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Pre or perioperative period: when is the best time to produce platelet-rich plasma in dogs?

[*Pré ou trans-operatório: qual o melhor momento para a produção do plasma rico em plaquetas em cães?*]

"Scientific Article/Artigo Científico"

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Abstract
Published studies about production of platelet-rich plasma (PRP), demonstrate differences related to the moment in when blood should be collected and processed. Blood collection could be performed before surgery, prior to the administration of drugs, fluids, or any surgical trauma, while others collect blood during the perioperative period. The present objective was to determine if blood collection and processing in the pre or perioperative periods would result in different platelet concentrations, and consequently, what period is recommended to prepare a best PRP. Blood samples were obtained from 20 healthy female dogs at two distinct moments: before surgery, prior to the administration of drugs and fluids (M0), and later, in the operation room, after administration of anesthetics and intravenous fluids (M1). To obtain PRP, blood was centrifuged at 1200 rpm for ten minutes, then at 1600 rpm for ten minutes. Results showed that blood collected at M1 had a 29% decrease in platelet count compared to M0 (highly significant statistical difference), what interferes directly in PRP quality, and therefore, it is concluded that blood collection and PRP processing should be realized at M0.

Keywords: surgery; platelet concentrate; growth factors.

Resumo
Trabalhos publicados sobre a produção de plasma rico em plaquetas (PRP) apresentam diferenças com relação ao momento de coleta e/ou processamento do sangue. Alguns autores relatam a coleta pré-operatória, antes da administração de fármacos, fluidos ou trauma cirúrgico, enquanto outros descrevem esses procedimentos sendo realizados durante o período transoperatório. Neste artigo se objetivou determinar se a coleta de sangue e processamento do PRP no período pré ou transcirúrgico pode resultar em uma diferente concentração plaquetária, e consequentemente, qual é o período recomendado para preparar um melhor PRP. Foram coletadas amostras de sangue de 20 cadelas hígidas em dois momentos distintos: na etapa pré-cirúrgica, antes das pacientes serem submetidas à anestesia e fluidoterapia (M0) e, posteriormente, na sala de cirurgia, após a administração dos fármacos anestésicos e fluido intravenoso (M1). Visando a obtenção do PRP, foi empregada uma metodologia em que o sangue foi centrifugado a 1200 rpm durante dez minutos e na sequência, a 1600 rpm por dez minutos. Como os resultados alcançados demonstraram que a coleta de sangue realizada no M1 apresentou uma redução de 29% na concentração plaquetária com relação à M0 (diferença estatística altamente significativa), o que interfere diretamente na qualidade do PRP, e portanto, conclui-se que a coleta de sangue e processamento do PRP deve ser realizada em M0.

Palavras-chave: cirurgia; concentrado de plaquetas; fatores de crescimento.

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Introduction

Platelet-rich plasma (PRP) is a biological product obtained from centrifugation of blood that has a platelet concentration two to five times higher than whole blood (Garcez et al., 2016; Alves and Grimalt, 2018), in which these PRP products present biotechnology with great results in stimulating regenerative processes (Imam, 2022).

The purpose in concentrating platelets is that they release growth factors (GFs), which induce angiogenesis, mitogenesis, chemotaxis, matrix synthesis, and tissue differentiation (Alves and Grimalt, 2018), being beneficial in the improvement of pain relief and function reestablishment (Thepsoparn, 2021). Another advantage is that PRP has the ability to neutralize invading pathogens, controlling bacterial growth, similar to the action of antibiotic therapy (Sethi et al., 2021).

Various protocols have been developed for obtaining PRP, but these protocols vary widely in amount of blood collected volume, number of centrifugations and speed, and equipment used (Franklin et al., 2015; Conceição et al., 2017; Miranda et al., 2019).

The ideal moment (pre or perioperative period) for production of PRP has not been previously discussed, but may exert some influence on platelet count of the final product. Some researchers prefer to draw and process blood prior to surgery (Singh et al., 2017; Kemper et al., 2018), while others opt to collect blood during surgery and/or with the patient under anesthesia (Franklin et al., 2015; Dalgin et al., 2017; Al-Dirawi et al., 2018). However, other factors other than time needed for preparing PRP should be taken into consideration, such as influence of drugs or fluids being administered.

The objective of this study was to analyze whether blood collection and processing in pre or perioperative periods would result in differences in platelet concentrations in PRP. To the authors' knowledge, this is the first study to perform this comparison.

