AUTOMATED PLASMAPHERESIS IN HORSES: REPORT OF TWO CASES

PLASMAFÉRESE AUTOMATIZADA EM EQUINOS: RELATO DE DOIS CASOS

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SUMMARY

Plasmapheresis is a procedure that consists of removing the blood from a donor, followed by subsequent plasma separation and reinfusion of the remaining elements. This procedure can be manual or automated, with a few reports of automated plasmapheresis in the equine specie. This article reports two cases of automated plasmapheresis in horses that were conducted to obtain hyperimmune plasma, while evaluating the complications inherent to the technique; as well as variation and recuperation of hematocrit and serum total proteins in the donors. The equipment *Fresenius* AS 104 was used to remove 20 % of total plasma with the initial sodium citrate ratio of 1:12 in the extracorporeal system. During the procedure, the reinfusion catheter was obstructed by blood clots and jugular vein phlebitis was observed in the donors. The recovery of hematocrit and total protein serum levels occurred in 96 hours. Despite the satisfactory recovery of hematimetric levels in the two animals, further research is needed into the suitability of automated plasmapheresis in horses, in an attempt to reduce the complications inherent to the technique.

KEY-WORDS: Automation. Complications. Equine Medicine. Hematimetrics values. Plasma collection.

RESUMO

A plasmaférese é um procedimento que consiste na retirada do sangue total de um doador e em seguida, separação do plasma com reinfusão dos elementos remanescentes. Este procedimento pode ser realizado de forma manual ou automatizada, com poucos relatos da técnica automatizada na espécie equina. Este artigo busca relatar a plasmaférese automatizada em dois equinos, para obtenção de plasma hiperimune, avaliando-se as complicações inerentes à técnica; e a variação e recuperação do volume globular e níveis séricos de proteínas totais nos animais doadores. Para os relatos foi utilizado o equipamento *Fresenius* AS 104, objetivando-se retirada de 20% do plasma total circulante com a proporção inicial de citrato de sódio de 1:12 no sistema extracorpóreo. Durante o procedimento observou-se obstrução por coagulação sanguínea no cateter de reinfusão e flebites jugulares nos doadores. A recuperação do volume globular e níveis séricos de proteínas totais ocorreu em 96 horas. Apesar da satisfatória recuperação hematimétrica nos dois animais, há a necessidade de novas pesquisas para a adequação da plasmaférese automatizada em equinos, na tentativa de diminuir as complicações inerentes à técnica.

PALAVRAS-CHAVE: Automação. Colheita de Plasma. Complicações. Medicina Equina. Valores hematimétricos.

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Plasmapheresis consists of removing whole blood from a patient using an anticoagulant bag (typically 20% sodium citrate) followed by plasma separation, which can be conducted by settling, centrifugation or specific filtering of the red cells. The remainder of the blood is infused back into the patient either manually or in an automated way, this procedure is indicated for removal of a substance or blood component present in excessive amounts in the circulation or simply to obtain plasma rich in antibodies specific to a given disease or poison (AABB, 2002). According to Marques Jr. et al. (2011), plasmapheresis can also be therapeutic, used to treat autoimmune diseases or to collect plasma.

Currently in Veterinary, the indications for plasma infusion include deficiency in hemostasis, transfer of passive immunity, immunosuppression, hypoproteinemia and hypovolemia. Also in horses, fresh or fresh frozen plasma is indicated for the treatment of diseases that cause hypoproteinemia, when acute blood volume expansion is needed; when passive immunity transfer fails; or to provide specific immunity against *Rhodococcus equi* and *Salmonella typhimurium* (HUNT & MOORE, 1990; SMITH & SHERMAN, 1994; DURHAM, 1996; COLLATOS, 1997; STONEHAM, 1997; POST, 2000). Therefore, plasmapheresis becomes increasingly necessary in the production of hyperimmune plasma for clinical treatment of immunosuppressed animals.

Automated plasmapheresis is widely performed in humans using a disposable sterile plastic loop attached to the apheresis equipment, which separates the plasma by centrifugation or microfiltration. The blood circulates exclusively in this circuit, not coming in direct contact with the equipment. Feige et al. (2003) compared automated plasmapheresis with the manual technique in horses and concluded that the former is well tolerated by the animals, faster and enables large scale production of good quality horse plasma.

This paper reports two cases of transfusion by automated plasmapheresis in horses, in order to obtain hyperimmune plasma while observing the complications that may arise during the procedure; as well as analyzing the recovery of total serum proteins (Pt; g/dL) and hematocrit (Ht; %) levels up to 30 days after the procedure.

Two male mongrel horses aged 7 (EQ1) and 12 (EQ2) years old, weighing 320 and 350 kg, respectively, were used. The animals were previously dewormed using a paste with 2% Moxidectin base (Equest - Fort DodgeAnimal Health-Iowa, USA) and after 7 days vaccinated against Eastern and Western Encephalomyelitis (dead virus) and tetanus toxoid (Equiloid - Fort Dodge Animal Health – Iowa, USA); rhinopneumonitis and Influenza (Fluvac Innovator EHV 4/1 - Fort Dodge Animal Health - Iowa, USA) and Rabies (Rubivac - Pfizer Saúde Animal - Guarulhos, Brazil). Reinforcement doses of the vaccines were given 28 days later, and 20 days after that the procedures were conducted, at the time when the immune response peaks according to Souza (2011).

