 was found to be highly correlated with platelet counts.[29] We suspect that as a result of the narrow range of platelet counts in the individual platelet-concentrate fractions, along with the high number of platelets, that our finding is related to an error inherent in platelet counting, particularly in horses. Although generally acceptable with regard to precision and accuracy when measuring platelet concentrates, hematology analyzers are calibrated for whole blood samples. Analytic errors can be introduced when platelet concentrate samples are diluted (necessary in our study for samples with platelet counts > 1,000,000/μL). Also, platelet aggregates can be formed during product sample collection.[33] In horses, platelets have been shown to aggregate easily, particularly when exposed to higher hematocrits or mechanical stimulation.[34] More advanced techniques for platelet enumeration such as fluorescent dyes and monoclonal antibodies could be used, but this would not help achieve the goal of developing a simple method for rapidly assessing the quality of platelet concentrate before surgery. When we looked for associations between platelet counts and TGF-β1 concentrations across all whole blood samples and platelet concentrates, we found a strong positive correlation (r_s = 0.680; $P < 0.001$). Therefore, it can be concluded from our data that if platelets are concentrated by use of the buffy coat or apheresis methods, TGF-β1 should also be concentrated. Comparative platelet counts between whole blood samples and platelet concentrate from the same sample is a crude screening test that can be performed by clinicians to assess the quality of the platelet concentrate prior to use. This association is supportive of the assumption that most of the TGF-β1 is platelet derived.

Significant positive correlations were found between WBC count and TGF-β1 concentrations in buffy coat platelet concentrates (r_s = 0.680). This suggests that WBC counts may be valuable in assessing the quality of platelet concentrates obtained by this method. One possible explanation for the strong, significant association between TGF-β1 concentration and WBC count in these concentrates may be the result of relative greater accuracy of counting WBCs, compared with platelets, in the platelet concentrate.

Although IGF-1 was present in platelet concentrates, IGF-1 concentrations were not increased, compared with IGF-1 concentrations in whole blood samples. In fact, concentrations of IGF-1 were significantly lower in buffy coat platelet concentrates, compared with apheresis platelet concentrates and whole blood samples. Results of several studies[21,23,24,30] in humans have revealed similar results. The plasma pool of IGF-1 is greater than the platelet pool in humans. Results of 1 study[35] revealed that the human platelet-derived IGF-1 accounts for < 0.1% of the total circulating concentration of IGF-1 in blood. Therefore, in our study, IGF-1 concentrations in platelet concentrate were assumed to be a function of how much plasma remained within the sample. The buffy coat technique removed most of the plasma from the sample in the last step of processing.

In our study, platelet concentrations were increased by 8.9-fold in apheresis platelet concentrates and by 5.2-fold in buffy coat platelet concentrates, compared with platelet concentrations in whole blood samples. Correspondingly, TGF-β1 was concentrated 2.8-fold and 3.1-fold. The TGF-β2 concentration was increased by 3.6-fold in apheresis platelet concentrates, compared with concentrations in whole blood. Further concentration of platelets obtained by use of the apheresis method is possible when followed by further filtration; this procedure resulted in a 10.5-fold increase in the TGF-β1 concentration. Platelet counts were positively associated with TGF-β1 concentrations in whole blood samples. Results of our study indicate that platelet counts can be compared between whole blood samples and platelet concentrates to indicate whether TGF-β1 has also been concentrated.

Overall, findings in our study indicate that platelet concentrates that are obtained from equine blood by use of the buffy coat or apheresis methods contain increased concentrations of growth factors, specifically TGF-β1, compared with whole blood. The buffy coat method has an advantage of less cost and time for processing, compared with the apheresis method. An advantage in concentration of TGF-β2 and IGF-1 can be obtained by use of the apheresis method. Filtration can additionally increase platelet counts and TGF-β1

concentrations in apheresis platelet concentrates. Platelet concentrate derived from either method may be useful in treating nonhealing wounds or enhancing bone grafts during surgery in horses. Future studies to assess the biological effects and further characterize platelet concentrates are warranted.

[a]Dynavet set 0.9, Vet Dynamics Inc, Templeton, Calif.
[b]Sequire kit, PPAI Medical, Ft Myers, Fla.
[c]Cell Saver 5, Haemonectics Corp, Braintree, Mass.
[d]Bowl set #261, Haemonectics Corp, Braintree, Mass.
[e]Burette Assembly, Haemonectics Corp, Braintree, Mass.
[f]Interpore filter #U100, Interpore Cross International, Irvine, Calif.
[g]Cell-Dyn 3500, Abbott Laboratories, Whippany, NJ.
[h]MPER, #78501, Pierce Biotechnology Inc, Rockford, Ill.
[i]Arm D, Interpore Cross International, Irvine, Calif: Personal communication, 2002.
[j]TGF-β1 Quantikine ELISA kit (#DB100), R & D Diagnostics, Minneapolis, Minn.
[k]TGF-β2 Quantikine ELISA kit (#DB250), R & D Diagnostics, Minneapolis, Minn.
[l]IGF-1 ELISA (#DSL-10-5600), Diagnostic System Laboratories, Webster, Tex.
[m]Sigma Stat, v2.06, SPSS Inc, Chicago, Ill.

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