Material and Methods

The experimental group was composed of 20 female dogs between two and five years of age and weighing among ten and 20 kg. Physical examination, hemogram, and serum biochemistry (ALT, AST, urea, and creatinine levels) were obtained for all dogs to confirm that they were healthy and able to undergo elective ovariohysterectomy (OH).

Blood was drawn from each patient at two distinct moments: in the preoperative period, prior to anesthesia or fluids (M0) and in the perioperative period, after the patient was anesthetized and receiving fluids, but before the start of surgery (M1). At each moment, 6.5 mL of blood were collected, via a vacuum collection system, into two tubes. One tube had sodium citrate as an anticoagulant for obtaining PRP and a platelet count (4.5 mL) and the other had ethylenediaminetetraacetic acid (EDTA) for obtaining a platelet count from whole blood (2 mL).

The blood sample was obtained from the cephalic vein while patient was still in the surgical preparation room. Blood collected for PRP production underwent centrifugation at 1200 rpm for ten minutes in a regular laboratory centrifuge (Benchtop Centrifuge Baby®, I Model 206 BL – Fanem®). Next, the plasma and buffy coat found above the red blood cells deposited at the bottom of the tube were transferred to a second sterile tube without additives using a precision pipet and sterile tip. This tube was then centrifuged at 1600 rpm for ten minutes. In sequence, approximately 80% of the supernatant was discarded, leaving only the final part of the plasma and the platelet pellet. This material was gently shaken to resuspend the platelets, resulting in PRP. A platelet count was then obtained from the PRP and from the whole blood using Rees-Ecker diluting fluid and Neubauer counting chamber.

Simultaneously, anesthetic procedures were performed to prepare the patient for surgery. The anesthetic protocol used was acepromazine hydrochloride at 0.1 mg/kg, given intramuscularly (IM) with 1 mg/kg of tramadol hydrochloride, also IM, as anesthetic pre-medication. After tranquilization, the patient was taken to the operating room and maintenance fluids (lactated Ringer's 3 mL/kg/h) and propofol (4 mg/kg) were given intravenously via the cephalic vein. Maintenance of the anesthetic plane was done with isoflurane in 100% free oxygen over a semi-closed circuit after orotracheal intubation.

The patient was placed in dorsal decubitus on the surgical table and the second blood collection from the cephalic vein was performed (M1), approximately 25 minutes after M0. The steps for production of PRP and for obtaining

platelet count from PRP and whole blood were the same as described for M0.

To calculate the necessary sample size, an entirely random process was chosen, with a stipulated error of 10%. Statistical analysis was done considering a completely random design, using Tukey's test at a significance level of 1%. Statistical analysis was performed using SYSTAT 10 software (demo version).

Results and Discussion

The methodology used to obtain and produce platelet-rich plasma directly influences its effects. Therefore, it is necessary to establish standardized and reliable protocols and methods for obtaining and applying PRP (Silva, 2021). Nonetheless the authors have not found published studies comparing blood collection and PRP production in the preoperative and perioperative periods. This is thus a novel research, though various studies have evaluated the influence of factors such as centrifugation speed, equipment, and type of anticoagulant or protocol used (Franklin et al., 2015; Carr et al., 2016; Conceição et al., 2017; Miranda et al., 2019). This study was developed based on the fact that there is no consensus between researchers, where some perform blood draw and PRP processing during the preoperative period (M0), and others during the perioperative period after the administration of fluid therapy and anesthetic drugs (M1), without the knowledge whether this can affect the final quality of the PRP.

The results obtained from Tukey's test (significance level of 1%), demonstrated a significant difference between platelet count in the PRP from M0 and M1 (Table 1). The same pattern was observed in platelet count in whole blood and PRP, where values obtained at M0 were higher than those at M1 (Figure 1). When the number of platelets in whole blood decreased at M1, the number of platelets in PRP also reduced.

Table 1. Platelets mean values for the analyzed treatments using Tukey's test at a significance level of 1%. Blood samples were obtained before surgery, prior to the administration of drugs and fluids (M0) and later in the operation room, after administration of anesthetics and intravenous fluids (M1).

	Whole blood M0	Whole blood M1	PRP M_0	PRP M_1
Mean	255700	186900	1353450	960100
SD	39126,1	25527,9	213376,0	145100,7
CV%	15,3	13,7	15,8	15,1

Where: Mean = arithmetic mean; SD = standard deviation; CV% = coefficient of variation in percent; PRP = platelet-rich plasma.