Upon pre-apheresis (M1), Ht and Pt of EQ1 and EQ2, were measured and reported as: Ht - 34% and 35%; and Pt – 8.4 and 9.2 g/dL, respectively, within the normal range for the species according to Souza Netto (2011). Ht and Pt were measured again at the following times: 24 hours (M2), 48 hours (M3), 72 hours (M4), 96 hours (M5) and 30 days (M6).

The measurement to evaluate Pt and Ht plasma recovery was conducted using the kit Pt Labtest® (Labtest Diagnóstica S/A - Lagoa Santa, Brazil) and the spectrophotometer Photonics 2000UV (Bel Equipamentos Analíticos Ltda. - Piracicaba, Brazil); while Ht was obtained by centrifuging the samples in tubes of micro-hematocrit at 14,000 g, for 5 minutes in a microhematocrit centrifuge (Microspin Gemmico Industrial Corp. – Taiwan, China), and further reading on a special scale.

Automated plasmapheresis was performed using a specific kit PL1 (Fresenius HemoCare do Brasil) coupled to the equipment *Fresenius* model AS104, which separates the plasma from the blood by automated centrifugation at 671 g. The PL1 kit is coupled to the equipment, together with 0.9% saline solution to replace the volume of the plasma removed, plus 20% sodium citrate to avoid extracorporeal blood clotting.

The left jugular vein was punctured with a 40X16 needle (Prod. Hospitalares DI - Belo Horizonte, Brazil), called the harvest vein, and the right jugular vein with the 14G catheter (Nipro Medical Ltda – Sorocaba, Brazil) was called the reinfusion vein. The recommended protocol, based on the procedures at the Blood Center of Unicamp and cited by Feige et al. (2003) and Feige et al. (2005), began at 1:12 ratio of anticoagulant solution (20% sodium citrate) in relation to whole blood processed.

At the end of plasmapheresis, the bags with the collected plasma were sent immediately to separate the plasma into sterile 500 mL individual bags (Fresenius HemoCare do Brasil Ltda. - Itapecerica da Serra, Brazil) under laminar flow hood (Filtracom - Valinhos, Brazil) and subsequently frozen at -18ºC.

The plasma volume to be collected was calculated considering 8% of total weight of the donor as blood absolute value and average hematocrit 35% (SOUZA NETTO, 2011). Thus, approximated total plasma value is 65% of total blood volume. Based on this, removal volume of 20% of total circulating plasma volume was calculated as 3,328 and 3,640 mL for EQ1 and EQ2, respectively.

Initially, the distribution of the response variables Pt and Ht were analyzed and descriptive statistics produced. Linear model repeated measures (SILVA & AZEVEDO, 2009) were used to compared the means Pt ou Ht at different times. The unstructured covariance structure was the most appropriate model for the repeated measures in the same animal. Tukey test (KLEINBAUM et al., 2007) was used to adjust P value resulting from multiple comparisons at different times. Statistical analysis was performed using SAS software (SAS INSTITUTE, 2009) at significance level $P< 0.05$.

The technique used presented two complications in the first repetition. The first one was the inability to keep the harvesting vein with the needle, which had to be handled eight times, thus causing mild phlebitis in EQ1. The second was the presence of blood clots in the reinfusion vein, which prevented blood flow. In an attempt to avoid obstruction of the vein, a diluted heparin solution was used, 25 000 UI heparin (Hepamax-s® - Blausiegel Ind. e Com. Ltda. - Cotia, Brasil**)** diluted in 500 mL of 0.9% saline solution (Fresenius Kabi Deutschland - Friedberg, Germany) that was injected directly in the catheter of the reinfusion vein every time 100 mL of blood was collected. Still phlebitis occurred in the right jugular vein, which became nonviable. At this time, it was necessary to puncture the right cephalic vein using a catheter 18G in order to return the blood. The volume of blood processed in EQ1 was 4,313 mL that yielded 2,580 mL of harvested plasma, and was replaced with 1,500 mL of 0.9% saline solution.

In EQ2, the anticoagulant ratio was increased to 1:10 (20% sodium citrate) and the needle was replaced by a catheter 14G in the harvesting vein; however, it was still necessary to use the heparin solution in the catheter in the reinfusion vein every 300 mL of harvested plasma, and yet the vein was obstructed three times. The processed blood volume of 6,350 mL yielded 2,578 mL of plasma, and was replaced with 1,600 mL of 0.9% saline solution. In EQ2, the higher blood fluidity allowed to process a volume 47.23% higher than in EQ1, which shortened the harvesting time 50 minutes. Harvesting times were 150 and 100 minutes for EQ1 and EQ2, respectively, with average processing of 5331,50 ± 1440,38 mL of blood and harvesting of 2,579 ± 1,41 mL of plasma.