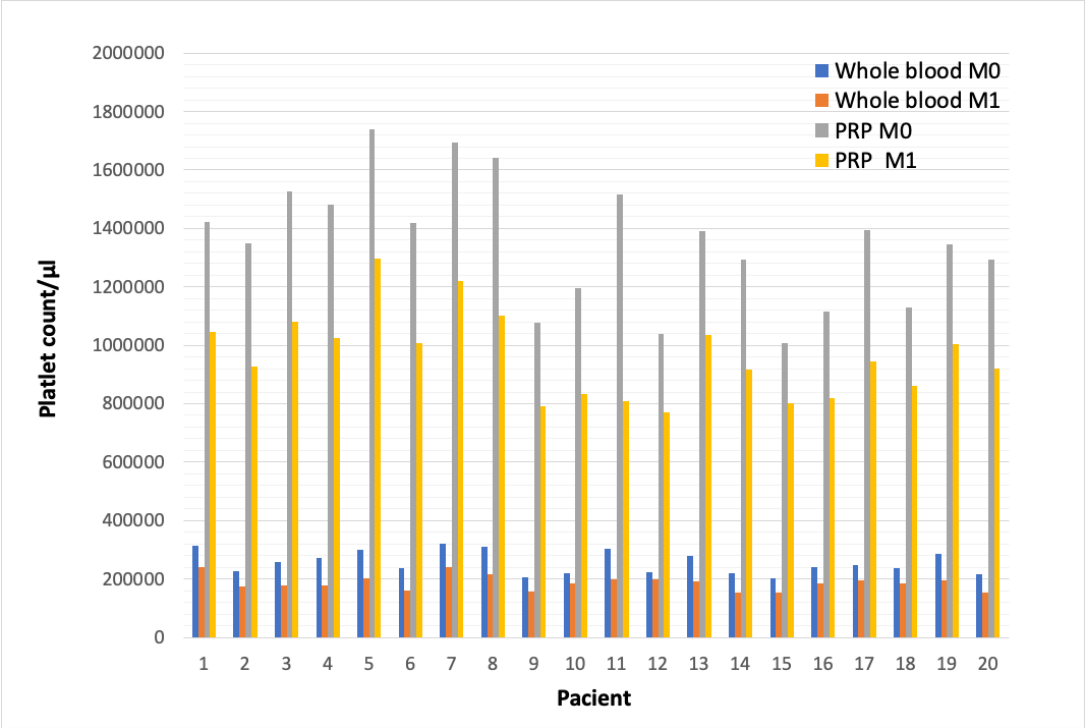

Figure 1. Platelet count for whole blood and platelet-rich plasma (PRP/µL) at M0 (blood samples obtained before surgery, prior to the administration of drugs and fluids) and at M1 (blood samples obtained later in the operation room, after administration of anesthetics and intravenous fluids.

According to Barbosa et al. (2008), platelet count in PRP is directly proportional to the concentration in whole blood, being usually two to five times the baseline value (Alves and Grimalt, 2018). The platelet count obtained on total blood and PRP in this research, demonstrated that the technique provided a four to five times increase on the count. A high level of platelets, remaining above baseline allows for rapid soft tissue regeneration with few adverse effects (Imam, 2022). When analyzing percentual values, blood collection at M1 had a 29% decrease in PRP platelet count compared to M0.

The evident decrease in PRP platelet count at M1 may compromise the therapeutic action of the product because, according to Foster et al. (2009), at least 1,000,000 platelets/µL are needed for PRP to exert its biological function. At M1, this value was not obtained in some dogs and therefore, if that PRP were used clinically and the results inadequate, it would be impossible to determine if those findings were related to the low platelet concentration (below recommended value) or if the product is inefficient.

The only differences between M0 and M1 which may have contributed to lower the platelet count in whole blood and PRP at M1 was the use of fluid therapy with lactated Ringer's and the drugs used during anesthetic procedure (acepromazine, tramadol, propofol, and isoflurane), since blood was drawn prior to any surgical trauma to reduce the number of variables that might interfere on platelet count. According to Adler and Kent (2002), surgical stimulus may initiate activation of platelets and coagulation factors, thus justifying that blood be collected before starting surgery, as done in the present study and by Pazzini et al. (2016).

Regarding studies in animals, blood has been collected from dogs after general anesthesia (Suaid et al., 2007) and from rabbits anesthetized with xylazine and ketamine (Vendramin et al., 2010). Patients will likely be receiving intravenous fluids, since fluid therapy is commonly used for maintaining a venous access and administrating drugs (Andrade, 2018). Fluid administration during surgery may interfere in platelet count and thus, in the final quality of PRP because it dilutes whole blood, which results in a decrease in platelet concentration (Chohan et al., 2011). According to Stockham and Scott (2011), administration of platelet-poor fluids (e.g. crystalloids) can result in a slight to moderate decrease in platelet count.

Some researchers choose to draw blood from the patients under anesthesia to facilitate collection, especially regarding restraint and stress during blood draw, and when protocols that need larger volumes of blood are used. However, according to Thrall et al. (2015), stress during blood collection may result in contraction of the spleen, which may be favorable for production of PRP, since the amount of circulating platelets should increase in this situation.

Elyazji et al. (2013), and Boyd et al. (2018) report several drugs may lead to a lower number of platelets and reduced function. In agreement with those authors, Fry (2010) reports there are several drugs that seem to inhibit platelet function in animals and humans, such as antibiotics (e.g. cephalosporins), antihistamines, barbiturates, halothane, heparin, and propranolol. There are various mechanisms through which different drugs may lead to thrombocytopenia, such as suppression of platelet synthesis, production of anti-platelet antibodies, platelet sequestering (O'rourke, 2010), inhibition of platelet release, increase in platelet consumption, and direct aggregation of platelets (Zimmerman, 2000), and there is little information on this topic in animals. It is known, however, that some of these mechanisms may take days to affect platelet count and, considering that thrombocytopenia at M1 was noted some minutes after drug administration, if this was indeed caused by drugs, it would have occurred due to a more immediate response (e.g. splenic sequestration).

Regarding the drugs used in the present study, Conner et al. (2012) report that acepromazine may alter platelet function by inhibiting ADP and AA. In a study by Sutil et al. (2017), between 15 and 720 minutes after administration of acepromazine in dogs, there was a decrease in hematologic values and a significant increase in size of the spleen. According to Tavares et al. (2014), when using acepromazine in dogs, after 15 minutes occurs a splenic dilation, that induced a reduction in hematocrit, probably due to splenic sequestration. In this perspective, possibly a splenomegaly after administration of acepromazine, could also result in a decrease on platelet count.

In a research performed by Elyazji et al. (2013), where tramadol was administered in rabbits for several days, there was a decrease in platelet count in almost 15% after 20 days. As for isoflurane, Ozgul et al. (2013) states that this general anesthetic decreases the amount of platelets

in humans in approximately 30%. While comparing platelet reduction in whole blood and PRP at M0 and M1, it was detected a decrease of 27% and 29%, respectively, corroborating with the results of the mentioned authors. Even if these drugs did not lower platelet count, blood collection after their administration is not recommended because they might lead to changes in platelet function, decreasing their aggregation and activation capabilities, which is not desirable for PRP, although blood collection has been done after anesthesia by several authors (Franklin et al., 2015; Pazzini et al., 2016; Dalgin et al., 2017; Al-Dirawi et al., 2018).

Another factor to consider is that the time from blood collection to use of the PRP in M0 may be directly related to the production method. Since automated devices take at most 30 minutes to produce PRP, blood can be collected between 30 minutes and an hour before surgery. Using this type of device, Conceição et al. (2017), reported a mean time of 14 minutes for production of PRP. When protocols using laboratory centrifuges are used, where the process of separating blood components is manual and thus, more arduous and time consuming, it is recommended to draw blood between two and three hours prior to surgery. With advancement of the learning curve, this time decreases, as reported by Pazzini et al. (2016), where the entire process between blood collection and obtaining the PRP took 40 minutes.

Even though blood collection at M0 may also have some use regarding the possibility of storing the material for later use, it is not recommended to be done too long before surgery. Hauschild et al. (2017), comparing different protocols of PRP, found that depending on the method, it was possible to use PRP up until six hours after production, which is within the limits suggested by Barroso et al. (2007), who states that blood derivatives processed in an open circuit should be used within a maximum of six hours. It is therefore suggested that the same recommendation should be followed for PRP, since during its storage, platelets may be activated, what would compromise the biologic function of the product (Landi and Marques Júnior, 2003). According to Weiser (2015), platelet concentration and their aggregation capacity are significantly decreased after 24 hours of storage, and it is therefore not advisable to use platelets after that.

Conclusion

In dogs the ideal moment for blood collection and processing to obtain platelet-rich plasma (PRP) with a higher platelet concentration is in the preoperative period (M0.)

Conflict of Interest

The authors report no conflicts of interest.

Ethics Committee

This research was approved by the Ethics Committee for the Use of Animals from the Federal Rural University of Pernambuco under license number 028/2015.

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