The average values of Ht at harvesting times during the procedure were not significantly different ($P<0.005$) (Figure 1).

Although the changes were not statistically significant, a maximum decrease of 14.28% of Ht was observed in EQ1 24 hours after the procedure; however, at time M3 the value was already close to the initial, different from the hemoconcentration balanced with hydration reported by Ângulo et al. (1997). Probably, this hemodilution is related to the hemolysis caused by the sodium citrate that returns to the animal with the blood components that were separated from the plasma.

Mild or severe anaphylactic events have been reported in the literature during automated plasmapheresis, mainly related to sodium citrate, such as itching, hives, blepharospam, tearing, dry cough, shortness of breath and rales in the airways (MARQUES JR. et al., 2011), however, none of these reactions were observed in the horses of this study.

The mean Pt values displayed in Figure 2, show significant changes at times M2, M3 and M4; non-significant values at times M5 and M6 ($P<0.001$) and Pt plasma recovery in 96 hours average.

According to Parra (2005), manual plasmapheresis in horses lasts 4 days, divided into three stages, and the recovery of the donor horse takes between 45 and 60 days after the procedure. In this report, the automated plasmapheresis yielded Pt recovery levels of 78.25% and 100% in EQ1and EQ2, respectively; only 96 hours after the procedure took place.

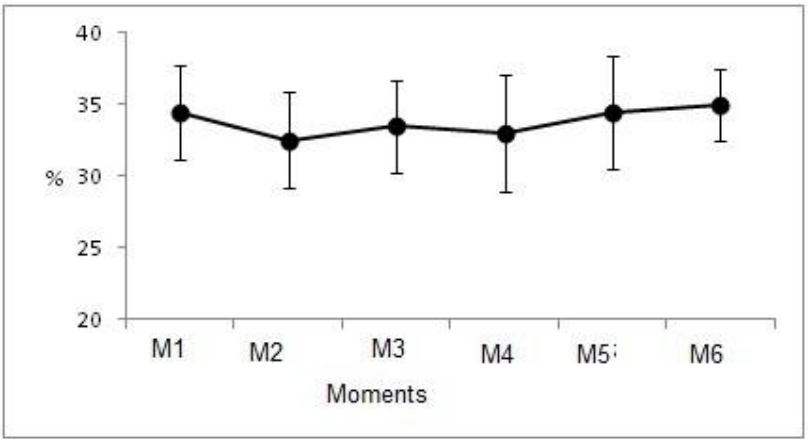

Figure 1 - Average hematocrit values - Ht (%) of horses during harvesting times. Pre-apheresis = M1, M2 = 24 hours later, M3 = 48 hours later, M4 = 72 hours later, M5 = 96 hours later, M6 = 30 days later.

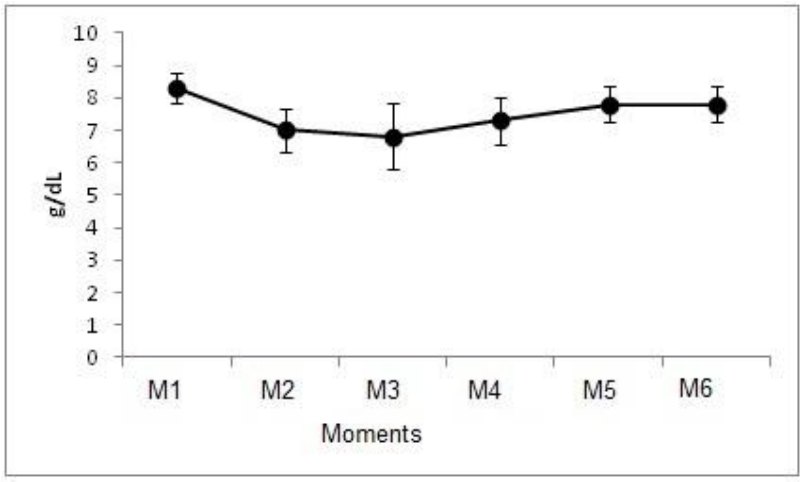

Figure 2 - Average total protein values - PT (g/dL) of horses at different times. M1 = Pre-apheresis, and, M2 = 24 hours after, M3 = 48 hours after, M4 = 72 hours after, M5 = 96 hours after, M6 = 30 days after.

Despite the rapid recovery of cell volume and Pt in donor horses, the operational complications such as phlebitis and decreased fluidity with obstruction of the reinfusion vein evidenced the non-reproducibility of the automated plasmapheresis technique reported by Feige et al. (2003) for Freiberger horses and by Feige et al. (2005) in blood donor horses in Switzerland. There may be differences in sodium citrate concentration and specific processing for different horse breeds. Therefore, the reports show the need for further research on automated equine plasmapheresis to seek the adequacy and standardization of the technique for commercial production of hyperimmune plasma or therapeutic procedure innovation in equine species.

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